How *Listeria* Exploits Host Cell Actin to Form Its Own Cytoskeleton. I. Formation of a Tail and How That Tail Might Be Involved in Movement

Lewis G. Tilney, David J. DeRosier,* and Mary S. Tilney

Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and
*Rosenstiel Center, Brandeis University, Waltham, Massachusetts 02254

Abstract. After *Listeria* is phagocytosed by a macrophage, it dissolves the phagosomal membrane and enters the cytoplasm. The *Listeria* then nucleates actin filaments from its surface. These actin filaments rearrange to form a tail with which the *Listeria* moves to the macrophage surface as a prelude to spreading. Since individual actin filaments appear to remain in their same positions in the tail in vitro after extraction with detergent, the component filaments must be cross-bridged together. From careful examination of the distribution of actin filaments attached to the surface of *Listeria* and in the tail, and the fact that during and immediately after division filaments are not nucleated from the new wall formed during septation, we show how a cloud of actin filaments becomes rearranged into a tail simply by the mechanics of growth. From lineage studies we can relate the length of the tail to the age of the surface of *Listeria* and make predictions as to the ratio of *Listeria* with varying tail lengths at a particular time after the initial infection. Since we know that division occurs about every 50 min, after 4 h we would predict that if we started with one *Listeria* in a macrophage, 16 bacteria would be found, two with long tails, two with medium tails, four with tiny tails, and eight with no tails or a ratio of 1:1:2:4. We measured the lengths of the tails on *Listeria* 4 h after infection in serial sections and confirmed this prediction. By decorating the actin filaments that make up the tail of *Listeria* with subfragment 1 of myosin we find (*a*) that the filaments are indeed short (maximally 0.3 μm in length); (*b*) that the filament length is approximately the same at the tip and the base of the tail; and (*c*) that the polarity of these filaments is inappropriate for myosin to be responsible or to facilitate movement through the cytoplasm, but the polarity insures that the bacterium will be located at the tip of a pseudopod, a location that is essential for spreading to an adjacent cell. Putting all this information together we can begin to unravel the problem of how the *Listeria* forms the cytoskeleton and what is the biological purpose of this tail. Two functions are apparent: movement and pseudopod formation.

SINCE actin was first recognized as a common constituent of nonmuscle cells (Ishikawa et al., 1969), an extraordinary amount of information has accumulated over the last 23 years on the cytoskeletons of animal and plant cells. We now know not only where to expect to find actin filaments in a certain cell type, but also what actin binding proteins are likely to be associated with them, how these proteins are related to other actin binding proteins, both in sequence and domain structure, and what is the function(s) of these actin binding proteins, at least in vitro. What is less understood is exactly how a certain cytoskeleton is formed at a prescribed location in a cell and at the appropriate time in the cell cycle.

To obtain information on what controls the formation of the cytoskeleton we have been studying the bacterial pathogen, *Listeria monocytogenes*, which invades macrophages (Tilney and Portnoy, 1989) and other mammalian cells. This bacterium enters the cell cytoplasm and nucleates actin filament assembly from its surface (Tilney et al., 1990). These filaments rearrange to form an actin tail with which the bacterium moves to the surface of the macrophage as a prelude to spreading. Being a definable unit of predictable size and shape, one can concentrate on what it does and how it does it. This simple cytoskeleton serves as a model system in which to try to determine how a cytoskeleton is formed and regulated.

The purpose of this manuscript is to describe how *Listeria* assembles its actin cytoskeleton and how it may use it. What we will describe here is how the filaments are rearranged to form its actin tail and from the length and polarity of the actin filaments how they might participate in movement of *Listeria* through the cytoplasm and into a pseudopod as a prelude to spreading.

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 3×10^4. 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.

Tissue Culture and Growth Medium

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

Infection

Listeria were grown overnight in BHI broth at 30°C to a density of 2×10^9/ml. 1 ml of culture was 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 (4×10^5) 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* (2×10^6). 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 and the addition of gentamicin sulfate to a final concentration of 5 μg/ml for 30 min. This concentration of gentamicin kills all extracellular *Listeria*, but does not harm intracellular *Listeria* (Portnoy et al., 1988).

Light Microscopy

To study movement, macrophages were plated onto 25-mm plastic petri plates which had a portion of the bottom removed and replaced with a cover slip (MatTek Corp., Ashland, MA). This allows examination of the cytoplasm of the macrophages growing in the petri plate with a Zeiss axiovert microscope (Carl Zeiss, Inc., Thornwood, NY) using a 95× phase contrast, oil immersion objective without "squashing" the macrophages between a slide and a coverslip. At varying times after infection of the macrophages in the petri plates, the petri plates could be mounted on a microscope stage that is maintained at 37°C (Irby Electronics, Norristown, PA) and the motion recorded using a Dage camera (Dage-MTI Inc., Michigan City, IN) connected to a video recorder. The movements could be displayed on a television monitor and, by using single frames could be quantitated. By using a "box" in concert the Dage light levels could be kept relatively low.

Decoration with Subfragment 1 of Myosin

Subfragment 1 of myosin (S1)[1] was prepared from rabbit skeletal muscle (Margossin and Lowey, 1973). To examine the polarity of the actin filaments in the tail, petri plates of infected macrophages were incubated for 3 1/2 h at 37°C, the growth medium decanted, and a solution containing 1% Triton X-100, 3 mM $MgCl_2$, and 50 mM phosphate buffer at pH 6.8 added. The solution was at 4°C and extraction was allowed to proceed for 10 min at 4°C. The Triton solution was decanted and 5 mg/ml S1 in 0.1 M phosphate buffer at pH 6.8, was added for 30 min. The macrophages were then washed in 0.1 M phosphate buffer for 10 min to remove unbound S1.

Electron Microscopy

All fixations were done in situ. Routine fixation of 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% OsO_4 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 3 times with water (4°C) to remove excess phosphate and en bloc stained with 0.5% uranyl acetate overnight. For fixation of material decorated with S1, we fixed with 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% OsO_4 in 0.1 M phosphate buffer, pH 6.3, for 30 min at 4°C, rinsed in water, and en bloc stained with uranyl acetate as mentioned above. For fixation with ruthenium red which, in our hands, tends to stabilize the actin filaments against the ravages of osmication, petri plates containing infected macrophages were detergent extracted with 1% Triton in 50 mM phosphate buffer at pH 6.8, and 1 mM $MgCl_2$ for 15 min at 4°C, then rinsed in 0.05 M cacodylate at pH 7.4. Fixation was carried out in 0.25% glutaraldehyde in 0.075 M cacodylate and 1% ruthenium red for 1 h, then rinsed in 0.05 cacodylate and postfixed in 1.5% OsO_4 and 1% ruthenium red in cacodylate buffer (Hayat, 1989). The specimen was rinsed and en bloc stained overnight in uranyl acetate.

In each case the specimens were dehydrated in ethanol 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).

Results

The Entry, Growth, and Division of Listeria

We have found *Listeria* in nearly all stages of its cell cycle in the phagosomes of macrophages, as might be expected if *Listeria* is phagocytosed at the direction of the host; independent of the cycle of the bacterium. By using mutants that are incapable of escaping from the phagosome because they lack hemolysin we know that *Listeria* do not divide while in the phagosome presumably because they lack nutrients. At 30 min after the initiation of infection only 2.6% of the wild-type *Listeria* had entered the cytoplasm, but by 60 min 25% of the *Listeria* were free of the phagosome. Taking into account the constant uptake of *Listeria* for 30 of the 60 min we estimate that on average it will take ~30–45 min for *Listeria* after entry into the macrophage to dissolve the membrane surrounding the phagosome and enter the cytoplasm.

From the multiplication data presented by Portnoy et al. (1988),Tilney et al. (1990), and Sun et al. (1990), we see that once the *Listeria* have entered the cytoplasm they double about every 50 min. If one extrapolates these multiplication curves taking into account the fact that it takes an average of 45 min for the *Listeria* to enter the cytoplasm, there is no perceptible lag in the initiation of growth. Since ~25% of the *Listeria* in a macrophage after 4 h are in the process of forming a new septum, septation must occur during 25% of the cell cycle.

The Appearance of Actin Filaments Associated with Listeria and the Growth of the Tail

Organization of the actin begins shortly after emergence from the phagosome. At 30 min after infection, only 2 out of 76 *Listeria* were free; neither had any indication of an actin "cloud." At 60 min, when 31 were free, eight had no associated actin, seven had a few filaments, and 16 were surrounded by a substantial cloud of actin filaments. Therefore, it seems to take ~15 min to nucleate a cloud of filaments. Furthermore, 75% of the Listeria that had entered the cytoplasm had actin filaments associated with them although tails were not found at this stage (60 min). By 2 h short tails were found on a number of *Listeria* while the remainder are covered by actin clouds. The first pseudopods containing *Listeria* were found 2 1/2 h after the beginning of infection or after two to three doubling times. By 3 h long tails could be found and at 3 1/2 h pseudopods containing the *Listeria* with long actin tails were common.

From these observations it seems reasonable to suspect that the length of the tail is proportional to the time the *Listeria* has spent in the cytoplasm. If only 1 h has passed, a cloud is formed, if 2 h, a few of the clouds have elongated into short tails, and if three or more hours have passed many have long tails. Since the tail is gradually growing longer,

1. *Abbreviation used in this paper*: S1, subfragment 1 of myosin.

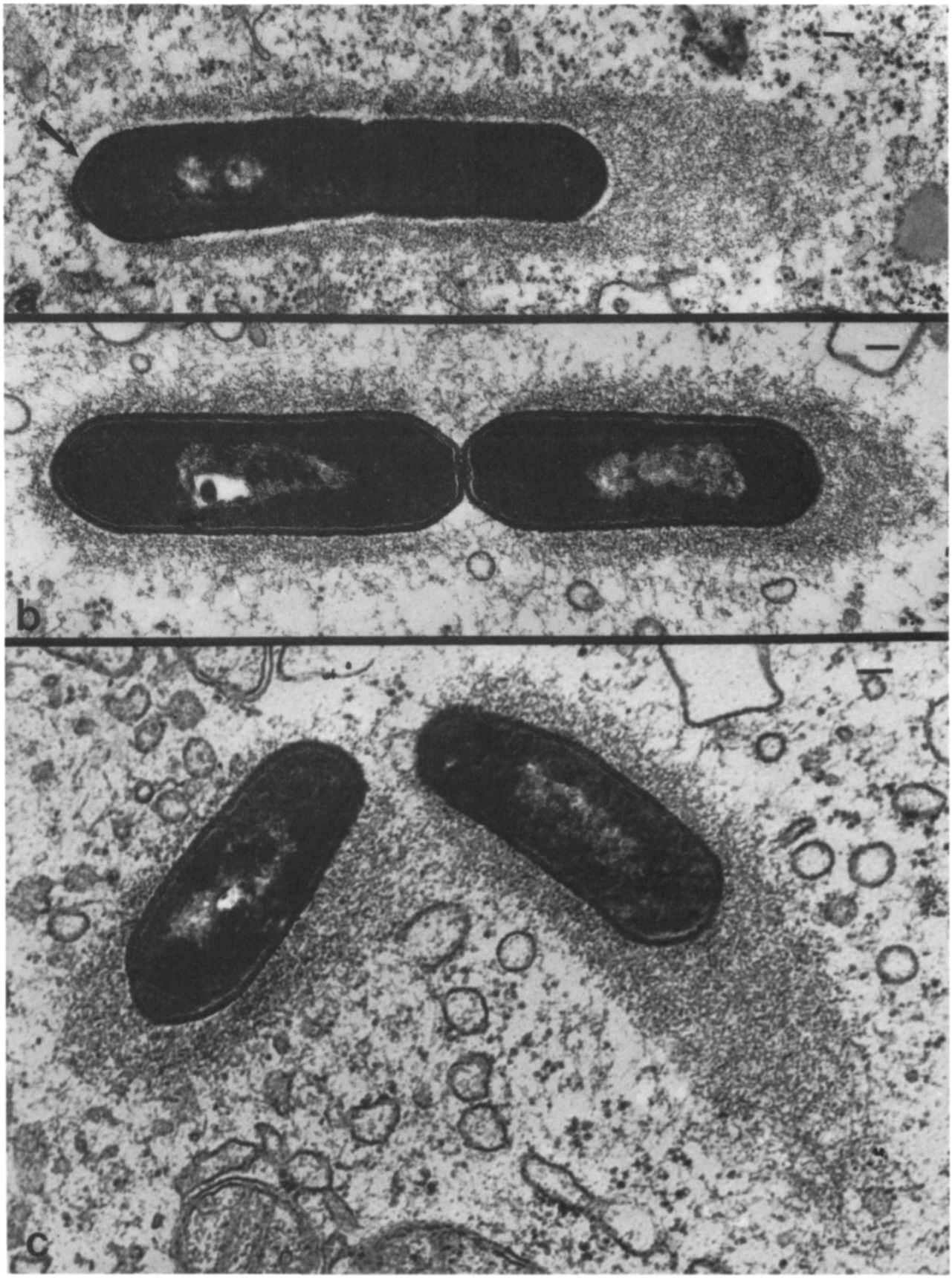


Figure 1. Thin sections through a *Listeria* in the cytoplasm of a macrophage. (*a*) A *Listeria* with a short tail. Actin filaments extend from the lateral and basal surfaces of the bacterium but are conspicuously absent from the anterior pole (*arrow*). (*b*) A *Listeria* in the final process of division. Whereas actin filaments extend from all surfaces of this dividing cell, they are sparse in the area where septation occurs. Notice that the right half of the *Listeria* has a thicker actin cloud and a more extreme tail than the left half. (*c*) A newly divided *Listeria*. Where division has just occurred the surfaces of the *Listeria* are free of actin filaments. The *Listeria* on the right has a much longer tail than that on the left. Bars, 0.1 μm.

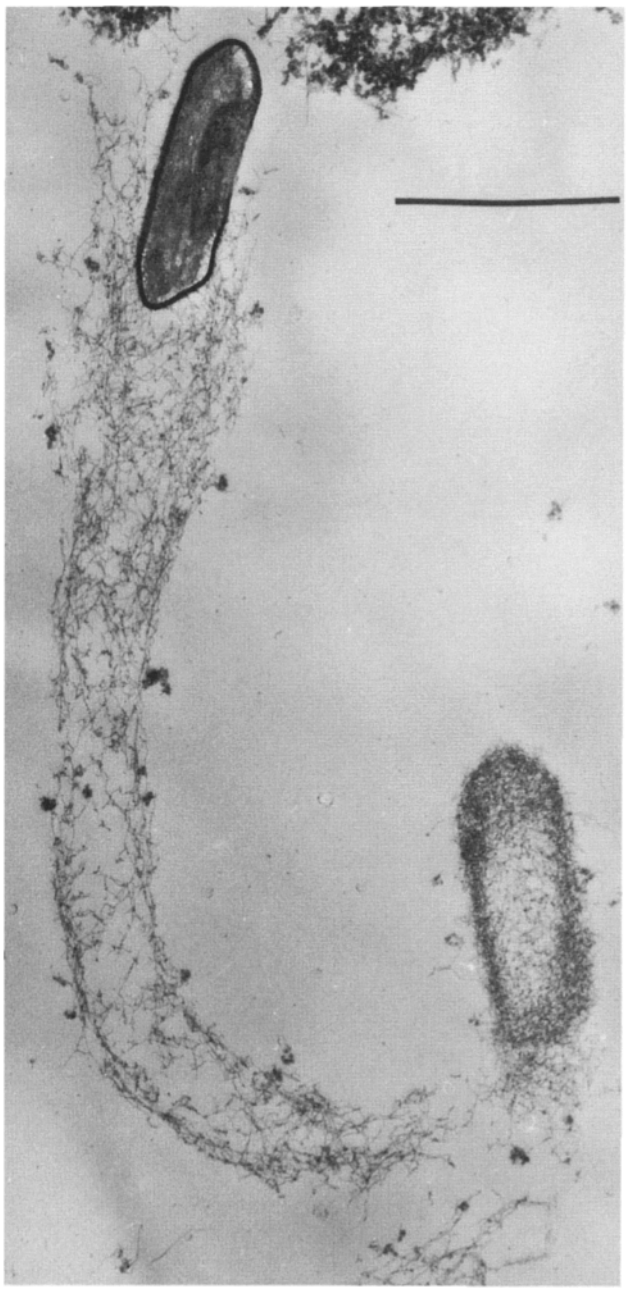


Figure 2. Thin section through a macrophage that had been detergent extracted then fixed with glutaraldehyde and osmium, each containing ruthenium red in the fixative. On the left of this micrograph is a *Listeria* with its long tail composed of actin filaments. On the right is an oblique section through a tail of another *Listeria*. In both cases the tail appears "hollow" with an abundance of actin filaments at the periphery and a lower density in the center. Also of interest is that the surface of the tail is not ragged, but presents a smooth contour. Bar, 1 μm.

yet the bacterium is dividing every 50 min, it seems reasonable to conclude that the older the surface of *Listeria*, the longer its attached tail should be.

There are no reports in the literature on where new cell wall material is inserted in *Listeria*. In other gram positive bacteria, however, (e.g., *Bacillus subtilis* or *Streptococcus*), determined by immunofluorescence or radioactive labeling of wall material, it is thought to be inserted at the equator, a region where ultimately the bacterium will separate (Cole and Hahn, 1962; Pooley et al., 1978). The distribution of actin filaments during septation (Fig. 1 *b*) and at the end of septation (Fig. 1, *a* and *c*) suggests that *Listeria* also produces new cell wall at its equator. In newly divided *Listeria* the only locations where there are no actin filaments on the surface of the *Listeria* are the two newly formed ends (Fig. 1 *c*) or at the exact point of septation. If cell wall and membranous material were inserted all over the surface of the bacterium, the actin cloud would be found all over the surface including the newly formed ends or ends in the state of formation (Fig. 1 *b*). Furthermore, in newly dividing or divided *Listeria* the tail on one is invariably longer than that on the other (Fig. 1, *b* and *c*).

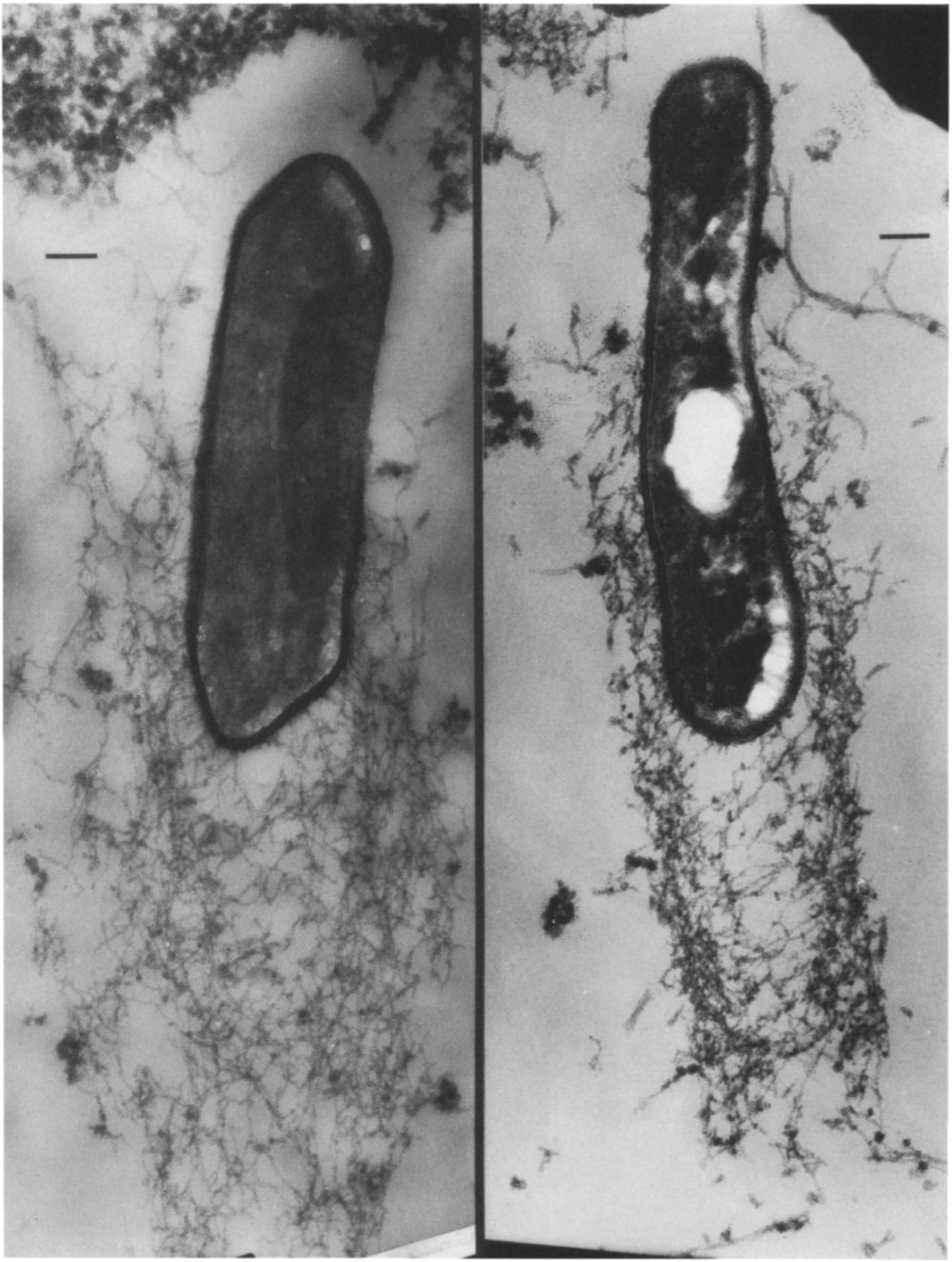
How Do the Actin Filaments Rearrange from the Cloud Form to the Tail Form?

In thin sections through infected macrophages, irrespective of whether they are extracted with detergent or not, one is impressed with the fact that the tails of *Listeria* have smooth contours (Figs. 1 *a* and 2, and see Figs. 4 and 10). It is almost as if they are covered by an invisible hair net, thereby keeping the short filaments that make up the tail confined and orderly. In fact, *Listeria* with the attached tails can be isolated by detergent extraction (Fig. 2) and the outline of the tail remains sharp and distinct, even for periods of more than 1 h. Because the component filaments do not drift away or become rearranged after the tail is isolated, the filaments in the tail must be connected together.

Equally striking is the observation that the tail is not homogeneous, but rather there is a high density of filaments at the margins of the tail with the center appearing somewhat hollow. This is particularly obvious in cross or oblique sections through the tail (Fig. 2). The filaments at the margins tend to be more frequently oriented parallel to the long axis of the tail than those in the center (see Fig. 4). Also, the filaments connected to the sides of the bacterium and to the end from which the tail extends are oriented perpendicular to the surface of the bacterium (Figs. 3 and 4), while all the other filaments are oriented at angles to this first population.

Based on the following observations, namely (*a*) that the filaments are located all around the bacterium except at the newly formed wall; (*b*) that they are crosslinked together; and (*c*) that monomers are added to the filaments at the surface of the bacterium (see the accompanying paper in this issue, Tilney et al., 1992), we will argue that the transformation from a cloud of actin filaments to a tail is due simply to the mechanics of growth. If the filaments elongated from the surface of the bug without cross-links they would tend to grow out radially as shown in Fig. 5 *a*. If, on the other hand, the filaments are cross-linked together as they elongate from the surface of the bug by cross-bridges that are a fixed length, the filaments will aggregate. Since there are no filaments at the newly septated wall, the cross-bridged filaments will pull the meshwork of cross-bridged filaments distally. In this way the filaments can elongate from the bug while still maintaining the cross-links in the meshwork (Fig. 5 *b*) and thus generate the tail we see extending from the distal end of the bacterium. Such a mechanism insures that the edges of the tail are not ragged, but delineated, which is what we observe.

Given that the density of filaments is roughly constant over



Figures 3 and *4*. Thin sections through *Listeria* with tails fixed with ruthenium red in the fixative. (Fig. 3, *left*) Higher magnification of the *Listeria* presented in Fig. 2. Notice that the filaments are all short and those connected to the surface of the *Listeria* extend at right angles to the bacterium. (Fig. 4, *right*) As in the preceding micrograph, the filaments that are connected to the *Listeria* extend at right angles to its surface. The filaments at the lateral margins of the tail are more abundant than those in the center which gives rise to the "hollow" appearance of the tail. These marginal filaments tend to be aligned parallel to the tail axis. Bars, 0.1 μm.

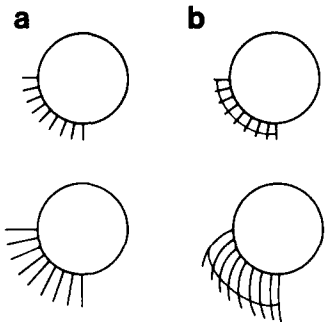


Figure 5. Drawing illustrating what happens to a radial distribution of filaments if they become connected together by bridges with fixed lengths. The filaments will be pulled together and if one pole of the cell is naked, they will naturally form a tail.

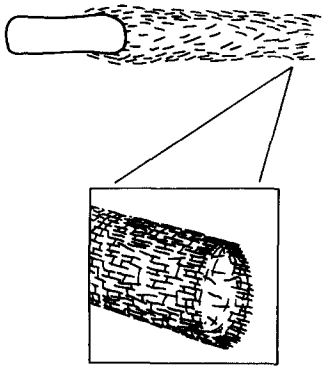


Figure 7. The "hollowness" of the tail of *Listeria* seen in a three dimensional drawing.

the bacterium surface except at the newly formed end (Fig. 1 *c*), the tail will necessarily have a less dense interior as there is more surface on the sides of the bacterium than on its end. To explain this we have drawn a set of "filaments" at a uniform density over a curved "cell" surface (in this case every 5 mm) (Fig. 6). As the filaments along the sides extend out they must curve backwards to maintain their cross-links with the rest of the tail. To do so they must crowd into a smaller volume. Thus the geometry of the bug's surface dictates the "hollow appearance" of the tail as illustrated in Fig. 7.

The Polarity of the Filaments in the Tail

Thin sections through the tail in which the component filaments were decorated with subfragment 1 of myosin allowed us to determine the polarity of many of the actin filaments. In Fig. 8 we present a micrograph and beside it a light print on which we have indicated many of those filaments whose polarity we could unequivocally determine. What is obvious is that the arrowheads point away from the bacterium, towards the tip of the tail; some point at an angle of 90° to the tip of the tail, but we do not find any that point towards the bacterium.

There are two additional facts that can be discerned by examination of these decorated filaments in the tail. First, the S1, by enlarging the filaments and at the same time stabilizing them against the ravages of osmication (Maupin-Szamier and Pollard, 1978) allow us to be certain that the filaments making up the tail are all short, seldom exceeding 0.2 μm in length. We demonstrated that this must be true in an earlier publication (Tilney and Portnoy, 1989) based upon observations that irrespective of the plane of section through the tail, transverse, oblique, or longitudinal, the filaments were always short. Here, by looking at all the filaments in sections through the tail and knowing their polarity and their orientation at the ends of the tail we can be sure that there is no possibility that the filaments are bent or twisted so as to give the appearance of short filaments when in fact they are somehow wound helically around the tail. Second, a comparison of the length of the filaments at the free end of the tail (tip) with those still attached to the surface of the *Listeria* (at the base of the tail) reveals that they are all approximately the same length.

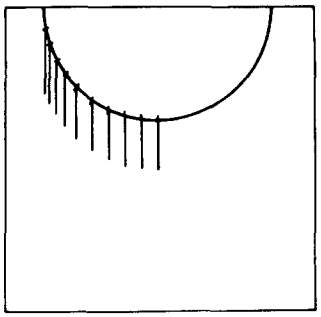
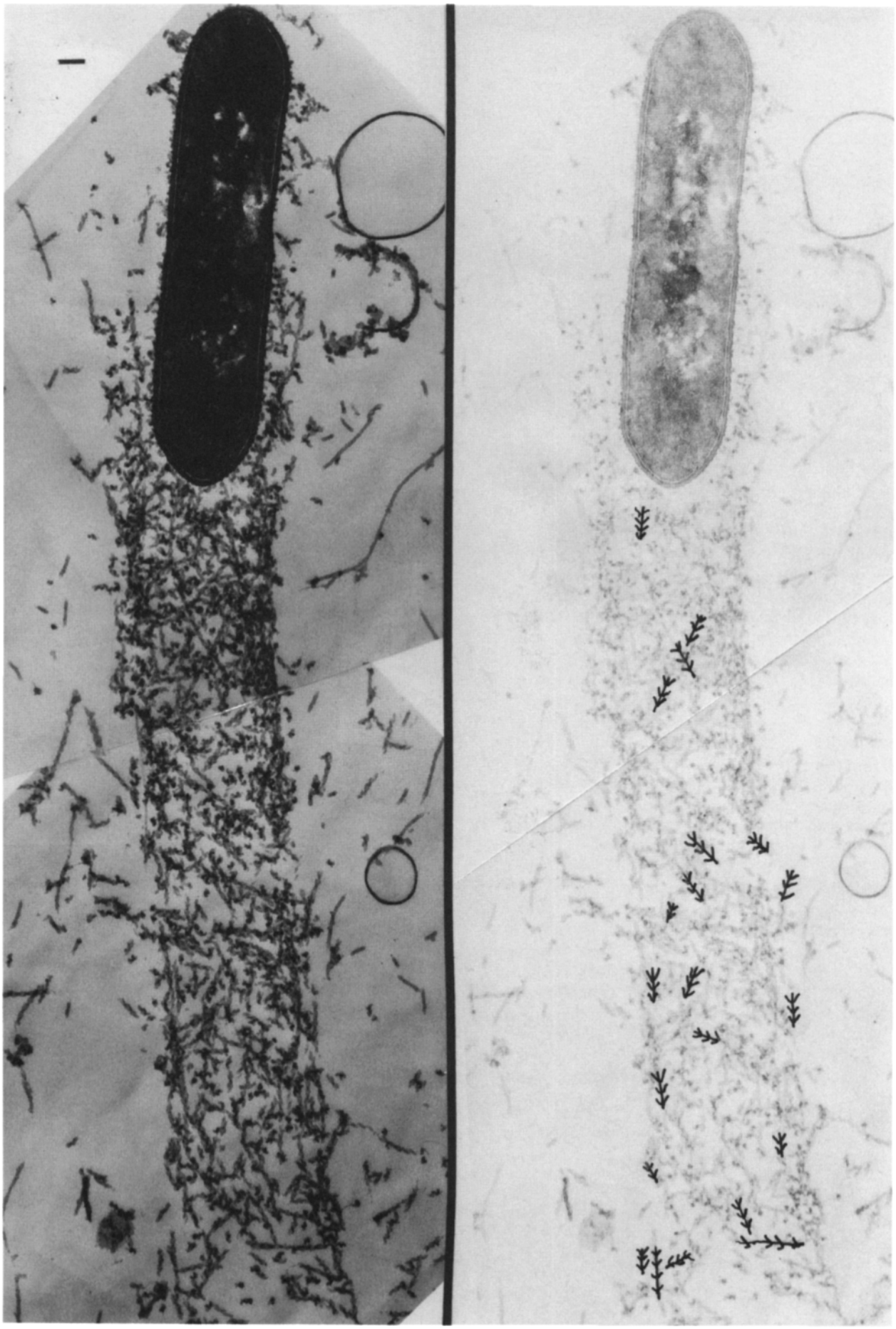


Figure 6. Filaments are drawn from a curved surface such as a bacterium at equal intervals. If these filaments all project in the same direction (because they are cross-bridged, see Fig. 5), the density of filaments at the edges will necessarily be higher than that in the center. This explains the apparent "hollowness" seen in cross sections through the tail of the *Listeria*.

Lineage: The Expected Length of Tails at Varying Times after Infection

As mentioned before, it seems reasonable to suspect that the length of the tail is proportional to the time the *Listeria* has spent in the cytoplasm. If only 1 h has passed, no tail is formed, if 2 h, a few have short tails, and if three or more have passed a number have long tails. But the bacterium is dividing every 50 min and the newly formed ends are free of actin filaments so that *Listerial* surfaces of varying ages will be found in a macrophage infected for 3–4 h. Thus, at this time we would expect to see a few bacteria with long tails, but also some with short tails and some with only a cloud of actin filaments around them. If the divisions of daughter bacteria in the same host macrophage are relatively synchronous, which they appear to be from the published multiplication curves (Tilney et al., 1990), a lineage map could be generated relating the age of the surface of *Listeria* to tail length (Fig. 9). This map would make quantitative predictions as to the percentage of long tails, short tails, no tails, etc., relative to the number of divisions *Listeria* has made in the cytoplasm of an infected cell (Fig. 9). These predictions can then be verified by determining the length of the

Figure 8. On the left is a thin section through a *Listeria* with a tail whose actin filaments have been decorated with subfragment 1 of myosin. The filaments are all short, not exceeding 0.3 μm in length, and the length of the filaments at the tip and base are comparable. On the right is a light print on which we have indicated filaments in ink whose polarity we can unequivocally determine from the left hand micrograph. Of interest is that in all cases the filaments have their pointed ends nearest the tip of the tail and their barbed ends nearest the *Listeria*. Bar, 0.1 μm.



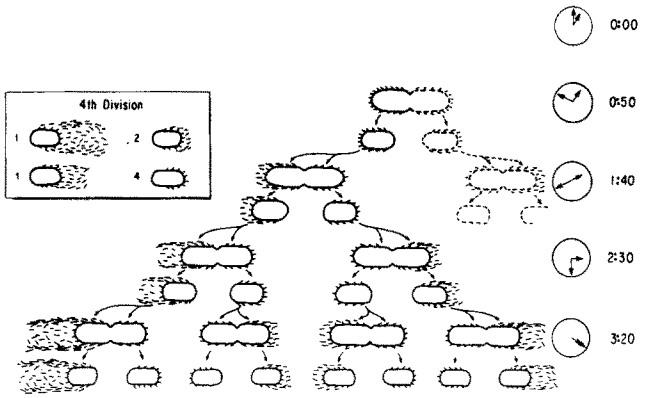


Figure 9. Drawing illustrating the lineage of a *Listeria* that had entered the cytoplasm of a macrophage. Since the *Listeria* divides every 50 min, by 4 h it should have divided 3 times (which includes 45 min for the *Listeria* to escape from the phagosome and enter the cytoplasm). After four divisions there should be 16 *Listeria*, two with a long tail, two with a shorter tail, four with tiny tails, and eight with no tails.

tails attached to all the *Listeria* that had infected an individual macrophage. For example, after four divisions or approximately 4 h after the beginning of infection, one should find 16 bacteria within the cytoplasm of the host macrophage, provided, of course, only one bacterium initially infected a macrophage. The ages of the surfaces would vary for these 16 bacteria as shown diagrammatically in Fig. 9. We should find two bacteria with long tails, two with somewhat shorter tails, four with tiny tails, and eight with no tails. Thus one should get a ratio of 1:1:2:4 as indicated in the drawing.

We cut serial sections of individual macrophages that had been infected for 4 h (an example is shown in Fig. 10) and measured the lengths of the tails (Fig. 11) of all the bacteria present. Since the *Listeria* and their tails do not necessarily lie parallel to the surface of a macrophage as it is bound to a petri plate even though the macrophages are very flat, to be certain of the length of a tail requires three-dimensional reconstruction of tail length from serial sections.

In Table I we document the tail lengths for a single macrophage infected for 4 h. Within the cytoplasm are 68 *Listeria* which must be the result of the incorporation of four bacteria during the infection period. What is obvious from this table is that, with four exceptions, tails grow from only one end of *Listeria*, the other being naked. Although tail lengths ranging from 0–3.1 μm are found, there are natural breaks in the distribution as indicated on the table. Using these breaks we plotted the data in the bar graph illustrated in Fig. 11 relating tail length to the number of individuals found with that length. The four individuals that had a tail extending from both ends were added to the category that would fit with the longest tail. We get a ratio of 1:1:2:4 (Fig. 11) which verifies experimentally our contention that the length of the tail is proportional to the age of the *Listerial* surface in the cytoplasm (Fig. 9).

From this same data we can calculate the rate of elongation of the tail per doubling. This value is 0.9 μm/cycle or a rate of elongation of the tail of 1.2 μm/h.

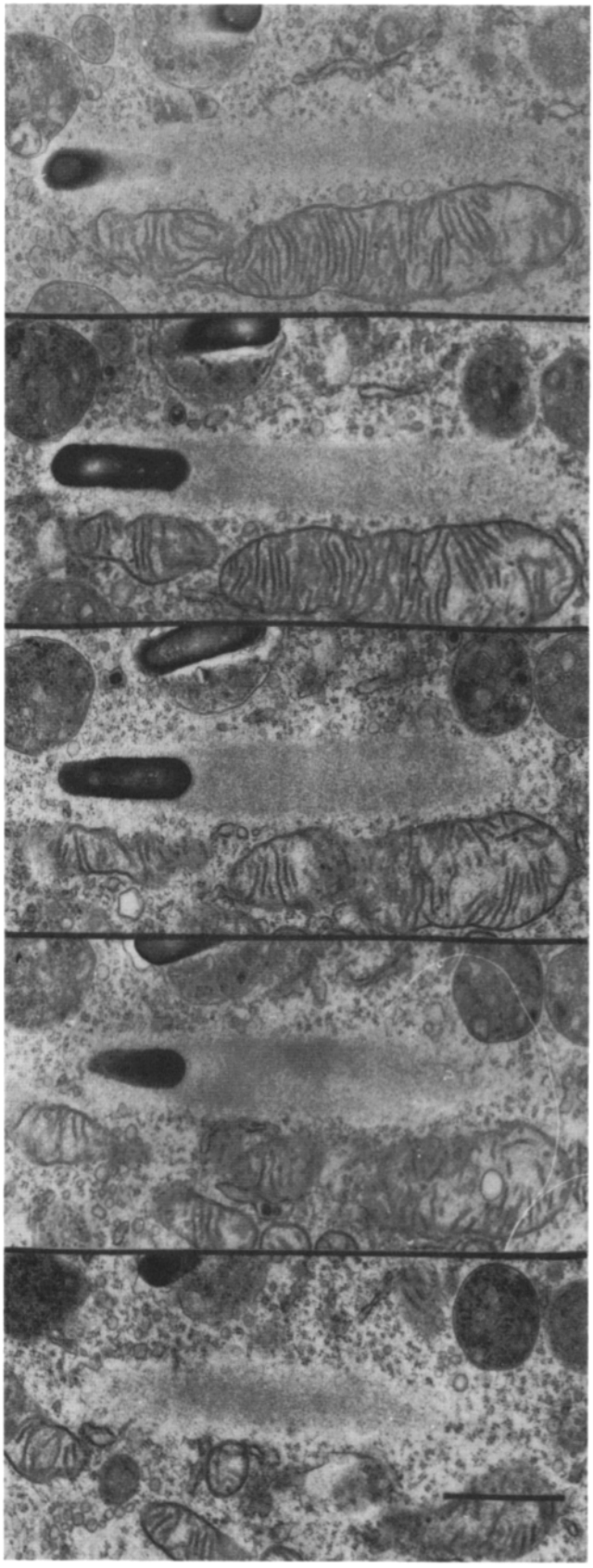


Figure 10. Five serial sections through a portion of a macrophage cytoplasm. The macrophage had been infected with *Listeria* 4 h previously. From these sections we can accurately determine the lengths of tails. Bar, 1 μm.

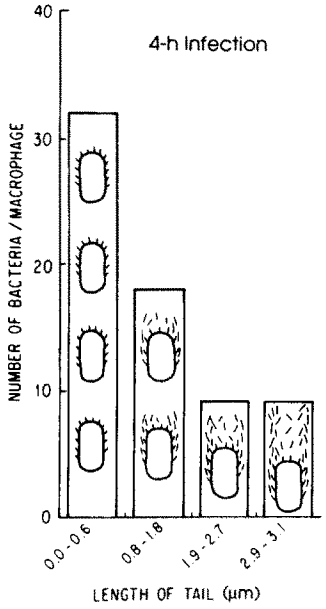


Figure 11. Serial sections were cut of a single macrophage fixed 4 h after infection by four *Listeria*. The length of the tails of all the *Listeria* (68) were measured (see Table I) The tail lengths of all 68 *Listeria* fall naturally into four groups as indicated on the bar graph. The ratio of tail lengths was 1:1:2:4 as predicted in the preceding lineage map shown in Fig. 9.

Table I. Length (μm) of Actin Tails Measured from 68 Listeria Present in One Macrophage (4 h after infection)

One end	Other end	One end	Other end	One end	Other end	One end	Other end
0.0	0.0	0.0	0.8	0.0	1.9	0.0	2.9
0.0	0.0	0.0	0.8	0.0	1.9	0.0	2.9
0.0	0.0	0.0	0.9	0.0	2.2	0.0	3.0
0.0	0.0	0.0	0.9	0.0	2.3	0.0	3.0
0.0	0.0	0.0	0.9	0.9	2.3	0.0	3.1
0.0	0.0	0.0	0.9	0.0	2.5	0.0	3.1
0.0	0.0	0.2	0.9	0.0	2.5	0.0	3.1
0.0	0.0	0.0	1.0	0.0	2.7	0.0	3.1
0.0	0.1	0.0	1.2	0.0	2.7	0.0	3.1
0.0	0.1	0.0	1.3				
0.0	0.1	0.0	1.5				
0.0	0.1	0.0	1.5				
0.1	0.1	0.0	1.5				
0.0	0.2	0.5	1.5				
0.0	0.2	0.0	1.6				
0.0	0.2	0.0	1.7				
0.0	0.2	0.0	1.8				
0.0	0.2	1.2	1.8				
0.0	0.2						
0.0	0.2						
0.0	0.2						
0.0	0.2						
0.0	0.2						
0.1	0.2						
0.2	0.2						
0.2	0.2						
0.0	0.5						
0.0	0.5						
0.0	0.5						
0.0	0.6						
0.0	0.6						

Movement of Listeria and Elongation of the Pseudopods

The movement of *Listeria* will be covered in a subsequent publication (Theriot et al., 1992). What is relevant to this report is that from video sequences Dabiri et al. (1990) have measured the rate of movement of *Listeria* in the cytoplasm of different tissue culture cells infected by *Listeria*. These rates are 0.2–1.0 μm/sec. Our values concur with these. Curiously, elongation of pseudopods containing *Listeria* is not linear. The initiation and the final stages of elongation occur at different rates than that of the bulk of the pseudopod elongation, but the average rate of elongation is 0.01 μm/sec.

We also found that if chloramphenicol, a potent inhibitor of bacterial protein synthesis, is added 15 min before pseudopods first appear (e.g., 2 1/2 h), no pseudopods form. If added at 2 1/2–3 h fewer pseudopods form than normally would be present at 4 h.

Discussion

How Listeria Forms a Tail of Actin Filaments from a Cloud

We demonstrated from lineage studies and other observations that a polar tail is generated from a cloud of actin filaments surrounding the bacterium simply by the mechanics of growth which includes actin filament assembly and interactions between filaments. Since nuclei must appear on the surface of the bacterium before actin filament assembly, and since the newly formed surfaces such as produced during septation are naked, the net result is that the cross-bridged filaments are shed in a polar fashion, essentially being pulled away from the bacterium by the filaments assembled at the tail end of the bacterium. The explanation for the high density of filaments at the margins of the tail relative to those in the center is that the marginal filaments are assembled from the sides of the bacterium while the only contribution to the center of the tail is from the end of the bacterium. Since there is more surface area on the sides of the bacterium than on the end, more filaments will be contributed to the margins, thus giving the tail its "hollow" appearance. In short, we now have an explanation of how a cloud of filaments is converted to a tail.

The Tail of Actin Filaments may Play a Key Role in Pseudopod Formation

Since actin filaments are often involved in motile mechanisms, one might suppose that the main reason that *Listeria* assembles a tail of actin filaments is to use this tail in some way as a means of translocating the bacterium to the margins of the cell as a prelude to spreading. This may be the case as without a tail the *Listeria* is incapable of movement, but an equally important function for the tail is to insure that the *Listeria* ends up in pseudopods that can reach out and be phagocytosed by a neighboring macrophage. Since projections of animal cells such as microvilli, microspikes, pseudopods, and stereocilia all contain a core of actin filaments necessary for growth and maintenance of these asymmetric processes, it seems reasonable to conclude that the actin tail of *Listeria* will be the core needed for the generation of a pseudopod. In essence all that is required is for the actin filaments at the margins of the tail to bind to the plasma membrane and by zippering these actin filaments to the plasma membrane, a pseudopod would be generated with the *Listeria* at the tip. In microvilli the connections that extend from the lateral surface of the filament bundle to the membrane are

myosin I (Coluccio and Bretscher, 1987, 1988; Mooseker et al., 1989). Yet actin filaments are polar and the polarity is identical in all these cell extensions with their barbed ends located at the tips of the process (Mooseker and Tilney, 1975; Tilney et al., 1980). In the decoration studies described in this report we also find that the short filaments that make up the tail are polarized in the same way as in the cell extensions just mentioned with their barbed ends nearest the bacterium which is located at the tip of the pseudopod. Biologically the bacterium should be located at the tip of the pseudopod, not the base, to facilitate spreading which occurs by a neighboring cell phagocytosing the pseudopod of the first cell. Thus the polarity of the filaments in the tail will insure that the *Listeria* will end up at the tip of a pseudopodial extension, an extension which can easily be phagocytosed by a neighboring macrophage.

We also demonstrated, using chloramphenicol, that continuous bacterial protein synthesis is necessary for *Listeria*-induced pseudopod formation. As yet we do not know what protein or proteins must be newly made for pseudopod formation. They may be essential for tail elongation or they may be used directly in pseudopod formation.

It Is Unlikely that Myosin Is Responsible for Movement of Listeria through the Cytoplasm

Although the actin filaments comprising the tail of *Listeria* are all short and not oriented strictly parallel to one another, when decorated with subfragment 1 of myosin, they all have their barbed ends located nearest the bacterium. Since the *Listeria* move like a comet (see Tilney and Portnoy, 1989), with the bacterium leading and the tail trailing behind, this polarity is inconsistent for a myosin based motor. It is as if the bacterium is located on the Z line of skeletal muscle. Active contraction only results in the movement of this Z line towards the thick filaments of myosin, never away from it as is necessary to occur here. Thus conventional myosin cannot account for the movement of *Listeria* in the cytoplasm.

What Might Account for the Movement of Listeria through the Cytoplasm?

Using fluorescent actin and fluorescent actin binding proteins (Sanger, J. M., F. S. Southwick and J.W. Sanger. 1990. *J. Cell Biol.* 111:390 Abstr.) it was concluded that the assembly of actin was responsible for the movement of *Listeria* through the cytoplasm at rates of 0.1–1 μm/s. Assembly appears to take place at the base of the bacterium and at the same time disassembly of the tail must occur at the tip as in most instances the tails remained at constant lengths. Additional evidence that actin assembly may account for the movement has come from Theriot et al. (1992). Cells were injected with "caged" actin and once the tails have formed and the *Listeria* are moving a thin bar of UV light was applied across the tail to produce a fluorescent bar. The *Listeria* moved away from the bar. Thus actin assembly seems to be occurring next to the bacterium and logically actin assembly induces movement. Coupled to this assembly must be a disassembly at the distal tip.

The Relation of the Age of the Listeria Surface to Actin Assembly

From the data provided by Theriot et al. (1992) we also know that the actin filaments in the tail turn over every 33 s regardless of tail length, yet the rate of movement of the *Listeria* through the cytoplasm is directly proportional to tail length. Thus *Listeria* with longer tails must be assembling actin at faster rates.

If we now combine the above information of Theriot et al. (1992) with the data presented in this paper in regard to the lineage and other observations, all of which demonstrate that the age of the *Listerial* surface is related to tail length, we come to the conclusion that older surfaces will induce faster assembly of actin either by increasing the rate of monomer addition or by increasing the rate of filament nucleation. If elongation is slow relative to nucleation, then the overall rate of actin assembly would be governed by the latter. The effective rate of motion would depend on surface age as if the older surfaces are transporting something to the outside of the cell, e.g., a nucleator, more efficiently than younger surfaces.

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