Actin-based Movement of Listeria Monocytogenes:
Actin Assembly Results from the Local Maintenance
of Uncapped Filament Barbed Ends at the Bacterium Surface

Jean-Baptiste Marchand,* Patrice Moreau,* Anne Paoletti, Pascale Cossart, Marie-France Carlier,* and Dominique Pantaloni*

Laboratoire d'Enzymologie and Centre de Génétique Moléculaire, CNRS, 91198 Gif-sur-Yvette Cedex; and CNRS URA 1300, Institut Pasteur, 75724 Paris

Abstract. The thermodynamic basis for actin-based motility of Listeria monocytogenes has been investigated using cytoplasmic extracts of Xenopus eggs, initially developed by Theriot et al. (Theriot, J. A., J. Rosenblatt, D. A. Portnoy, P. J. Goldschmidt-Clermont, and T. J. Mitchison. 1994. Cell. 76:505–517) as an in vitro cell-free system. A large proportion (75%) of actin was found unpolymerized in the extracts. The amount of unassembled actin (12 μM) is accounted for by the sequestering functions of TI3 xen (20 μM) and profilin (5 μM), the barbed ends being capped. Movement of Listeria was not abolished by depletion of over 99% of the endogenous profilin. The proline-rich sequences of ActA are unlikely to be the target of profilin. All data support the view that actin assembly at the rear of Listeria results from a local shift in steady state due to a factor, keeping filaments uncapped, bound to the surface of the bacterium, while barbed ends are capped in the bulk cytoplasm. Movement is controlled by the energetic difference (i.e., the difference in critical concentration) between the two ends of the filaments, hence a constant ATP supply and the presence of barbed end capped F-actin in the medium are required to buffer free G-actin at a high concentration.

The role of membrane components is demonstrated by the facts that: (a) Listeria movement can be reconstituted in the resuspended pellets of high speed-centrifuged extracts that are enriched in membranes; (b) Actin-based motility of endogenous vesicles, exhibiting the same rocketing movement as Listeria, can be observed in the extracts.

Although it is recognized that actin assembly plays a key role in cell motility, the exact mechanism by which actin assembly occurs, to produce movement, in response to extracellular stimuli, is far from understood (10, 11, 42, 61). The phenomenon of local transient actin assembly in living cells would appear to require two independent key steps: (a) a complex assembly of proteins at the plasma membrane to form a site-directed nucleator of actin filaments; reference 47; and (b) for actin filaments to effectively elongate onto these specified sites, a local shift in the steady state of actin assembly must occur (5). The main regulatory proteins which can control the steady state of actin assembly, i.e., the steady-state concentration of free G-actin coexisting with filaments, are capping proteins which increase the critical concentration by blocking the barbed end, and profilin, which, via its participation in actin assembly (34, 37), decreases the contribution of free G-actin itself (30), hence, antagonizes capping protein action. It has been shown that changes in free G-actin concentration result in amplified corresponding changes in the concentration of G-actin in complex with sequestering proteins. Capping of filaments increases the pool of (sequestered) unpolymerized actin at the expense of the F-actin pool, while uncapping of barbed ends leads to F-actin assembly from the pool of sequestered actin, an effect which is enhanced by profilin (5, 30).

Observations of the actin dynamics involved in the propulsive movement of the pathogenic intracellular bacterium Listeria monocytogenes in the host cytoplasm (48, 49) have shown evidence for a striking similarity between this process and the protrusion of the lamellipodia of locomoting cells. These elegant experiments demonstrated that actin assembly itself is directly linked to movement and indicated that Listeria monocytogenes is a useful tool for probing the components of the cellular motile machinery present at the leading edge. Genetic studies demonstrated that the expression of ActA protein at the surface of Listeria is necessary to induce actin assembly and propulsion of the bacterium (25). Similarly, the expression of IcsA, a protein unrelated to ActA, at the surface of Shi-
gella flexneri, another pathogenic bacterium, induces actin assembly and intracellular propulsion (2), the characteristics of the movement being very similar to that of Listeria. It is highly likely that these two surface proteins bind differently to components of the same transduction cascade leading to actin assembly.

The recent design of a cell-free system, the Xenopus egg cytoplasmic extract, capable of reconstituting the motility of Listeria monocytogenes in vitro, constitutes a breakthrough (50) that now facilitates the identification of the proteins involved in site-directed actin assembly and a test of the function of ActA and IcsA via genetic constructions. In particular, we have recently shown that heterologous expression of ActA in the non-pathogenic L. innocua strain, or of IcsA in Escherichia coli is sufficient to induce actin assembly and bacterial propulsion in these extracts (Kocks, C., J.-B. Marchand, E. Gouin, H. d’Hauteville, P. J. Sansonetti, M.-F. Carlier, and P. Cossart, manuscript submitted for publication).

In the present work, cytoplasmic extracts of Xenopus eggs were used to analyze the roles of different cytoplasmic components thought to be involved in Listeria movement.

The following questions have been addressed: what is the thermodynamic basis for actin assembly at the rear of Listeria? Is an energy supply necessary for movement and by which biological system is this energy used? Does movement require profilin? Are soluble or insoluble components of the cytoplasm involved in movement?

Materials and Methods

Proteins

Actin was purified from rabbit muscle and isolated as CaATP-G-actin by Sephadex G200 chromatography (30). Actin was rhodamine labeled, in the F-actin form, using S and 6 carb oxytetramethyl rhodamine succinimidyl ester (NHS-R) (20), and stored as CaATP-G-Rh-actin (~60 mM) at ~80°C. Thymosin β and profilin were purified from bovine spleen as described (30). DNaseI GRADE II was from Boehringer-Mannheim Biochemicals (Indianapolis, IN). The actual concentration of DNaseI was calculated by G-actin titration.

Cytoplasmic Extracts of Xenopus Eggs

Interphase extracts of Xenopus eggs were prepared as described (27) but omitting cytochalasin D. Briefly, the eggs were activated by electric shock (12 V, 2 s) and spun after 15 min, first at 1000 g, 4°C, for 1 min with a ml layer of silicon oil AR 200 (density = 1.049; Fluka AG, Buchs, Switzerland) to eliminate buffer, then crushed at 15,000 g, 4°C, for 20 min. The cytoplasmic layer was removed, the extract was supplemented with protease inhibitors (27), 0.2 mM EGTA, 150 mM sucrose, 100 μg/ml cycloheximide, rapidly frozen in 50 μl aliquots in liquid nitrogen and stored at ~80°C.

Bacterial Strains

L. monocytogenes strain Lut12 (pactA3) (Collection name BUG666) overexpressing ActA (25) was mainly used in the experiments reported here. Bacteria were grown at 37°C in brain–heart infusion (Difco Laboratories, Detroit, MI) with aeration in the presence of 7 mg/ml chloramphenicol. Overnight cultures were diluted 10-fold in fresh medium and regrown to stationary phase (A600nm = 2.0 to 2.5), and then supplemented with an equal volume of 60% glycerol. Aliquots of 100 ml were frozen in liquid nitrogen and stored at ~80°C.

Notes

1. Abbreviations used in this paper: DCCD, N,N'-dicyclohexylcarbodi-imide; GTP–S, guanosine 5’–(3-thiotriphosphate); NEM, N-ethylmaleimide; NHS-R, 5 and 6 carb oxytetramethyl rhodamine succinimidyl ester; PI, phosphatidylinositol; PLC, phospholipase C.

Anti-Xenopus Egg Profilin Antibody

Initial experiments showed that a serum raised against bovine brain profilin failed to recognize Xenopus egg profilin. Therefore a serum was raised against Xenopus egg profilin as follows. Profilin was purified by poly-L-proline affinity chromatography of 18 ml of Xenopus egg cytoplasmic extract treated with 1% Triton X-100 (100,000 g supematant). Pure profilin was eluted with 6 M ura, the peak fractions (0.75 mg) were dialyzed against 5 mM Tris, pH 7.5, for 48 h. The purified profilin was injected intradermally four times at 2/3-week intervals. In the first immunization, 100 μg profilin, with complete Freund's adjuvant, was injected in six spots (100-μl each); subsequent injections consisted of 50 μg profilin with incomplete Freund's adjuvant. The serum used in this study was prepared by bleeding the rabbits 2 wk after the fourth injection. This serum recognized Xenopus egg profilin specifically and rabbit 5S profilin at 1/5,000 dilution in Western blots using ECL (Amersham Corp., Arlington Heights, IL) detection. It did not cross-react with up to 50 ng bovine spleen profilin, at 1/1,000 dilution. Conversely the serum raised against bovine spleen profilin did not cross-react with Xenopus egg profilin.

Fluorescence and Phase Contrast Video Microscopy Observation of Listeria Movement in Cytoplasmic Extracts: Data Acquisition and Movement Analysis

Aliquots of Xenopus egg extracts were thawed and supplemented with 1 mM ATP, 30 mM creatine phosphate, ~3 μM rhodamine-actin, and bacteria (2.108/ml). The bacteria were spun at 4,000 g for 5 min at 20°C, resuspended in XB buffer (10 mM Hepes, pH 7.7, 100 mM KCl, 1 mM MgCl2, 0.1 mM CaCl2, 50 mM sucrose) before addition to the extracts. The total volume of all components added to the extract did not exceed 20% of the original volume. Bacterial motility was also measured in the pellets of Xenopus egg extracts sedimented at 400,000 g for 30 min at 4°C in the ultracentrifuge (TL100; Beckman Instruments, Palo Alto, CA). In this case the pellet was gently resuspended in the original volume of XB buffer containing creatine phosphate and rhodamine actin before addition of bacteria.

The mixture was brought to room temperature for 5 min and 2.5 μl were squeezed between a slide and a 22 × 22 mm coverslip. The thickness of the solution between the two glass surfaces was 5 μm. Motility of bacteria was observed, after 1-h incubation, at 20°C, on a Zeiss III RS microscope equipped with a silicon-intensified camera (LHESA LHIA036), which allowed observation of bacteria by phase contrast and of fluorescent actin tails simultaneously, using a 100 W tungsten-iodine lamp for phase contrast and a 200 W mercury arc for fluorescence. Images were recorded in real time or in time lapse on a video tape recorder (Panasonic). Measurements were therefore made after the endogenous F- and G-actin pools in the extracts were homogeneously labeled by rhodamine actin.

An Optimas software (Biocam) attached to a PC computer was used to record the movement of bacteria from the images, in terms of space (x, y) and time (t) coordinates, by clicking on the center of the bacterium at time intervals of a few seconds. The time window was adjusted to bacterium speed so that the minimum elementary displacement recorded was ~2 μm. The displacement versus time was derived for each bacterium over periods of a few minutes, corresponding to travelled distances of 15–60 μm (10–20 time points), from which the average speed was derived. For each sample, the speeds of 10–20 bacteria were measured, and the average speed and standard deviation were calculated.

Only the motile bacteria were selected for speed measurements. When applicable, the fraction of motile bacteria was also measured. Comparison of rates of movement under different conditions or upon addition of various amounts of inhibitors implies, to be meaningful, that the percent of bacteria moving remains constant. Biases introduced in the data by changes in the fraction of bacteria moving are noted and discussed in Results where necessary.

Analysis of the fluorescence decay in fluorescent actin tails was performed using an image analysis Optimas software. The fluorescence intensity was integrated along the median line of the comet, over a width of 0.8 μm. The half-life of actin filaments was derived from the combined measurements of bacterium speed and exponential fluorescence decay.

Quantitation of Actin and Profilin in Different Cellular Fractions by Western Blotting

Fractions of extracts containing different amounts of protein were denatured in SDS and submitted to SDS-PAGE (10–15% acrylamide) in parallel with standard known amounts of pure actin and profilin in a range in
which the linearity of the ECL immunodetection was first established. The electrophoresed samples were transferred onto nitrocellulose and probed with either monoclonal anti-actin (IgM N350 from Amersham Corp.; at 1/1,000 dilution) or anti-Xenopus egg profilin antiserum at 1/5,000, and detected by chemiluminescence using the ECL kit. The amount of actin or profilin in the different fractions was evaluated by comparing the integrated intensity of the densitometered bands of each sample with the calibration standards. This technique was also used to evaluate the amount of profilin remaining in extracts following depletion by poly-L-proline-agarose chromatography, and in mock-depleted extracts, chromatographed on undervariatized agarose in parallel.

**Quantitation of T\(^{\beta_4}\)en in Xenopus Egg Extracts by HPLC**

Extracts were treated with 0.5 \(N\) \(\text{HClO}_4\) on ice. Precipitated proteins were eliminated by centrifugation (20,000 \(g\), 0°C, 10 min). The supernatant was brought to pH 4 by \(\text{KOH}\). After elimination of \(\text{KClO}_4\) by centrifugation, the material was analyzed by reverse phase HPLC (Beckman Instruments, Gold system) on Lichrospher RP select B (5 \(\mu\)m; Merck Sharp and Dohme, St. Louis, MO) using a 5 to 50% acetonitrile gradient and 214 nm absorbance monitoring. \(T\(^{\beta_4}\)en\) eluted at the same position as bovine spleen \(T\(^{\beta_4}\) (17). The amount of \(T\(^{\beta_4}\)en\) in the assayed sample was derived from the comparison of the integrated area of the peak of \(T\(^{\beta_4}\)en\) on the elution pattern with the area of the corresponding peak of \(T\(^{\beta_4}\) standards, and comparison of its UV spectrum with that of bovine \(T\(^{\beta_4}\).

**Mass Spectrometry Identification of T\(^{\beta_4}\)en**

The peak fraction eluting from reverse phase HPLC of Xenopus egg perchloric extracts at the position of the bovine \(T\(^{\beta_4}\) standard was identified by mass spectrometry (Fison, Manchester, UK).

**Gel Electrophoresis under Non-denaturing Conditions**

Non-denaturing gel electrophoresis was used as described (41) to monitor the formation of a complex between G-actin and \(T\(^{\beta_4}\)en\) in Xenopus egg extracts. Gels were Coomassie blue stained.

**Treatment of Extracts by Clostridium Toxins**

Pellets of high speed-sedimented extracts were resuspended in the original volume of 20 mM Hepes, pH 7.5, 0.1 mM DTT, 2 mM MgCl\(_2\), and either 100 \(\mu\)M GDP and 50 mM eoxenolyme C\(_2\) of *Clostridium botulinum* toxin, or 5 \(\mu\)M guanosine 5'-O-(3-thiotriphosphate) (GTP-\(\gamma\)-S), and 10 nM B toxin. Control samples were prepared without toxins. Samples were incubated at 20°C for 30 min and then processed for motility assay by addition of rhodamine-actin, ATP-regenerating system, and bacteria.

**Results**

**Amounts of Assembled and Unassembled Actin, Thymosin \(\beta_4\), and Profilin in Xenopus Egg Cytoplasmic Extracts**

Extracts (100 \(\mu\)l) were centrifuged at 400,000 \(g\), 4°C for 20 min in the TL 100 Beckman ultracentrifuge. The pellet was resuspended in a volume of XB buffer equal to the volume of the supernatant (i.e., ~75% of the total volume of the extract). The amounts of actin and profilin in the total extract and in the supernatant and pellet fractions were derived from western blot analysis of the different fractions, as displayed in Fig. 1. The corresponding data, summarized in Table I, show that the extracts contain a total amount of 16 \(\mu\)M actin, of which 12 \(\mu\)M (75%) is unpolymerized. Profilin was present at 5 ± 1 \(\mu\)M in the extracts, and most of it (>80%) was recovered in the supernatant, indicating that it was essentially soluble. However the amount of ~1 \(\mu\)M profilin found in the pellet seems too high to be accounted for by simple contamination suggesting that a small proportion (~10%) of profilin may be in part associated with membrane components, consistent with immunofluorescence localization studies (3). Evidence for the presence of \(T\(^{\beta_4}\)en\), the known \(17\) Xenopus variant of \(T\(^{\beta_4}\) in Xenopus egg extracts, was provided by the HPLC elution pattern of the perchloric extracts (data not shown). Mass spectrometry of the clean peak fraction eluting at the same position as the \(T\(^{\beta_4}\) standard showed evidence for a single component of mass 5,006 D identical to the calculated mass of \(T\(^{\beta_4}\)en\). From the area of the \(T\(^{\beta_4}\)en\) peak in the HPLC pattern (three determinations), the concentration of \(T\(^{\beta_4}\)en\) was evaluated at 20 \(\mu\)M ± 2 \(\mu\)M in Xenopus egg extracts, and the same amount (within 10%) was found in the supernatant, consistent with this protein acting as a purely soluble, G-actin sequestering agent (8, 56). To confirm the interaction between G-actin and \(T\(^{\beta_4}\)en\) in the extracts, the soluble cytoplasmic fraction was spun at 400,000 \(g\) and analyzed by non-denaturing gel electrophoresis (41). Because actin is a major protein in Xenopus egg extracts, it was prominent in Coomassie blue-stained non-denaturing gels. Fig. 2 shows that unpolymerized actin in the supernatant of centrifuged extracts
Interphase cytoplasmic extracts of Xenopus eggs were spun at 400,000 x g, 4°C, for 20 min. The amounts of actin and of profilin present in whole extracts and in the supernatant and pellet of centrifuged extracts was derived from the analysis of Western blots as shown in Fig. 1. The amount of Tβ4 was derived from the HPLC elution pattern of perchloric extracts (see Materials and Methods).

Table 1. Amounts of Assembled and Unassembled Actin, of Profilin and of Tβ4 in Cytoplasmic Extracts of Xenopus Eggs

| Protein | Whole extract | Supernatant | Pellet |
|---------|---------------|-------------|--------|
| Actin   | 16 ± 2 μM     | 12 ± 1.5 μM | 4 ± 1 μM |
| Profilin| 5 ± 1 μM      | 4 ± 0.8 μM  | ≤1 μM  |
| Tβ4     | 20 ± 2 μM     | 20 ± 2 μM   | -      |

Table 2 shows that as long as Kt > [Aa] the amount of sequestered actin is greater when [Aa] is higher, i.e., when barbed ends are capped. The large proportion of actin found un polymerized in the extracts suggests that in fact [Aa] is high, i.e., that barbed ends must be capped. Indeed if we a contrario assume that barbed ends are free, then [Aa] = 0.1 μM (the critical concentration at the barbed end) and the amount of actin sequestered by Tβ4, the major G-actin sequestering protein, can be calculated, taking the equilibrium dissociation constant for the Tβ4–actin complex as Kt = 1 μM (56). Eq. 1 shows that the amount of Tβ4–actin complex would be 20 × 0.1 = 1.8 μM. Profilin does not sequester G-actin when barbed ends are free but rather causes a decrease in As to a value 10-fold lower than 0.1 μM (30) so that the amount of 1.8 μM Tβ4–actin calculated above is greatly overestimated, if barbed ends are free. Assuming free barbed ends, in the presence of profilin, [Aa] = 0.01 μM, hence, Eq. 1 shows that accounting for the presence of 10 μM un polymerized actin in the cytoplasm would require a total amount of 100 μM sequestering agent of affinity 107 M−1 for G-actin, or of 1 μM sequestering agent of affinity 106 M−1 for G-actin. Even summing up all possible G-actin binding proteins known other than the most abundant one (Tβ4), it is not possible, if barbed ends are free, to account for the 12 μM un polymerized actin measured in the extracts. On the other hand, if barbed ends are capped, both profilin and Tβ4 act as G-actin sequestering proteins, and using [Aa] = 0.6 μM (the critical concentration at the pointed end), and a value of 0.2 μM for the equilibrium dissociation constant Kp for the profilin–actin complex (30–32), one can calculate

\[
[Aa] = [As] + \sum_i [S_{10}] \frac{[Aa]}{[As] + K_i}
\]

where [S10] is the total concentration of the G-actin binding protein S10 and K1 is the equilibrium dissociation constant for the binding of S10 to G-actin. Eq. 1 shows that as long as Kt > [Aa] the amount of sequestered actin is greater when [Aa] is higher, i.e., when barbed ends are capped. The large proportion of actin found un polymerized in the extracts suggests that in fact [Aa] is high, i.e., that barbed ends must be capped. Indeed if we a contrario assume that barbed ends are free, then [Aa] = 0.1 μM (the critical concentration at the barbed end) and the amount of actin sequestered by Tβ4, the major G-actin sequestering protein, can be calculated, taking the equilibrium dissociation constant for the Tβ4–actin complex as Kt = 1 μM (56). Eq. 1 shows that the amount of Tβ4–actin complex would be 20 × 0.1 = 1.8 μM. Profilin does not sequester G-actin when barbed ends are free but rather causes a decrease in As to a value 10-fold lower than 0.1 μM (30) so that the amount of 1.8 μM Tβ4–actin calculated above is greatly overestimated, if barbed ends are free. Assuming free barbed ends, in the presence of profilin, [Aa] = 0.01 μM, hence, Eq. 1 shows that accounting for the presence of 10 μM un polymerized actin in the cytoplasm would require a total amount of 100 μM sequestering agent of affinity 107 M−1 for G-actin, or of 1 μM sequestering agent of affinity 106 M−1 for G-actin. Even summing up all possible G-actin binding proteins known other than the most abundant one (Tβ4), it is not possible, if barbed ends are free, to account for the 12 μM un polymerized actin measured in the extracts. On the other hand, if barbed ends are capped, both profilin and Tβ4 act as G-actin sequestering proteins, and using [Aa] = 0.6 μM (the critical concentration at the pointed end), and a value of 0.2 μM for the equilibrium dissociation constant Kp for the profilin–actin complex (30–32), one can calculate

\[
[Aa] = [As] + [Po] \frac{[Aa] + [To]}{[As] + Kp + [To]} + [Tao] \frac{[Aa]}{[As] + Kt}
\]

where [Po] and [To] represent the total concentrations of profilin and Tβ4, respectively.

Using the measured values [Po] = 5 μM, [To] = 20 μM, one finds [Aa] = 11.3 μM, in good agreement with the experimentally determined value of 12 μM. Hence available data are consistent with the view that filament barbed ends are capped in Xenopus egg extracts, which accounts for the large amount of un polymerized actin, and the amounts of Tβ4 and profilin are sufficient to account for most of the un polymerized pool.

Role of Profilin in the Movement of Listeria in Xenopus Egg Extracts

The movement of Listeria was successfully observed in Xenopus egg extracts supplemented with 1 mM ATP, creatine phosphate and fluorescently labeled actin, as previously described (50). The percent of motile bacteria was greater in the presence of the ATP regenerating system (80%) than in its absence (10%). The rates of movement were variable (up to twofold) among different prepara-
Figure 3. Movement of Listeria monocytogenes (strain Lut 12 + p ActA) in Xenopus egg extracts under different conditions. The same field was observed either in fluorescence only (d-g) or simultaneously in phase contrast, to show the bacterium as a dark spot, and in fluorescence, to show the bright actin tails (a-c). (a) Standard image of Listeria moving in whole extract; (b) bacteria moving in resuspended 400,000 g pellet; (c) movement is clearly visible in extracts 99% depleted in profilin; (d-f) role of the ATP supply in movement. Typical comets observed in the absence of creatine phosphate after 1 h (d) and 3 h (e) incubation, and in the presence of creatine phosphate after 3 h incubation (f). (g) Effect of BeF₃ on the movement. Comets were photographed after 1 h incubation in the presence of 100 μM BeF₃. Note the absence of fluorescence decay and very long actin tails. Bar, 10 μm.

Propulsion rates ranged from a low of 5.7 ± 1.5 μm/min (n = 14) to optimum rates of 14 μm/min (SD = 4, n = 10) at 20°C with the Lut 12 (pactA3) bacteria. Analysis of the exponential fluorescence decay of assembled actin in the comet tails yielded a filament half-life of 55 s, in satisfactory agreement with the values of 42 and 33 s previously found in Xenopus egg extracts and in infected PtK₂ cells, respectively (50).

Listeria monocytogenes was unable to move in the high-speed supernatant of extracts obtained by centrifugation at 400,000 g. On the other hand, as illustrated in Fig. 3 (a and b), motility was restored in the resuspended pellets, with or without the addition of 3 μM fluorescently labeled rhodamine–actin. Movement also was observed, but at appreciably slower rates, in resuspended pellets of extracts that had been incubated at 4°C for 30 min in the presence of up to 1% Triton X-100 before centrifugation (see Table III). The rate of Listeria movement in the resuspended pellet was ~5-7 μm/min, and routinely showed a lower variability than the rate measured in the whole extract. The observation of movement in the resuspended pellet provided a first indication that neither profilin nor Tβ₄, which fractionate mainly in the supernatant, are absolutely critical for Listeria movement. Further work was carried out to assess the role of profilin as follows. Profilin was depleted from total extracts by treatment with poly-L-proline-agarose beads. Mock-depleted extracts were obtained by an identical treatment, carried out in parallel with underivatized agarose (50). Depletion of profilin was carried out either by a “batch” procedure, at 4°C as described by Theriot et al. (50), or by micro-chromatography of the extract (100 μl) on a poly-L-proline affinity column made of a capillary tube containing 100 μl poly-L-proline-agarose. In most cases, both procedures (batch + chromatography), were used consecutively. The amount of profilin remaining in the treated extracts was measured by Western blots. The following observations, summarized in Table II, were made. (a) Treatment of the extracts by either underivatized agarose or poly-L-proline-agarose led to a dilution of about twofold of the material (from protein assay), which caused a decrease in the average rates of Listeria movement in mock-depleted controls as compared to the rates measured in the original extract. (b) The
“batch” method provided the same depletion in profilin whether the incubation of the extract (with stirring) was carried out for 10 min or for 2–4 h. (c) The batch method provided ~90% profilin depletion, the chromatographic method led to over 99.5% profilin depletion, according to western blotting determination (Fig. 4). (d) The rate of movement following extensive profilin depletion was between 100 and 75% of the rates in mock-depleted controls; the percent of motile bacteria was about 50% of the mock-depleted control. Adding back 10 μM profilin, immediately after depletion, in the motility assay did not change the rate of movement nor the percent of bacteria moving. On the other hand, addition of 5 μM F-actin to profilin-depleted extracts resulted in 30% increase in rate of movement (Table II). These results lead us to conclude that profilin is not absolutely required for the movement of Listeria in Xenopus egg extracts, a conclusion opposite to the one drawn by Theriot et al. (50). In an effort to understand the possible source of the discrepancy between the two laboratories, a supplementary profilin depletion experiment was carried out choosing an egg extract that initially worked poorly (few motile bacteria, rate of movement in untreated extract: 7.6 ± 2.7 μm/min, n = 72). The mock-depleted control showed ~10% of bacteria moving at slow rate (1.6 ± 0.7 μm/min, n = 17). The profilin-depleted sample showed no movement although bacteria were surrounded by a red cloud of polymerized actin. Movement was not restored by adding back 5 μM profilin, in agreement with the above listed experiments; however movement was restored (rate 1.4 ± 0.3 μm/min, n = 6) by addition of 5 μM F-actin. Movement was also restored by the simultaneous addition of 5 μM F-actin and 5 μM profilin. This last piece of data indicates that the absence of movement in the profilin-depleted sample was not due to profilin depletion per se, but to the removal of actin which remains bound to profilin in the poly-L-proline chromatography. This result suggests that a low actin content might have been a limiting factor in the poor motility displayed by Listeria in this extract.

In the model presented for the role of profilin in Listeria movement (50; for review see references 12, 13, 45), the fourfold repeated proline-rich sequence of ActA was proposed to be the site for binding profilin. If this was the case, extracts containing large amounts of poly-L-proline should not support Listeria movement due to competition between ActA and poly-L-proline for binding to profilin. In our hands, addition of up to 1.2 mg/ml poly-L-proline (10,000–30,000 mol wt; Sigma Chemical Co., St. Louis, MO) to extracts failed to inhibit movement of Listeria. This amount of poly-L-proline is saturating for profilin or profilin-actin (31). Moreover, addition of up to 10 μM of the proline-rich 21-amino acid peptide of sequence 268–287 of ActA, failed to impede the movement of Listeria. The first result is in agreement with our data showing that profilin is not strictly necessary in Listeria propulsion; the second result shows that: (a) the proline-rich sequence in ActA is not used for binding profilin, in agreement with our previous in vitro data (31) showing that this peptide does not bind profilin; (b) if another putative protein binds...
to the proline-rich sequence in ActA, it must recognize the repeated sequence with a much higher affinity than the single proline-rich peptide, to account for the failure of peptide 268-287 of ActA to compete with ActA.

**Requirement of an Energy Source for Listeria Movement**

The role of ATP as a source of energy fueling the propulsion of Listeria in extracts, and the potential involvement of motor ATPases in the movement was investigated as follows.

A preliminary experiment showed that pretreatment of the resuspended pellets by either 25 U/ml apyrase or 15 U/ml hexokinase and 10 mM glucose for 15 min at 20°C abolished movement of bacteria, and no actin assembly could be detected at the surface of Listeria.

The rate of movement was evaluated in the presence of 1 mM ATP added at time zero, with or without creatine phosphate as an ATP-regenerating system. Rates and actin tail lengths were measured on the same samples at different time intervals over a period of >8 h. Fig. 5 shows that Listeria moved faster and movement was sustained over a longer period of time in the presence than in the absence of an ATP-regenerating system. In the absence of creatine phosphate, the rate of movement decreased with time and bacteria eventually stopped moving by 4 h; the decrease in rate was delayed in the presence of creatine phosphate, indicating that ATP is required for movement. The length of the actin tails also changed with time. In the absence of creatine phosphate, the tails grew very long as the bacteria slowed down upon ATP depletion. The tail length increased from an average size of ~30 μm after 2-h incubation, to 200 μm after 4-h incubation, and 300 μm after 6-h incubation. In the presence of creatine phosphate, the tail length remained constant (~50 μm) for a longer time.

![Figure 5. Role of the energy supply in Listeria movement. The rate of movement and the length of the actin tail (inset) were measured at different time intervals on the same preparations, incubated in the absence (closed symbols), and in the presence (open symbols) of 30 mM creatine phosphate.](image)

**Table III. Effect of Metabolic Inhibitors on the Movement of Listeria in Xenopus Egg Extracts**

| Experiment number | Compound          | Target                        | Rate, μm/min |
|-------------------|-------------------|-------------------------------|--------------|
| 1                 | AMP PCP          | 1 mM ATPases (V, mb, mt) kinases | 7.1 ± 1.7    | 6.6 ± 2.1 |
|                   | Genistein        | 30 μM ATPases (ser-thre) kinases | 6.5 ± 2.8    | 6.6 ± 2.1 |
|                   | Vanadate         | 100 μM ATPases (V, mb) myosin  | 10.1 ± 4     | 6.6 ± 2.1 |
| 2                 | DCCD             | 1 mM ATPases (V, mb)          | 5.3 ± 2.2    | 6.2 ± 2.6 |
| 3                 | NEM              | 20 μM ATPases (V, mb)         | 8.4 ± 3.9    |           |
| 4                 | Baflomycin       | 1 μM ATPases (V)              | 11.4 ± 3.6   | 9.6 ± 2.8 |
| 5                 | Herbinycin A     | 1 μM Tyrosine kinases         | 8.7 ± 3.3    | 8 ± 2.7   |
| 6                 | Heparin          | 10 μM Casein Kinase II        | 8.5 ± 3.8    | 7.9 ± 1.6 |
| 7**               | (BeF₃, H₂O)      | 100 μM ATPases, GTPases       | 9.5 ± 1.8    | 6.7 ± 3   |
| 8**               | Wortmannin       | 1 μM PI3-kinase               | 7.9 ± 4.2    | 5.2 ± 1.6 |
| 9**               | Triton X100      | 0.2% G-proteins              | 4.3 ± 0.7    | 5.4 ± 1.1 |
|                   | Triton X100      | 1%                           | 4.9 ± 0.9    |           |
|                   | Triton X100      | 1%                           | 4.9 ± 0.9    |           |
|                   | Triton X100      | 1%                           | 4.5 ± 1.4    | 5.4 ± 2.3 |

Each compound was added to extract at the indicated concentration together with the bacteria, rhodamine-actin ATP, and creatine phosphate. Movement was assayed after 1-h incubation at 20°C. V, mb, mt refer to vacuolar, plasma membrane, and mitochondrial species of ATPases respectively.

*, Indicates that movement was assayed in the resuspended high speed pellet in the presence of the inhibitor.

**, The extract was treated by Triton X-100 for 30 min at 4°C before being sedimented at 400,000 g. The motility assay was carried out on the resuspended pellet without Triton.
time and the increase in length was slowed down. The changes in tail length observed with different sources of ATP are shown in Fig. 3 (d-f). The following conclusions can be derived from these results. A constant length of the actin tail indicates that the rate of assembly of actin (i.e., the rate of Listeria movement) is equal to the rate of F-actin disassembly in the tail in a typical steady-state process. The increase in tail length that occurs upon ATP depletion, while the rate of movement slows down, indicates that the rate of filament disassembly in the tail gradually becomes lower than the rate of actin filament assembly behind Listeria.

The possible involvement of different types of ATPases in Listeria movement was assayed using several metabolic inhibitors, including AMPPNP, vanadate, N,N'-dicyclohexylcarbodiimide (DCCD), N-ethylmaleimide (NEM), or bafilomycin. As summarized in Table III, none of these agents inhibited Listeria movement. The failure of 100 μM vanadate or BeF₃ to inhibit Listeria movement argues against the participation of myosin motors in movement (the different myosin species have similar ATPase head motor domain, and different targetting tails). Inhibitors of kinases (6-dimethylaminopurine, genistein, herbimycin A, heparin), and of phosphatases (okadaic acid) also failed to inhibit Listeria movement. Of particular interest is the effect of BeF₃ ·H₂O, a structural analog of inorganic phosphate which blocks ATPases and GTPases in the NDP-P intermediate state of NTP hydrolysis. The addition of BeF₃ did not inhibit Listeria movement, rather it caused a significant increase in the rate of movement (1.5- to 2-fold). In addition, the actin tails, stabilized by BeF₃ in the F-ADP-P₇ state of the actin filament (4, 9), never disassembled and showed no fluorescence decay. Actin tails several millimeters in length were routinely observed in the presence of 100 μM BeF₃ as illustrated in Fig. 3 g. The formation of long stable tails is easily explained by the inhibition of F-actin disassembly by BeF₃. On the other hand, the faster rate of movement could result from a direct or an indirect effect of BeF₃ on actin assembly, as follows. The net rate of assembly, which controls the movement, is the algebraic sum of the rate of association of actin subunits to filament ends (positive term) and of the rate of dissociation of subunits from filament ends (negative term); in the absence of BeF₃, dissociation being inhibited, an increase in the net rate of assembly, hence in the speed of bacteria, is observed. Alternatively, BeF₃ may maintain an essential GTPase transducer in the GDP-P₇-like state, that may activate a putative effector molecule playing a crucial role in inducing actin assembly. The role of small G proteins Rho and Rac in the regulation of actin assembly and Listeria movement has indeed been demonstrated (1, 38), and a G-protein is also known to be involved in actin assembly in stimulated neutrophils (16). To test this possibility, the effect on movement of GTP-γS, a non-hydrolyzable analog of GTP which acts by maintaining G-proteins in an active GTP state, was studied. Addition of up to 2 mM GTP-γS to the preparation of resuspended pellets did not appreciably change the rate of Listeria movement, in a reproducible fashion. Cumulated measurements from three independent experiments showed that the bacteria moved at similar rates in the control and GTP-γS-containing samples respectively (see Table III).

Accordingly, treatment of the resuspended pellets by exoenzyme Cₙ of C. botulinum or by toxin B of Clostridium difficile (see Materials and Methods), two toxins known to specifically abolish rho function (22, 23) did not affect the movement of Listeria. This result differs from the one obtained in infected cells (1), which may indicate that the target of Listeria is downstream rho in the transduction cascade leading to actin assembly. This target would be active in Xenopus egg extracts, but would have to be rho-activated in the whole living cell.

Assays of Listeria Movement in High Speed Pellets of Xenopus Egg Extracts: Effect of Tβ₄, DNaseI, Profilin, and Phalloidin

The fact that Listeria movement can be reconstituted in the resuspended pellet of extracts centrifuged at 400,000 g makes this material, which contains very low amounts of profilin, Tβ₄ and other soluble proteins, useful for the analysis of Listeria movement in response to the addition of different proteins.

The amount of endogenous actin in the resuspended pellet was 4 μM, to which 2 μM rhodamine-actin was added. Bacteria moved in this medium at 60-70% of their optimum rate in the original extract (i.e., at 5-7 μm/min). Movement also was observed using phase contrast microscopy without addition of rhodamine-actin. Addition of exogenous F-actin resulted in a small increase in the rate, which reached a maximum value of 9-10 μm/min at 10 mM F-actin. Movement was assayed in resuspended pellets supplemented with 23 μM Tβ₄. In the absence of exogenous F-actin, the rate of movement was appreciably inhibited by Tβ₄ (1-1.5 μm/min), but increased notably upon re-addition of F-actin (Fig. 7). It should be noted that the decrease in rate was accompanied by a decrease in the number of motile bacteria; about ten times fewer bacteria moved at 1 μm/min in the presence of Tβ₄ than in the absence of Tβ₄. The data shown in Fig. 6, support the view that: (a) Tβ₄ depolymerizes F-actin; (b) no movement can be observed when all F-actin has been depolymerized by Tβ₄; (c) about 5 μM actin can be depolymerized by 23 μM Tβ₄, consistent with a steady-state concentration of G-actin of 0.5 μM, indicating that barbed ends are capped in the resuspended pellets, as they are in the original extract.

The effect of DNaseI on the movement of bacteria in resuspended pellets supplemented with 20 μM F-actin is shown in Fig. 7. No change was observed in the rate of movement nor in the fraction of motile bacteria up to 15-20 μM DNaseI, but above that concentration both the rate and percent of moving bacteria decreased abruptly to zero. These data confirm that total depolymerization of F-actin leads to abolishment of actin-based motility, and that a minimum amount of F-actin is necessary for Listeria movement, above which the rate of movement and actin tail width are independent of the concentration of F-actin. The role of F-actin is to maintain the high critical concentration (0.6 μM) of the pointed ends in the medium, thus allowing actin assembly at the barbed ends at the surface of Listeria.
Figure 6. Actin-dependence of the rate of Listeria movement in the resuspended high speed pellets of Xenopus egg extracts. Listeria movement was assayed in C_{60} resuspended in XB buffer and supplemented with the indicated concentrations of actin, and with (open symbols) or without (closed symbols) 23 μM T_{34}. The lowest actin concentration (6 μM) corresponds to 4 μM endogenous actin in C_{60} and 2 μM added rhodamine-actin. Rates of movement were measured, over a 1-h period, after 1-h incubation. Each point is the average of 8–15 rate measurements on different bacteria. The error bar represents the standard deviation.

In a third experiment, the resuspended pellets were supplemented with 40 μM F-actin and 46 μM T_{34}, and increasing amounts of bovine spleen profilin (mammalian profilin I) were added. The rate of movement of Listeria increased upon addition of profilin, from ~6–70μm/min, in several independent experiments cumulated in Fig. 8. The fact that profilin did not inhibit movement, as did the F-actin-depolymerizing proteins T_{34} and DNaseI supports the view that profilin sequesters G-actin in the medium, where barbed ends are capped, however, the profilin–actin complex participates in actin assembly at uncapped barbed ends of filaments created available for elongation at the rear of Listeria.

Finally, movement of Listeria and induction of actin assembly were completely inhibited after preincubation of the extracts with 10 μM phallolidin which leads to total polymerization of endogenous actin, as shown by centrifugation and non-denaturing gel electrophoresis showing the absence of actin in the supernatant. Hence G-actin subunits, not filaments, are recruited by the bacteria.

Movement of Vesicles in Xenopus Egg Cytoplasmic Extracts

In the absence of Listeria, observation of Xenopus egg extracts supplemented with rhodamine-actin and the ATP regenerating system revealed the movement of spherical vesicles of variable diameter (typically 0.5–2.5μm diam). These appeared dark in phase contrast, displayed fluorescent actin tails, and exhibited rocketing movements at speeds comparable to those of Listeria. Photographs of rocketing vesicles are shown in Fig. 9. This observation is very similar to the actin-based movement of polycationic beads bound to the growth cone membrane (15) or to the rocketing movement of endosomes in Zn^{2+} ion-treated cells, reported by J. Heuser and J. H. Morisaki (1992. Mol. Biol. Cell 3:172a). The relative abundance of rocketing vesicles in Xenopus egg extracts was variable among preparations and we have thus far been unable to reproducibly isolate these vesicles.

The observations showing that the movement of Listeria was reconstituted in the resuspended high speed pellet of the extract, a material rich in both membrane and cytoskeletal components, and that endogenous vesicles in the cytoplasm were capable of spontaneous actin-based movement suggested that lipid-associated components might be the first targets used by Listeria to direct actin assembly at...
its surface. The involvement of phosphoinositide metabolism in signalling processes leading to actin assembly is well known (7, 24 for review). In vitro studies have demonstrated that the binding of PI-4P and PI-4,5P₂ to actin-associated proteins such as profilin and capping proteins like gelsolin, capZ, and gCap 39 (19, 21, 26, 59) can prevent their binding to actin; it is not clear however, whether binding of PIP₂ to capping proteins might be the first step causing uncapping of filament ends and subsequent actin assembly onto the uncapped barbed ends (4, 18). On the other hand, several data indicated that changes in the levels of PI3,4P₂ and PI3,4,5P₃, the products of the stimulus-activated PI-3 kinase, correlate temporally with actin assembly in stimulated cells (53, 60). The possibility that components of the surface of the bacteria stimulate the activity of a latent membrane-associated PI-3 kinase, was tested using Wortmannin, a specific inhibitor of PI-3 kinase (29). Pretreatment of resuspended high speed pellets by 1 μM Wortmannin for 30 min at 20°C before proceeding to the motility assay did not affect the movement of Listeria. Thus, neither PI-3 kinase nor the 3D-phosphoinositides are involved in promoting actin assembly at the surface of Listeria.

Discussion

Listeria Movement Is Due to Local Generation of Uncapped Barbed Ends While the High Critical Concentration of the Pointed Ends Is Maintained in the Medium

The propulsive movement of Listeria monocytogenes in interphasic cytoplasmic extracts of Xenopus eggs has been used in this work as an in vitro approach to elucidate the thermodynamic basis of actin based motility in living cells.

The following conclusions have been reached. Actin, a major protein of the egg cytoplasm (0.7 mg/ml), is found essentially (75%) unpolymerized as in most motile cells. Profilin was present at 5 μM, and TBP₄ in extracts. These two proteins were found essentially in the soluble part of the extract. Profilin and TBP₄ account for the 12 μM of unassembled actin only if the filament barbed ends are capped, thus buffering the steady-state concentration of G-actin to the high critical concentration of the pointed ends (0.6 μM). It can be calculated that if barbed ends were not capped, profilin would not sequester G-actin, and sequestering agents (TBP₄ and others like ADF) would have to be present at concentrations one to two orders of magnitude higher than the actual cellular amounts to account for the 12 μM unassembled actin. Since TBP₄ (6) and profilin (30, 32) preferentially bind ATP-G-actin and not ADP-G-actin under physiological conditions, this result indicates that actin is sequestered as ATP-G-actin. A recent report (39) also came to the conclusion that monomeric actin carries ATP as the bound nucleotide in Xenopus egg extracts. Hence, all available data supports a model in which the assembly of actin filaments, via barbed end elongation at the surface of Listeria (52) is due to a local site-directed shift in steady-state whereby the critical concentration for F-actin assembly decreases locally from 0.6 μM (C₄ at the pointed end), found in the bulk cytoplasm to 0.1 μM (C₄ at the barbed end) most likely due to local uncapped barbed ends. The rate of actin assembly during this relaxation process is k₄₅ (C₄ at the pointed end) - C₄ at the barbed end). This mechanism accounts for the observations that actin assemblies onto filament barbed ends localized at the bacteria surface, and depolymerizes within the comet tail, a phenomenon described in terms of "nucleation-release" or "treadmilling" appearance (see reference 43 for a recent review of these). The mechanism we propose here differs appreciably from the treadmilling mechanism as originally described by Wegner (57). In the treadmilling mechanism, both filament ends are free, and a single critical concentration, intermediate between the barbed end and the pointed end critical concentrations, is maintained in solution, which implies that a net slow actin disassembly from the pointed ends is compensated by an identical net slow assembly (0.05 μm/min) at the barbed ends. The present mechanism in contrast relies on a local change in steady state of actin assembly, i.e., of critical concentration (5). The same mechanism may have a more general role in other actin-based processes such as movement of the leading edge, phagocytosis or stress fiber assembly at focal contacts. According to this model, movement requires: (a) the presence of F-actin and active capping proteins in the bulk medium (to buffer free G-actin concentration at a high enough concentration); (b) the presence of ATP in the medium (to ensure the difference in critical concentrations between the two ends, which fuels the movement); (c) a local signal mediating assembly, from G-actin subunits of anchored, uncapped barbed ends at the bacterium surface. This "leaky capper" function is

2. For more details on leaky cappers, see Carlier, M.-F., and D. Pantaloni. 1995. In Actin and Actin Binding Proteins. A. Bremer and V. Aebi, editors. Landes Biomedical Publishers, CRC Press. Boca Raton, FL. In press.

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most likely fulfilled by ActA protein (25), in association with an unknown cytoplasmic component. In agreement with these requirements we have observed that F-actin depolymerizing proteins (DNaseI, T₈₄) inhibit the movement of Listeria, because when all actin is disassembled the high critical concentration of the pointed end is no longer buffered in the cytoplasm (Figs. 6 and 7); conversely, when all actin is polymerized and stabilized by phalloidin, no difference in \( C_P \) can be established between the environment of the bacteria and the bulk cytoplasm, hence no movement occurs; finally, movement stops when ATP has been exhausted from the medium (Fig. 5), because in the presence of ADP, no difference in critical concentration at the two ends exists.

**Profilin Is Not Necessary for Listeria Movement and Does Not Bind to the Proline-rich Sequence of ActA**

Profilin represents a special case of G-actin-binding protein since it sequesters G-actin actively in the bulk cytoplasm where barbed ends are capped, but participates in actin assembly at the barbed ends created at the rear of Listeria. Hence, consistent with the proposed model, profilin does not inhibit movement and may even accelerate it somewhat (Fig. 8), because, in decreasing the steady-state concentration of G-actin in regions where barbed ends are formed (30), it increases the difference \( C_P - C_C \) which controls the rate of assembly. Note that the two apparently opposed activities of profilin (sequestration of actin, and participation in assembly) coexist here in a synergic fashion to promote Listeria movement. Therefore, while profilin is not strictly necessary for actin-based Listeria movement, it appears to be necessary in some experimental systems and in other organisms.

**Why Listeria Movement Can Be Observed in the Absence of a Large Pool of Sequestered Actin**

In most reactions of actin assembly in response to extra-cellular signals, e.g., platelet activation or neutrophil response to chemotactic agents, a large reservoir of unpolymerized actin is necessary to amplify the uncapping effect and create a massive local increase in F-actin (e.g., 10⁻⁵⁻⁵⁻⁰ M). We were therefore surprised to observe that Listeria movement can be fully reconstituted in resuspended pellets of high speed centrifuged extracts, which do contain F-actin and capping proteins, but do not contain a pool of unassembled actin, since F-actin polymerized in the comet tails of a typical sample observed in videomicroscopy is very small: in a typical preparation containing \( 10^7 \) bacteria/ml extract, assuming that each bacterium has a 50-μm-long actin tail, and an average density of F-actin in the tail of \( \sim 200 \) filaments of 100 subunits per mm of tail length (51), the molarity of F-actin assembled in tails is calculated to be only 0.2 μM, an amount at least two orders of magnitude lower than the one formed upon stimulation of motile cells. Hence, a large pool of unassembled actin is not necessary to fuel Listeria movement in the in vitro assays, and these bacteria can be considered as simple "tracers," or "indicators" of the shift in steady state occurring upon stimulation. The situation might be different in infected cells, in which at some degree of proliferation bacteria might eventually drive all the endogenous F-actin pool into the comet tails, at which point movement is expected to stop.

**Actin Assembly Is Necessary for Listeria Movement. Is It Sufficient?**

Our results support the view (49) that actin assembly itself could be the motor of Listeria movement. The fact that neither vanadate nor other ATPase inhibitors affect the...
movement does not support the involvement of myosin motors as well as the suggested possibility (6) that a local depletion in ATP by non-myosin like ATPase could cause desquestration of G-actin and consequent filament assembly and movement. However, the simple statements that actin assembly is the motor of movement and that this occurs by a difference between the bulk and the local concentration for assembly does not appear sufficient to account for all the characteristics of the propulsive mechanism. Indeed, if our model is correct, the rate of actin assembly at the rear of Listeria is \( k_2 (C^ F_2 - C^ G_2) \times 10^{-5} \), corresponding to a rate of filament elongation of 1 \( \mu \text{m/min} \), which is 5- to 10-fold lower than the actual rate of propulsion. Hence, either the value of \( k_2 \) at the anchored barbed ends is actually larger than the value of 10 \( \mu \text{M}^{-1} \text{s}^{-1} \) obtained for free barbed ends from in vitro solution studies (35), or the rate of movement depends on the structural organization of the tail by actin cross-linking proteins such as \( \alpha \)-actinin or fimbrin (36). Support for this view is provided by the observation that in the absence of \( \alpha \)-actinin, the simple cloud of actin around the bacterium (36) suggests that in the absence of a-actinin, the simple cloud of actin generated by the bacteria does not promote appreciable movement (28). On the other hand, the value of \( \sim 1 \mu \text{m/min} \) calculated for the rate of filament elongation within our model is perfectly consistent with the observed rate of movement of the leading edge of locomoting fibroblasts (55). In this case filaments appear long enough for the movement to be strictly due to the incorporation of actin subunits at the level of membrane-associated barbed ends (48). In addition, the large fluctuations in the speed of bacteria which have been reported in living cells (28) and which we confirm here in Xenopus extracts are unlikely to be accounted for by fluctuations in actin concentration, since the diffusion of monomeric actin in the cytoplasm is much faster than the movement of the bacteria; the availability of an unidentified cytoplasmic component responsible for the uncapping of barbed ends may be rate limiting in some instances, especially if this is a membrane-linked, insoluble component.

Is Phosphatidylinositol Metabolism Involved in Listeria Movement?

Two pieces of data suggest that membrane components are candidates for the signal transducing element(s) which are subverted by Listeria to induce local actin assembly: first, movement is supported by the resuspended pellets of high speed centrifuged extracts, which contain membrane material; second, endogenous vesicles are capable of spontaneous rocketing movement driven by actin assembly. Although the rocketting movement of Listeria in Xenopus egg cytoplasm shares similarity with other actin-based motility processes like platelet or neutrophil activation, the rho-PI-3 kinase pathway (60) does not seem directly involved in Listeria movement in these acellular extracts, according to the present data. Further biochemical fractionation studies are now underway to identify and characterize the lipid-bound and cytoplasmic components responsible for local uncapping of barbed ends at the surface of Listeria.

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Note Added in Proof: Very recent deletion analyses of the ActA gene (Pistor, S., T. Chakraborty, U. Walter, and J. Wehland. 1995. Current Biol. 5:517–525) have shown that the poly-L-proline region of ActA is not required for actin assembly, in agreement with the present data.

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