How the *Listeria monocytogenes* ActA protein converts actin polymerization into a motile force

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The ActA protein is an essential determinant of pathogenicity that is responsible for the actin-based motility of *Listeria monocytogenes* in mammalian cells and cell-free extracts. ActA appears to control at least four functions that collectively lead to actin-based motility: (1) initiation of actin polymerization, (2) polarization of ActA function, (3) transformation of actin polymerization into a motile force and (4) acceleration of movement mediated by the host protein profilin.

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Eight years have passed since the role of actin filaments in mediating the intracellular movement and cell-to-cell spread of *Listeria monocytogenes*, a Gram-positive, facultative, intracellular bacterial pathogen, was first described[1–3]. During this time, the *actA* gene has been identified as the primary (and potentially only) bacterial factor required for actin-based motility. Mutants lacking ActA are able to enter and grow in the cytosol of eukaryotic cells but are unable to polymerize host actin and are consequently unable to move by actin-based motility or to spread from cell to cell[4–6]. In addition, ActA null mutants are 1000-fold less virulent than wild type in a murine model of infection[5]. Several review articles that focus on the ActA protein and the actin-based motility of *L. monocytogenes* have recently been published[7–12]. Rather than restate much of what has already been written, we will focus on five issues that have been matters of controversy; we will discuss how the ActA protein may function to mediate the complex phenotype of bacterial actin-based motility and attempt to clarify some of the discrepancies in the literature.

ActA acts alone

The ActA protein is clearly required for the actin-based motility of *L. monocytogenes* in eukaryotic cells[4–6], and several studies demonstrate that ActA is sufficient to direct actin polymerization. Transfection of the *actA* gene into eukaryotic cells results in actin polymerization at sites of ActA expression[13,14]. In addition, purified ActA coupled to beads induces actin polymerization in cytoplasmic extracts of *Xenopus laevis*, but the ActA-coupled beads fail to move[15]. Similarly, ActA bound to the surface of *Streptococcus pneumoniae* induces actin polymerization around the diplococci when placed in *X. laevis* extracts. If the ActA protein is distributed on the *S. pneumoniae* surface asymmetrically, actin-based motility is observed[15]. Thus, these data show that ActA is the sole bacterial factor required for motility and implicate ActA asymmetry, in addition to actin polymerization, as a prerequisite for the generation of this motility. This is supported by the finding that expression of the *actA* gene in *Listeria innocua*, a nonpathogenic strain naturally lacking the *actA* gene, confers an actin-based motility phenotype on these bacteria[16].

Consequently, current research has focused on the ActA protein and its mode of action. Understanding how ActA generates actin-based motility is of interest to microbiologists and cell biologists because, for the first time, they have a system for actin-based motility available that is known to revolve around a single protein. However, the motility produced by ActA is likely to be complex and probably results from the coordinated orchestration of multiple host cellular functions. In addition, we must stress that while ActA is sufficient, the possible existence of other unidentified *L. monocytogenes* proteins that contribute to actin-based motility cannot be ruled out. The issue of ActA asymmetry in *L. monocytogenes* may involve unknown factors. Furthermore, no current studies address the mechanism of host cell filopod formation, although it is often assumed that the force of actin-based motility is sufficient to push bacteria out into these cellular extensions.

Variation of ActA expression

Several strains of *L. monocytogenes* are commonly studied by laboratories investigating the pathogenesis of these bacteria. While this fact is seldom mentioned beyond the 'Materials and methods' of most research publications, successful interpretation of many results requires an understanding of how isolates differ. Strains of *L. monocytogenes* vary in several characteristics, including serotype, rate of growth, hemolytic activity and, most importantly for this review, ActA expression (Table 1). There are 12 serotypes of *L. monocytogenes*, three of which (1/2a, 1/2b and 4b) are responsible for >90% of reported human infection[17]; the five most common strains studied belong to serotypes 1/2a, 1/2b and

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1/2c. The regulation of ActA expression from these strains is variable and is an important consideration for studies of ActA function. For example, strain SLCC-5764 was used in an early study to reconstitute the movement of *L. monocytogenes* in cell-free cytoplasmic extracts because strain 10403S did not express enough ActA to provide reproducible activity[18]. In a subsequent report, LO28 was successfully used in extracts, but only when ActA was expressed from a multicopy plasmid[19]. These differences are noteworthy as some of the conclusions of these two reports are contradictory (see below). Importantly, all of these strains appear to express the ActA protein at high levels during intracellular infection.

Table 1. *Listeria monocytogenes* wild-type isolates and activities

Strain	Serotype	Hemolysis[a]	ActA (broth)[b]	ActA (cells)[c]
EGD	1/2a	+	+	++++
LO28	1/2c	++	++	++++
10403S	1/2b	+	+/–	++++
SLCC-5764	1/2a	++++	++++	++++
NCTC-7973	1/2a	++++	++++	++++

[a]Based on lysis of sheep red blood cells in suspension by bacterial culture supernatants.
[b]Based on western blots of detergent-solubilized bacterial surface proteins after separation on SDS-polyacrylamide gel electrophoresis.
[c]Based on [^{35}S]methionine incorporation during infection of J774 cells.

The nature of asymmetry

As stated above, directional motility of *S. pneumoniae* coated with ActA is dependent upon an asymmetric ActA distribution across its surface[15]. Two studies have examined ActA localization on the surface of *L. monocytogenes* and have reported contradictory results: one, that the protein uniformly covers the bacterial surface[20] and, the other, that there is a polar distribution of ActA (Ref. 21). The latter report is consistent with the requirement for asymmetry noted with *S. pneumoniae*. However, if ActA is distributed symmetrically around the surface of *L. monocytogenes*, any requirement for asymmetry could be met by modifications that regulate ActA activity. For example, we have reported that ActA is phosphorylated during intracellular infection[5]. The sites of phosphorylation map to multiple residues in the repeat region of ActA (Ref. 22). Although we have reported that deletion of this repeat region does not inhibit bacterial actin-based motility, mutant bacteria move less frequently than wild type. We do not yet know whether this decrease in initiating motility is caused by increasingly symmetrical ActA activity and whether ActA phosphorylation occurs predominantly at one bacterial pole.

There are two additional considerations when evaluating contradictory results of ActA distribution. First, the above studies used different strains of *L. monocytogenes*: one used EGD (Ref. 23) while the other used LO28 (Ref. 21). These two isolates differ in serotype, reflecting variation in bacterial surface composition. Perhaps differences in the dynamics or structure of the cell wall, which presumably limits diffusion of the ActA protein on the bacterial surface, account for the observed variation. Second, the studies used different antibodies. If expression of ActA on the *Listeria* surface is symmetric, modulation of asymmetrical activity by protein modification may also alter the antigenicity of ActA.

In our laboratory, we have frequently observed that 10403S possesses an apparently uniform distribution of ActA while moving by actin-based motility. However, dividing bacteria always appear to be devoid of ActA at the septum (for example, see Ref. 5). If ActA is absent, at least transiently, from the new pole of the daughter bacteria, an asymmetry in polymerized actin filaments may be established that, in turn, may direct continued actin-based motility. Such a mechanism could be enhanced by the ActA repeats and their phosphorylation.

Clouds and tails

How many steps contribute to the movement of *L. monocytogenes*? We have addressed two already: actin polymerization and asymmetry of ActA function. Evidence for a third step dates back to 1989. The use of transmission electron microscopy to examine cell lines infected with *L. monocytogenes* revealed that bacteria were associated with two different types of actin assemblies: actin clouds and actin tails[1,2]. By video microscopy, bacteria with tails, but not clouds, were found to move by actin-based motility[3]. Why do some bacteria induce clouds, while others induce tails and move? While asymmetry may be part of the answer, it is clearly not the entire story. The cloud-to-tail transition is observed with dead *L. monocytogenes* in cell-free cytoplasmic extracts, ruling out cell wall dynamics or other bacterial metabolic processes[18]. Furthermore, when purified ActA is distributed to only one pole of *S. pneumoniae* before their introduction into cell-free cytoplasmic extracts, these bacteria form transient cloud structures before the onset of motility and tail formation (G. Smith and J. Theriot, unpublished). The fact that *S. pneumoniae* expressing ActA in an asymmetric fashion still make the transition from cloud to tail indicates that the ActA protein performs the function(s) necessary for this transition.

In a recent study, we found that two repeat motifs in the ActA protein increase the number of moving intracellular bacteria[22]. One of the repeat motifs, the 11-amino-acid proline-rich repeats (present in four copies), binds directly to the host vasodilator-stimulated phosphoprotein (VASP), localizes host profilin to the bacterial surface, increases the velocity of bacteria and increases the number of bacteria moving within an infected cell. The second repeat motif, the long repeats (present in three copies), also increases the proportion of moving bacteria, but by a mechanism that does not involve VASP, profilin or changes in velocity. Neither type of repeat is required to initiate actin polymerization, which is directed by a domain that is nearer to the amino terminus than the repeats[24,25]. The proline-

Table 2. Differences in profilin-depletion studies

Study	Strain	ActA source	Extract source	Conclusion
Theriot *et al.*[18]	SLCC-5764	Chromosomal	Meiotically arrested eggs	Profilin depletion destroys motility
Marchand *et al.*[19]	L028	Multicopy plasmid	Activated (interphase) eggs	Profilin depletion does not affect motility

rich repeats and long repeats are good candidates for elements directing the cloud-to-tail transition. Why actin polymerization and asymmetry of ActA function are not sufficient to drive directional motility is not obvious; therefore, the role of the repeats in initiating motility may be subtle. To begin movement in the viscous environment of a host cell cytosol requires, in theory, overcoming friction or yield stress. The proline-rich repeats, which produce a force resulting in increased bacterial velocity, may also provide the additional force necessary to break the yield stress of the host cytosol and thereby increase the number of moving bacteria.

A second factor in the cloud-to-tail transition has been revealed by a mutant strain of *L. monocytogenes* that forms clouds but not tails[26]. Fluorescent polarization studies show that this mutant polymerizes actin filaments in arrays that are significantly more disordered than those polymerized by wild-type bacteria, which to a large degree form in parallel[27]. The authors hypothesize that the inability of this mutant to initiate movement is a direct consequence of filament disorder. Deletion of the entire repeat region of ActA results in a similar decrease in filament alignment[28]. Whether this phenotype is attributed to the ActA proline-rich repeats, long repeats or both has yet to be determined. It is tempting to speculate about an association between this phenotype and the phosphorylation of the long repeats. If phosphorylation of ActA is somehow favored at one bacterial pole, but not the other, this mechanism may enhance the functional ActA asymmetry required for the initiation of bacterial motility (a factor that may be of greater significance in some strains than others). We must emphasize the word 'enhance' in this hypothesis because *actA* alleles lacking all the repeats are capable of initiating movement, albeit at a frequency significantly lower than the wild type[22,25].

A role for profilin?

Profilin is a 15-kDa actin-binding protein that was first implicated in ActA function in 1994 (Ref. 18). By indirect immunofluorescence microscopy, profilin can be detected at the interface of actin tails and the moving *L. monocytogenes*. Profilin has the unique ability to bind actin monomers without inhibiting their addition to the kinetically active end of an actin filament[29]. In the presence of the actin-monomer-sequestering protein, thymosin β4, profilin desequesters actin monomers from thymosin, resulting in filament elongation[30]. The observed concentration of profilin at the bacteria–tail interface indicates that this is a site of rapid filament elongation which may in turn increase the velocity of bacterial motility[31]. Although this is an attractive model, studies addressing the role of profilin in *L. monocytogenes* motility have produced conflicting results. Using cell-free cytoplasmic extracts to reconstitute bacterial motility, depletion of profilin has been reported either to inhibit bacterial motility completely, but leave actin polymerization around the bacteria intact[18], or have no effect on bacterial motility at all[19]. This surprising paradox probably reflects differences in experimental design (Table 2). Both reports do agree that, at least under appropriate conditions, profilin can increase bacterial velocity.

In intact eukaryotic cells, *L. monocytogenes* moves much faster than in extracts depleted of profilin [3–7 times faster in the potoroo kidney epithelial cell line (PtK2) and 19–46 times faster in the mouse macrophage-like cell line (J774)][3,19,32]. While profilin may not be required for the slow rates of movement observed in some profilin-depleted extracts, the enhanced rates of movement attained in intact cells appear to be dependent upon this protein. It is now clear that ActA binds VASP and at least one VASP-related protein (Mena) and, in turn, VASP binds profilin[33–35]. Furthermore, the VASP-binding domain in ActA maps to the proline-rich repeats[22,24]. Sequential removal of these repeats decreases the localization of VASP and profilin to the surface of moving *L. monocytogenes* and decreases the rate of movement of these bacteria by ~2–3 μm min^{-1} per repeat in PtK2 cells[20]. When all the proline-rich repeats are deleted, the bacteria move at rates of 4.0–4.5 μm min^{-1}, which is similar to the rates observed with *L. monocytogenes* in cell-free extracts depleted of profilin[19,22]. Strains expressing ActA isoforms that lack all of the proline-rich repeats show similar decreases in velocity in cell-free extracts, although different strains of bacteria and different extract types may influence the degree to which these decreases manifest themselves[22,25]. In theory, these rates could be attained in the absence of profilin, providing the host cell cytosol has an unsequestered actin monomer pool of ~2 μM (based on a filament elongation rate of 11.6 μm s^{-1})[36].

Attempts to address the function of profilin directly in intact cells, by microinjecting ligands potentially competitive to the VASP–profilin or VASP–ActA interactions, result in inhibition not only of the ability of *L. monocytogenes* to move by actin-based motility but also of the ability of the bacteria to polymerize actin[37,38]. As bacteria lacking the proline-rich repeats in ActA move by actin-based motility but fail to bind profilin, these results are difficult to interpret.

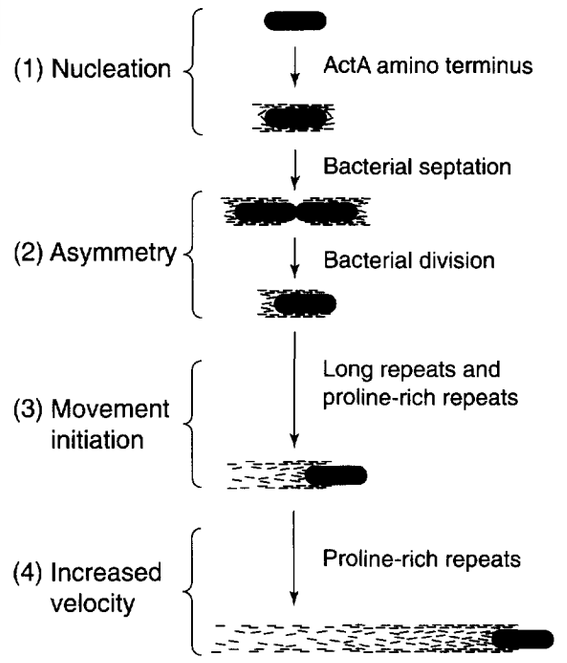

Fig. 1. Four steps contributing to bacterial motility. A coherent picture is beginning to emerge of the mechanism of *Listeria monocytogenes* actin-based motility. The four steps shown here are now known to contribute to motility, although details of their mode of action are not fully understood. (1) Actin polymerization is initiated by a region in the amino terminus of the ActA protein. Little is known of how nucleation occurs. (2) Directional motility suggests that ActA only functions at one pole of the bacterium. While the dynamics of the cell wall may provide the mechanism of ActA polarization (analogous to experiments performed with *Streptococcus pneumoniae*), the nature of asymmetry in *L. monocytogenes* remains controversial. Nevertheless, bacterial septation and division are implicated in this process. (3) In addition to asymmetric actin polymerization, at least one additional step is necessary for motility to begin. Nothing is known about this step, although separate roles for the ActA long repeats and ActA proline-rich repeats are implicated. Perhaps the bacteria overcome a cytoplasmic yield stress before the onset of motility. (4) The bacterial velocity is increased by the action of the ActA proline-rich repeats, which recruit vasodilator-stimulated phosphorylation (VASP) and profilin to the site of actin filament assembly. Reprinted from Ref. 20 with permission from Rockefeller University Press.

Questions for future research

- What are the signals that induce expression of ActA during intracellular infection? Is there an advantage to strains that can upregulate ActA expression in an intracellular environment?
- How do the ActA long repeats promote motility?
- How is ActA function limited to one pole of the bacteria? Is ActA asymmetrically distributed over the bacterial surface?
- How could *L. monocytogenes* move in a directional manner if the ActA protein is uniformly distributed over the bacterial surface?
- What other host factors participate in bacterial motility?
- Why has *L. monocytogenes* evolved to move at relatively high speeds? What advantage does speed provide?
- Is actin-based motility sufficient to produce stable filopod structures? Is it sufficient for cell-to-cell spread?

Concluding remarks

Historically, much attention has been paid to the ability of *L. monocytogenes* to nucleate actin polymerization[1,39,40]. Whether these bacteria perform *de novo* actin nucleation or initiate actin polymerization by one of several other possible mechanisms is unknown. A recent report has identified a complex of eight human platelet proteins, related to a profilin-binding complex first identified in *Acanthamoeba castellanii*[41], that is sufficient to nucleate actin clouds around *L. monocytogenes in vitro*[42]. Whether this complex is responsible for actin nucleation in intact host cells and whether it binds to ActA are not yet known. If the complex binds profilin in the same way as the related *A. castellanii* complex, binding to the ActA proline-rich repeats indirectly via a VASP–profilin tethering mechanism may occur in intact cells. However, it is important to remember that the proline-rich repeats of ActA are not essential for actin nucleation around *L. monocytogenes* and that a domain nearer the amino terminus than the repeats contains the critical region for the recruitment of this activity[22,24,25]. It will be very interesting to see if actin nucleation occurs around *L. monocytogenes* in extracts and cells in which this complex is depleted.

Although the mechanism by which *L. monocytogenes* initiates actin polymerization is still in question, we now know that ActA performs several functions that together culminate in bacterial actin-based motility. Among these are (1) initiation of actin polymerization mediated by a sequence at the amino terminus of ActA and independent of the repeat region[22,24,25], (2) polarization of ActA function, (3) transformation of actin polymerization into a motile force and (4) acceleration of velocity mediated by the proline-rich repeats, which indirectly mediate the binding of profilin (Fig. 1). Whether other unidentified functions are performed or whether other bacterial proteins contribute to aspects of *L. monocytogenes* motility, such as asymmetry or filopod formation, are yet to be determined. Furthermore, the mechanistic details of these functions are largely undescribed and will undoubtedly shed light on the cell biology of actin polymerization and actin-based motility, as well as the cell biology of the pathogenesis of *L. monocytogenes*. In this respect, ActA appears to be a highly specialized protein that is exploited in several ways by bacteria to get them from one host cell to another as efficiently as possible.

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Antiviral activity of cyclopentenone prostanoids

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Cyclopentenone prostanoids inhibit virus replication by turning on an intracellular defence response that involves the induction of cytoprotective heat-shock proteins, the modification of viral glycoprotein maturation and the control of NF-κB activation. These molecules represent an interesting model for the development of novel antiviral drugs that can affect different targets during the virus life cycle.

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In an age when so many antibiotics are available to combat almost any bacterial infection, advances in the control and cure of viral diseases are still limited. Toxicity and lack of therapeutic efficacy in long-term treatments are the major disadvantages of the antiviral drugs now in use. The failure to develop nontoxic antiviral drugs has traditionally been blamed on the intracellular parasitic nature of viruses. Consequently, most of the drugs that affect virus replication also interfere with important metabolic processes in uninfected cells. Although more selective antiviral compounds that inhibit the function of specific viral proteins are being developed, the high mutation rate seen in several viruses represents a further obstacle for long-term effective treatment. One successful approach in combating viral diseases appears to be the simultaneous use of two or more drugs that affect different targets during the virus life cycle.

A group of prostaglandins (PGs) and PG derivatives are able to interfere with virus replication at multiple levels while triggering the synthesis of cytoprotective proteins by the host cell. They appear to act differently from any other known antiviral agents and offer the prospect of novel strategies to combat viral infections.

Prostaglandins control virus replication

Prostaglandins are a class of naturally occurring cyclic C_{20} fatty acids with potent biological properties. In eukaryotic cells, they are synthesized from arachidonic acid and other polyunsaturated fatty acid precursors derived from the phospholipid pool of the cell membrane, and they function as intracellular signal mediators in the regulation of physiological and pathological processes, including inflammation and the febrile response, the immune response, cell proliferation and differentiation, and cytoprotection[1].

In the early 1980s, the use of virus models to investigate the molecular events that follow the exposure of mammalian cells to prostaglandins led to the serendipitous discovery that specific arachidonic acid derivatives

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