ELSEVIER

Biochimica et Biophysica Acta 1495 (2000) 140–149

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Reconstitution of *Listeria* motility: implications for the mechanism of force transduction

Donald J. Olbris, Judith Herzfeld *

Department of Chemistry (MS #015) and Keck Institute for Cellular Visualization, Brandeis University, Waltham, MA 02454-9110, USA

Received 10 August 1999; received in revised form 11 November 1999; accepted 22 November 1999

Abstract

Listeria monocytogenes and some other infectious bacteria polymerize their host cell's actin into tails that propel the bacteria through the cytoplasm. Here we show that reconstitution of this behavior in simpler media resolves two aspects of the mechanism of force transduction. First, since dilute reconstitution media have no cytoskeleton, we consider what keeps the tail from being pushed backward rather than the bacterium being propelled forward. The dependence of the partitioning of motion on the friction coefficient of the tail is derived. Consistent with experiments, we find that the resistance of the tail to motion is sensitive to its length. That even small tails are stationary in intact cells is attributed to anchoring to the cytoskeleton. Second, the comparatively low viscosity of some reconstitution media magnifies the effects of diffusion, such that a large gap will develop between the bacterium and its tail if they are unattached. At the viscosities of diluted platelet extracts, steady-state gaps of several bacterium lengths are predicted. Since such gaps are not observed, we conclude that *Listeria* must be attached to their tails. We consider what purposes such attachments might serve under physiological conditions. The implications for related pathogens and amoeboid locomotion are also discussed.

Keywords: *Listeria*; Motility; Actin; *Shigella*; *Ascaris*

1. Introduction

Listeria monocytogenes is a Gram-positive infectious bacterium that moves within a host cell by usurping control of its cytoskeleton [1–6]. By assembling actin behind themselves in 'comet' tails, *Listeria* travel at speeds generally about 0.1–0.2 μm/s, but sometimes exceeding 1.0 μm/s [7]. The tail is composed of short, cross-linked actin filaments which apparently remain stationary while the bacterium moves [8]. Actin monomers are added to the tail at the rear of the bacterium (i.e., at the front of the tail), where ActA, the sole bacterial protein necessary for motility, is localized [9–12]. When the bacterium reaches the plasma membrane, it may penetrate a neighboring cell within a filopod-like extension. If the neighboring cell phagocytoses the extension, *Listeria* can escape from the phagosome into the cytoplasm and infect the neighboring cell without having encountered the extracellular environment. From the bacterium's point of view, motility through subversion of the existing actin cytoskeleton is efficient. Building materials (actin) and tools to manipulate them (a variety of actin binding proteins) are already present in the cytoplasm. This strategy is also used by a number of unrelated microorganisms, including

* Corresponding author. Fax: (781) 7362516;
E-mail: herzfeld@brandeis.edu

0167-4889/00/$ – see front matter
PII: S0167-4889(99)00156-1

Shigella flexneri [6], members of the *Rickettsia* family [13], and the vaccinia virus [14].

The mechanism by which the energy of polymerization is transduced into physical force is an open question. One way to examine the requirements for force transduction is to exploit the recent reconstitution of *Listeria* motility in simpler media [15–19]. We focus on two (somewhat related) differences between cytoplasm and simpler reconstitution media: the cytoskeletal organization of the medium and its viscosity.

Removing the cytoskeleton from the system is important in answering a straightforward question: independent of the mechanism of force generation, why does the bacterium move forward instead of the tail backward? In cytoplasm, the answer could be trivial, because the tail may be attached by cross-linking proteins to the cell's cytoskeleton, which then provides a platform from which to propel the bacterium. However, such cytoskeletal attachments probably do not exist in diluted cell extracts and may not even exist in all regions of whole cells. To address the question, we consider a simple model based on Newton's equations of motion. We find that the density and length of the tail are crucial determinants of the partitioning of motion between the tail and bacterium in the absence of a cytoskeletal platform. This explains the dependence of *Listeria* motility on tail length in reconstituted systems. On the other hand, it implicates anchoring to the cytoplasm as the basis for the observation that even small tails are stationary in intact cells.

Reducing the viscosity of the medium is also important in determining the role of diffusion in the motility mechanism. *Listeria* motility has already been reconstituted, for other purposes, in media of widely varying viscosities. We conservatively estimate the diffusion coefficients in those experimental systems and calculate lower bounds on the expected size of the steady-state gap between a freely diffusing bacterium and its tail. In highly diluted platelet extracts, this gap would be several bacterium lengths long. Since no such gaps are visible in published micrographs, we conclude that the bacteria must be attached to their tails. We also comment on the possibility of extending this analysis to other systems with polymerization-based motility, including crawling cells.

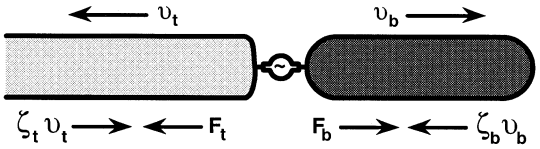

Fig. 1. Schematic of forces acting on *Listeria* and its tail. The bacterium and the tail are pushed apart by a motor or other mechanism for force generation that is associated with actin polymerization. Each body (the bacterium and the tail) feels a force F due to the motor that is opposed by a drag force $\zeta\upsilon$ that depends on the friction coefficient ζ and the speed υ of the body.

2. Theory

2.1. Partitioning of motor-based motion between tail and bacterium

To calculate to what degree *Listeria*'s tail would be expected to move backward as the bacterium moves forward, a force-based model need only include the bacterium, its tail, and the force that pushes them apart. That force will be attributed to a motor, although for the present purposes it could just as well result from any other mechanism that produces a real force. The tail is composed of many short actin filaments which are assumed to be cross-linked into a single body by, for example, α-actinin, which *Listeria* requires to form tails and generate motility [20]. A schematic of the system is shown in Fig. 1. In addition to the force F exerted by the motor, the tail and bacterium each also feel a speed-dependent frictional drag force $-\zeta\upsilon$ [21], where ζ is the friction coefficient and υ is the speed of the moving body. The speed of the tail υ_t is the speed at which a particular (e.g., photobleached) element of the tail moves backward relative to the substrate, although the front edge of the tail generally does not move backward because of continued polymerization.

In the subcellular world, the drag force $-\zeta\upsilon$ is many orders of magnitude larger than the inertial term ma, so the latter term is dropped from Newton's equations of motion. The equations of motion for the bacterium-tail system in Fig. 1 are then

$$\begin{aligned} -\zeta_b \upsilon_b + F_b &= 0 \\ -\zeta_t \upsilon_t + F_t &= 0 \end{aligned} \tag{1}$$

where the subscripts b and t refer to the bacterium

and tail, respectively. By Newton's third law, the two forces are equal in magnitude: $F_b = F_t = F_m$, where F_m is the force generated by the motor. The speeds can be calculated by substituting the motor force into Eq. 1:

$$\begin{aligned} v_b &= \frac{F_m}{\zeta_b} \\ v_t &= \frac{F_m}{\zeta_t} \end{aligned} \tag{2}$$

The speed of the tail is thus related to the speed of the bacterium by

$$\frac{v_t}{v_b} = \frac{\zeta_b}{\zeta_t} = r \tag{3}$$

This ratio is independent of the motor force and the viscosity of the medium.

It is also instructive to consider how motion due to actin polymerization is partitioned into movement of the bacterium vs. the tail. Polymerization must occur at the rate v_t+v_b. The fraction of this speed that translates into motion of the tail is

$$f_t = \frac{v_t}{v_t + v_b} = \frac{\zeta_b}{\zeta_t + \zeta_b} = \frac{r}{1+r} \tag{4}$$

while the fraction that translates into motion of the bacterium is

$$f_b = \frac{v_b}{v_t + v_b} = \frac{\zeta_t}{\zeta_t + \zeta_b} = \frac{r}{1+r} \tag{5}$$

When $r = 1$, the bacterium and elements within the tail move in opposite directions at equal speeds (Eq. 3). The effect of actin polymerization is thus partitioned half to backward motion of the tail (Eq. 4) and half to forward motion of the bacterium (Eq. 5). The bacterium is propelled forward most efficiently (i.e., with least waste in backward tail motion) when r is small.

2.2. Partitioning of diffusion-based motion between tail and bacterium

The dependence of v_b/v_t on the ratio of friction coefficients r is the same even if the separation of the bacterium and tail is due to diffusion instead of a force. In one dimension, a particle with diffusion coefficient D diffuses an average distance

$$x = \sqrt{\frac{4Dt}{\pi}} \tag{6}$$

away from a barrier in a time t [22]. It is sufficient to consider one-dimensional diffusion for *Listeria* because the bacterium can be assumed to be prevented from moving laterally by the 'cup' that the actin filaments form around its rear end [23,24]. The progress of the particle can then be characterized by

$$v_{diff} = \frac{\Delta x}{\Delta t} \approx \frac{2D}{\pi x} \tag{7}$$

where the effective diffusive 'speed' v_{diff} represents the limit of distance diffused divided by time rather than an instantaneous speed defined along a kinematic trajectory. v_{diff} decreases with increasing distance away from the barrier because when the particle is near the barrier, it is more likely to diffuse away from it, while diffusion farther away from the barrier is more uniformly bidirectional.

In the bacterium-tail system, each diffuses away from the barrier represented by the other. From Eq. 7 the diffusive speed of each at a bacterium-tail separation of Γ is

$$v_b = \frac{2D_b}{\pi\Gamma} \tag{8a}$$

$$v_t = \frac{2D_t}{\pi\Gamma} \tag{8b}$$

The ratio of the speeds is then

$$\frac{v_t}{v_b} = \frac{D_t}{D_b} \tag{9}$$

Since the diffusion coefficient is inversely related to the friction coefficient by [21]

$$D = k_B T / \zeta \tag{10}$$

where k_B is Boltzmann's constant, and T is the absolute temperature, the ratio of speeds is again given by Eq. 3.

2.3. Gap between diffusing bacterium and tail

If the bacterium and tail are independently diffus-

ing, decreasing the solvent viscosity will allow them to wander farther apart. However, since the tail is simultaneously elongating, the distance between the tail and bacterium depends on the rates of both polymerization and diffusion. The mean speed at which the separation of the bacterium and tail grows will be given by the sum of Eqs. 8a and b. As Γ increases, this speed falls, and the bacterium and tail eventually separate no faster than actin is added to the tail. If p is the rate of actin polymerization, the equation

$$p = \frac{2D_t}{\pi\Gamma^*} + \frac{2D_b}{\pi\Gamma^*} = \frac{2(D_t + D_b)}{\pi\Gamma^*} \tag{11}$$

defines the steady-state bacterium-tail separation, Γ*.

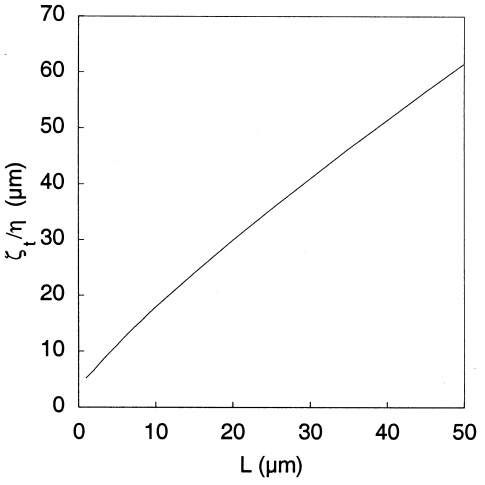

Fig. 2. Maximum friction coefficient ζ_t/η (μm) for the tail as a function of tail length L (μm), as given by Eq. 13, for the case $d_t = d_b$.

3. Results

All of our results depend on either the friction coefficient or the diffusion coefficient of *Listeria*. Friction coefficients can only be easily calculated for simple geometric shapes. A hemisphere-capped cylinder of total length L_b and diameter d_b that is moving parallel to its length has a friction coefficient of [25]

$$\zeta_b = \frac{2\pi\eta L_b}{\ln(L_b/d_{b)}} \tag{12}$$

where η is the medium's viscosity. The diffusion coefficient can then be calculated using Eq. 10. For *Listeria*, at $T = 300$ K, with length $L_b \approx 1$ μm, and with diameter $d_b \approx 0.3$ μm [2,5,24,26], $\zeta_b/\eta = 5.2$ μm, and the diffusion coefficient is 7.9×10^{-9} cm^2/s in water ($\eta = 0.01$ poise). A slightly larger result is obtained when the bacterium is treated as an ellipsoid [27] instead of a sphero-cylinder.

Knowing the bacterium's friction coefficient allows us to use Eq. 2 to estimate the force F_m that motors (or other mechanisms) would need to generate to produce the observed speeds. *Listeria* has been observed to move in cytoplasm at speeds exceeding 1.0 μm/s [7]. Estimates for the viscosity of the cytoplasm vary over several orders of magnitude [28]; the value of 30 poise used by Mogilner and Oster [29] lies in the middle of the range. At $\eta = 30$ poise, the bacterium would have a friction coefficient of about $\zeta_b = 1.57\times10^{-2}$ dyne·s/cm (Eq. 12) and a drag force at $\upsilon_b = 1$ μm/s of $F_{drag} = \zeta_b\upsilon_b = 15.7$ piconewtons. If that force were distributed over 15 actin filaments (a conservative number), a motor on each need only generate about 1 piconewton to sustain the motion, a value within the range of forces generated by myosin and RNA polymerases [30,31]. A larger number of motors (or other mechanisms) would require less force from each to maintain the same speed.

3.1. Partitioning of motion between tail and bacterium

The friction coefficient of *Listeria*'s tail is difficult to estimate because of its complexity. A start may be made by observing that the resistance to movement of the tail is governed by the resistance to fluid movement around the tail and the resistance to fluid movement through the tail. The flow of fluid through the tail is expected to be minimal due to the tail's density. However, any fluid flow through the tail will decrease the resistance relative to that due to flow around the tail alone. Therefore the friction coefficient of the tail considered as a solid object (with no internal fluid flow) provides an upper limit for the actual friction coefficient of the tail, and estimates of tail motion based on a solid tail represent a lower limit for actual tail motion.

The friction coefficient of a tail modeled as a hemisphere-capped cylinder is then

$$\zeta_t/\eta \leq 2\pi L_t/\ln(L_t/d_t) \tag{13}$$

where the deviation from equality depends on the

extent of fluid flow through the tail. Fig. 2 shows the dependence of the maximum value of ζ_t/η on tail length for a tail with a diameter equal to that of the bacterium ($d_t = d_b = 0.3$ μm). Occasionally $d_t > d_b$, but the dependence of ζ_t/η on d_t is weak (see Eq. 13). For typical tails, $L = 7$ μm [1,4,26], and Eq. 13 gives $\zeta_t/\eta \leq 14$ μm. But tails as long as 40 μm have been reported [3,7,18], and for these tails Eq. 13 gives $\zeta_t/\eta \leq 51.4$ μm. On the other hand, for a tail only as long as the bacterium ($L_t = L_b = 1$ μm), Eq. 13 gives $\zeta_t/\eta \leq 5.2$ μm. For still shorter tails, Eq. 13 becomes unreliable. However, the friction coefficient for a solid sphere $\zeta_t = 3\pi d_t\eta$ provides an estimate of $\zeta_t/\eta \leq 2.8$ μm for a tail of $L_t \approx d_t$. Overall, the friction coefficient is determined strongly by length.

Fig. 3 shows a plot of the partitioning ratio r (solid line, Eq. 3) and the bacterium's fraction of the motion f_b (broken line, Eq. 5) as a function of ζ_t/η (μm). (We are still assuming that the tail is unanchored; if the tail is anchored to an extended cytoskeleton, the effective $\zeta_t/\eta \to \infty$, so that $r \to 0$, and $f_b \to 1$.) For a tail of length and diameter equal to that of the bacterium, $\zeta_t/\eta \leq \zeta_b/\eta = 5.2$ μm, and $r \geq 1$ and $f_b \leq 0.5$. If actin is added to such a tail at 0.2 μm/s, then, the bacterium would move forward at no more than 0.1 μm/s; the tail would move backward at at least that speed, and this backward motion of the tail relative to the substrate would be easily detectable. For shorter, nascent tails, the tail motion would be greater, and the bacterium motion would be less. On the other hand, longer tails would move more slowly. A tail with $d_t = d_b$ and $L_t = 20$ μm has $\zeta_t/\eta \leq 30$ μm. In that case, up to 85% of the polymerization rate would be apportioned to the bacterium. If actin is polymerized at 0.2 μm/s, this tail would move backward at at least 0.03 μm/s unless it were anchored.

Short *Listeria* tails have been obtained by Carlier et al. [18] by adding actin depolymerizing factor to diluted platelet extracts. At high concentrations of actin depolymerizing factor, they observed that the tails did not become long enough to allow the bacterium to move forward. The absence of forward bacterial motion implies that for these very short tails, essentially all of the effect of actin polymerization is partitioning into backward motion of the elements of the tail, as predicted in the forgoing analysis. More recently, appreciable drift in tails lacking α-actinin has been observed, presumably because the tails were no longer cross-linked to the surrounding medium [32].

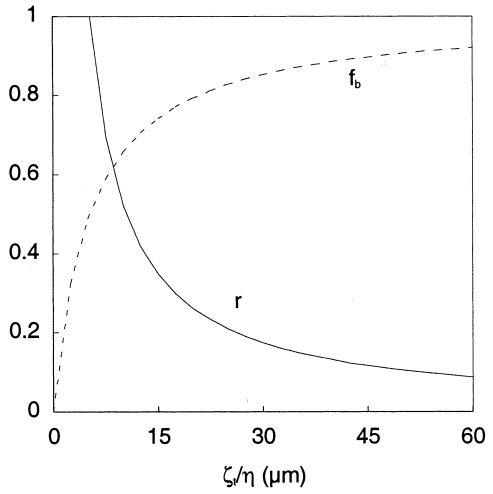

Fig. 3. Partitioning of motion as a function of tail friction coefficient ζ_t/η (μm). The solid line is the ratio of tail speed to bacterium speed (Eq. 3); the broken line is the fraction of the actin polymerization rate that is apportioned to movement of the bacterium (Eq. 5). When the tail's friction coefficient is small (left side of graph), the tail moves more than the bacterium, if neither is anchored. When the tail (and its friction coefficient) grows, it resists motion more than the bacterium, and polymerization moves the bacterium forward farther than it moves the tail backward (right side of graph).

Theriot et al. [8] report that filaments in 1–15 μm long tails of *Listeria* moving at $\upsilon_b = 0.02$–0.2 μm/s remain stationary relative to the cytoplasm. Calculation of r and υ_t for the length/speed pairs in their Fig. 3c reveals that if the tails were unanchored, their speeds would be greater than 0.02 μm/s, the lowest reported bacterium speed. Therefore we assume that tail movement would have been detected were it present, and the tails were probably anchored in the cytoplasm in that case.

Zhukarev et al. [24] have observed the presence of actin filaments extending perpendicularly from the tail into the cytoplasm. These filaments may provide the anchors which prevent the tail from diffusing in the cytoplasm. Alternatively, they may merely increase the effective diameter of the tail, therefore decreasing the tail's diffusion coefficient (according to Eq. 13) and making tail motion smaller and less observable.

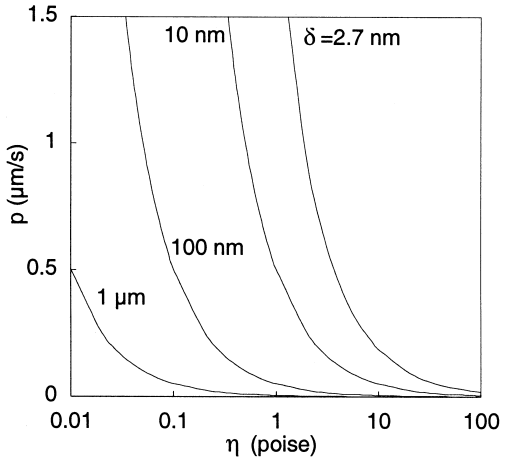

Fig. 4. Minimum steady-state bacterium-tail gap Γ^*_{min} in the absence of attachment. Lines of constant Γ^*_{min} (based on Eqs. 10–12) are plotted as a function of polymerization rate p (μm/s) and viscosity η (poise). In the upper right region, diffusion is slow enough (due to high viscosity), and polymerization is fast enough, that freely diffusing *Listeria* stay close to their tails. As p or η is reduced, the minimum gap widens. The viscosity of water is 0.01 poise, and cytoplasmic viscosities are about 30 poise.

3.2. Steady-state gap for diffusing bacteria

Since the diffusion coefficient varies inversely with viscosity, the mean bacterium-tail gap Γ* given by Eq. 11 increases as either the viscosity or the polymerization rate decreases. The gap Γ* also depends on D_t, which itself depends on tail length. Independent of the length of the tail, $\Gamma^* \geq 2D_b/\pi p = \Gamma^*_{min}$, where Γ^*_{min} is dependent on the diffusion coefficient of the bacterium alone. (The equality applies to very long tails or tails anchored to an extended cytoskeleton. For unanchored tails of short to moderate length, $D_t \geq D_b$, and Γ* will be larger.)

Fig. 4 shows the variation of Γ^*_{min} with polymerization speed p (μm/s) and viscosity η (poise). The relevant scale for comparison is the increment δ by which actin polymerization occurs. For actin filaments, monomer addition to the double strand causes filament elongation in increments of half a monomer length, so δ = 2.7 nm [33]. The contours in Fig. 4 are lines of constant steady-state tail-bacterium separation. In the upper right corner of the graph, $\Gamma^*_{min} < \delta$ (gap smaller than the polymerization increment), and in the lower left corner of the graph, $\Gamma^*_{min} > \delta$ (gap much larger than the polymerization increment).

Where do experimental observations fall on this diagram? For the commonly observed tail polymerization speeds of $p \approx 0.1$–0.2 μm/s [3,8] in the cytoplasm (η = 30 poise), the expected steady-state gap Γ^*_{min} is less than 0.7 δ.

The predicted gap is larger for *Listeria* motility in *Xenopus* extracts [15,16]. *Xenopus* extract is a structureless fluid that can be drawn from a centrifuge tube through a 16 gauge needle (J.A. Theriot, personal communication, 1997). This would be difficult if the extract had a viscosity of more than 10 poise. (By comparison, olive oil has a viscosity of about 1 poise, glycerol has a viscosity of about 10 poise, and honey has a viscosity of about 100 poise [34].) It is also observed that *Listeria* in extracts show visible Brownian motion before becoming motile, while those in cytoplasm do not (J.A. Theriot, personal communication, 1997). By characterizing visible Brownian motion as motion over a distance of at least 5% of *Listeria*'s length in less than 1 s, we estimate that *Listeria*'s diffusion coefficient must be at least 1.2×10^{-11} cm^2/s and, using Eqs. 10 and 12, that the viscosity of *Xenopus* extracts must therefore be less than 7 poise. At $\eta \approx 7$ poise and polymerization rates of $p = 0.1$–0.2 μm/s [15,16], the expected minimum steady-state bacterium-tail gap is about 1.3–2.7 δ (= 3.5–7.3 nm).

Listeria motility has also been reconstituted in diluted platelet extracts [18,19]. The extracts, already 6-fold diluted from platelet cytoplasm, were further diluted 48-fold with a buffer of 5 mM ATP-Mg, 6 mM DTT, and 3.25 μM rhodamine-labeled G-actin. The viscosity can be estimated given the volume fraction of solute φ:

$$\frac{\eta}{\eta_s} = \left(1 - \frac{\phi}{\phi_m}\right)^{-2} \tag{14}$$

where η_s is the viscosity of the solvent, and ϕ_m is the volume fraction at which the suspension undergoes a transition to an ordered or glassy state [35]. For hard spheres, $\phi_m = 0.63$, but for a charged protein like actin, a smaller value is appropriate. We take $\phi_m = 0.1$, a conservative number for our purposes (since a larger ϕ_m would only give a still smaller estimate for η and a larger diffusive gap). Because the buffer has a volume fraction of protein of only 2×10^{-4}, its viscosity is essentially that of the solvent, water, and adding buffer to the platelet extract is therefore as-

sumed to be the same as simply reducing the volume fraction of the solute in the platelet extract. If the viscosity of the undiluted platelet extract were 30 poise, also a conservative number for our purposes, then a 48-fold dilution would imply, using Eq. 14, that the diluted platelet extract has a viscosity only 1.04 times the solvent viscosity, or 0.0104 poise. This extract's viscosity would be nearly 1000 times less viscous than we estimate *Xenopus* extracts to be. We can also calculate an upper limit to the viscosity (yielding a lower limit for the diffusive gap) without trying to estimate the viscosity of the undiluted platelet extract. If we assume that the original extract was 100% solute, which is clearly an overestimate, a 48-fold dilution results in a solution with a 2% volume fraction of solute and Eq. 14 predicts a viscosity of 1.6 times the viscosity of the solvent, or 0.016 poise. We take this value as an upper limit to the viscosity of the 48-fold diluted platelet extract.

Listeria were observed to move in diluted platelet extracts at an average speed of 4 μm/min, or about 0.07 μm/s [18]. At a viscosity of no more than 0.016 poise and a polymerization rate of 0.07 μm/s, the steady state tail-bacterium gap is predicted to be at least 4.7 μm, or no less than 4 times the length of the bacterium. No such gaps are evident in micrographs of motility in these extracts. We conclude that if the bacterium can so easily diffuse away from the tail but does not, then it must be attached to the tail.

So far we have assumed that $D_t \ll D_b$ (i.e., considered Γ^*_{min}), as appropriate for long tails. However, as shown in the previous section, the shorter the tail, the larger the value of D_t, and the larger the gap size predicted by Eq. 11. Therefore, in the absence of a cytoskeleton to anchor new actin filaments, short nascent tails will become separated from the bacteria even at higher viscosities than long tails. Thus the development of mature actin tails in cytoskeleton-free media also requires attachment of the growing filaments to the bacteria.

4. Discussion

4.1. Listeria motility

Listeria motility has recently been reconstituted outside of the cell. We have performed two simple analyses that allow us to interpret the results of those experiments so as to shed some light on the underlying mechanisms of *Listeria* motility. First, we demonstrated that when *Listeria* tails are not anchored in the cytoplasm, they will exhibit some backward motion relative to the substrate as the *Listeria* move forward. We calculate that tails with lengths in the upper end of the previously observed range may move backward slowly enough to appear stationary. However, friction between the cytoplasm and tail is not great enough to immobilize short tails, and reports that even short tails are stationary in vivo presumably reflect anchoring to the cytoskeleton. Second, we calculate that *Listeria* bacteria must be attached to their actin tails. Some reconstitution media are of sufficiently low viscosity that if the tail and bacterium were not attached, diffusive motion would rapidly separate them. Models for *Listeria* motility which depend on free motion of the bacterium relative to the tail can therefore be ruled out.

Any model for *Listeria* motility needs to explain how *Listeria* nucleates actin filament formation, promotes continued actin polymerization, and transduces the energy of polymerization into a physical force that propels the bacterium. These issues have been examined separately. Recently the minimal set of proteins necessary to reconstitute *Listeria* motility has been identified, and roles for those proteins in nucleation and promotion of actin polymerization have been proposed [32]. However, no model has been proposed that assembles all of the pieces (nucleation, polymerization, and force transduction) necessary for *Listeria* motility into a unified whole. Even so, the calculations performed above, coupled with existing experimental observations, definitively remove one class of models from consideration: those in which *Listeria* is not attached to its tail. It is not necessary that the mechanisms for filament nucleation, continued polymerization, and force transduction all depend on tail-bacterium attachment, but each mechanism must be able to accommodate attachment. For force transduction, the original and elastic Brownian Ratchet models [29,36], which assume that the bacterium is freely diffusing, would have to be modified to include tethering.

Although tethers are required for the motility observed in diluted extracts, the physiological significance of the tethers is unclear. In the cytoplasm,

high viscosity prevents bacteria from diffusing far from their actin tails, and the cytoskeleton might prevent nascent tails from diffusing away from their bacteria. Therefore tethers may not be necessary to keep bacteria and their tails together under physiological conditions. A plausible physiological role for the tethers is in filament nucleation, which requires binding to nascent actin filaments. This binding may persist during growth. Persistent binding during filament growth suggests a second function, such as the maintenance of uncapped, polymerization-capable filament ends.

In the foregoing analysis, we have focused on *Listeria* because it has received a great deal of experimental scrutiny. However, the great similarities between *Listeria* motility and the locomotion of other pathogens such as *Shigella*, *Rickettsia*, and vaccinia virus suggest that the preceding analyses may be applied to those systems. Studies of these pathogens in low viscosity media would provide analogous evidence as to whether they are attached to their tails or freely diffusing.

4.2. Amoeboid locomotion

One might speculate on how similar *Listeria* motility is to a cell's own use of actin for the extension of filopodia and lamellipodia. The same tools and materials are being used; are the blueprints the same? Lamellipodial extension and *Listeria*-like motility have both been observed in neuronal growth cones of *Aplysia* [37]. In addition to extension of radial actin filaments in the lamellipodium, polycationic microbeads brought into contact with the surface of the lamellipodium can induce actin assembly into a *Listeria*-like tail behind the microbead. The actin polymerization in these 'inductopodia' may be caused by concentration of a cell adhesion molecule in the membrane [38], indicating the possibility of a connection between the underlying mechanisms of motility for the extension of lamellipodia and inductopodia. However, since the membrane of the lamellipodium cannot stretch far from the actin network it encloses, the viscosity arguments presented above cannot be applied directly to the intact cell.

The role of membrane-based control of actin assembly has also been studied in *Xenopus* extracts. Here endogenous membrane vesicles and phosphoinositide containing lipid vesicles are both propelled by actin comet tails similar to those of *Listeria* [39]. The viscosity of these systems has not been reported, but observation of the motility in a range of viscosities could determine whether the proteins recruited to the vesicles are bound.

The role of membrane components in amoeboid locomotion has been examined in more detail in another system. Unlike mammalian sperm, the amoeboid sperm of the nematode *Ascaris suum* are not swimmers, but crawlers. Much like crawling epithelial goldfish keratocytes [40], the sperm extend broad, flat lamellipodia in front of themselves [41,42]. At the leading edge of the lamellipodium, protein is polymerized into a network, and the protein network remains stationary while the cell pulls forward over it. Although the polymerizing protein in *Ascaris* sperm is the 14 kDa major sperm protein (MSP), which has no obvious homology to actin [41], the movements of *Ascaris* worm sperm appear quite similar to those of fish keratocytes. Nature has apparently solved the motility problem with the same blueprint, but different materials.

The MSP-based motility of *Ascaris* sperm has also been reconstituted in vitro [43]. ATP was added to an extract prepared from the supernatant of centrifuged, freeze-thawed sperm. The MSP in the extract polymerized and bundled into multifilament tails, which were observed by phase-contrast video microscopy. At the end of each growing tail was a vesicle composed of membrane from the sperm. The vesicles were pushed ahead of the tails as they grew, at speeds of 0.03–0.5 μm/s.

The *Ascaris* vesicles and their trailing MSP fibers are strikingly similar to *Listeria* and their actin tails. Although the vesicles appear somewhat larger than *Listeria* (1–2 μm spheres vs. 1 μm×0.3 μm cylinders), the vesicle diffusion coefficients would be within a factor of 4–6 of *Listeria*'s diffusion coefficient at the same viscosity and temperature. The *Ascaris* extracts also appear to have low viscosity, as indicated by the absence of appreciable actin, the absence of detectable MSP filament assembly other than that associated with the vesicle tails, and the rapid Brownian motion of vesicles without associated fibers (T.M. Roberts, personal communication, 1997). The last observation in itself supports the hypothesis that the tail-associated vesicles are in fact attached to

the tails. However, if the motility could be reconstituted in a system with viscosity demonstrably less than 1 poise, the vesicles would visibly separate from the MSP tails if the vesicles were freely diffusing, and continued tail-vesicle association would indicate attachment.

Attachment of the vesicles to their tails has implications for motility of the intact sperm cells. The same study [43] reported attempts to isolate the factors in the *Ascaris* membrane needed for motility. In immunofluorescence assays, an antibody directed against phosphotyrosine labeled membrane at the leading edge of the crawling sperm's pseudopod. The same antibody labeled the motile membrane vesicles associated with MSP, but not vesicles without MSP tails. In addition, MSP polymerization did not occur in an extract where the membrane vesicles have been removed by further centrifugation. This evidence suggests that the same mechanism underlies both the original crawling motility and the vesicle motion. Thus, if MSP fibers are attached to the membrane vesicles in the cell-free system, it is probable that MSP filaments are also attached to the forward membrane of intact *Ascaris* sperm undergoing amoeboid locomotion. Similar studies of vesicles in actin-based systems would reveal whether this reasoning can be generalized.

Acknowledgements

We would like to thank Julie A. Theriot, Thomas M. Roberts, and Marie-France Carlier for helpful discussions of their experiments, and Jeff Gelles for discussions of Brownian Ratchets. This work was supported by the National Science Foundation under Grant No. HRD-9021929 and the National Institutes of Health under Grant No. HL-36546.

References

[1] L.G. Tilney, D.A. Portnoy, Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*, J. Cell Biol. 109 (1989) 1597–1608.

[2] L.G. Tilney, D.J. DeRosier, M.S. Tilney, How *Listeria* exploits host cell actin to form its own cytoskeleton. I. Formation of a tail and how that tail might be involved in movement, J. Cell Biol. 118 (1992) 71–81.

[3] J.M. Sanger, J.W. Sanger, F.S. Southwick, Host cell actin assembly is necessary and likely to provide the propulsive force for intracellular movement of *Listeria monocytogenes*, Infect. Immun. 60 (1992) 3609–3619.

[4] F.S. Southwick, D.L. Purich, Dynamic remodeling of the actin cytoskeleton: lessons learned from *Listeria* locomotion, BioEssays 16 (1994) 885–891.

[5] P. Cossart, Actin-based bacterial motility, Curr. Opin. Cell Biol. 7 (1995) 94–101.

[6] J.A. Theriot, The cell biology of infection by intracellular bacterial pathogens, Annu. Rev. Cell Dev. Biol. 11 (1995) 213–239.

[7] G.A. Dabiri, J.M. Sanger, D.A. Portnoy, F.S. Southwick, *Listeria monocytogenes* moves rapidly through the host-cell cytoplasm by inducing directional actin assembly, Proc. Natl. Acad. Sci. USA 87 (1990) 6068–6072.

[8] J.A. Theriot, T.J. Mitchison, L.G. Tilney, D.A. Portnoy, The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization, Nature 357 (1992) 257–260.

[9] C. Kocks, E. Gouin, M. Tabouret, P. Berche, H. Ohayon, P. Cossart, *L. monocytogenes*-induced actin assembly requires the *actA* gene product, a surface protein, Cell 68 (1992) 521–531.

[10] S. Pistor, T. Chakraborty, K. Niebuhr, E. Domann, J. Wehland, The ActA protein of *Listeria monocytogenes* acts as a nucleator inducing reorganization of the actin cytoskeleton, EMBO J. 13 (1994) 758–763.

[11] C. Kocks, J.-B. Marchand, E. Gouin, H. d'Hauteville, P.J. Sansonetti, M.-F. Carlier, P. Cossart, The unrelated surface proteins ActA of *Listeria monocytogenes* and IcsA of *Shigella flexneri* are sufficient to confer actin-based motility on *Listeria innocua* and *Escherichia coli* respectively, Mol. Microbiol. 18 (1995) 413–423.

[12] G.A. Smith, D.A. Portnoy, J.A. Theriot, Asymmetric distribution of the *Listeria monocytogenes* ActA protein is required and sufficient to direct actin-based motility, Mol. Microbiol. 17 (1995) 945–951.

[13] R.A. Heinzen, S.F. Hayes, M.G. Peacock, T. Hackstadt, Directional actin polymerization associated with spotted fever group Rickettsia infection of Vero cells, Infect. Immun. 61 (1993) 1926–1935.

[14] S. Cudmore, P. Cossart, G. Griffiths, M. Way, Actin-based motility of vaccinia virus, Nature 378 (1995) 636–638.

[15] J.A. Theriot, J. Rosenblatt, D.A. Portnoy, P.J. Goldschmidt-Clermont, T.J. Mitchison, Involvement of profilin in the actin-based motility of *L. monocytogenes* in cells and cell-free extracts, Cell 76 (1994) 505–517.

[16] J.-B. Marchand, P. Moreau, A. Paoletti, P. Cossart, M.-F. Carlier, D. Pantaloni, Actin-based movement of *Listeria monocytogenes*: actin assembly results from the local maintenance of uncapped filament barbed ends at the bacterium surface, J. Cell Biol. 130 (1995) 331–343.

[17] M.D. Welch, A. Iwamatsu, T.J. Mitchison, Actin polymer-

ization is induced by Arp2/3 protein complex at the surface of *Listeria monocytogenes*, Nature 385 (1997) 265–269.
[18] M.-F. Carlier, V. Laurent, J. Santolini, R. Melki, D. Didry, G.-X. Xia, Y. Hong, N.-H. Chua, D. Pantaloni, Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implications in actin-based motility, J. Cell Biol. 136 (1997) 1307–1323.
[19] V. Laurent, M.-F. Carlier, Use of platelet extracts for actin-based motility of *Listeria monocytogenes*, in: J. Celis (Ed.), Cell Biology: a Laboratory Handbook, vol. 2, Academic Press, San Diego, CA, 1998.
[20] F.G. Dold, J.M. Sanger, J.W. Sanger, Intact alpha-actinin molecules are needed for both the assembly of actin into the tails and the locomotion of *Listeria monocytogenes* inside infected cells, Cell Motil. Cytoskeleton 28 (1994) 97–107.
[21] F. Reif, Fundamentals of Statistical and Thermal Physics, McGraw-Hill, New York, 1965.
[22] J. Crank, The Mathematics of Diffusion, Clarendon Press, Oxford, 1975.
[23] L.G. Tilney, D.J. DeRosier, A. Weber, M.S. Tilney, How *Listeria* exploits host cell actin to form its own cytoskeleton II. Nucleation, actin filament polarity, filament assembly, and evidence for a pointed end capper, J. Cell Biol. 118 (1992) 83–93.
[24] V. Zhukarev, F. Ashton, J.M. Sanger, J.W. Sanger, H. Shuman, Organization and structure of actin filament bundles in *Listeria*-infected cells, Cell Motil. Cytoskeleton 30 (1995) 229–246.
[25] M. Doi, S.F. Edwards, The Theory of Polymer Dynamics, Oxford University Press, Oxford, 1986.
[26] D. Nanavati, F.T. Ashton, J.M. Sanger, J.W. Sanger, Dynamics of actin and alpha-actinin in the tails of *Listeria monocytogenes* in infected PtK_2 cells, Cell Motil. Cytoskeleton 28 (1994) 346–358.
[27] H.C. Berg, Random Walks in Biology, Princeton University Press, Princeton, 1993.
[28] P.A. Valberg, H.A. Feldman, Magnetic particle motions within living cells, Biophys. J. 52 (1987) 551–561.
[29] A. Mogilner, G. Oster, Cell motility driven by actin polymerization, Biophys. J. 71 (1996) 3030–3045.
[30] H. Yin, M.D. Wang, K. Svoboda, R. Landick, S.M. Block, J. Gelles, Transcription against an applied force, Science 270 (1995) 1653–1657.
[31] J.T. Finer, R.M. Simmons, J.A. Spudich, Single myosin molecule mechanics: piconewton forces and nanometre steps, Nature 368 (1994) 113–119.
[32] T.P. Loisel, R. Boujemaa, D. Pantaloni, M.-F. Carlier, Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins, Nature 401 (1999) 613–616.
[33] D.J. DeRosier, L.G. Tilney, The form and function of actin, in: J.W. Shay (Ed.), Cell and Muscle Motility, vol. 5, Plenum Press, New York, 1984, pp. 139–169.
[34] H.A. Barnes, J.F. Hutton, K. Walters, An Introduction to Rheology, Elsevier, Amsterdam, 1989.
[35] W.B. Russel, D.A. Saville, W.R. Schowalter, Colloidal Dispersions, Cambridge University Press, Cambridge, 1989.
[36] C.S. Peskin, G.M. Odell, G.F. Oster, Cellular motions and thermal fluctuations: the Brownian Ratchet, Biophys. J. 65 (1993) 316–324.
[37] P. Forscher, C.H. Lin, C. Thompson, Novel form of growth cone motility involving site-directed actin filament assembly, Nature 357 (1992) 515–518.
[38] C. Thompson, C.-H. Lin, P. Forscher, An *Aplysia* cell adhesion molecule associated with site-directed actin filament assembly in neuronal growth cones, J. Cell Sci. 109 (1996) 2843–2854.
[39] L. Ma, L.C. Cantley, P.A. Janmey, M.W. Kirschner, Corequirement of specific phosphoinositides and small GTP-binding protein Cdc42 in inducing actin assembly in *Xenopus* egg extracts, J. Cell Biol. 140 (1998) 1125–1136.
[40] J.A. Theriot, T.J. Mitchison, Actin microfilament dynamics in locomoting cells, Nature 352 (1991) 126–131.
[41] T.M. Roberts, M. Stewart, Nematode sperm locomotion, Curr. Opin. Cell Biol. 7 (1995) 13–17.
[42] J.A. Theriot, Worm sperm and advances in cell locomotion, Cell 84 (1996) 1–4.
[43] J.E. Italiano Jr., T.A. Roberts, M. Stewart, C.A. Fontana, Reconstitution in vitro of the motile apparatus from the amoeboid sperm of Ascaris shows that filament assembly and bundling move membranes, Cell 84 (1996) 105–114.