Arrest of *Listeria* movement in host cells by a bacterial ActA analogue: Implications for actin-based motility

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ABSTRACT Upon entering the host cell's cytoplasm, the pathogen *Listeria monocytogenes* can subvert the normal contractile system of the host cell; subsequent assembly of polar actin-filament structures is likely to provide the force for rapid intracellular bacterial movement and its cell-to-cell spread. We have now investigated the functional consequences of microinjecting *Listeria*-infected PtK2 cells with a synthetic peptide, CFEPPPTDE. This peptide represents one of four related oligoproline stretches in ActA, a bacterial surface protein necessary for *Listeria*-induced actin assembly. Over an estimated intracellular concentration range of 80 nM to 0.8 μ M, this analogue rapidly blocks the formation of the actin-filament tails and arrests intracellular bacterial motility. Over the same time scale and concentration range, introduction of the ActA analogue also causes host cell membrane retraction. Bodipy-phalloidin staining reveals that microinjection of the ActA analogue results in massive retraction of the actin cytoskeleton. Microinjection of 1–20 μ M poly(L-proline) (intracellular concentration) fails to block *Listeria* intracellular movement or polar actin-filament assembly. As observed with ActA, however, poly(L-proline) does cause membrane retraction. Our findings demonstrate the efficacy of low molecular weight peptides in efforts to distinguish mechanistic features in *Listeria* motility and PtK2 host cell membrane reorganization. These observations also suggest that a cytoskeletal component sensitive to specific oligoproline peptides may participate in protein-protein interactions essential for both of these actin-associated processes.

The intracellular pathogen *Listeria monocytogenes* has generated considerable interest among contractile protein investigators because of this bacterium's ability to induce directional host-cell actin assembly that propels the bacterium rapidly through the cytoplasm (1–3). This organism is capable of escaping from the phagolysosome and replicating in the host cell (4–8). Intracellular movement allows the bacteria to migrate to the periphery of the cytoplasm where they form filopods laden with bacteria, which are thought to be ingested by adjacent host cells. This overall process allows *Listeria* to spread from cell to cell without ever coming in contact with the extracellular milieu, enabling this pathogen to circumvent the harmful consequences of the host's humoral immune defenses.

All evidence indicates that formation of polar actin-filament structures is required for *Listeria* intracellular movement and cell-to-cell spread. Time-lapse video and studies using a caged actin monomer probe indicate that each polar actin structure becomes anchored in the cytoplasm (9, 10), acting as a platform that creates a physical boundary that confines the bacterium to forward movement (11). New actin monomers add to the bacterial-actin platform interface as the filamentous actin structure progressively elongates, forcing

the bacterium to move in a forward direction (9). Cytochalasin D blocks further elongation of these actin structures, results in marked shortening of the tails, and stops both bacterial intracellular movement (9) and cell-to-cell spread (1).

Unlike pseudopod and lamellipod formation (12), *Listeria* provide a simpler model for understanding actin-based motility. Since actin assembly is induced only after the bacterium is intracellular, the complex events associated with host membrane receptor occupancy are bypassed. Nonetheless, the same proteins thought to play important roles in controlling interactions of actin filaments in the peripheral cytoplasm of motile cells are also found in the *Listeria*-induced actin structures, including α -actinin (2), tropomyosin (9), and ABP-280 (J. M. Sanger, B. Mittal, F.S.S., and J. W. Sanger, unpublished data).

How then do *Listeria* induce the polymerization of actin filaments at such discrete sites? ActA, a bacterial protein containing a cluster of several oligoproline stretches, is absolutely required for *Listeria*-induced actin assembly (13, 14). Despite the presence of ActA on its surface, when this bacterium is combined with purified actin monomers, actin assembly is not stimulated (15). It has also been found that ActA fails to bind to globular actin (13). Therefore, ActA must stimulate actin assembly by binding to, and perhaps activating, one or more of the host cell's actin regulatory protein(s).

To explore the possibility that the polypoline repeats in ActA (13) serve as binding sites for such an actin regulatory protein, we synthesized a peptide representative of one of these repeat oligoproline sequences and microinjected this analogue into *Listeria*-infected cells. At protein concentrations that might be expected in the cell, this peptide blocked *Listeria* movement and also caused marked reorganization of the actin filament structure of the peripheral cytoplasm of host cells with resulting massive peripheral membrane retraction. These findings emphasize the likely importance of this specific class of oligoproline peptides in both intracellular *Listeria* motility and host-cell motility.

EXPERIMENTAL METHODS

L. monocytogenes 10403S was used to infect PtK2 cells as described (2, 9) with one modification. Culture dishes containing bacteria were first centrifuged for 15 min at 400 $\times g$ at room temperature and then incubated for 45 min at 37°C. Cells were then washed with phosphate-buffered saline (PBS) and resuspended in medium containing gentamicin sulfate (10 μ g/ml) to prevent extracellular growth of bacteria (2, 8, 9). Cells were fixed and stained as described (2, 9).

Individual cells were microinjected with peptide using a micromanipulator and microinjector (models 5171 and 5242,

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respectively; Eppendorf) and Eppendorf Femtotip micropipettes. Temperature was maintained at 37°C with an MS-200D perfusion microincubation system (Narishige, Tokyo). Peptides were synthesized by the automated Merrifield method and diluted to a stock concentration of 1–1.8 mg/ml in sterile PBS (pH 7.2).

We used a Nikon Diaphot inverted microscope with an image intensifier and charge-coupled device camera (Dage-MTI, Michigan City, IN). Digital images were obtained and processed, and lengths were measured using an Image-1 video image analyzer (Universal Imaging, West Chester, PA). Differences between rocket tail lengths and migration speeds were analyzed using the unpaired Student *t* test.

Purified actin was conjugated with pyrene and actin assembly was monitored by fluorescence intensity (16). Profilin-actin complexes were purified from calf spleen by using poly(L-proline) affinity chromatography (17). Solutions containing purified profilin-actin complexes and ActA peptide in S2 buffer (10 mM imidazole hydrochloride, pH 7.4/0.5 mM ATP/1 mM dithiothreitol/1 mM MgCl₂/0.1 M KCl) were incubated overnight at 25°C and then centrifuged at 160,000 × *g* for 30 min to sediment actin filaments (Airfuge, Beckman). Starting material, supernatants, and pellets were boiled in sample buffer containing 1% SDS/2% (vol/vol) 2-mercaptoethanol/10% (wt/vol) sucrose, subjected to electrophoresis through a 15% polyacrylamide slab gel, and stained with Coomassie blue.

RESULTS

Microinjection of an ActA Synthetic Peptide Analogue into Infected PtK2 Cells. ActA contains a series of oligoproline regions, each bracketed by one or several acidic side-chain amino acids (13). These regions may bind one or more actin regulatory proteins in a way that stimulates actin polymerization resulting in the formation of the polar actin structures required for intracellular movement of *Listeria* in host cells. A critical test of this hypothesis is the ability of low molecular weight oligoproline analogues to block bacterial movement by dispersing the putative actin regulatory protein(s) from the bacterial surface. Accordingly, we explored the consequences of introducing, by microinjection, a synthetic peptide (CFEFPPPTDE) as an analogue of one of ActA's oligoproline repeats[§] (amino acid residues 270–278) to which we added a cysteine residue at the N terminus for future conjugation studies.

In Fig. 1, multiple bacteria can be seen migrating in a PtK2 cell, and each displays a trailing phase-dense rocket tail. The arrowhead points to a single bacterium enlarged in the lower montage. Bacteria eventually reached the peripheral cytoplasm where they protruded outwardly from the peripheral cytoplasm to form filopodia. A number of organisms were seen in such filopods (see in the lower left of the larger panels of Fig. 1). A small volume of the peptide (10 µg/ml, needle concentration) was microinjected at *t* = 170 s. By assuming that the volume delivered represented 5–10% of the cell volume (18), this would yield an approximate intracellular concentration of 0.4–0.8 µM. Within 35 s, all bacterial movement ceased. In addition, the phase-dense actin filament rocket tails disassembled (Fig. 1, *t* = 205 s). Note the lack of any phase-dense material on either end of the enlarged bacterium after injection (see Fig. 1, *t* = 259 s). In some experiments, longer phase-dense tails persisted for 30–60 s after movement had ceased (see Fig. 3B, partially divided bacteria at the upper left). Because the half-life of tail

FIG. 1. *Listeria* movement and actin rocket tail formation in PtK2 host cells before and after microinjection of the synthetic ActA peptide. Arrowheads point to the bacterium that has been enlarged in the montage at the bottom and analyzed in Fig. 2A and B. A needle concentration of 10 µg/ml has been microinjected near the right side of the nucleus at *t* = 170 s. Within 35 s all bacterial movement has ceased and the majority of the phase-dense actin rocket tails have disappeared (205-s frame). Also note the marked retraction of the peripheral membrane and cytoplasm. An electronically enlarged view of the single bacterium is shown in the montage at the bottom at various times before (29 s, 88 s, and 146 s) and after microinjection (259 s) of the ActA peptide analogue. (Bars = 30 µm.)

disassembly is 70–80 s (9), tails would be expected to persist for brief periods after movement ceases if the ActA peptide does not affect actin depolymerization.

The effects of microinjection of ActA peptide on bacterial motility and rocket tail length were also quantified by direct measurement. The speeds of bacterial movement in PtK2 cells during these experiments varied from 0.05 µm/s to 0.15 µm/s. In all instances, after microinjection of ActA peptide (10 µg/ml, needle concentration), bacterial intracellular migration was arrested. The speed of the particular bacterium designated by the arrowhead in Fig. 1 was determined at each time. Prior to the microinjection of the ActA peptide, the migration rate varied between 0.11 and 0.08 µm/s. Similarly, the length of the phase-dense tail remained relatively constant varying between 2.2 and 2.8 µm. On addition of peptide, both the rate of bacterial migration and tail length rapidly decreased to 0 (Fig. 2A and B).

We also explored the concentration dependence of ActA peptide inhibition. Microinjection of a lower concentration of the ActA peptide (1 µg/ml; estimated intracellular concentration, 80 nM) into infected PtK2 cells also caused the rapid cessation of bacterial movement and the dissolution of the phase-dense rocket tails within 30–60 s (see Fig. 2C). A needle concentration of 0.1 µg/ml (estimated intracellular concentration, 8 nM) generally failed to impair *Listeria* movement. Repeated microinjection resulted in the intracellular accumulation of the peptide that resulted in effects identical to single injections of higher concentrations of the peptide (see Fig. 2D).

[§]For reference, the corresponding proline-rich sequences in *Listeria* ActA protein are 235-DFPPPTDE, 269-FFPPPTDE, 304-FFPPPTDE, and 350-DFPPIPTTE, where the number preceding each sequence designates the first amino acid position (13).

FIG. 2. Bacterial speeds (A, C, and D) and actin rocket tail lengths (B) in PtK2 host cells before and after microinjection of ActA peptide analogue. Three concentrations of the peptide analogue were microinjected into cells. The concentration in parentheses is the estimated final intracellular ActA peptide concentration, assuming the peptide was evenly distributed throughout the entire volume of the cell. Arrow indicates when the ActA peptide analogue was added. A and B correspond to the experiment depicted in Fig. 1 (bacterium marked by the arrowhead and seen in the montage). C and D show the effects of lower concentrations of the ActA peptide analogue (needle concentrations, 1 $\mu\text{g/ml}$ and 0.1 $\mu\text{g/ml}$, respectively). Three injections of the peptide at 0.1 $\mu\text{g/ml}$ were required to inhibit *Listeria* movement.

Microinjection of the peptide also caused all of the bacteria-containing filopodia to retract, with the bacteria collapsing back into the cytoplasm. Compare, for example, the bottom left region of the preinjection image at $t = 146$ s to the postinjection micrograph at $t = 205$ s illustrated in the large frames in Fig. 1. To our surprise, microinjection of the synthetic ActA peptide also caused a rapid retraction of the peripheral membrane (Fig. 1, 205 s). Membrane was seen to retract up to 20 μm in 35 s and up to 30 μm in 100 s. Cessation of bacterial movement, actin tail disassembly, and membrane retraction took place within the same time frame.

Membrane retraction was also found to be concentration dependent. Microinjection of a higher concentration (1 mg/ml , needle concentration; yielding an estimated intracellular concentration of 8 μM) resulted in the instantaneous retraction of the peripheral membrane and the cessation of all bacterial movement. Some segments of the membrane remained adherent to the surface resulting in some fragmentation of the peripheral membrane and cytoplasm. Microinjection of a lower concentration of the ActA peptide (1 $\mu\text{g/ml}$) into infected PtK2 cells caused peripheral membrane retrac-

tion of a similar magnitude (16–17 μm) to that observed at 10 $\mu\text{g/ml}$ (Fig. 3). Host-cell membrane retraction, however, was not generally associated with the membrane fragmentation consistently noted with higher concentrations of ActA peptide (compare Fig. 1, 205 s, to Fig. 3B).

Staining with the filamentous actin probe bodipy-phalloidin revealed marked changes in the actin-filament morphology after microinjecting ActA analogue (Fig. 4). Before microinjection typical actin-filament rocket tails and stress fibers were seen (Fig. 4A). Approximately 10 min after microinjection with ActA peptide (needle concentration, 10 $\mu\text{g/ml}$), phalloidin-stained rocket tails and stress fibers were no longer seen (Fig. 4B). The edge of the microinjected cell exhibits increased fluorescence, suggesting actin filaments have accumulated near the retracting membrane.

ActA analogue added to the extracellular medium at a much higher concentration (final concentration, 25 μM) failed to cause membrane retraction, indicating that the

FIG. 3. Effects of intracellular ActA peptide on the host-cell peripheral cytoplasm and membrane. (A) The infected PtK2 cell on the left has a broad lucent veil of cytoplasm. At $t = 17:38:00$ (hours:minutes:seconds), the ActA peptide analogue at 1 $\mu\text{g/ml}$ (needle concentration) (estimated intracellular concentration, 80 nM) was microinjected just to the right of the nucleus. (B) Then 30 s after microinjection of the peptide, massive retraction of the peripheral cytoplasm and membrane was seen. All bacterial movement ceased, and over the ensuing 30 s, the phase-dense actin rocket tails disassembled. (Bars = 30 μm .)

FIG. 4. Effects of microinjection of ActA analogue on the host-cell actin cytoskeleton. (A) Bodipy-phalloidin-stained PtK2 cells before microinjection of the ActA peptide analogue. A typical infected cell is seen in the upper left. Two actin-filament rocket tails are marked by the arrowheads. The bacteria cannot be seen in this fluorescent micrograph. On phase-contrast microscopy, however, a bacterium is located in front of the wider higher-intensity region of each rocket tail. The lower right shows a typical uninfected cell with an extensive network of stress fibers. (B) Cells were fixed and stained ≈ 10 min after microinjection of the ActA analogue (10 $\mu\text{g/ml}$, needle concentration). The bright cell in the center was microinjected with the peptide. Note the loss of stress fibers. The cell appears shrunken, the peripheral cytoplasm of the cell retracting toward the cell center. The fluorescence intensity in the region of retraction is brighter, suggesting a higher concentration of actin filaments in this region. No bacteria-associated rocket tails are seen. (Bar = 10 μm .)

membrane and peripheral cytoplasm changes induced by intracellular injection of the peptide are unlikely to represent a generalized cytotoxic effect (data not shown).

Microinjection of Poly(L-proline) into *Listeria*-Infected PtK2 Cells. To further explore the structure–function relationship of the ActA protein synthetic analog, we also microinjected poly(L-proline) (mean M_r , 5000). Injection of poly(L-proline) at a needle concentration of 50 $\mu\text{g}/\text{ml}$ (estimated intracellular concentration, 1 μM) failed to impair *Listeria* movement. The bacterial speed before injection ($0.11 \pm 0.05 \mu\text{m}/\text{s}$, mean $\pm$ SEM, $n = 16$) was not significantly different than the speed after injection ($0.13 \pm 0.01 \mu\text{m}/\text{s}$, $n = 27$; $P = 0.077$). Adding a second equal dose of this polypeptide also failed to block *Listeria* movement. In fact, this second injection resulted in a significant increase in speed compared to *Listeria* movement in untreated cells [$0.18 \pm 0.01 \mu\text{m}/\text{s}$ ($n = 29$) vs. $0.11 \pm 0.05 \mu\text{m}/\text{s}$ ($n = 16$); $P < 0.001$]. Similarly a single injection of poly(L-proline) failed to alter bacterial actin rocket tail length [tail length before injection, $3.5 \pm 0.4 \mu\text{m}$ (mean $\pm$ SEM, $n = 20$) vs. $3.6 \pm 0.3 \mu\text{m}$ ($n = 33$) after injection; $P = 0.79$] while a repeat injection (Fig. 5B) was associated with a near doubling of tail length [$6.3 \pm 0.5 \mu\text{m}$ ($n = 28$); $P < 0.001$]. Microinjection of a higher needle concentration of 1 mg/ml (estimated intracellular concentration, 20 μM) also failed to block bacterial movement. Although poly(L-proline) did not impair *Listeria* movement, this polypeptide caused peripheral host-cell membrane retraction similar to that observed after microinjection of the synthetic ActA peptide analogue. Membrane retractions of up to 25 μm were observed when needle concentrations of 1 mg/ml were injected and up to 14 μm were observed after injection of needle concentrations of 50 $\mu\text{g}/\text{ml}$ into infected PtK2 cells (Fig. 5B).

As an additional control, we separately microinjected a high concentration (estimated intracellular concentration, 94 μM) of an unrelated synthetic peptide VKSKIGSTDNIKYZ-PKGG, itself corresponding to the first repeated sequence in microtubule-associated protein 2 (19). This peptide contained a single proline, rather than a stretch of four adjacent prolines, and was synthesized and purified in a manner identical to the ActA peptide. This peptide failed to impair *Listeria* migration or rocket tail formation and did not alter peripheral membrane morphology (data not shown). This

suggests that the effects observed at much lower concentrations of ActA peptide cannot be attributed to chemical impurities persisting through solid-phase peptide synthesis and subsequent purification. Similarly, microinjection of the PBS alone failed to affect *Listeria* movement or cell morphology.

Effects of the ActA Peptide on Actin Assembly and on the Profilin–Actin Complex. Final concentrations of 10 and 25 μM ActA had no detectable effect on the assembly rate (1.2 fluorescent units/min, $\pm$ ActA) or the final steady-state filament concentration of 1.5 μM pyrenylactin (data not shown). As assessed by an ultracentrifugation assay, ActA (final concentrations, 30–60 μM) also failed to dissociate spleen profilin–actin complexes (final concentrations, 1.7–3 μM) alone or combined with sonicated actin filaments (final concentration, 0.1 μM) (data not shown).

DISCUSSION

Listeria intracellular motility and actin rocket tail formation have been used in the present studies to probe the critical role of oligoproline peptides in regulating actin filament assembly. The synthetic peptide analogue, representing the likely functional domain of a bacterial surface protein ActA, completely blocks *Listeria*-induced actin assembly when added to the cytoplasm of infected cells. The intracellular concentrations of ActA analogue used in our experiments with mammalian cells, 10^{-8} to 10^{-6} M, are in a concentration range common to many intracellular proteins. Even at 10^{-8} M intracellular concentrations, this bacterial peptide analogue caused permanent inhibition of *Listeria*-induced actin assembly and bacterial movement, suggesting that this oligopeptide sequence is a very effective competitive inhibitor. Nonetheless microinjection of cells is semiquantitative at best, and we cannot as yet estimate the cellular concentration of protein(s) capable of binding our ActA analogue with high affinity.

The ActA analogue has two important structural features: (i) it contains a string of four prolines and (ii) the peptide is bordered by negatively charged groups (Asp and Glu). It is noteworthy that poly(L-proline) fails to block *Listeria* movement, suggesting stretches of oligoproline alone cannot effectively compete with the bacterial product responsible for inducing actin assembly. At high intracellular concentrations, however, microinjection of this polypeptide did result in membrane retraction. Apparently, peripheral membrane spreading and bacterial intracellular movement can be independently inhibited, and this finding opens the way for further mechanistic exploration of these processes. The poly(L-proline) experiments also indicate that ActA-mediated inhibition of *Listeria* movement is unlikely to be secondarily caused by fragmentation of the host-cell peripheral membrane.

Although we had anticipated that microinjection of the ActA peptide analogue should affect the actin-rich *Listeria* rocket tails, much to our surprise, the host cell cytoplasm was also reorganized over the same concentration range of these agents. In retrospect, this finding should have been anticipated if *Listeria* motility reflects the salient features of actin-based host-cell motility. This peptide analogue is likely to compete with an oligoproline-containing membrane-bound protein involved in regulating host-cell actin assembly at the peripheral membrane.

We still cannot present a complete model for how ActA and a putative oligoproline-rich integral membrane protein might work in a mechanistically analogous manner, but some essential features are emerging. Intracellular bacterial motility appears to occur through the binding of an actin regulatory protein to one or more of the nonidentical oligoproline repeats on ActA. Likewise, we envision cortical assembly of actin filaments to be the consequence of the anchoring of an actin regulatory protein at oligoproline-containing sites lo-

FIG. 5. Effects of microinjection of poly(L-proline) on *Listeria* movement and actin rocket tail formation. (A) Multiple bacteria are migrating within the cell. Phase-dense rocket tails are readily seen. (B) At $t = 18:21:00$ (hours:minutes:seconds), poly(L-proline) (needle concentration, 50 $\mu\text{g}/\text{ml}$; estimated intracellular concentration, 1 μM) was microinjected into the infected cell. No effect on bacterial migration or tail formation was seen. A second microinjection of the same concentration of the peptide was performed at $t = 18:25:00$. Two minutes after the second microinjection, long actin-filament rocket tails persisted and bacterial migration speeds actually increased (see Results). Note the large holes in the cytoplasm at the sites of microinjection (right side of the micrograph). The sites progressively enlarged and represent membrane retraction. (Bars = 30 μm .)

calized on the cytoplasmic surface of the host cell membrane. To our knowledge, profilin is the only actin-associated protein known to bind to polyproline with high affinity, and we have found significant quantities of profilin in the host PtK2 cells (data not shown) by SDS electrophoretic analysis of proteins adsorbed to poly(L-proline) covalently coupled to Sepharose beads (20). Profilin also forms a one-to-one complex with monomeric actin and could serve as a carrier protein to transport actin monomers to proline-rich sites on the cell's inner membrane surface, much as actin appears to concentrate on the bacterial surface. Profilin has been found to facilitate nucleotide exchange *in vitro* (21, 22); therefore, this protein may also increase the rate of conversion of ADP to ATP-actin monomers, a condition that could stimulate actin polymerization to produce the localized expansile forces necessary for motility. We should also note that Goddette and Frieden (23) postulated that cytochalasin D's action in cells arises from this compound's ability to slow the rate of exchange of ATP with actin-bound ADP relative to nucleotide hydrolysis leading to an accumulation of ADP-containing monomers. Cytochalasin D blocks the formation of *Listeria* actin rocket tails further supporting the supposition that nucleotide exchange may be rate limiting in *Listeria*-induced actin assembly.

Our ActA protein analogue may act to displace profilin from both the bacterial cell wall and the inner cell membrane surfaces, thereby blocking assembly of nearby actin filaments. This possibility is supported by filamentous actin staining of microinjected cells with bodipy-phalloidin, which reveals loss of stress fibers and accumulation of filamentous actin near the retracting peripheral membrane. Such disruption of the normal actin filament architecture would also be expected to result in a reduction of peripheral cytoplasm rigidity, in turn leading to retraction of the peripheral membrane. The dramatic retraction of peripheral membranes associated with our ActA analogue suggests that a proline-rich profilin-anchoring protein on the inner surface of cells may play an important role in promoting cortical actin cytoskeleton assembly. Possible candidates include vinculin (24), human synapsin I (25), and the transmembrane protein CD2 (26); each contains oligoproline sequences bordered by negatively charged amino acids in their cytoplasmic domains. In any case, this hypothetical model predicts the basic features of what may be a class of proline-rich membrane proteins and/or peptides with structural elements designed for controlling actin-dependent processes.

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1. Tilney, L. G. & Portnoy, D. A. (1989) *J. Cell Biol.* **109**, 1597–1608.
2. Dabiri, G. A., Sanger, J. M., Portnoy, D. A. & Southwick, F. S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6068–6072.
3. Mounier, J. A., Ryter, M., Coquis-Rondon, M. & Sansonetti, P. J. (1990) *Infect. Immun.* **58**, 3477–3486.
4. Racz, P., Tenner, K. & Mero, E. (1972) *Lab. Invest.* **26**, 1694–1700.
5. Havel, E. (1986) *Infect. Immun.* **54**, 787–792.
6. Gaillard, J.-L., Berche, P., Mounier, J., Richard, S. & Sansonetti, P. (1987) *Infect. Immun.* **55**, 2822–2829.
7. Kathariou, S., Metz, P., Hof, H. & Goebel, W. (1987) *J. Bacteriol.* **169**, 1291–1297.
8. Portnoy, D. A., Jacks, P. S. & Hinrichs, D. J. (1988) *J. Exp. Med.* **167**, 1459–1471.
9. Sanger, J. M., Sanger, J. W. & Southwick, F. S. (1992) *Infect. Immun.* **60**, 3609–3619.
10. Theriot, J. A., Mitchison, T. J., Tilney, L. G. & Portnoy, D. A. (1992) *Nature (London)* **357**, 257–260.
11. Peskin, C. S., Odell, G. M. & Oster, G. F. (1993) *Biophys. J.* **65**, 316–324.
12. Stossel, T. P. (1993) *Science* **260**, 1086–1094.
13. Kocks, C., Goulin, E., Tabouret, M., Berche, P., Ohayon, H. & Cossart, P. (1992) *Cell* **68**, 521–531.
14. Kocks, C., Hellio, R., Gounon, P., Ohayon, H. & Cossart, P. (1993) *J. Cell Sci.* **105**, 699–710.
15. Tilney, L. G., Connelly, P. S. & Portnoy, D. A. (1990) *J. Cell Biol.* **111**, 2979–2988.
16. Young, C. L., Southwick, F. S. & Weber, A. (1990) *Biochemistry* **29**, 2232–2240.
17. Lindberg, U., Shutt, C. E., Hellsten, E., Tjader, A.-C. & Hult, T. (1988) *Biochim. Biophys. Acta* **967**, 391–400.
18. Wan, K., Feramisco, J. R. & Ash, J. F. (1982) *Methods Enzymol.* **85**, 514–562.
19. Joly, J. C., Flynn, G. & Purich, D. L. (1989) *J. Cell Biol.* **109**, 2289–2294.
20. Tanaka, M. & Shibata, H. (1985) *Eur. J. Biochem.* **151**, 291–297.
21. Mochrin, S. C. & Korn, E. D. (1980) *Biochemistry* **19**, 5359–5362.
22. Goldschmidt-Clermont, P. J., Machesky, L. M., Doberstein, S. K. & Pollard, T. D. (1991) *J. Cell Biol.* **113**, 1081–1089.
23. Goddette, D. W. & Frieden, C. (1986) *J. Biol. Chem.* **261**, 15974–15980.
24. Weller, P. A., Orgryzko, E. P., Corben, E. B., Zhidkova, N. I., Patel, B., Price, G. J., Spurr, N. K., Koteliaksky, V. E. & Critchley, D. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5667–5671.
25. Sudhof, T. C. (1990) *J. Biol. Chem.* **265**, 7849–7852.
26. Sayre, P. H., Chang, H.-C., Hussey, R. E., Brown, N. R., Richardson, N. E., Spagnoli, G., Clayton, L. K. & Reinherz, E. I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2941–2945.