SECRETS OF ACTIN-BASED MOTILITY REVEALED BY A BACTERIAL PATHOGEN

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Actin-based cell motility is a complex process involving a dynamic, self-organizing cellular system. Experimental problems initially limited our understanding of this type of motility, but the use of a model system derived from a bacterial pathogen has led to a breakthrough. Now, all the molecular components necessary for dynamic actin self-organization and motility have been identified, setting the stage for future mechanistic studies.

AMOEBOID MOTILITY
A distinctive form of cell crawling typified by *Amoeba proteus*, which involves extension of pseudopodia and cytoplasmic streaming.

FIBROBLAST
Common cell type found in connective tissue in many parts of the body, which secretes an extracellular matrix rich in collagen and other macromolecules and connects cell layers.

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The ability to move in a directed, purposeful manner is one of the properties we most closely associate with living cells. Many forms of cell motility, such as the intracellular movement of organelles by molecular motors, rely on discrete and stable protein machines. By coupling energy release to a protein conformational change, myosin and kinesin carry their cargo a single step along their substrate, and larger-scale movement is simply the linear addition of many such discrete steps. These types of motility have been extensively studied in purified or semi-purified systems, and a great deal is known about the molecular and biophysical requirements for movement[1].

In contrast, AMOEBOID MOTILITY is not driven by discrete machines acting additively. Instead, it is a complex process involving an interconnecting network of non-equilibrium, dynamic, whole-cell events. Although detailed descriptive studies of amoeboid motility have graced the cell biological literature for over fifty years, it could not be easily investigated at the molecular level using classic biochemical or genetic techniques. However, the past ten years have seen remarkable advancements in our understanding of the molecular basis of amoeboid motility. The breakthrough came, oddly, from a bacterial pathogen called *Listeria monocytogenes*. Like many cell biologists, this pathogen chose actin-based motility as its field of study, but it has had the advantage of millions of years of evolutionary experimentation. This review tells the story of how the secrets of amoeboid motility known to this tiny bacterium have been revealed.

Actin dynamics in locomoting cells

Crawling cells, such as epithelial cells, FIBROBLASTS or neurons, have at their front a broad, flat region, usually less

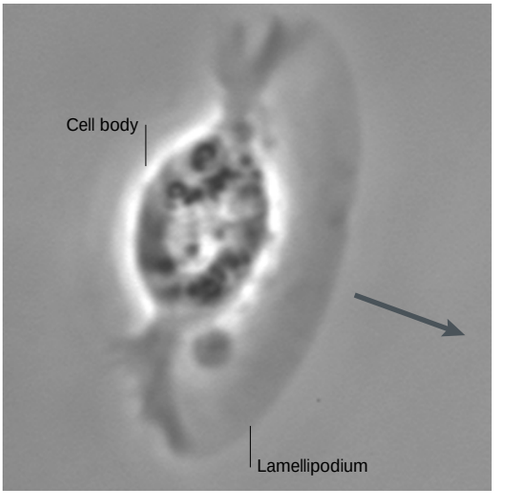


Figure 1 | **A rapidly moving cell; a keratocyte from the skin of a fish.** This is a phase-contrast micrograph, a single frame from a video sequence. The lamellipodium and cell body are labelled. This cell is moving in the direction of the large arrow.

Movie online

KERATOCYTE
A small, motile cell type found in the epidermis of fish and amphibians.

LEADING EDGE
The thin margin of a lamellipodium spanning the area of the cell from the plasma membrane to about 1 μm back into the lamellipodium.

GROWTH CONE
Motile tip of the axon or dendrite of a growing nerve cell, which spreads out into a large cone-shaped appendage.

than one micrometre thick, filled with a dense meshwork of actin filaments. Referred to as a lamellipodium, this region of the cell contains all of the machinery necessary for amoeboid motility. Rapidly moving cells, such as the fish epidermal KERATOCYTE, consist basically of a large lamellipodium that carries the cell body on its dorsal surface (FIG. 1) (Movie 1). Small lamellipodial fragments sliced off from a cell body can crawl on their own, essentially forming a tiny nucleus-free cell[2,3]. The crawling process can be broken down into three subprocesses: the assembly of actin into a coherent meshwork at the LEADING EDGE of the lamellipodium, the coupling of this meshwork to the external substrate, and the controlled depolymerization of the meshwork for recycling and reuse of the actin monomer. Understanding how each of these subprocesses is regulated and how they interconnect and work together is critical to the study of how cells crawl.

Several experiments established the principle that actin assembly (BOX 1) occurs primarily at the front of lamellipodia. In fibroblasts and neuronal GROWTH CONES, a spot photobleached in the actin meshwork of the lamellipodium translocates backwards slowly and moves rearward relative to the leading edge in a coherent fashion[4,5]. When a stationary neuronal growth cone is allowed to recover after the actin meshwork is completely depolymerized by treatment with the toxin cytochalasin, the actin network reforms exclusively at the leading edge and then moves rearward[6].

Across cell types, the rate of rearward flux of the actin meshwork is negatively correlated with the speed of forward protrusion. In rapidly moving fish keratocytes, photoactivated spots of fluorescent actin that have incorporated into the lamellipodium remain stationary with respect to the substrate as the leading edge moves forward[7]. In slowly moving fibroblasts, the actin meshwork moves rearward with respect to the substrate, even as the leading edge continues to move forward[8]. To use the treadmilling activity of actin assembly in the lamellipodium for efficient forward extension of its leading edge, the cell must anchor the actin meshwork through the plasma membrane to the underlying substrate. A keratocyte has an efficient 'clutch' mechanism that allows rapid forward protrusion and little or no rearward flux, whereas a fibroblast has a 'slippery clutch' that results in significant rearward flux and slow forward protrusion. Within a given cell type, there is no correlation between the rate of centripetal movement of actin and the rate of lamellipodial protrusion, so the components that control the rate of protrusion by regulating actin dynamics must be localized at the leading edge[8].

The depolymerization of actin from the meshwork seems to be tightly controlled. Depending on the cell type, the average lifetime of actin filaments in the lamellipodium is very short — around 20 seconds to 2 minutes[7,8]. The rate of filament loss is correlated with cell speed: rapidly moving cells have more labile actin filaments in their lamellipodia, whereas filaments in slowly moving cells are more stable[9]. But most importantly, in all cells, the turnover of actin filaments is at least two orders of magnitude faster than the turnover of pure actin filaments in solution, indicating that other proteins inside the cell must be actively disassembling the filaments in the lamellipodia.

Such cell-based experiments established organizational principles for making a functional lamellipodium

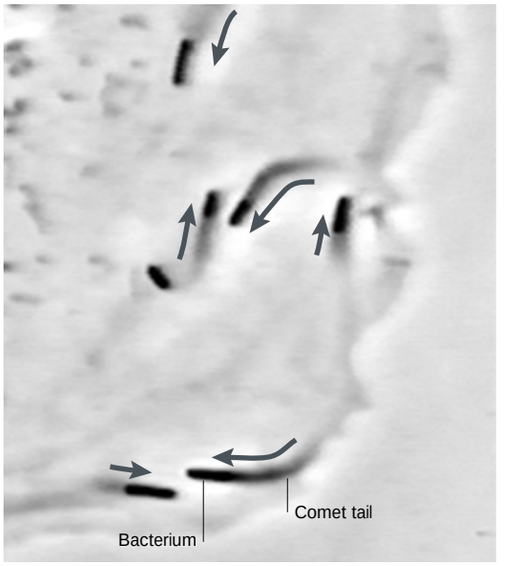


Figure 2 | **Movement of *Listeria monocytogenes* in an infected host cell.** This is a phase-contrast micrograph, a single frame from a video sequence. The kidney epithelial cell was infected about five hours before the acquisition of this video sequence. All of the bacteria in this cell are clonal descendants of a single individual. A bacterium and its associated comet tail are labelled. Bacteria are moving in the direction of the blue arrows.

Movie online

Box 1 | Actin filament dynamics

Actin is one of the most abundant proteins in eukaryotic cells, and is a primary determinant of cell shape and cytoplasmic structure. It exists in two forms, G-actin (for globular), the soluble 43 kDa protein subunit, and F-actin (for filamentous), a helical polymer of arbitrary length where individual subunits self-associate in a head-to-tail fashion. About half the actin in a typical cell (up to 50 μM) is in the form of G-actin, and the other half is in the form of F-actin. Actin is an ATPase, and ATP hydrolysis affects the kinetics of polymerization.

The rate-limiting step in the formation of F-actin from a solution of pure G-actin is the formation of a stable 'nucleus'. When two molecules of G-actin collide in solution, they will form a dimer, but the dimer comes apart rapidly and no filament can grow. When three or four molecules collide simultaneously, they form a more stable trimer or tetramer, which can be rapidly elongated by further collisions of individual subunits with either end of the growing filament. In cells, spontaneous nucleation is rare. Cells regulate the location of new F-actin formation by regulating nucleation.

Within the cell, the dynamic behaviour of F-actin and G-actin is modified and regulated by a group of over 100 actin-binding proteins. These include proteins that bind to G-actin and prevent it from polymerizing, proteins that bind to F-actin and prevent it from depolymerizing, accessory proteins that affect the rate of nucleotide hydrolysis, proteins that sever long filaments into smaller bits, proteins that bypass the slow steps of nucleation, myosin motors that carry cargo along filaments … in short, proteins to speed up or slow down every dynamic behaviour of this remarkable polymer. In addition, F-actin crosslinking proteins can assemble multiple filaments into larger-scale structures, including bundles where all the filaments align in parallel and meshworks where the filaments cross orthogonally.

that could not have been predicted from *in vitro* properties of actin polymerization. First, actin filaments are nucleated and grow primarily at the leading edge, immediately adjacent to the plasma membrane. Second, filaments are crosslinked into a coherent meshwork that either remains stationary with respect to the substrate as the cell moves forward (in rapidly moving cells) or moves rearward towards the cell body (in stationary or slowly moving cells). And last, actin filaments in the bulk of the meshwork, away from the leading edge, depolymerize rapidly so that steady-state, self-organized movement can be maintained. Cell motility requires that these three processes be properly coordinated in space and time.

In a lamellipodium, there must be modulatory factors that govern these phenomena. This raises several specific molecular questions. What factors catalyse nucleation and elongation of actin filaments at the leading edge? How are filament nucleation and elongation suppressed elsewhere? What is causing the older filaments to depolymerize so rapidly? What holds the meshwork together, and is it important for motility that this meshwork be coherent?

Technical problems have impeded attempts to answer these questions. Genetic systems have been of limited use in identifying the full complement of components that make up the machinery necessary for actin-based motility. Yeast, unfortunately, do not crawl. Genetic and reverse genetic approaches in model metazoans and in *Dictyostelium* successfully defined the cellular functions of some of the individual components of the motility apparatus. However, because the actin polymerization machinery necessary for amoeboid motility is so critical for other aspects of cellular behaviour, many cells with lesions in important cytoskeletal loci are inviable and therefore difficult to evaluate for motility phenotypes. In addition, functional redundancy is rampant in the actin cytoskeleton, so many null mutants in genes that encode interesting proteins have no detectable phenotype. Biochemical reconstitution of amoeboid motility has been hindered by the need for an intact cell plasma membrane, which must serve to localize filament nucleation[10] and might contribute to force generation[11]. A decade ago, the identification of a genetically manipulable model system that could mimic the actin filament dynamics of lamellipodial protrusion without the requirement for a plasma membrane was desperately needed to understand actin-based motility at the molecular level.

Actin-based motility of bacterial pathogens

In the late 1980s, several research groups found that F-actin is responsible for the intracellular movement of two unrelated bacterial pathogens, *Listeria monocytogenes*[12,13] and *Shigella flexneri*[14,15], which live within the cytoplasm of the host cell. Because *L. monocytogenes* is less virulent and easier to handle experimentally than *S. flexneri*, most laboratories investigating this form of actin-based motility have chosen to focus on *L. monocytogenes*, and we will concentrate on the *L. monocytogenes* model system in this review.

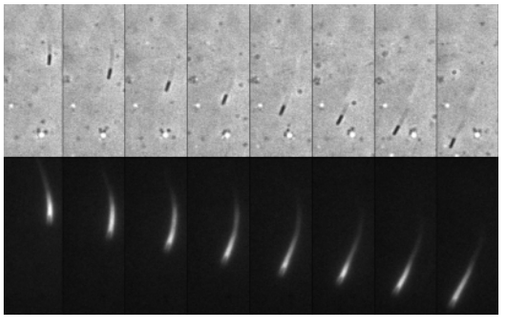


Figure 3 | **Reconstitution of *Listeria monocytogenes* motility in a cytoplasmic extract.** Top row | Phase-contrast images showing the position of the bacterium. Adjacent frames are separated by ten-second intervals. Bottom row | Fluorescent signal arising from rhodamine–actin added as a tracer. The fluorescent images were captured less than one second after the corresponding phase image. The online movie shows a polystyrene bead coated with ActA moving in a similar extract.

Movie online

Actin-based motility is essential in the *L. monocytogenes* life cycle. In a natural food-borne infection, the bacteria induce phagocytosis by the epithelial cells lining the small intestine, an event that can be replicated in the laboratory in a wide variety of tissue-culture lines. The bacteria then secrete a pore-forming toxin (listeriolysin O) that degrades the enclosing membrane, and escape into the cytoplasm of the host cell. After a few hours of growth and division, host-cell actin filaments begin to form a dense cloud on the surface of the bacteria. Subsequently, the actin cloud becomes polarized into a comet tail made up of an oriented, crosslinked network of actin filaments with their barbed ends pointing towards the bacterium[12]. Bacteria associated with comet tails move very rapidly within the host cytoplasm, at rates of up to 1 μm per second[16] (FIG. 2) (Movie 2). Finally, the infection spreads as the bacteria push their way into neighbouring cells through plasma membrane projections[17,18].

The *L. monocytogenes* comet tail resembles a simplified lamellipodium, and the bacterial surface imitates the plasma membrane at the leading edge. Labelled exogenous actin monomers preferentially incorporate into the actin tail near the bacterial surface in living and permeabilized cells[19,20], recapitulating the behaviour in lamellipodia, where new incorporation is primarily at the leading edge. Fluorescence photoactivation experiments reveal that filaments in the tail of a moving bacterium remain stationary and only the bacterium moves forward[21], as in rapidly moving lamellipodia. The rate of filament depolymerization in the comet tail is independent of either position in the tail or bacterial speed, and the filaments have very short half-lives, of the order of 30 seconds[21].

In contrast to amoeboid motility, bacterial actin-based motility does not require the host cell plasma membrane and could therefore be reconstituted in a cell-free cytoplasmic extract, which has facilitated biochemical approaches to study the regulation of actin

Box 2 | Special features of the lamellipodium

In general, it is thought that the dynamic behaviour of the actin-binding proteins described here is comparable between comet tails and lamellipodia. However, there are important differences. For example, ActA is not found in eukaryotic systems. The search for the eukaryotic equivalent of ActA led to the characterization of a new protein family called WASP/Scar. Wiskott–Aldrich syndrome protein (WASP) is expressed only in human haematopoietic cells and contains a GTPase binding domain[69] that binds the small GTPases, Cdc42 and Rac, known to be involved in regulating the triggering of actin polymerization in fibroblasts[70]. Its close relative N-WASP is expressed widely in vertebrate cells[71], and causes filopodial formation when co-expressed with Cdc42 in cultured cells[72]. The more distantly related protein Scar (for suppressor of cyclic AMP receptor mutation) was discovered in *Dictyostelium*, where its deletion causes cytoskeletal defects[73]. WASP and Scar interact with the p21 subunit of Arp2/3[74] and, like ActA, Scar activates Arp2/3 to nucleate actin filaments[75]. Finally, polystyrene beads coated with WASP are capable of forming actin comet tails and moving in cytoplasmic extracts, in a manner apparently identical to the movement of *L. monocytogenes* or ActA-coated beads[76].

Much of what is known about cell motility and lamellipodial protrusion has come from descriptive observations. Two of the most visually striking behaviours of lamellipodia include ruffling and rearward flux. Ruffling is a phenomenon where the protruding leading edge detaches from the substrate and folds back on the dorsal surface of the lamellipodium. Rearward flux, described in the section 'Actin dynamics in locomoting cells', requires myosin[77]. Neither of these characteristic behaviours can be investigated using *L. monocytogenes* as a model system.

dynamics[22] (FIG. 3). The exclusive localization of actin filament growth close to the bacterial surface indicated that factors either secreted by the bacterium or expressed on the bacterial surface must trigger actin polymerization. Because *L. monocytogenes* continue to move in cells in the presence of drugs that inhibit bacterial protein synthesis[20], the key factor was probably a stable protein on the surface of the bacterium rather than a factor secreted continually by the bacterium.

The bacterial factors involved in actin polymerization are required for the spread of bacteria from cell to cell. To identify the gene(s) required for actin assembly, screens were designed to identify mutant bacteria deficient in the ability to spread from cell to cell, but capable of normal initial cell invasion, membrane lysis and bacterial division. The only gene ever isolated in such *L. monocytogenes* screens is *actA*[23,24]. Furthermore, ActA confers actin-based motility on normally immotile bacteria, for example if *act*A is expressed in the non-pathogenic strain *Listeria innocua*[25], or if purified ActA protein is attached asymmetrically to *Streptococcus pneumoniae*[26]. Polystyrene beads coated with purified ActA protein have been shown to form comet tails and move in cytoplasmic extracts, proving that no other bacterial surface components are required for motility[27] (Movie 3).

Dissection of the comet tail and lamellipodium

A flurry of experimental work followed the identification of ActA and the reconstitution of motility in cytoplasmic extracts. Today, researchers in the field largely agree on the identities and the functions of all the main molecules required for regulating actin filament dynamics in the self-organized motile system of the comet tail. The similarities, and differences, between the simplified bacterial comet tail and the far more complicated, dynamic lamellopodial structure have given great insight into the organization and regulation of these systems at many levels.

Factors catalysing nucleation and elongation. Although ActA is sufficient to cause polymerization at the bacterial surface, it does not interact directly with actin to form a comet tail, indicating that it probably interacts with other host-cell factors. As ActA is a surface protein, initial efforts focused on host-cell factors that localize to the bacterial surface and not throughout the comet tail. Two proteins that fulfil this criterion were initially identified by immunofluorescence surveys: the G-actin-binding protein profilin[22] and an F-actin associated protein called vasodilator-stimulated phosphoprotein (VASP)[28]. Both proteins require ActA to associate with the bacterial surface. VASP binds directly to ActA[28], and profilin binds to VASP[29]. It seemed unlikely that these factors were responsible for localized actin nucleation, however, as cytoplasmic extracts depleted of profilin could still support nucleation of actin clouds by *L. monocytogenes*[22,30]. Furthermore, VASP binds to F-actin[29] but does not show nucleating activity, and profilin significantly inhibits nucleation[31]. Profilin can serve as a nucleotide exchange factor for actin[32], and it can also lower the effective critical concentration for actin polymerization in a cellular environment[33]. So, it was concluded that the combination of VASP and profilin may accelerate filament elongation at the bacterial surface, but neither one is the nucleator.

Systematic ActA deletion studies carried out in bacteria indicated that VASP and profilin are localized by a central polyproline-rich region in the middle of the protein, and that four consensus FPPPP repeats act in an additive fashion to bind multiple molecules of VASP[34]. Bacteria containing an ActA construct that lacks the VASP-binding domain still mediate actin nucleation, although they move more slowly than wild-type bacteria in both cytoplasmic extracts[35] and in infected cells[34]. Interestingly, the rate of motility is linearly related to the number of proline-rich repeats present[34]. Immunolocalization shows that the central proline-rich domain recruits VASP[36], and biochemical experiments show that the consensus motif is sufficient for VASP binding[37]. Subsequently, the VASP-related proteins mammalian Enabled (Mena) and Ena/VASP-like protein (Evl) were found to act interchangeably with VASP in bringing profilin to the bacterial surface and in

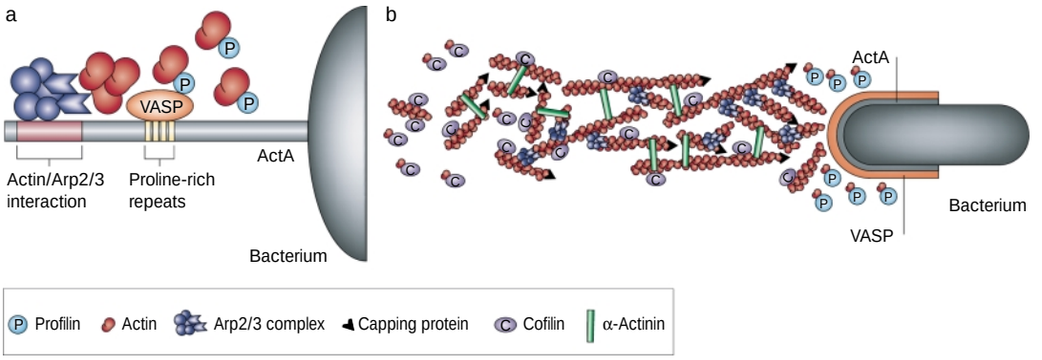


Figure 4 | **Diagram of the molecular components required for actin-based motility of *Listeria monocytogenes*.** **a** | Interactions between host-cell proteins and ActA at the bacterial surface. Two domains of ActA are required for normal motility. The amino-terminal domain activates actin filament nucleation through Arp2/3. The central proline-rich domain binds VASP and profilin interacts with VASP, enhancing filament elongation. **b** | Host protein functions throughout the comet tail. In addition to the factors that act at the bacterial surface, capping protein binds to the barbed end of actin filaments to prevent elongation of older filaments, α-actinin crosslinks filaments to stabilize the tail structure, and ADF/cofilin disassembles old filaments. (VASP, vasodilator-stimulated phosphoprotein.)

enhancing the speed of actin-based motility[38,39]. Profilin tagged with green fluorescent protein (GFP–profilin) associates with moving bacteria in infected cells and, strikingly, the concentration of GFP–profilin at the bacterial surface is closely correlated with bacterial speed[40]. This indicates that the proline-rich repeats, VASP and profilin may act together as an accelerator for bacterial movement.

VASP and profilin may also have independent effects on filament elongation. Experiments using human platelet extracts show that movement of *L. monocytogenes* is still enhanced in the presence of a mutant profilin that does not bind proline-rich sequences and therefore cannot associate with VASP. Conversely, VASP still accelerates *L. monocytogenes* motility in profilin-depleted extracts[39]. Surprisingly, overexpression of members of the Ena/VASP family in mammalian cells causes them to move at less than half the speed of wild-type cells, and removal of Ena/VASP proteins by sequestration to the mitochondrial surface causes cells to move faster than wild-type cells[41]. This observation indicates that enhancement of the rate of filament elongation by VASP may not directly translate into an increase in crawling speed in mammalian cells, possibly highlighting an important difference between lamellipodia and *L. monocytogenes* motility (for other differences, see BOX 2).

But regardless of the nature of the accelerator, what turns the ignition key by catalysing F-actin nucleation in the first place? The answer to this question came from a biochemical study. Platelet extracts, rich in actin-associated cytoskeletal proteins and easy to obtain in large quantities, were fractionated, and the fractions examined using a visual assay to determine which could support the formation of F-actin clouds around *L. monocytogenes*. The most purified active fraction contained a tightly associated protein complex consisting of seven polypeptide chains[42]. This complex, named Arp2/3 after two of its members (actin related proteins 2 and 3), had initially been isolated as a profilin-binding complex by affinity chromatography of *Acanthamoeba castellanii* cytosol[43], but its function in the regulation of actin dynamics had previously been unclear. The nucleating activity of Arp2/3 can be measured *in vitro*[44], but it is

Table 1 | Actin-based motility in other systems

System	Structure formed	Host factors	Special features	References
Shigella flexneri	Comet tail similar to *Listeria*	N-WASP, Arp2/3, profilin	• Bacterial factor IcsA (VirG) has no homology with ActA • Actin dynamics are identical to *L. monocytogenes*	14, 15, 25, 50, 80, 81
Rickettsia spp.	Comet tail, made of long twisted bundles		• Bacterial factor not identified • Tails are straight • Dynamics are distinct from *L. monocytogenes* and *S. flexneri*	82–85
Enteropathogenic *Escherichia coli*	Pedestal	WASP, Arp2/3	• Signalling from bacterium occurs across host cell membrane • Bacterial factors intimin and Tir required	86, 87
Vaccinia virus	Comet tail similar to *Listeria*	N-WASP, Nck, WIP, Src-family tyrosine kinase	• Intracellular enveloped form moves • Actin-based movement may contribute to viral budding	88–90
Vesicles	Transient comet tail similar to *Listeria*	PtdIns(4,5)P_2, Cdc42, N-WASP, Arp2/3	• Endosomal rocketing induced by phorbol esters, metal ions • May occur normally with nascent endosomes	91–95

Nck, non-catalytic region of tyrosine kinase; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; N-WASP, neural Wiskott–Aldrich syndrome protein; WASP, Wiskott–Aldrich syndrome protein; WIP, Wiskott–Aldrich syndrome protein interacting protein.

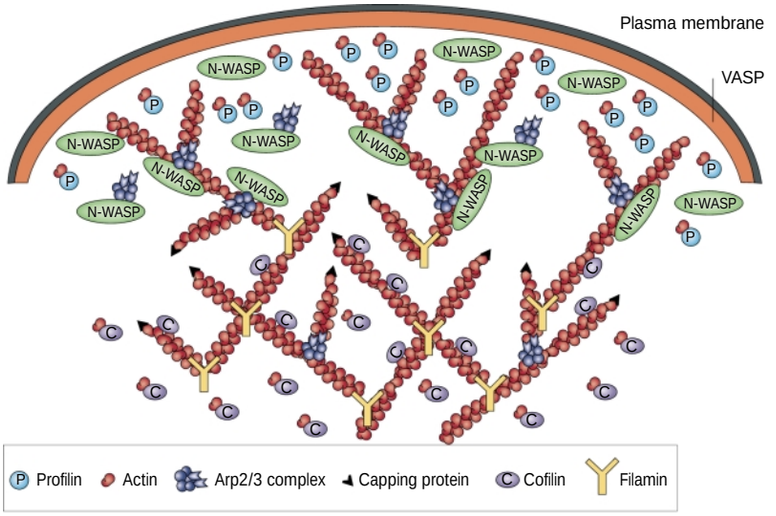


Figure 5 | **Functions of similar proteins in the lamellipodium.** N-WASP activates Arp2/3 to nucleate actin filaments. VASP and profilin, which are localized to the leading edge, facilitate elongation. Capping protein caps the barbed ends of older filaments. Filamin crosslinks filaments into an actin network. Finally, ADF/cofilin accelerates depolymerization throughout the lamellipodium, except for a cofilin-free zone at the immediate leading edge (reviewed in REF. 78). The localization of N-WASP is not well known. Here we show it at the leading edge, binding and activating Arp2/3 in the cytoplasm, and associated with Arp2/3 at filament branches. (VASP, vasodilator-stimulated phosphoprotein; N-WASP; neural Wiskott–Aldrich syndrome protein.)

markedly activated by the presence of ActA (REF. 45). The amino-terminal domain of ActA, which was implicated by the deletion studies as being sufficient to cause actin nucleation inside of cells, is also sufficient for full activation of Arp2/3 (REF. 46). Arp2/3 can also bind to the side of a pre-existing actin filament and initiate nucleation of a new filament at that location, creating a branch at a 70° angle from the original filament[44]. Such branches are found throughout the lamellipodium[47] with Arp2/3 at the branch points. Arp2/3 is localized to the leading edge of several cell types[42,43,48], and it is found throughout the actin comet tail associated with *L. monocytogenes*[49]. So, activated Arp2/3 is responsible for the nucleation of actin polymerization at the bacterial surface (FIG. 4a).

Activation of Arp2/3 by ActA is essential for *L. monocytogenes* motility. No full-length homologues of ActA exist in mammalian cells, so how is Arp2/3 activated in lamellipodia? Similarly, IcsA (VirG), the bacterial surface protein required for *S. flexneri* actin-based motility, does not interact directly with Arp2/3, indicating that some other factor mediates the activation of Arp2/3 in this system. Recently, it has been found that Wiscott–Aldrich syndrome protein (WASP) and its relatives, N-WASP and Scar (for suppressor of cyclic AMP receptor mutation), have a function similar to ActA, activating Arp2/3 downstream of signalling through small GTPases (BOX 2). In the case of *S. flexneri* motility, N-WASP binds IcsA (VirG) and activates Arp2/3 at the bacterial surface[50] (TABLE 1).

Factors suppressing nucleation and elongation. New actin filaments are continuously nucleated and elongated exclusively at the bacterial surface or at the leading edge of the cell, suggesting that some mechanism exists to prevent the continuing elongation of old filaments. The simplest mechanism to achieve this would involve capping the growing barbed ends of the older filaments. Several proteins with barbed-end F-actin capping activity are known, including capping protein (also known as CapZ) and gelsolin. Biochemical studies indicate that ActA may suppress capping close to the bacterial surface[30], although ActA probably exerts this effect indirectly. Capping protein is strongly associated with comet tails[51]. Gelsolin is localized throughout the tail and, paradoxically, is enriched at the bacterial surface[52], but it may be inactive there. The combination of barbed-end capping suppression at the bacterial surface, exclusive localization of the elongation enhancers VASP and profilin at the bacterial surface, and potent activation of Arp2/3 by ActA, seems to be sufficient to enable nucleation and elongation only at the front of the comet tail.

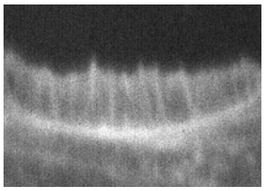
Factors causing filament depolymerization. An important feature of both lamellipodial and bacterial motility dynamics is the rapid depolymerization of the actin meshwork far from the leading edge and the bacterial surface, suggesting the existence of depolymerizing factors. Another functional study using *L. monocytogenes* elucidated the specific role of the protein that controls filament depolymerization. This protein, called ADF (actin depolymerizing factor) or cofilin, was first identified in biochemical assays as a factor that accelerates actin depolymerization. ADF/cofilin binds cooperatively to the sides of actin filaments and increases the twist of F-actin[53], destabilizing the filament structure and causing an increase in the rate of spontaneous filament breakage[54] and a significant acceleration of subunit dissociation from the filament pointed end[55]. It has a higher affinity for ADP-containing filaments, and so preferentially accelerates the turnover of old filaments after nucleotide hydrolysis has occurred, rather than the newest filaments, which still contain ATP. This combination of activities makes cofilin the leading candidate as the factor responsible for the 10–100-fold higher actin turnover rate in cells compared with the turnover rate of pure actin.

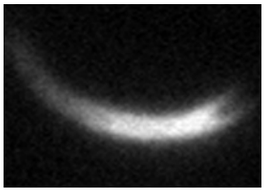
Immunodepletion of ADF/cofilin from cytoplasmic extracts supporting *L. monocytogenes* motility alters the morphology of the comet tails, making them five times longer than normal[56]. Conversely, addition of excess exogenous ADF/cofilin to extracts causes shortening of the actin tail and increases the rate of bacterial motility[55]. ADF/cofilin is localized throughout the *L. monocytogenes* comet tail[56], consistent with the previous finding that depolymerization occurs uniformly everywhere in the tail[21].

Experiments done in intact cells also show that ADF/cofilin is important for acceleration of actin turnover[57]. ADF/cofilin is localized to the leading edge and ruffling membrane of motile cells[58]. Rapidly moving keratocytes show an ADF/cofilin-free zone at the very leading edge of the lamellipodium[47]. This narrow ADF/cofilin-free margin may be the geometrical correlate of the hydrolysis kinetics of ATP; the newest ATP-containing filaments at the front are briefly protected from rapid disassembly by ADF/cofilin. The spatial separation between nucleation and elongation at the front,

Table 2 | Similarities between lamellipodia and comet tails

Protein	Function	Localization in lamellipodia	Localization in comet tails
F-actin*	Cell shape and cytoplasmic structure	Enriched in stress fibres and focal contacts	Throughout tail[16]
Arp2/3*	Nucleation Filament crosslinking Pointed-end capping	Leading edge and throughout lamellipodium[47,49] Filament branches[47]	Bacterial surface and throughout tail[42]
Capping protein*	Barbed-end capping	Cytoplasm and cell–cell contacts[96]	Throughout tail[51]
Gelsolin‡	Barbed-end capping, severing	Cytoplasm and focal contacts[97]	Bacterial surface and throughout tail[52,56]
VASP	Binds F-actin and profilin, binds proline-rich region of ActA	Leading edge[98] and focal contacts[29]	Bacterial surface[34]
Profilin	Actin monomer binding	Leading edge, focal contacts[40]	Bacterial surface[22]
ADF/cofilin*	Depolymerizes ADP filaments	Lamellipodium[58] (1 μm from edge[47])	Throughout tail[56]
α-actinin	Crosslinking	Focal contacts and stress fibres[99]	Throughout tail[16]


Lamellipodium


Comet tail

* Absolutely required for motility in the reconstituted system. ‡ Can be substituted for capping protein.

and disassembly further back, helps to maintain the steady-state organization of the motile F-actin meshwork in both lamellipodia and comet tails.

Factors that crosslink filaments. Because Arp2/3 frequently binds to the side of a pre-existing filament as it nucleates the growth of a new filament, the meshwork forming at the front of the comet tail or the leading edge of the lamellipodium is effectively crosslinked at birth, in a dendritic web[44,47]. In addition, numerous F-actin crosslinking proteins are found throughout the comet tail, including fimbrin[59] and α-actinin[16]. Microinjection of a dominant-negative fragment of α-actinin, which inhibits crosslinking by the endogenous protein, causes *L. monocytogenes* in infected cells to stop moving[60]. This observation indicates that strong crosslinking is important for movement through the highly viscous cytoplasm of a living cell, although its mechanical contribution may be less important in cytoplasmic extracts (see below).

Fimbrin and α-actinin, which crosslink F-actin to form tight parallel bundles, are not generally found in lamellipodia. Instead, lamellipodia are enriched in a different type of crosslinking protein, filamin (also called ABP-280), which tends to crosslink filaments at right angles to form a web. Mutant melanoma cells that fail to express filamin show very poor motility[61]. These complementary results in comet tails and in lamellipodia seem to indicate that crosslinking is indeed important for mechanical stability of a protrusive self-organized actin structure, but that the nature of the crosslinker required is probably different depending on the details of filament organization in each case.

Establishment of a purified system. Using the molecular information provided by the studies detailed above, we might hypothesize that *L. monocytogenes* motility could be reconstituted *in vitro* with a mixture of the following host proteins: actin, Arp2/3, VASP, profilin, capping protein, ADF/cofilin and α-actinin, along with a steady supply of ATP. This impressive feat has recently been accomplished[62] (FIG. 4b). Of these proteins, only actin, Arp2/3, ADF/cofilin and capping protein are absolutely required for motility. VASP and profilin increase the rate of movement, and α-actinin stabilizes the tail. The success of this purified system definitively establishes the minimal components necessary for this type of self-organized actin-based motility. In addition, it clearly shows that this form of force generation does not require a myosin motor[62], and that actin polymerization alone can act as a bona fide molecular motor[63]. Moreover, the functional conservation between the lamellipodium and the comet tail is striking (TABLE 2, FIG. 5), proving that *L. monocytogenes* is an excellent tiny cell biologist.

Open questions

The establishment of a purified protein motility system that includes all of the bacterial and host components necessary and sufficient for actin-based movement[62] represents a satisfying culmination of the past decade of molecular research in this field. Work from numerous laboratories has brought us to the point where we now know most of the critical molecules involved in actin regulation at the leading edge of cells. However, despite this list of molecules, we still cannot formulate the set of rules needed to generate a motile cell, or a lamellipodium, or even a comet tail. The purified system has confirmed the supposition that actin polymerization alone must produce the force necessary for motility, but provides no further information about the actual microscopic mechanism of force generation. It will, however, provide a useful experimental system for investigating some of the open mechanistic questions about cell movement.

Translating the minimal requirements for bacterial actin-based motility to a mechanism of cell motility or even the protrusion of a lamellipodium requires a significant increase in complexity, the most important difference being the presence of a plasma membrane. Further understanding of cell motility will also require knowledge of how the regulation of cell adhesion and nuclear translocation are integrated with lamellipodial protru-

BROWNIAN RATCHET MODEL (ELASTIC)
A proposed model for actin-based motility in which actin filaments are thought to flex away from the bacterial surface to allow addition of monomer at the end of the filament. When the filament flexes back, it is one subunit longer and pushes the bacterium forward that distance.

BULK ELASTIC MODEL
A proposed model for actin-based motility, which treats the actin comet tail as a cohesive elastic gel that responds elastically to deformation. This indicates that the energy from actin polymerization may be stored as elastic energy in the actin gel to produce force that propels the bacterium forward.

sion to move the entire cell. But even within the comparatively simple system of the actin comet tail, many basic mechanistic questions remain to be addressed. We suggest that the answers to the following questions will begin to reveal the rules needed to organize an actin comet tail, and may lead to insights into how to address the more difficult questions of whole-cell locomotion.

How is force generated? In theory, motile force for actin-based motility can come solely from the chemical potential of actin polymerization[63,64]. Several models for the mechanism by which this conversion takes place have been proposed, including BROWNIAN RATCHET-TYPE MODELS[65] and BULK ELASTIC MODELS[66]. Current data are inconclusive about which of these models, if any, are correct, and have been limited in large part by the natural variations in the extract and cultured-cell preparations used to assay motility. With the purified protein system, force generation by the self-organizing actin polymerization machinery should now be amenable to detailed biophysical experimentation analogous to the studies that defined the protein conformational changes responsible for force generation by the discrete motor proteins myosin and kinesin[1].

How is movement initiated? Actin-associated bacteria in cells seem to exist in two states: moving with a comet tail, or stationary with a uniform cloud[12]. The same two states are seen in cytoplasmic extracts, both for bacteria and for ActA-coated polystyrene beads[27]. These two states can readily interconvert in a classic bistable system. What makes a bacterium or a bead start moving? Stochastic modelling based on the elastic Brownian ratchet mechanism for force generation has suggested that this symmetry breaking event might be caused by a form of dynamic positive feedback. Small variations in the polymerization rate on one side of the bacterium versus the other side can be amplified to cause large-scale symmetry breaking[67]. The quantitative theoretical predictions of this model could be confirmed or refuted using the purified system.

What causes variations in speed? Within a population of genetically identical bacteria moving in a single host-cell type, there are wide variations in average speed from one bacterium to another[17,21,34]. This wide variation is not due to sampling error, but rather to the fact that some individual bacteria are intrinsically faster than other individual bacteria (P.A.G and J.A.T., unpublished observations). What is responsible for these intrinsic differences? Changes in ActA surface density do not affect speed[27,34], so the source of variation must lie elsewhere. Even within the trajectory of an individual bacterium, there are significant variations in speed over time, often over an order of magnitude within a period of a few minutes. Are variations in the subcellular environment responsible for this? If so, can this property be used to map out the positions of biochemically distinct microenvironments within a living cell?

What causes curvature in trajectories? It is extremely rare to find a bacterium that moves in a straight line; most bacterial tails are gently curved (FIG. 2, FIG. 3). What causes these curves? Is there a correlation between variations in curvature and variations in speed? Some bacterial strains carrying point mutations in ActA show curvature behaviours that are very different from wild-type bacteria, including one mutant that makes tighter smooth curves and one mutant that can 'skid', making occasional sharp turns at apparently random intervals (P. Lauer, S. Rafelski, D. Portnoy and J.A.T, unpublished observations). How do these mutations affect interactions with the host-cell proteins that govern actin self-organization and movement?

These mechanistic questions, and many others, can now be definitively addressed in the purified protein system[62], exploiting the ability of ActA to confer motility on artificial particles whose geometry can be controlled[27]. So, with a set of proteins in hand, and a simple system in which to study these interactions, the time is ripe for a powerful convergence of molecular, biochemical and physical techniques on a single area: the organization and control of the actin cytoskeleton.

This review has detailed an experimental success story, in which an unusual bacterial system has been successfully exploited as a robust, reproducible proxy for eukaryotic actin-based amoeboid motility. Using immunofluorescence, biochemical and genetic techniques, proteins putatively involved in motility were identified and found to be necessary and sufficient for motility, as shown by a minimal reconstituted system. As a whole, this experimental journey can serve as a lesson on how to approach molecular mastery of a dynamic, self-organizing cellular system. Now that the molecular basis of this type of motility is fairly well understood, attention can be focused on the biophysical mechanisms[68], bringing us one step closer to a detailed understanding of the beautiful and complex process of amoeboid motility.

Links

DATABASE LINKS actin | *act*A | profilin | VASP | Mena | Evl | Ena | Arp2/3 | VirG | WASP | N-WASP | Scar | CapZ | gelsolin | | cofilin | fimbrin | α-actinin | filamin
FURTHER INFORMATION *L. monocytogenes* | Theriot lab homepage
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