Actin Filament Nucleation by the Bacterial Pathogen, *Listeria monocytogenes*

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**Abstract.** Shortly after *Listeria* is phagocytosed by a macrophage, it dissolves the phagosomal membrane and enters the cytoplasm. 1 h later, actin filaments coat the *Listeria* and then become rearranged to form a tail with which the *Listeria* moves to the macrophage surface as a prelude to spreading. If infected macrophages are treated with cytochalasin D, all the actin filaments associated with the *Listeria* break down leaving a fine, fibrillar material that does not decorate with subfragment 1 of myosin. This material is associated with either the surface of the *Listeria* (the cloud stage) or one end (the tail stage). If the cytochalasin-treated infected macrophages are detergent extracted and then incubated in nuclei-free monomeric actin under polymerizing conditions, actin filaments assemble from the fine, fibrillar material, the result being that each *Listeria* has actin filaments radiating from its surface like the spokes of a wheel (cloud form) or possesses a long tail of actin filaments formed from the fine, fibrillar material located at one end of the *Listeria*. Evidence that the fine fibrillar material is involved in nucleating actin assembly comes from a *Listeria* mutant. Although the mutant replicates at a normal rate in macrophages, actin filaments do not form on its surface (cloud stage) or from one end (tail stage), nor does the bacterium spread. Furthermore it does not form the fine fibrillar material. Evidence that the nucleating material is a secretory product of *Listeria* and not the macrophage comes from experiments using chloramphenicol, which inhibits protein synthesis in *Listeria* but not in macrophages. If chloramphenicol is applied 1 h after infection, a time before actin filaments are found attached to the *Listeria* in untreated macrophages, actin filaments never assemble on the *Listeria* even when fixed 3 h later. Furthermore the fine fibrillar material is absent, although there is a coat of dense granular material.

A major control over cell shape and nonmuscle motility is the regulation of the length and distribution of actin filaments composing the cytoskeleton. Unfortunately little is known about this regulation in vivo, largely because a biological system that provides access to these controls has not been available. What is needed is a system in which actin filament assembly occurs at a distinct site(s) in the cell that can be isolated in sufficient quantity to identify and biochemically characterize the components involved in actin nucleation.

Several actin-binding proteins that will nucleate actin filaments in vitro have been purified from cells. Most of these (with the exception of poncutilin [Shariff and Luna, 1990]) make up a subclass of actin-binding proteins called "cappers." Examples include gelsolin (Wang and Bryan, 1981) and villin (Weber et al., 1987). Although these cappers may play a role in actin filament nucleation in some cells, we think they are probably not the nucleators present in many cell types for several reasons. First, in certain cells these molecules are present in prodigious amounts, being major components, not just one capping molecule per actin filament. For example, in intestinal epithelial cells the villin/actin ratio is 1:2–1:3, not, as one might expect, 1:1,000 or 1:10,000. Second, a nucleator logically should reside at the end of the filament, not along its length, which is not the case for villin (Drenckhahn and Dermietzel, 1988). Third, in some cells such as the hair cells of the cochlea, no villin or gelsolin is present as assayed immunologically and by western analysis (Tilney et al., 1989), yet there are enormous numbers of actin filaments in organized arrays.

We have stumbled upon a biological system in which we have an excellent chance of isolating, purifying, and characterizing a natural actin filament nucleator. This system involves the growth and spread of the pathogenic bacterium, *Listeria monocytogenes*, in macrophages (Tilney and Portnoy, 1989; Mounier et al., 1990). Briefly, subsequent to being phagocytosed by a macrophage, the *Listeria* escapes into the cytoplasm by dissolving the membrane of the phagosome, an event mediated by the secretion of hemolysin (Gaillard et al., 1987; Portnoy et al., 1988; Tilney and Portnoy, 1989). The *Listeria* then begins to grow and divide using the host's nutrients. At the same time a cloud of actin filaments assembles around the bacterium. The actin filaments then reorganize to form a long tail of actin which can be up to 5
μm long. The Listeria with its actin tail then migrates to the cell surface like a comet with the Listeria at its head. There it forms a long pseudopod that is phagocytosed by a neighboring macrophage. Thus, within the phagosome of the second macrophage is the Listeria with much of its actin tail, surrounded by the plasma membrane of the pseudopod which in turn is surrounded by the phagosome membrane. The doubly encapsulated Listeria escapes to the cytoplasm by dissolving both membranes, again probably mediated by hemolysin, and the cycle is repeated.

We will demonstrate in this paper that Listeria nucleates the assembly of actin off a fine fibrillar material that appears on its surface while in the cytoplasm of a macrophage. We are now in a position to try to isolate this material (the actin nucleator), a task that can be accomplished with a combination of genetics and molecular biological techniques.

**Materials and Methods**

**Bacterial Strains and Growth Conditions**

Listeria monocytogenes strain 10403S (Bishop and Hinrichs, 1987) was used in this study. This strain belongs to serotype 1. It is resistant to 1 mg/ml of streptomycin and its LD₅₀ for mice is 3 × 10⁷. Bacteria were grown in suspension in brain–heart infusion broth (BHI) (Difco Laboratories Inc., Detroit, MI) at 30°C. Stock cultures were kept as suspensions of cells at −70°C in 50% glycerol. For actin nucleation studies bacteria were grown at 37°C in BHI broth.

**Formation, Isolation, and Growth of Listeria Mutants**

DP-L1049 is a transposon-derived insertion mutant of the wild-type 10403S. This mutant was isolated as a small-plaque forming mutant in monolayers of mouse L2 cells. Its growth is normal both intracellularly and extracellularly, but it is incapable to cell-to-cell spread. Accordingly, it is avirulent with an LD₅₀ >3 logs higher than the parental strain. (The LD₅₀ of 10403S is 3 × 10⁷, whereas 5 × 10⁹ of the mutant did not kill any of the infected mice.) A full description on the formation of this mutant will be presented elsewhere.

The chloramphenicol-resistant strain was derived from 10403S and transformed with pAM 401, a chloramphenicol-resistant plasmid (Wirth et al., 1986).

**Tissue Culture and Growth Medium**

The macrophage cell line J774 (Ralph et al., 1975) obtained from J. Unkeless (Mount Sinai Medical School, New York, NY) were grown in spinner flasks in DME (Gibco Laboratories, Grand Island, NY) supplemented with 5% FCS and maintained in the presence of 100 U/ml penicillin and 10 mg/ml streptomycin.

**Infection**

Listeria were grown overnight in BHI broth at 30°C to a density of 2 × 10⁹/ml of culture were sedimented in a microfuge tube (14,000 g) for 1 min; the supernatant was discarded; and the pellet was washed once in 1 ml of PBS, pH 7.4. Macrophages (4 × 10⁶) were plated in plastic tissue-culture petri dishes (30 mm diam) in the absence of antibiotics the evening before use. To the macrophage plates were added the Listeria (2 × 10⁶). After the initial 30-min infection, monolayers were washed three times with 37°C PBS followed by the addition of 2 ml of prewarmed growth medium containing gentamicin sulfate added to a final concentration of 5 μg/ml. This concentration of gentamicin kills all extracellular Listeria, but does not harm intracellular Listeria (Perlnoy et al., 1988).

**Chloramphenicol Treatment**

The infections were performed as outlined in the preceding section, except that four times the number of bacteria were added to the dishes that received chloramphenicol after 1 h of infection and twice the number of bacteria added to the dishes that received chloramphenicol after 2 h of infection. In both cases, chloramphenicol (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 20 μg/ml.

**Cytochalasin**

Cytochalasin D (Sigma Chemical Co., St. Louis, MO) was dissolved in DMSO. Macrophages were infected with Listeria for 30 min and washed three times with PBS at 37°C, and 1 h after the beginning of the initial infection cytochalasin D at 0.5 μg/ml was added together with gentamicin sulfate. 3 h later, the macrophages, still in the cytolysin, were either fixed or detergent extracted.

**Detergent Extraction, Addition of Actin, and Decoration with Subfragment 1**

Macrophages were infected for 30 min. After an additional 60 min, the macrophages were put in cytolysin for 2.5 h. At this time the plates were washed for 20 min in 1% Triton-X 100 containing 3 mM MgCl₂ and 30 mM Tris at pH 7.5 at 4°C. To the plate that would serve as a control, we added a solution containing 1 mM MgCl₂, 60 mM KCl, and 50 mM phosphate buffer at pH 6.8. The experimental plate contained the same solution plus 5 μM G-actin. Both the experimental and control plates were then placed on a rocker table at room temperature. After 20 min had elapsed the salt solution, with or without exogenous actin, was decanted off and the petri plates washed with 0.1 M phosphate buffer at pH 6.8. The plates were then incubated in the solution containing 5 μM subfragment 1 of rabbit myosin in 0.1 M phosphate at pH 6.8 for 10 min at room temperature and then 20 min at 4°C. After decoration with subfragment 1 the petri plates were washed and then fixative was added.

The G-actin was obtained by extracting acetone powders of rabbit skeletal muscle by the method of Spudich and Watt (1971) and gel filtering it (Weber et al., 1987). The actin was stored in aliquots in liquid nitrogen until needed at a concentration of 7 mg/ml. The morning before experimentation, the actin solution was thawed and centrifuged at 120000 g for 2 h to remove any small aggregates or tiny F-actin oligomers that had spontaneously formed during freezing that would act as nuclei and induce polymerization. This centrifugation technique gives the same results as gel filtering the actin solution just before use and is much more rapid. Only the top half of the centrifuged actin solution was used and it was pipetted out carefully. The S1 was prepared from rabbit skeletal muscle (Morgonis and Lowey, 1973) and quick frozen in liquid nitrogen in small aliquots at high concentration, 70 mg/ml. These aliquots were stored in liquid nitrogen and thawed just before use.

To test whether Listeria grown in liquid media nucleate actin assembly, we grew Listeria in suspension at 37°C in BHI medium. The Listeria were then carefully pipetted on grids coated with formvar with a light carbon coat for 2 min. The excess fluid was removed with a small piece of filter paper and a drop of filter aid added which contained 5 μg G-actin, 1 mM MgCl₂, 60 mM KCl, and 50 mM phosphate buffer at pH 6.8. After 15 min incubation in this medium, the grids were negatively stained with 1% uranyl acetate. To be sure that the monomeric actin was polymerizable we added to the monomeric actin Limulus acrosomes which act as nuclei to induce polymerization of actin (Coluccio and Tilney, 1984). These polymerized actin filaments off their ends as would be expected.

**Electron Microscopy**

All fixations were done in situ. Fixation of cytolysin treated cells was carried out by the addition of a freshly prepared solution containing 1% glutaraldehyde (from an 8% stock supplied by Electron Microscopy Sciences, Fort Washington, PA), 1% OsO₄, and 0.05 M phosphate buffer at pH 6.3. Fixation was carried out on ice (4°C) for 30 min. The preparation was then washed three times with water (4°C) to remove excess phosphate and then en bloc stained in 0.5% uranyl acetate overnight. The specimens were then dehydrated in alcohol and embedded in Epon. Thin sections were cut with a diamond knife, picked up on uncoated grids, and stained with uranyl acetate and lead citrate. The sections were examined with a Philips 200 electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ). For fixation of decorated material we added a solution containing 1% glutaraldehyde, 2% tannic acid, and 0.05 M phosphate buffer at pH 6.8 for 30 min at room temperature. The preparation was then washed in 0.1 M phosphate buffer and postfixed in 1% OsO₄ in 0.1 M phosphate buffer, pH 7.4.
6.3, for 30 min at 4°C, rinsed in water and dehydrated and embedded as mentioned above.

Results

The Effect of Cytochalasin D

Tilney and Portnoy (1989) demonstrated that treatment of infected macrophages with cytochalasin D had no effect on multiplication of *Listeria* in the macrophages, but prevented their spread to neighboring macrophages. These *Listeria* possessed neither a cloud of actin filaments nor "comet tails" although "some had some sparse fuzz around them." We reinvestigated the effect of cytochalasin studying macrophages infected for 1 h with *Listeria*, then incubated in cytochalasin D for 3 h before fixation. The fuzz consists of fine fibrils, 30-40 Å in diameter, smaller and more irregular than actin filaments. These fine fibrils do not decorate with subfragment 1 of myosin as will be documented in the next section. Particularly interesting is that in some *Listeria* the fuzz is attached exclusively to one end of the *Listeria*. Here the fuzz can be substantial (Fig. 1), extending up to 0.3 μm from that end. This is not a comet's tail, which is composed of actin filaments that produce a tail that can extend up to 5 μm from that end. The accumulation of fuzz at one end of the *Listeria* does not seem to be the result of division of the *Listeria*. This will be the topic of a subsequent publication.

Actin Filament Assembly Induced by *Listeria*

To address the question of whether *Listeria* nucleates actin assembly, we infected parallel macrophage monolayers with *Listeria* and added cytochalasin D 90 min after the beginning of infection. Incubation in cytochalasin D was continued for 2 1/2 h. At that time the monolayers were detergent extracted and 5 M of nuclei-free G-actin was added to one dish. After 20 min under polymerizing conditions, the dishes were washed and to each we added a solution of subfragment 1 of myosin. After decoration in subfragment 1, the petri plates were washed and fixative was added. Thus the only difference between the two monolayers is that to one no exogenous actin was added (Fig. 2). Both were treated identically with detergent, cytochalasin D, and subfragment 1 of myosin.

Fig. 2 illustrates representative examples of *Listeria* which were treated with cytochalasin D, detergent, and subfragment 1 of myosin, but no exogenous actin. A few of the bacteria (Fig. 2, a and b) appear naked with little adherent material, many more (Fig. 2, c and e) have a small amount of amorphous and/or fine fibrillar material attached directly to their surfaces, and still others (Fig. 2, d and f) have a mass of fine fibrillar material attached to only one end of the bacterium. In a few instances we find filaments, but most of these are intermediate filaments as seen by their smooth, undecorated surface (Fig. 2, b and e). There is an occasional filament that might be decorated (Fig. 2 e), although we cannot be sure. With the dosage of cytochalasin D used here (0.5 μg/ml) some filaments remain in the cortex of the cell (Fig. 2 a), but the majority have disappeared. We find no cases in which decorated actin filaments appear connected to the *Listeria* and the number of "possible" actin filaments located even near a *Listeria* is very low indeed. In short, although most of the *Listeria* are coated all over their surfaces or at one end with a fine fibrillar material that does not decorate with subfragment 1 of myosin, few, if any, actin filaments are associated with the *Listeria*.

When we examined the cultures that had been incubated in exogenous actin under polymerizing conditions after cytochalasin treatment and detergent extraction (Fig. 3), we found a strikingly different image. At low magnification the *Listeria* appear as fuzzy units, some with long tails (compare...
Figure 2. Thin sections of Listeria treated with cytochalasin D, detergent, and subfragment 1 of myosin but no exogenous actin. (a) Low magnification of a portion of a macrophage with many Listeria. The cortex of the macrophage (C) contains a few filaments. Otherwise the cytoplasm is devoid of filaments. (b) A Listeria that appears virtually naked with no adherent fine, fibrillar material. (c) Longitudinal section of a Listeria with a small amount of fine, fibrillar material attached to its surface. (d) Longitudinal section of a Listeria showing that the fine, fibrillar material is associated with one end of the Listeria. (e) Cross-section through a Listeria showing its associated amorphous, fine, fibrillar material. (f) Longitudinal section illustrating the fine, fibrillar material associated with one end of a Listeria. Two intermediate filaments can be seen (F).
Figure 3. Thin sections of *Listeria*-infected macrophages that had been incubated in exogenous actin after treatment with cytochalasin D and detergent. (a) Low magnification showing the *Listeria* with a mass of fuzzy material around them. Some have tails of fuzz. Otherwise the background seems clear. (b) Grazing section through a *Listeria* showing decorated actin filaments around the *Listeria*. (c) Longitudinal section through a *Listeria* showing the extreme density of the material near the surface of the *Listeria* with decorated filaments extending out in all directions.
Figure 4. Cross-section of a *Listeria* illustrating decorated actin filaments extending radially from the surface of the *Listeria*. Note that the decorated actin filaments can be several microns long. (b) Longitudinal section of a *Listeria* showing the decorated actin filaments extending from only one end of the *Listeria*, a region denoted by X. The focus of the actin filaments is actually behind the *Listeria*.
Listeria material that is located not along the body of the bacterium, but off to one end (compare Figs. 2f and 4b). The filaments radiate in all directions from this focus, some extending back wards to parallel the body of the Listeria, but not intimately associated with it. This focus is extremely dense and as already mentioned consists in part of the fine fibrillar material present in the controls and in part of the decorated actin filaments (Figs. 3a and 4a). Thus, if the fibrillar material is present attached to the body of Listeria, one finds actin "clouds" (Figs. 3c and 4a) and if associated with one end of the Listeria, one finds actin "tails" (Fig. 4b).

To determine if Listeria that had been grown outside macrophages would nucleate the assembly of actin filaments or whether nucleation is only induced when Listeria are grown within the cytoplasm of a macrophage, we incubated Listeria in nuclei-free, monomeric actin under polymerizing conditions and examined the bacteria by negative staining. No actin filaments were seen attached to the Listeria. All that was seen was a flagellum attached to the surface of the Listeria. There was no fine, fibrillar material (Fig. 5).

**Mutant Listeria Lack the Fine Fibrillar Material: This Shows That This Material Is Essential for Actin Filament Assembly**

We isolated a mutant of Listeria (DP-L1049) that, when added to macrophages, enters, grows, and multiplies (Fig. 6), but is unable to spread to adjacent macrophages. Examination of thin sections of macrophages infected with this mutant for 4 h before fixation reveals that the mutant Listeria dissolve the phagosome as in the wild type, but do not assemble actin filaments around them to form clouds or comet tails of actin filaments (Fig. 7). Equally significant is that there is no fine fibrillar material on their surfaces. The observations on this mutant substantiate the conclusions of the preceding section, namely that the fine fibrillar material is instrumental in nucleating actin filament assembly from Listeria.

**Is the Nucleating Material Around Listeria a Bacterial Product? The Effect of Chloramphenicol**

To determine if the fine fibrillar material off which the actin filaments appear to nucleate is a product of the Listeria or if it is synthesized by the macrophage and secondarily bound to the Listeria, we applied an inhibitor of bacterial protein.

![Figure 5](image.png)

*Figure 5. Listeria grown in liquid media were incubated for 25 min in a solution containing monomeric actin and salts optimal for assembly. The Listeria were then negatively stained. No actin filaments are nucleated by the Listeria. Instead all that is associated with the Listeria is its flagellum (F). This shows that Listeria must reside in the macrophage cytoplasm to nucleate actin assembly.*

![Figure 6](image.png)

*Figure 6. Graph depicting the multiplication of Listeria in the cytoplasm of the macrophage cell line, J774. DP-L1049 is a mutant cell line of Listeria that does not nucleate actin filament assembly. CM T = 1 is a culture of infected macrophages to which chloramphenicol was added 1 h later. CM T = 2 is a culture of macrophages infected for 2 h before the addition of chloramphenicol. A chloramphenicol resistant strain was examined after chloramphenicol was added at t = 0. The growth curve was identical to that of the wild type.*
Figure 7. Thin section through a macrophage infected with the mutant strain of *Listeria* (DP-LJ049). The *Listeria* were allowed to grow in the cytoplasm for 4 h before fixation.

synthesis (chloramphenicol) to infected macrophages. Chloramphenicol does not affect the growth of the macrophage cell line, but almost immediately after the application to infected macrophages, inhibits *Listeria* growth and replication (Fig. 6). We also infected macrophages with chloramphenicol-resistant *Listeria* and then added chloramphenicol. These *Listeria* doubled and behaved like wild-type *Listeria*.

We infected macrophages with *Listeria* and after 1 h applied chloramphenicol. (By this time many of the *Listeria* will have escaped from the phagosome using their newly synthesized hemolysin, but none will have yet surrounded themselves with actin filaments [see Tilney and Portnoy, 1989].) The chloramphenicol infected macrophages were fixed 3 h later. Examination of thin sections of the *Listeria* in the chloramphenicol treated macrophages revealed that they did not have a coating of actin filaments. Instead attached to their surfaces were dense granules (Fig. 8, a and b), markedly distinct from the fine fibrillar material or actin filaments.

In contrast, if macrophages were infected with *Listeria* and 2 h later chloramphenicol added and examined at *t* = 4, numerous actin filaments emanate from their surfaces (Fig. 8, c and d). By 2 h of infection, untreated *Listeria* would be surrounded by a cloud of actin filaments, so this result is what one would expect.

The simplest interpretation of our results is that the nucleating material is a product of the *Listeria*, not the macrophage.

**Discussion**

In this paper we have demonstrated that associated first with the surface of *Listeria* and subsequently attached to one end is a fine fibrillar material from which actin filaments assemble. A mutant was isolated that enters, grows, and multiplies normally in infected macrophages, yet does not assemble actin around it and does not spread. It lacks the fine fibrillar material as well. Thus, the fine fibrillar material seems to be involved in the nucleation of actin filaments.

A common explanation, and certainly a logical one, for the presence of actin filaments encircling the *Listeria* in a
Figure 8. (a and b) Thin sections of macrophages that were infected with *Listeria*. Chloramphenicol was added 1 h later and the macrophages fixed 3 h after the addition of chloramphenicol. No actin assembly occurs from the surface of the *Listeria*, although there is a coat of a dense granular material that is different from the fine fibrillar material. (c and d) Thin sections through macrophages infected with *Listeria* for 2 h before the addition of chloramphenicol. In this case a cloud of actin filaments can be seen attached to the *Listeria*, presumably having formed before the addition of the chloramphenicol.

Macrophage, is that the macrophage is responding to a foreign intracytoplasmic invader by “walling it off” using its cytoskeleton. It is obvious from the data presented in this report that this is not the explanation in this case. First, when actin is added to detergent-extracted macrophages infected with *Listeria*, the actin specifically assembles from the surface of the *Listeria*. If this were an invasion response by the macrophage, one would expect to see actin assembling off other assemblies of the host’s cytoskeleton, not directly off the *Listeria*. Second, a mutant *Listeria* (DP-L1049) fails to become surrounded by actin filaments, although there is no difference to the macrophage between the mutant and any other invading pathogen. Third, when protein synthesis is inhibited in *Listeria*, but not the macrophage, e.g., by chloramphenicol, no actin filaments encircle the *Listeria*. Fourth, when *Bacillus subtilis*, expressing the hemolysin gene of *Listeria*, is fed to macrophages, it escapes from the phagosome and enters the cytoplasm, but no actin filaments surround it (Bielecki et al., 1990). Thus, instead of the macrophage responding to the invading *Listeria*, our studies indicate that the *Listeria* is actively taking advantage of its host. The simplest explanation for these results is that the fine fibrillar material, the material that appears to be involved in the nucleation of actin filament assembly is a secretory product of the *Listeria*. Thus, the reason why the mutant cannot spread is that this gene product(s) is not expressed.

There are a number of unanswered questions that have been uncovered by this study. First, it is not clear what the mechanism is for the rearrangement of the fine fibrillar material from the surface of the *Listeria* to its accumulation at one pole. In short, how does the actin distribution change from the cloud to form the “comet’s tail”? This subject will be covered in a subsequent publication as its answer can best be approached through the study of a mutant not yet described.

Second, we know that the actin filaments either in the cloud or in the tail in untreated macrophages are all short (Tilney and Portnoy, 1989). In contrast when we examined the filaments nucleated from the fine fibrillar material after the macrophages are detergent extracted we find that they can be and usually are quite long. In fact in cross-sections of *Listeria* they look like the rays, from a sun (Fig. 4 a). Apparently we have removed, in our extracted preparation, components that function to control filament length, e.g., putative cappers. Obviously it would be interesting to know
what these components are, how they are regulated, and who synthesizes them, the bacterium or the macrophage. This is a problem of general interest to cell biologists as there are now a number of actin cappers described, but we still do not know what regulates their distribution. More specifically, in untreated macrophages there are short actin filaments associated with *Listeria*, but in the cortex of the cell, only a micron or so away, there are long actin filaments, e.g., in the microvilli.

Third, it would be interesting to know what the signal is that stimulates the *Listeria* to secrete this fine fibrillar nucleating material. The signal must be a substance present in the cytoplasm of the macrophage because *Listeria* grown in liquid culture do not have the fine fibrillar material associated with them. Furthermore, it takes at least 30 min after the escape of the *Listeria* from the phagosome for this substance to first appear on the surface. Newly escaped *Listeria* are invariably "naked," with no actin filaments and/or fine fibrillar material associated with them (Tilney and Portnoy, 1989). Parenthetically, we should mention that we have not rigorously proved that the fine, fibrillar material is a secreted product of the *Listeria* and not a product of the cytoplasm of the macrophage that attaches to the *Listeria* and is subsequently redistributed. For this interpretation, the *Listeria* must secrete an additional substance on its surface that acts to "glue" the fine fibrillar material to it. This "glue" would not be produced by the mutant and must be blocked by chloramphenicol. Although this interpretation is possible, it is unlikely, as no such material is ever seen in macrophages not infected by *Listeria* and this material is seen only close to or attached to the *Listeria*, never in other parts of the cytoplasm of the macrophage.

Finally, we would like to find out more about the fine fibrillar material and to determine mechanistically how it nucleates actin filament assembly at the molecular level. We believe that using mutants like the one described here gives us the opportunity to do just that. Thus, in the near future, we should be able to isolate the gene or genes responsible for the production of the fine fibrillar material that in turn is responsible for actin nucleation.

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