Actin-based motility of intracellular pathogens

Edith Gouin[1], Matthew D Welch[2] and Pascale Cossart[1]

The actin cytoskeleton is harnessed by several pathogenic bacteria that are capable of entering into non-phagocytic cells, the so-called 'invasive bacteria'. Among them, a few also exploit the host actin cytoskeleton to move intra- and inter-cellularly. Our knowledge of the basic mechanisms underlying actin-based motility has dramatically increased and the list of bacteria that are able to move in this way is also increasing including not only *Listeria*, *Shigella* and *Rickettsia* species but also *Mycobacterium marinum* and *Burkholderia pseudomallei*. In all cases the central player is the Arp2/3 complex. Vaccinia virus moves intracellularly on microtubules and just after budding, triggers actin polymerization and the formation of protrusions similar to that of adherent enteropathogenic *Escherichia coli*, that involve the Arp2/3 complex and facilitate its inter-cellular spread.

Addresses
[1] Unité des Interactions Bactéries-cellules, Institut Pasteur; INSERM U604; 28 Rue du Docteur Roux, Paris 75015, France
[2] Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

Corresponding author: Cossart, Pascale (pcossart@pasteur.fr)

Current Opinion in Microbiology 2005, **8**:35–45

This review comes from a themed issue on
Host–microbe interactions: bacteria
Edited by Pascale Cossart and Jorge Galán

Available online 7th January 2005

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2004.12.013

Introduction

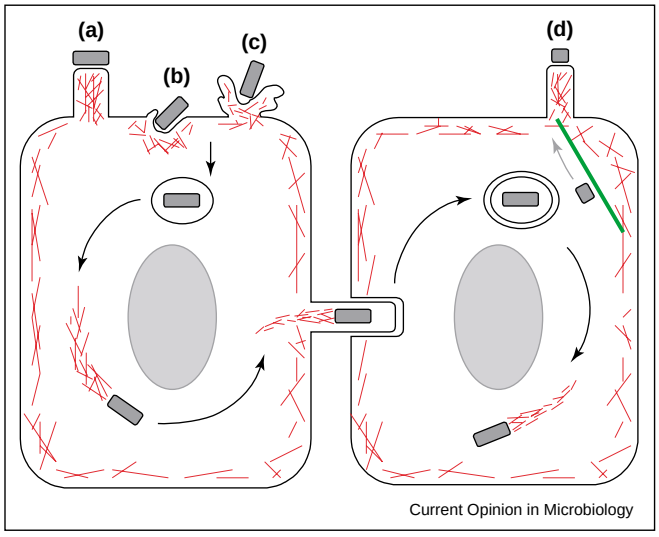
Several intracellular bacterial pathogens share the ability to enter the host cytoplasm during infection, where they induce actin polymerization at their surface and initiate actin-based motility. The force generated by actin assembly propels the bacteria through the cytoplasm and into neighboring cells, promoting cell-to-cell spread (Figure 1). Direct spread from cell to cell allows these pathogens to circumvent host innate and adaptive immune responses [1–4]. Bacterial pathogens such as *Listeria monocytogenes*, *Shigella flexneri*, and multiple *Rickettsia* species, have been known for some time to exhibit intracellular actin-based motility [4]. Recent studies indicate that *Burkholderia pseudomallei* and *Mycobacterium marinum* can be added to this list. In addition to pathogens that use actin for intracellular movement, others such as enteropathogenic *Escherichia coli* (EPEC) and Vaccinia virus use actin polymerization at the plasma membrane to form motile pedestals on the surface of infected cells (Figure 1). These structures result from attachment (EPEC) or function in cell-to-cell spread (Vaccinia virus). Interestingly, all pathogens studied so far promote actin polymerization by exploiting a host protein complex called the Arp2/3 complex, which is a conserved molecular machine used by host cells to nucleate actin filaments and organize them into branched arrays. Nevertheless, it appears that different pathogens have adapted different strategies for interacting with and activating the host Arp2/3 complex, suggesting that they have independently evolved the capacity to undergo actin-based motility.

Here, we review recent advances in our understanding of actin-based pathogen motility. We describe the basic machinery that regulates actin polymerization in eukaryotic host cells. Moreover, we survey advances in our understanding of how pathogens exploit the host actin polymerization machinery, focusing on *L. monocytogenes*, *S. flexneri*, *Rickettsia* species, and vaccinia virus, as well as on pathogens recently found to undergo actin-based motility, including *B. pseudomallei* and *M. marinum*.

Regulation of actin polymerization in host cells

The actin cytoskeleton of eukaryotic cells plays a critical role in whole-cell motility [5], and in the intracellular movement of organelles such as endosomes [6]. Strikingly, pathogens have evolved the capacity to exploit the host actin cytoskeleton to promote their own motility during infection. Actin itself is one of the most abundant proteins in eukaryotic cells, constituting upwards of 5% of total protein in some cell types. It is an ATP-binding protein that exists in two forms, monomers (G-actin), and double helical polymers or filaments (F-actin). The spontaneous nucleation of actin filament assembly from pure monomers is kinetically unfavorable, but once nucleation takes place, filaments undergo rapid elongation by reversible endwise association of monomers. Within each filament, monomers are arranged head to tail, imparting a molecular polarity on the filament and defining two biochemically distinct ends. One end is called the plus or barbed end and the other the minus or pointed end. *In vitro*, ATP-bound monomers polymerize onto the barbed end with a rate constant that is 10 times greater than for the pointed end, and in cells polymerization also occurs predominantly at barbed ends. Following addition of the monomer onto filament ends, ATP is hydrolyzed to ADP, and inorganic phosphate is released. Filaments depolymerize by endwise monomer dissociation, which primarily

Figure 1



Schematic drawing of various stages of the infectious process by **(a)** EPEC, **(b)** *Listeria* and *Rickettsia*, **(c)** *Shigella* and *Burkholderia pseudomallei* and **(d)** vaccinia virus. EPEC adhere to the mammalian cells via interaction between the Tir protein after its translocation and insertion in the mammalian cell membrane and the bacterial surface protein intimin. This interaction leads to the formation of pedestals. *Listeria* and *Rickettsia* enter non phagocytic cells via a zipper mechanism i.e. with tight apposition of the plasma membrane around entering bacteria. *Shigella* enter non-phagocytic cells via a trigger mechanism i.e. the formation of large membrane extensions that result in macropinocytosis. *Burkholderia* induce large membrane invaginations. *Listeria*, *Rickettsia*, *Shigella* and *Burkholderia* are internalized in a vacuole from which they rapidly escape. Free in the cytosol, they recruit and start to polymerize actin, thereby inducing their own movement. In some cases, they reach the membrane, induce the formation of protrusions, that result in the formation of two membrane vacuoles that are lyzed and allow a new cycle of infection to begin. Note that *Mycobacterium marinum* (see text) only infects macrophages and does not induce ruffles. Vaccinia virus (the IEV form) moves intracellularly on microtubules (green). After virus release from the infected cell, the virus (the ECV form, see text) is propelled on a pedestal following Src recruitment and a Src mediated switch between microtubule-dependent movement and actin-based motility.

occurs by loss of ADP-bound monomers from pointed ends. In cells, the processes of actin polymerization and depolymerization are regulated by an enormous variety of actin binding proteins that perform numerous functions including nucleating filament assembly, binding monomers, capping filament ends, severing and depolymerizing filaments, and organizing filaments into different arrays. These proteins allow cells to modulate actin assembly in response to external or internal stimuli.

Pathogens have evolved the ability to exploit the host machinery that controls actin polymerization and depolymerization to generate actin arrays that function in motility. To initiate motility, pathogens first stimulate the nucleation of actin filament assembly at their surface, or at the plasma membrane, by activating host actin nucleating factors. All pathogens evaluated so far have been shown to utilize the Arp2/3 complex, one of two major actin nucleating activities in cells [7]. The Arp2/3 complex consists of the actin related proteins (Arps) Arp2 and Arp3, as well as five other subunits. Because Arp2 and Arp3 are structurally similar to conventional actin [8], it has been hypothesized that in the active complex, the barbed ends of these Arps may serve as a template for polymerization. However, on its own the Arp2/3 complex has no actin nucleation activity. It is activated by a class of cellular proteins called nucleation promoting factors (NPFs) [9] of which one example are the members of the Wiskott–Aldrich syndrome protein (WASP) family. Most NPFs contain several conserved domains including a proline-rich region, a C-terminal WASP homology 2 (W) region that binds actin monomers, and C-terminal central and acidic regions (CA) that bind to the Arp2/3 complex. Activation of the Arp2/3 complex by NPFs involves a conformational change in the complex [10••], which is thought to facilitate binding to the side of an existing actin filament and initiation polymerization of a new filament at a 70° angle, generating characteristic Y-branched structures that can be observed by electron microscopy at the leading edge of motile cells [5,11,12]. Intracellular pathogens have adopted two main strategies for activating the Arp2/3 complex: either they express mimics of WASP family NPFs (e.g. *L. monocytogenes* ActA /*L. ivanovii* IactA, or *Rickettsia* RickA), or they express surface proteins that recruit host family WASP proteins (e.g. *S. flexneri*). Interestingly, no pathogens evaluated thus far have been demonstrated to utilize a second set of actin nucleating proteins in host cells, the formin family. Formins are an evolutionarily conserved set of proteins that nucleate actin assembly and remain

tethered to the barbed end of growing filaments, where they permit polymerization and inhibit filament capping and termination [13]. In cells, formins promote the formation of parallel bundles of actin filaments, which are geometrically distinct from the branched arrays generated by the Arp2/3 complex. Because formins have not been shown to be utilized by pathogens, we will not discuss them at length.

Once filaments are nucleated and organized into Y-branched networks by the Arp2/3 complex at the pathogen surface, filament elongation occurs, generating a force that drives motility. Force generation has been proposed to involve several biophysical mechanisms, including a tethered Brownian ratchet mechanism that takes into account filament behavior at the molecular level, and an elastic model that considers the behavior of actin networks at the macroscopic level [14]. Regardless of the precise mechanism of force generation, elongation is eventually terminated at the ends of older filaments by capping protein CapZ [15], leaving only the ends of newly nucleated filaments at the pathogen surface free to polymerize. As filaments age and hydrolyze ATP, older ADP-containing filaments in the array are severed and depolymerized via the activity of ADF/cofilin proteins [16]. The set of host proteins described above, including those involved in nucleation (Arp2/3 complex and NPFs), capping (CapZ) and depolymerization (ADF/cofilin), have been shown to constitute the minimal set of proteins sufficient to drive actin-based motility *in vitro* [17]. Interestingly, while mechanisms of depolymerization appear similar between pathogens, mechanisms for promoting actin nucleation are more variable, and each pathogen studied to date has evolved a slightly different strategy, as discussed below.

The actin-based motility of pathogenic bacteria

Listeria monocytogenes

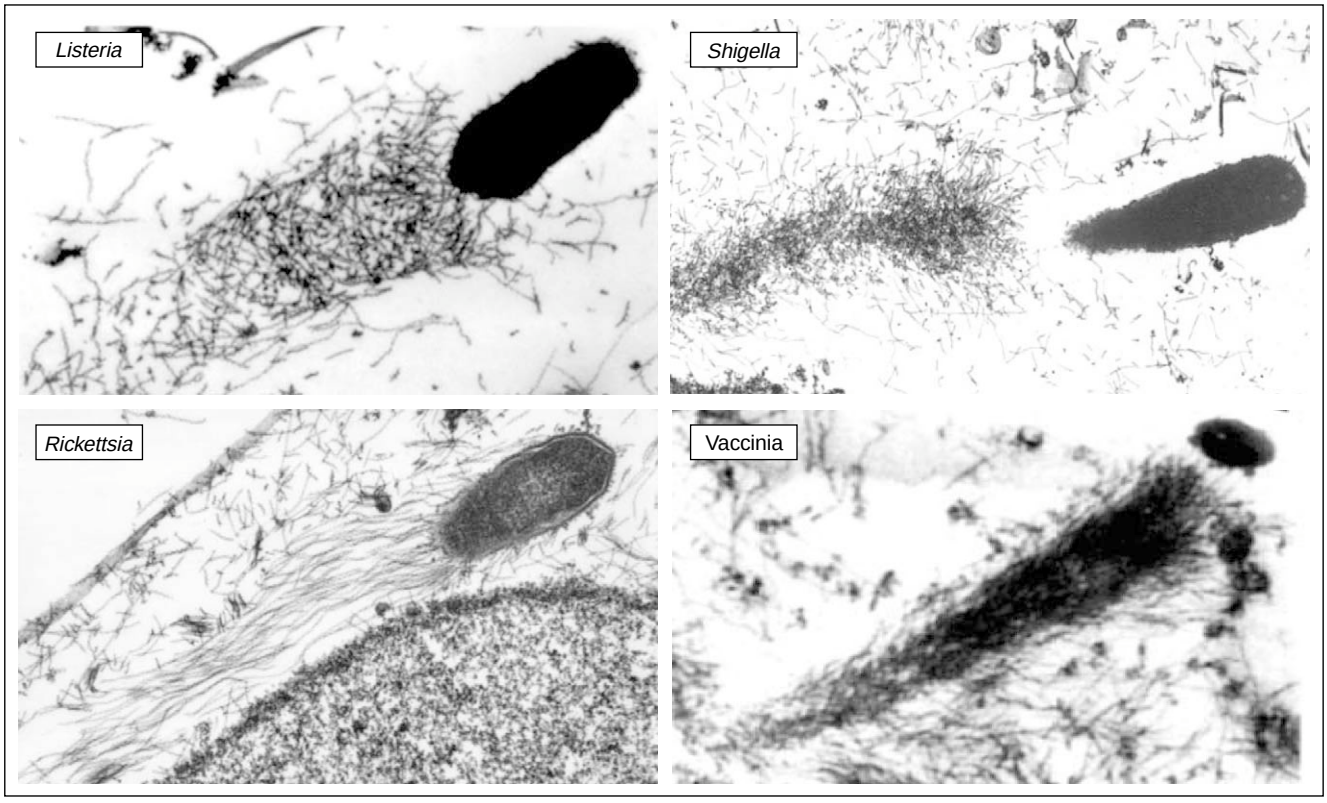
L. monocytogenes is responsible for foodborne infections, during which bacteria spread from the intestinal lumen to the liver and spleen, and ultimately, via a hematogenous route, to the brain and the placenta. Symptoms include gastroenteritis, encephalitis and abortion. A related pathogen, *L. ivanovii*, primarily infects animals. Once ingested, bacteria are internalized into host cells [2,18] and then rapidly escape from the internalization vacuole into the cytosol, where they polymerize actin on their surface and initiate actin-based motility. Moving bacteria form characteristic comet tails consisting of filaments oriented with their fast-growing barbed ends facing the bacterial surface [19,20] (Figure 2). The velocity of motility averages 10–15 μm/min, but can be as fast as 36 μm/min in some cell types [21,22]. Recently, the velocity of *L. monocytogenes* motility was also shown to vary as bacteria pass through regions of a single cell with different cytoarchitectures [23]. The mechanistic role of motility in cell-to-cell spread (Figure 1), originally proposed based on electron microscopy, has now been confirmed by real time observation in tissue culture cells [24].

Actin assembly at the *L. monocytogenes* surface is mediated by the ActA protein [25,26] (Figure 3). *L. ivanovii* has a related protein called IactA, whose expression can complement a *L. monocytogenes actA* mutant [27,28]. ActA is necessary for bacterial motility in tissue culture cells, and is critical for pathogenesis in the murine model of infection [29,30]. Moreover, ActA is sufficient for motility in the absence of other bacterial factors, as demonstrated by the observation that ActA-coated latex beads polymerize actin and undergo actin-based motility in cell extracts [31]. The protein is secreted and is tethered to the bacterial surface by a transmembrane domain, where it displays a polarized distribution, with maximal expression at the pole from which the comet tail forms [32].

At the molecular level, a central function of ActA is to act as an NPF for the Arp2/3 complex [33,34] (Figure 4). The N-terminal portion of the protein is sufficient for this activity [34], and is also necessary for actin polymerization and actin-based motility in infected cells [35,36]. Interestingly, the N-terminal region of ActA contains consensus elements present in eukaryotic WASP family NPFs, including an actin monomer-binding region as well as Arp2/3 binding C and A regions. A series of mutagenesis studies have demonstrated that activation of the Arp2/3 complex by these elements, and in particular the C region, plays an essential role in actin polymerization and a critical role in pathogenesis [36–40]. One key difference between ActA and host NPFs is that ActA lacks elements that bind to regulatory proteins such as Rho family GTPases, which is likely to be advantageous for pathogenesis because it allows for constitutive actin nucleation activity that is independent of host regulation.

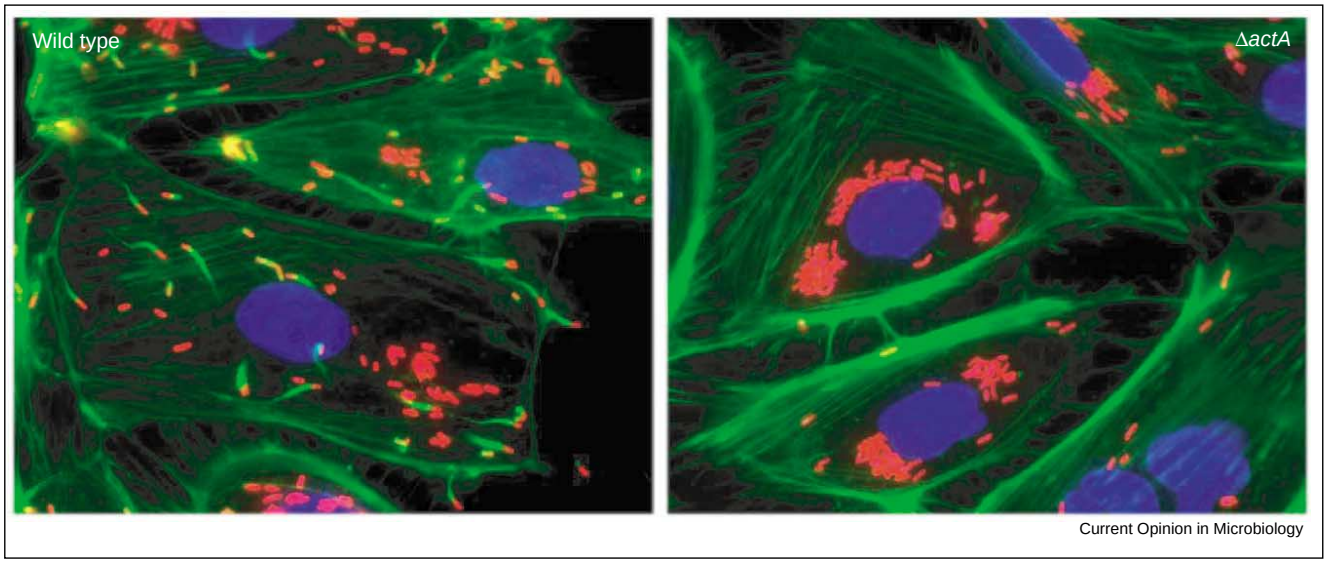
Under normal circumstances, the N-terminus of ActA appears to continuously activate the Arp2/3 complex during *L. monocytogenes* motility, as the complex is located throughout comet tails [33], and filaments throughout tails are organized in a Y-branched arrangement with the characteristic 70° branch angle [41,42]. However, a recent report has demonstrated that, at least *in vitro*, if Arp2/3 activity is inhibited after the initial nucleation event, *L. monocytogenes* motility can continue by Arp2/3-independent filament elongation [43••]. The elongation phase involves the formation of tails consisting of long, parallel filaments, and requires the presence of an actin-bundling protein such as fascin as well as an unknown factor that binds to the bacterial surface. Interestingly, a similar (but not identical) filament architecture is observed in those comet tails formed within membrane protrusions resulting from the collision of a bacterium with the plasma membrane [44]. This suggests that bacteria may naturally transition between phases of motility that are more or less dependent on Arp2/3-mediated nucleation.

Figure 2



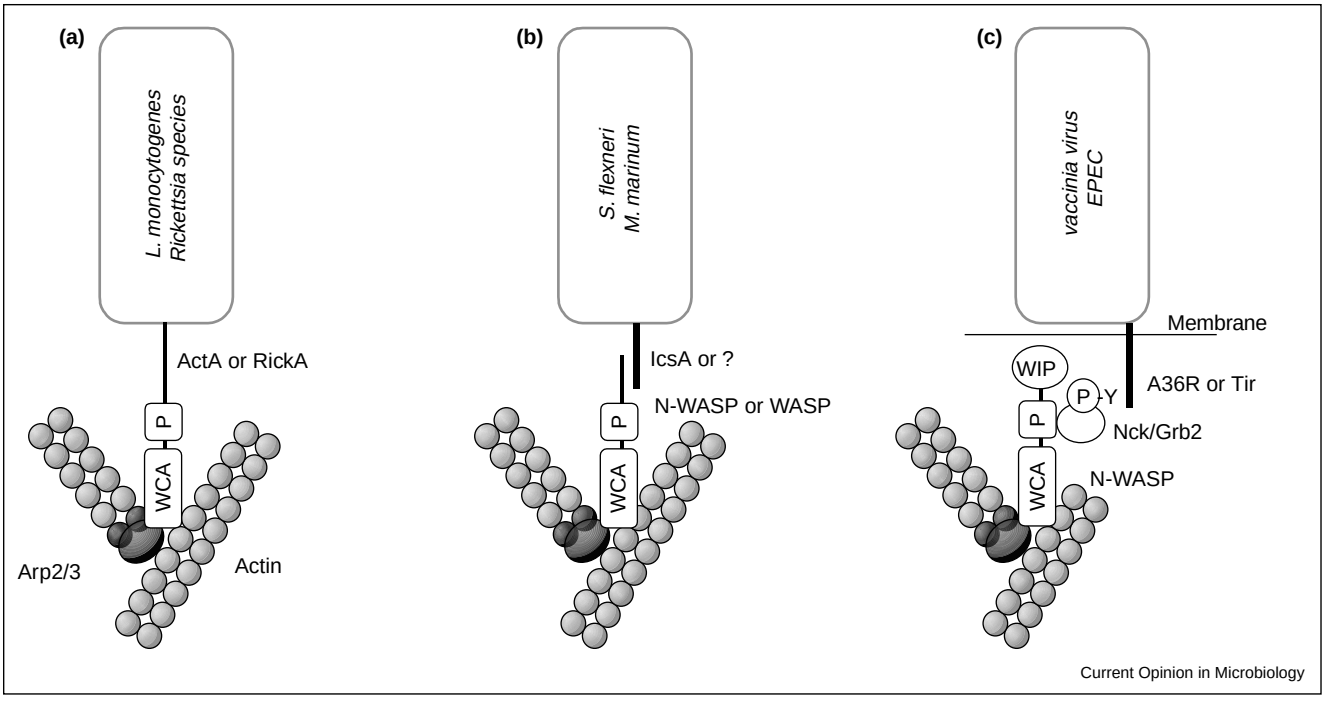
Electron microscopy of actin tails analyzed after myosin S1 decoration. For *Shigella, Listeria* and *Rickettsia*, see [41]; reprinted with permission. Copyright 1999 The company of biologists Ltd. For vaccinia see [85]; reprinted with permission, Copyright 1995 Nature publishing group. Strikingly, *Rickettsia* tails are made of long and unbranched filaments. It is not the case for *Listeria*, *Shigella* tails and vaccinia pedestals which display the characteristic Y branched structures.

Figure 3



PtK2 cells infected by *Listeria* wild type (left)or the *actA* deletion mutant (right panel). Infected cells were fixed and labeled with DAPI (blue) to label DNA in the nucleus and in bacteria, FITC phalloidin (green) to label actin and anti-*Listeria* antibodies (red) for fluorescence microscopy analysis. The *actA* mutant forms microcolonies inside cells.

Figure 4



Schematic representation of three types of Arp2/3 activation by various pathogens **(a)** *Listeria monocytogenes* and *Rickettsia* species, **(b)** *Shigella flexneri* and *Mycobacterium marinum* and **(c)** EPEC and vaccinia virus. The surface proteins ActA of *L. monocytogenes* and RickA of *Rickettsia* species, directly activate the Arp2/3 complex. IcsA of *Shigella* recruits N-WASP which then activates the Arp2/3 complex. *M. marinum* recruits WASP and by doing so probably activates the Arp2/3 complex. However, the mycobacterial protein(s) or component(s) involved are unknown. Vaccinia virus and EPEC trigger the recruitment of N-WASP and thus induce the activation of the Arp2/3 complex. Note that in these two cases the pathogen is separated from the actin cytoskeleton machinery by the plasma membrane.

In addition to the role of the N-terminal region and Arp2/3 in nucleation, the central proline-rich region of ActA plays an important role in promoting efficient motility [35]. These proline-rich sequences serve as binding sites for the host vasodilator-stimulated phosphoprotein (VASP) [45], whose mechanistic contribution to motility is just now beginning to be understood. One function of VASP is to bind and recruit profilin, an actin monomer-binding protein that itself promotes polymerization at barbed-ends [45]. Additionally, more recent work suggests that VASP promotes the release of Arp2/3 complex branch junctions from ActA [46•], which may accelerate motility by allowing for more rapid growth of the actin network at the bacterial surface. Moreover, VASP appears to influence the geometry of networks formed by the Arp2/3 complex. In particular, VASP reduces the frequency of Y-branch formation [47,48], and has recently been shown to increase the proportion of filaments organized in a parallel alignment in comet tails formed by beads *in vitro* [49•]. The interaction with VASP is important for pathogenesis, as ActA mutants that do not interact with VASP exhibit alterations in motility parameters such as directionality and persistence, and are defective in cell-to-cell spread and virulence in mice [50•].

Although mammals and mammalian tissue culture cells have thus far served as primary models for studying *L. monocytogenes* pathogenesis, recent work suggests that other model systems can also be employed. For example, *L. monocytogenes* has now been shown to infect *Drosophila melanogaster* [51•] and cell lines derived from fly [51•,52•]. Moreover, actin-based motility occurs in the cytosol of insect cells. This opens up the possibility that non-mammalian model systems may contribute to our understanding of *L. monocytogenes* motility and pathogenesis in the near future.

Shigella flexneri

Shigella flexneri is a Gram-negative, foodborne pathogen that invades the colonic mucosa, causing inflammation and diarrhea. The infection cycle strongly resembles that of *L. monocytogenes*. After internalization by phagocytosis, bacteria escape from the internalization vacuole and begin actin-based motility at rates of 10–15 μm/min [41,53] (Figure 1), similar to *L. monocytogenes*. Actin polymerization is mediated by a bacterial protein called IcsA (or VirG), which is both necessary for [54–56], and is the only bacterial protein that is sufficient for this process [53,57]. IcsA is localized to the outer membrane in a

polarized distribution, with the highest concentration at the pole from which actin polymerizes [58]. Although the mechanism of polarization is not well understood, it is thought to involve polar secretion [59]. IcsA, as the protein AIDA (adhesion involved in diffuse adherence) that shares similarity with IcsA, is a member of a family of autotransporters that mediate their own translocation across the outer membrane [60,61].

In contrast to ActA, which evolved to mimic WASP family NPFs, IcsA has evolved the ability to bind a host NPF called N-WASP and recruit it to the bacterial surface [62,63] (Figure 4). Host N-WASP co-localizes with IcsA at the bacterial pole from which actin polymerizes, and it is essential for *S. flexneri* motility in cell extracts [62] and in infected cells [64,65]. Binding of N-WASP to IcsA occurs via N-terminal autoregulatory regions within N-WASP [65]. These autoregulatory regions can bind to the C-terminal WCA element of N-WASP when it is present in the autoinhibited conformation, preventing Arp2/3 complex activation. Activation of N-WASP in host cells normally occurs upon binding of the Rho family GTPase Cdc42 together with other factors, which causes a conformational change that exposes the WCA region, leading to Arp2/3 activation [66,67]. IcsA appears to function like Cdc42 in that it promotes the conformational change that activates N-WASP and subsequently the Arp2/3 complex. Thus, rather than mimicking the host NPF molecule, as ActA, IcsA appears to mimic an activator of a host NPF.

In addition to host N-WASP, host Arp2/3 complex is also essential for bacterial motility in cell extracts [63], and it is present throughout actin tails [41]. At the ultrastructural level, *S. flexneri* tails appear morphologically similar to those formed by *L. monocytogenes* [41]. Despite the common use of the Arp2/3 complex for actin nucleation by *S. flexneri* and *L. monocytogenes*, there are interesting differences between these bacteria with regard to the function of other actin binding proteins. For example, it has recently been observed that VASP and the related Mena and Evl proteins play no apparent functional role in *S. flexneri* motility, as bacteria move at normal velocity in cells deficient in these proteins [68•]. Nevertheless, VASP does localize to the bacterial surface, and is found in actin tails [41,68•]. Additionally, the focal adhesion protein vinculin has been postulated to play a role in *S. flexneri*, but not *L. monocytogenes* motility, although this is controversial [4]. Thus, although *S. flexneri* uses an Arp2/3-based nucleation mechanism, it has evolved a distinct mechanism for activating the Arp2/3 complex, and it utilizes a distinct but overlapping set of actin binding proteins during motility.

Rickettsia species

Rickettsia species are obligate intracellular, Gram-negative bacteria that fall into two groups, the spotted fever group and the typhus group. Members of the spotted fever group, including *R. conorii*, the agent of the Mediterranean spotted fever, and *R. rickettsii*, the agent of Rocky Mountain spotted fever, exhibit actin-based motility in the cytosol of host cells. The velocity of motility is about 5–8 μm/min [41,69–71], which is half the rate of *L. monocytogenes* and *S. flexneri*. Of the typus group *Rickettsia*, only *R. typhi* has been observed to exhibit erratic actin-based motility, and to form actin tails that are shorter than those of *R. conorii* or *R. rickettsii* [72]. Interestingly, *Rickettsia* can also grow within the nucleus of infected cells, and actin-based bacterial motility has been observed in the nuclear compartment [69].

Despite superficial similarities with other pathogens, *Rickettsia* exhibit some striking differences from *L. monocytogenes* and *S. flexneri*, the most pronounced of which relates to the organization of actin filaments in their comet tails [41] (Figure 2). At the resolution of the light microscope, *R. rickettsii* tails comprise two or more distinct bundles that wrap around each other in a helical fashion [72] (this feature has not been observed with *R. conorii*). At the ultrastructural level, *Rickettsia* tails comprise long filaments arranged in nearly parallel arrays [41,72], distinct from the branched organization seen in tails formed by *L. monocytogenes*. Consistent with the long and unbranched filament architecture, Arp2/3 complex is not detected along the length of *Rickettsia* tails by immunofluorescence microscopy [41,72,73]. In addition, overexpression of the WCA fragment of N-WASP, which interferes with Arp2/3 complex function and *S. flexneri* motility in cells, has only a modest effect on *R. rickettsii* motility [73]. It was, however, not the case for *R. conorii*, for which motility was impaired in cells expressing the WCA fragment of Scar, a WASP-family protein [74••]. These observations led to the speculation that *Rickettsia* motility may not involve the activity of the Arp2/3 complex.

Despite these observations, recent work suggests that *Rickettsia* do use the Arp2/3 complex for actin-based motility. The first clue implicating Arp2/3 complex came from genome sequence information, which revealed the existence of a gene in *R. conorii* that encodes a protein with sequence similarity to the human WASP family of NPFs [75]. This gene and its protein product, called RickA, are present in several spotted fever group *Rickettsia* species, but are absent from the genomes of typhus group species such as *R. prowazekii* and *R. typhi* [76,77••]. RickA is expressed on the bacterial surface [74••], but it lacks a signal sequence, and the mode of secretion is unknown. Importantly, RickA behaves like *L. monocytogenes* ActA and other NPF in its ability to activate the Arp2/3 complex *in vitro* [74••,77••] (Figure 4). Moreover, overexpression of the RickA WCA domain inhibits actin-based bacterial motility in infected cells [74••], suggesting that RickA activation of the Arp2/3 complex is critical for this process. Finally, the Arp2/3 complex was

detectable around intracellular bacteria before tails formation [74••].

Several interesting questions about the mechanism of *Rickettsia* motility remain to be addressed. For example, why is the Arp2/3 complex absent from *Rickettsia* tails, and how is the parallel architecture of filaments generated? One possibility is that RickA and Arp2/3 are used only transiently to initiate actin polymerization bacterial surface. Once polymerization is initiated, the Arp2/3 complex may not be necessary for actin filament elongation and sustained motility. This idea is in agreement with the recent observation that the initiation of *L. monocytogenes* motility *in vitro* requires the Arp2/3 complex, but that sustained motility can be driven by Arp2/3-independent filament elongation [43••]. Understanding how *Rickettsia* may transition from Arp2/3-dependent to Arp2/3-independent actin polymerization, and why they may have evolved this strategy, are questions for future investigation.

Mycobacterium marinum

Mycobacteria are responsible for human diseases including tuberculosis, a respiratory infection caused by *M. tuberculosis*, and leprosy, a peripheral nerve infection caused by *M. leprae*. The related species *M. marinum* causes a systemic tuberculosis-like disease in animals such as fishes and frogs, and a localized disease in humans. Like *M. tuberculosis*, *M. marinum* grows and replicates in macrophages. Persistent macrophage infection results in the aggregation of infected cells and can lead to granuloma formation. Until recently, it was thought that pathogenic mycobacteria did not escape from the phagosome into the cytosol of macrophages. However, a recent report demonstrates that a fraction of *M. marinum* can in fact escape from the phagosome [78••]. Intriguingly, once in the cytosol, bacteria are propelled using actin-based motility at rates of about 11 μm/min [78••]. This striking observation suggests a role for actin-based motility in cell-to-cell spread of *M. marinum*.

The mechanism of actin polymerization by *M. marinum* appears to be most similar to *S. flexneri*. *M. marinum* appears to initiate actin polymerization by recruiting WASP, a host NPF that is specifically expressed in macrophages and other blood cells [78••]. WASP is concentrated at the bacterial pole from which actin polymerizes, suggesting a role in actin nucleation. Similarly, the Arp2/3 complex is localized throughout actin tails, as is the actin-binding protein VASP. Although these observations strongly suggest that *M. marinum* uses WASP and the Arp2/3 complex to nucleate actin assembly during motility, this hypothesis has not yet been directly tested. Nevertheless, it appears that *M. marinum* and *S. flexneri* have evolved the same strategy for promoting actin nucleation by recruiting a host NPF to the bacterial surface (Figure 4).

Because there is only a single report documenting *M. marinum* actin-based motility, several questions remain to be addressed. For example, which *M. marinum* gene(s) and protein(s) are involved in the recruitment of WASP? Do other mycobacterial species exhibit actin-based motility? What is the functional importance of actin-based motility during infection? Future work that addresses these questions will expand on this intriguing new development in the mycobacterium field.

Burkholderia pseudomallei

B. pseudomallei is a Gram-negative bacterium that causes melioidosis, a disease that affects humans and animals in tropical areas. Infection by environmental organisms generally occurs via cutaneous inoculation. The clinical manifestations are diverse and range from localized subacute infections to pneumonia and sepsis. It has been known for some time that *B. pseudomallei* is internalized into host cells, where it escapes from the phagosome and grows in the cytosol. However, it was only recently reported that *B. pseudomallei* can induce actin polymerization and form actin tails [79], tell-tale signs of actin-based motility. Bacteria also form actin-rich membrane protrusions, and can spread from cell to cell in tissue culture models of infection.

The molecular mechanism of actin polymerization by *B. pseudomallei* is largely unknown. It was recently shown that the Arp2/3 complex is localized throughout actin tails formed by this bacterium, suggesting a role for the complex in actin-based motility [80••]. However, a functional role for the Arp2/3 complex is uncertain, as overexpression of an Arp2/3 binding WCA fragment of Scar, which inhibits *Listeria*, *Shigella* and *R. conorii* motility, does not block *B. pseudomallei* tail formation. Moreover, it is not clear whether *B. pseudomallei* expresses its own NPF for the Arp2/3 complex (like *L. monocytogenes* and *Rickettsia*), recruits a host NPF to its surface (like *S. flexneri* and *M. marinum*), or uses some other mechanism for actin polymerization. Bacteria do not utilize the host NPF N-WASP, as actin tails form normally in N-WASP$^{-/-}$ cells. Similarly, tails form normally in cells deficient in VASP/Ena/Mena, indicating that these proteins are not essential for actin polymerization. Future work will reveal whether *B. pseudomallei* has evolved a strategy for polymerizing actin that is similar to other pathogens, or whether it uses a so far undescribed mechanism of actin-based motility.

Vaccinia virus

Vaccinia virus is a relative of the smallpox virus, and is used as the vaccine for smallpox. After entry into cells, replication and assembly of intracellular mature virus (IMV) particles occur in cytoplasmic virus factories. A subset of IMVs are then transported along microtubules towards the centrosome, where they are wrapped by a double membrane to generate the intracellular enveloped virus (IEV). Although it was originally thought that IEVs

exhibited intracellular actin-based motility [81], it has subsequently been shown that IEVs are transported to the plasma membrane along microtubules via the activity of kinesin motor proteins [82]. Upon fusion of IEVs with the plasma membrane, an infectious cell associated enveloped virus (CEV) remains associated with the extracellular surface of the host cell. At this stage, actin polymerization is initiated beneath the CEV particle, resulting in the formation of actin tails that support membrane protrusions with CEVs at their tips [83]. This form of actin-based motility has been proposed to facilitate cell-to-cell spread during infection [81,82] (Figure 2).

Vaccinia virus has evolved a strategy for inducing actin polymerization that is mediated by Src tyrosine kinase signaling. A viral protein called B5R, which is associated with the surface of the CEV, interacts with an unknown host-cell protein to promote Src activation and subsequent tyrosine phosphorylation of the viral A36R protein [84••]. Phosphorylated A36R in turn recruits the adaptor proteins Nck and Grb2, which bind to and activate a complex of WIP (WASP interacting protein) and the NPF N-WASP [85–87] (Figure 4). Actin polymerization is presumed to involve the Arp2/3 complex, and inhibition of Arp2/3 activity by expression of the WCA fragment of the NPF N-WASP was recently shown to inhibit the viral infection cycle [88]. Interestingly, A36R also binds directly to the kinesin light chain [89], and is critical for microtubule-based movement of IEVs [83]. Moreover, it was recently observed that tyrosine phosphorylation of A36R by Src can act as a molecular switch between microtubule and actin-based motility, as Src mediates dissociation of the virus from kinesin and association with the actin polymerization machinery [84••]. The mechanism used by vaccinia virus to trigger actin polymerization and protrusion formation shares many similarities with pedestal formation by enteropathogenic *Escherichia coli* (EPEC) [90]. In the case of EPEC, tyrosine phosphorylation of a type III secreted protein called Tir, which is inserted in the mammalian cell membrane causes recruitment of Nck and N-WASP to induce Arp2/3-mediated actin polymerization and pedestal formation [91]. Interestingly, *S. flexneri* exploits also the Src signaling pathway to induce actin polymerization at the plasma membrane during infection [92].

Conclusions

Our knowledge of actin-based motility has in 15 years reached a degree of in-depth knowledge that was totally unsuspected when bacterial motility was first reported. What are the remaining black boxes? One first question is how long actin filaments are generated during *Rickettsia* movement? Is there another factor involved or has RickA other activities? Another issue concerns the switch between the formation of branched filaments and the generation of long filaments observed upon *Listeria* or *Shigella*-induced protrusion formation. Another critical question is how Arp2/3 switches from an inactive form to an active form. One may hope that the X-ray structure of a co-crystal of Arp2/3 with one of its activators, a bacterial one or another will shed light on an event which is at the heart of so many critical events for the cell and for pathogenic bacteria.

Acknowledgements

Work on actin-based motility in P Cossart's laboratory received financial support from Institut Pasteur, EU, ARC and DGA, and in M Welch's laboratory from NIH (Grant number GM59609). P Cossart is an HHMI international investigator.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cossart P: **Actin-based motility of pathogens: the Arp2/3 complex is a central player**. *Cell Microbiol* 2000, **2**:195-205.

2. Cossart P, Sansonetti PJ: **Bacterial invasion: the paradigms of enteroinvasive pathogens**. *Science* 2004, **304**:242-248.

3. Frischknecht F, Way M: **Surfing pathogens and the lessons learned for actin polymerization**. *Trends Cell Biol* 2001, **11**:30-38.

4. Goldberg MB: **Actin-based motility of intracellular microbial pathogens**. *Microbiol Mol Biol Rev* 2001, **65**:595-626.

5. Pollard TD, Borisy GG: **Cellular motility driven by assembly and disassembly of actin filaments**. *Cell* 2003, **112**:453-465.

6. Engqvist-Goldstein AE, Drubin DG: **Actin assembly and endocytosis: from yeast to mammals**. *Annu Rev Cell Dev Biol* 2003, **19**:287-332.

7. Weaver AM, Young ME, Lee WL, Cooper JA: **Integration of signals to the Arp2/3 complex**. *Curr Opin Cell Biol* 2003, **15**:23-30.

8. Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S, Pollard TD: **Crystal structure of Arp2/3 complex**. *Science* 2001, **294**:1679-1684.

9. Welch MD, Mullins RD: **Cellular control of actin nucleation**. *Annu Rev Cell Dev Biol* 2002, **18**:247-288.

10. •• Goley ED, Rodenbusch SE, Martin AC, Welch MD: **Critical conformational changes in the Arp2/3 complex are induced by nucleotide and nucleation promoting factor**. *Mol Cell* 2004, **16**:269-279.
The authors have used FRET(fluorescence resonance energy transfer) to probe the conformational dynamics of the Arp2/3 complex upon activation. They show that nucleotide binding promotes a substantial conformational change in the complex. They demonstrate that nucleotide binding to each Arp is critical for activity and is coupled to nucleation promoting factor (NPF) binding. The binding of Wiskott-Aldrich syndrome protein (WASP) family NPFs induces further conformational reorganization of the Arp2/3 complex and the ability to promote this conformational reorganization correlates with activation efficiency.

11. Svitkina TM, Bulanova EA, Chaga OY, Vignjevic DM, Kojima S, Vasiliev JM, Borisy GG: **Mechanism of filopodia initiation by reorganization of a dendritic network**. *J Cell Biol* 2003, **160**:409-421.

12. Svitkina TM, Borisy GG: **Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia**. *J Cell Biol* 1999, **145**:1009-1026.

13. Zigmond SH: **Formin-induced nucleation of actin filaments**. *Curr Opin Cell Biol* 2004, **16**:99-105.

14. Mogilner A, Oster G: **Polymer motors: pushing out the front and pulling up the back**. *Curr Biol* 2003, **13**:R721-R733.

15. Wear MA, Cooper JA: **Capping protein: new insights into mechanism and regulation**. *Trends Biochem Sci* 2004, **29**:418-428.

16. Maciver SK, Hussey PJ: **The ADF/cofilin family: actin-remodeling proteins**. *Genome Biol* 2002, **3**:reviews3007.1-3007.12.

17. Loisel TP, Boujemaa R, Pantaloni D, Carlier MF: **Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins**. *Nature* 1999, **401**:613-616.

18. Cossart P, Pizarro-Cerda J, Lecuit M: **Invasion of mammalian cells by *Listeria monocytogenes*: functional mimicry to subvert cellular functions**. *Trends Cell Biol* 2003, **13**:23-31.

19. Tilney LG, DeRosier DJ, Weber A, Tilney MS: **How *Listeria* exploits host cell actin to form its own cytoskeleton. II. Nucleation, actin filament polarity, filament assembly, and evidence for a pointed end capper**. *J Cell Biol* 1992, **118**:83-93.

20. Tilney LG, Portnoy DA: **Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes***. *J Cell Biol* 1989, **109**:1597-1608.

21. Dabiri GA, Sanger JM, Portnoy DA, Southwick FS: ***Listeria monocytogenes* moves rapidly through the host-cell cytoplasm by inducing directional actin assembly**. *Proc Natl Acad Sci USA* 1990, **87**:6068-6072.

22. Sanger JM, Dabiri G, Mittal B, Kowalski MA, Haddad JG, Sanger JW: **Disruption of microfilament organization in living nonmuscle cells by microinjection of plasma vitamin D-binding protein or DNase I**. *Proc Natl Acad Sci USA* 1990, **87**:5474-5478.

23. Lacayo CI, Theriot JA: ***Listeria monocytogenes* actin-based motility varies depending on subcellular location: a kinematic probe for cytoarchitecture**. *Mol Biol Cell* 2004, **15**:2164-2175.

24. Robbins JR, Barth AI, Marquis H, de Hostos EL, Nelson WJ, Theriot JA: ***Listeria monocytogenes* exploits normal host cell processes to spread from cell to cell**. *J Cell Biol* 1999, **146**:1333-1350.

25. Kocks C, Gouin E, Tabouret M, Berche P, Ohayon H, Cossart P: ***L. monocytogenes*-induced actin assembly requires the actA gene product, a surface protein**. *Cell* 1992, **68**:521-531.

26. Domann E, Wehland J, Rohde M, Pistor S, Hartl M, Goebel W, Leimeister-Wachter M, Wuenscher M, Chakraborty T: **A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin**. *EMBO J* 1992, **11**:1981-1990.

27. Karunasagar I, Krohne G, Goebel W: ***Listeria ivanovii* is capable of cell-to-cell spread involving actin polymerization**. *Infect Immun* 1993, **61**:162-169.

28. Gouin E, Dehoux P, Mengaud J, Kocks C, Cossart P: **iactA of *Listeria ivanovii*, although distantly related to *Listeria monocytogenes* actA, restores actin tail formation in an *L. monocytogenes* actA mutant**. *Infect Immun* 1995, **63**:2729-2737.

29. Goossens PL, Marchal G, Milon G: **Transfer of both protection and delayed-type hypersensitivity against live *Listeria* is mediated by the CD8+ T cell subset: a study with *Listeria*-specific T lymphocytes recovered from murine infected liver**. *Int Immunol* 1992, **4**:591-598.

30. Brundage RA, Smith GA, Camilli A, Theriot JA, Portnoy DA: **Expression and phosphorylation of the *Listeria monocytogenes* ActA protein in mammalian cells**. *Proc Natl Acad Sci USA* 1993, **90**:11890-11894.

31. Cameron LA, Footer MJ, van Oudenaarden A, Theriot JA: **Motility of ActA protein-coated microspheres driven by actin polymerization**. *Proc Natl Acad Sci USA* 1999, **96**:4908-4913.

32. Kocks C, Hellio R, Gounon P, Ohayon H, Cossart P: **Polarized distribution of *Listeria monocytogenes* surface protein ActA at the site of directional actin assembly**. *J Cell Sci* 1993, **105**:699-710.

33. Welch MD, Iwamatsu A, Mitchison TJ: **Actin polymerization is induced by Arp2/3 protein complex at the surface of *Listeria monocytogenes***. *Nature* 1997, **385**:265-269.

34. Welch MD, Rosenblatt J, Skoble J, Portnoy DA, Mitchison TJ: **Interaction of human Arp2/3 complex and the *Listeria monocytogenes* ActA protein in actin filament nucleation**. *Science* 1998, **281**:105-108.

35. Lasa I, David V, Gouin E, Marchand JB, Cossart P: **The amino-terminal part of ActA is critical for the actin-based motility of *Listeria monocytogenes*; the central proline-rich region acts as a stimulator**. *Mol Microbiol* 1995, **18**:425-436.

36. Lasa I, Gouin E, Goethals M, Vancompernolle K, David V, Vandekerckhove J, Cossart P: **Identification of two regions in the N-terminal domain of ActA involved in the actin comet tail formation by *Listeria monocytogenes***. *Embo J* 1997, **16**:1531-1540.

37. Skoble J, Portnoy DA, Welch MD: **Three regions within ActA promote Arp2/3 complex-mediated actin nucleation and *Listeria monocytogenes* motility**. *J Cell Biol* 2000, **150**:527-538.

38. Pistor S, Grobe L, Sechi AS, Domann E, Gerstel B, Machesky LM, Chakraborty T, Wehland J: **Mutations of arginine residues within the 146-KKRRK-150 motif of the ActA protein of *Listeria monocytogenes* abolish intracellular motility by interfering with the recruitment of the Arp2/3 complex**. *J Cell Sci* 2000, **113**:3277-3287.

39. Lauer P, Theriot JA, Skoble J, Welch MD, Portnoy DA: **Systematic mutational analysis of the amino-terminal domain of the *Listeria monocytogenes* ActA protein reveals novel functions in actin-based motility**. *Mol Microbiol* 2001, **42**:1163-1177.

40. Boujemaa-Paterski R, Gouin E, Hansen G, Samarin S, Le Clainche C, Didry D, Dehoux P, Cossart P, Kocks C, Carlier MF *et al.*: ***Listeria* protein ActA mimics WASp family proteins: it activates filament barbed end branching by Arp2/3 complex**. *Biochemistry* 2001, **40**:11390-11404.

41. Gouin E, Gantelet H, Egile C, Lasa I, Ohayon H, Villiers V, Gounon P, Sansonetti PJ, Cossart P: **A comparative study of the actin-based motilities of the pathogenic bacteria *Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia conorii***. *J Cell Sci* 1999, **112**:1697-1708.

42. Cameron LA, Svitkina TM, Vignjevic D, Theriot JA, Borisy GG: **Dendritic organization of actin comet tails**. *Curr Biol* 2001, **11**:130-135.

43. •• Brieher WM, Coughlin M, Mitchison TJ: **Fascin-mediated propulsion of *Listeria monocytogenes* independent of frequent nucleation by the Arp2/3 complex**. *J Cell Biol* 2004, **165**:233-242.

Using successive steps of bacterial incubations in cell extracts with or without the Arp2/3 complex, it was shown that *Listeria monocytogenes* motility can be separated into an Arp2/3 dependent nucleation phase and an Arp2/3 independent elongation phase. The nucleation reaction assembles a comet tail of branched actin filaments directly behind the bacterium. The elongation-based reaction generates a hollow cylinder of parallel bundles that attach along the sides of the bacterium and requires at least a bundling protein such as fascin.

44. Sechi AS, Wehland J, Small JV: **The isolated comet tail pseudopodium of *Listeria monocytogenes*: a tail of two actin filament populations, long and axial and short and random**. *J Cell Biol* 1997, **137**:155-167.

45. Geese M, Loureiro JJ, Bear JE, Wehland J, Gertler FB, Sechi AS: **Contribution of Ena/VASP proteins to intracellular motility of *Listeria* requires phosphorylation and proline-rich core but not F-actin binding or multimerization**. *Mol Biol Cell* 2002, **13**:2382-2396.

46. • Samarin S, Romero S, Kocks C, Didry D, Pantaloni D, Carlier MF: **How VASP enhances actin-based motility**. *J Cell Biol* 2003, **163**:131-142.

The authors propose an interesting role for VASP which is not to compete with capping proteins at barbed ends. VASP would increase the rate of dissociation of the branch junction in Arp2/3 complex mediated actin polymerization.

47. Skoble J, Auerbuch V, Goley ED, Welch MD, Portnoy DA: **Pivotal role of VASP in Arp2/3 complex-mediated actin nucleation,**

actin branch-formation, and *Listeria monocytogenes* motility. *J Cell Biol* 2001, **155**:89-100.

48. Bear JE, Svitkina TM, Krause M, Schafer DA, Loureiro JJ, Strasser GA, Maly IV, Chaga OY, Cooper JA, Borisy GG *et al.*: **Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility**. *Cell* 2002, **109**:509-521.

49. • Plastino J, Olivier S, Sykes C: **Actin filaments align into hollow comets for rapid VASP-mediated propulsion**. *Curr Biol* 2004, **14**:1766-1771.
Using beads coated with VCA of WASP and the proline-rich region of ActA, the authors analyze the interconnected roles of the Arp2/3 complex and VASP. VASP would enhance speed and hollowness of the tail. The authors also provide evidence that a highly branched network is not necessarily associated with optimized movement, explaining why lamellipodia extend more slowly than filopodia.

50. • Auerbuch V, Loureiro JJ, Gertler FB, Theriot JA, Portnoy DA: **Ena/VASP proteins contribute to *Listeria monocytogenes* pathogenesis by controlling temporal and spatial persistence of bacterial actin-based motility**. *Mol Microbiol* 2003, **49**:1361-1375.
The authors generated a very large series of *Listeria* mutants affected in VASP binding sites and tested them in cell lines expressing different Ena/VASP derivatives thereby succeeding to detect in VASP, regions affecting either speed or directionality.

51. • Mansfield BE, Dionne MS, Schneider DS, Freitag NE: **Exploration of host–pathogen interactions using *Listeria monocytogenes* and *Drosophila melanogaster***. *Cell Microbiol* 2003, **5**:901-911.
This is the first paper describing the use of *Drosophila* as an animal model for evaluating *Listeria* virulence.

52. • Cheng LW, Portnoy DA: ***Drosophila* S2 cells: an alternative infection model for *Listeria monocytogenes***. *Cell Microbiol* 2003, **5**:875-885.
The authors show that drosophila S2 cells can be successfully infected by *Listeria* and allow intracellular actin-based motility paving the way to the use of RNAi in the study of actin-based motility. It is important to note that these cells are grown at lower temperatures than mammalian cells and thus cannot be used for infections with bacteria which requires an elevated temperature like *Shigella* and also that these cells have some phagocytic properties precluding studies on invasion.

53. Goldberg MB, Theriot JA: ***Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility**. *Proc Natl Acad Sci USA* 1995, **92**:6572-6576.

54. Makino S, Sasakawa C, Kamata K, Kurata T, Yoshikawa M: **A genetic determinant required for continuous reinfection of adjacent cells on large plasmid in *S. flexneri* 2a**. *Cell* 1986, **46**:551-555.

55. Lett MC, Sasakawa C, Okada N, Sakai T, Makino S, Yamada M, Komatsu K, Yoshikawa M: **virG, a plasmid-coded virulence gene of *Shigella flexneri*: identification of the virG protein and determination of the complete coding sequence**. *J Bacteriol* 1989, **171**:353-359.

56. Bernardini ML, Mounier J, d'Hauteville H, Coquis-Rondon M, Sansonetti PJ: **Identification of icsA, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin**. *Proc Natl Acad Sci USA* 1989, **86**:3867-3871.

57. Kocks C, Marchand JB, Gouin E, d'Hauteville H, Sansonetti PJ, Carlier MF, Cossart P: **The unrelated surface proteins ActA of *Listeria monocytogenes* and IcsA of *Shigella flexneri* are sufficient to confer actin-based motility on *Listeria innocua* and *Escherichia coli* respectively**. *Mol Microbiol* 1995, **18**:413-423.

58. Goldberg MB, Barzu O, Parsot C, Sansonetti PJ: **Unipolar localization and ATPase activity of IcsA, a *Shigella flexneri* protein involved in intracellular movement**. *J Bacteriol* 1993, **175**:2189-2196.

59. Robbins JR, Monack D, McCallum SJ, Vegas A, Pham E, Goldberg MB, Theriot JA: **The making of a gradient: IcsA (VirG) polarity in *Shigella flexneri***. *Mol Microbiol* 2001, **41**:861-872.

60. Benz I, Schmidt MA: **AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic *Escherichia coli* strain 2787 (O126:H27), is synthesized via a precursor molecule**. *Mol Microbiol* 1992, **6**:1539-1546.

61. Brandon LD, Goehring N, Janakiraman A, Yan AW, Wu T, Beckwith J, Goldberg MB: **IcsA, a polarly localized autotransporter with an atypical signal peptide, uses the Sec apparatus for secretion, although the Sec apparatus is circumferentially distributed**. *Mol Microbiol* 2003, **50**:45-60.

62. Suzuki T, Miki H, Takenawa T, Sasakawa C: **Neural Wiskott–Aldrich syndrome protein is implicated in the actin-based motility of *Shigella flexneri***. *EMBO J* 1998, **17**:2767-2776.

63. Egile C, Loisel TP, Laurent V, Li R, Pantaloni D, Sansonetti PJ, Carlier MF: **Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility**. *J Cell Biol* 1999, **146**:1319-1332.

64. Lommel S, Benesch S, Rottner K, Franz T, Wehland J, Kuhn R: **Actin pedestal formation by enteropathogenic *Escherichia coli* and intracellular motility of *Shigella flexneri* are abolished in N-WASP-defective cells**. *EMBO Rep* 2001, **2**:850-857.

65. Suzuki T, Mimuro H, Suetsugu S, Miki H, Takenawa T, Sasakawa C: **Neural Wiskott–Aldrich syndrome protein (N-WASP) is the specific ligand for *Shigella* VirG among the WASP family and determines the host cell type allowing actin-based spreading**. *Cell Microbiol* 2002, **4**:223-233.

66. Millard TH, Sharp SJ, Machesky LM: **Signalling to actin assembly via the WASP (Wiskott–Aldrich syndrome protein)-family proteins and the Arp2/3 complex**. *Biochem J* 2004, **380**:1-17.

67. Suzuki T, Mimuro H, Miki H, Takenawa T, Sasaki T, Nakanishi H, Takai Y, Sasakawa C: **Rho family GTPase Cdc42 is essential for the actin-based motility of *Shigella* in mammalian cells**. *J Exp Med* 2000, **191**:1905-1920.

68. • Ally S, Sauer NJ, Loureiro JJ, Snapper SB, Gertler FB, Goldberg MB: ***Shigella* interactions with the actin cytoskeleton in the absence of Ena/VASP family proteins**. *Cell Microbiol* 2004, **6**:355-366.
By using cells deficient in Ena/VASP family proteins, the authors seem to definitively establish that although VASP is recruited around the surface and all along the tails of intracellular bacteria, it is not essential for actin-based motility.

69. Heinzen RA, Grieshaber SS, Van Kirk LS, Devin CJ: **Dynamics of actin-based movement by *Rickettsia rickettsii* in vero cells**. *Infect Immun* 1999, **67**:4201-4207.

70. Heinzen RA, Hayes SF, Peacock MG, Hackstadt T: **Directional actin polymerization associated with spotted fever group *Rickettsia* infection of Vero cells**. *Infect Immun* 1993, **61**:1926-1935.

71. Teysseire N, Chiche-Portiche C, Raoult D: **Intracellular movements of *Rickettsia conorii* and *R. typhi* based on actin polymerization**. *Res Microbiol* 1992, **143**:821-829.

72. Van Kirk LS, Hayes SF, Heinzen RA: **Ultrastructure of *Rickettsia rickettsii* actin tails and localization of cytoskeletal proteins**. *Infect Immun* 2000, **68**:4706-4713.

73. Harlander RS, Way M, Ren Q, Howe D, Grieshaber SS, Heinzen RA: **Effects of ectopically expressed neuronal Wiskott–Aldrich syndrome protein domains on *Rickettsia rickettsii* actin-based motility**. *Infect Immun* 2003, **71**:1551-1556.

74. •• Gouin E, Egile C, Dehoux P, Villiers V, Adams J, Gertler F, Li R, Cossart P: **The RickA protein of *Rickettsia conorii* activates the Arp2/3 complex**. *Nature* 2004, **427**:457-461.
The authors demonstrate that RickA of *Rickettsia conorii* is expressed on the bacterial surface and when purified *in vitro* can activate the Arp2/3 complex. More importantly the authors show that the Arp2/3 complex is detectable on intracellular bacteria before actin tail formation, suggesting that the Arp2/3 complex is used transiently at the onset of actin tail formation, in agreement with the lack of detection of the Arp2/3 complex along the tail.

75. Ogata H, Audic S, Renesto-Audiffren P, Fournier PE, Barbe V, Samson D, Roux V, Cossart P, Weissenbach J, Claverie JM *et al.*:

Mechanisms of evolution in *Rickettsia conorii* and *R. prowazekii*. *Science* 2001, **293**:2093-2098.

76. McLeod MP, Qin X, Karpathy SE, Gioia J, Highlander SK, Fox GE, McNeill TZ, Jiang H, Muzny D, Jacob LS *et al.*: **Complete genome sequence of *Rickettsia typhi* and comparison with sequences of other rickettsiae**. *J Bacteriol* 2004, **186**:5842-5855.

77. •• Jeng RL, Goley ED, D'Alessio JA, Chaga OY, Svitkina TM, Borisy GG, Heinzen RA, Welch MD: **A *Rickettsia* WASP-like protein activates the Arp2/3 complex and mediates actin-based motility**. *Cell Microbiol* 2004, **6**:761-769.
The authors from [74••,77••] provide evidence that RickA, as ActA acts as a WASP family protein and activates the Arp2/3 complex. They also show that actin tails generated by RickA-coated beads comprise a Y-branched filament network suggesting that RickA or host factors reorganize the Y-branched networks into parallel arrays in a manner similar to a model proposed for filopodia formation.

78. •• Stamm LM, Morisaki JH, Gao LY, Jeng RL, McDonald KL, Roth R, Takeshita S, Heuser J, Welch MD, Brown EJ: ***Mycobacterium marinum* escapes from phagosomes and is propelled by actin-based motility**. *J Exp Med* 2003, **198**:1361-1368.
The authors have gone against the dogma! They convincingly show that in macrophages, *Mycobacterium marinum* can trigger the formation of actin tails and spread from cell to cell directly. They show that this process is probably due to the recruitment of WASP.

79. Kespichayawattana W, Rattanachetkul S, Wanun T, Utaisincharoen P, Sirisinha S: ***Burkholderia pseudomallei* induces cell fusion and actin-associated membrane protrusion: a possible mechanism for cell-to-cell spreading**. *Infect Immun* 2000, **68**:5377-5384.

80. •• Breitbach K, Rottner K, Klocke S, Rohde M, Jenzora A, Wehland J, Steinmetz I: **Actin-based motility of *Burkholderia pseudomallei* involves the Arp 2/3 complex, but not N-WASP and Ena/VASP proteins**. *Cell Microbiol* 2003, **5**:385-393.
This is a very complete study in which the authors have used a panoply of tools previously developed for the study of other motile bacteria to analyze the so far intriguing actin-based motility of *Burkholderia pseudomallei*. While the Arp2/3 complex is detectable in the actin tails, overexpression of an Arp2/3 binding fragment does not seem to affect motility.

81. Cudmore S, Cossart P, Griffiths G, Way M: **Actin-based motility of vaccinia virus**. *Nature* 1995, **378**:636-638.

82. Smith GL, Murphy BJ, Law M: **Vaccinia virus motility**. *Annu Rev Microbiol* 2003, **57**:323-342.

83. Rietdorf J, Ploubidou A, Reckmann I, Holmstrom A, Frischknecht F, Zettl M, Zimmermann T, Way M: **Kinesin-dependent movement on microtubules precedes actin-based motility of vaccinia virus**. *Nat Cell Biol* 2001, **3**:992-1000.

84. •• Newsome TP, Scaplehorn N, Way M: **SRC mediates a switch from microtubule- to actin-based motility of vaccinia virus**. *Science* 2004, **306**:124-129.
An in-depth study using various mutant cells, mutated virus and a variety of approaches including video microscopy, in which the authors show beautifully that localized recruitment and activation of Src play an important role in regulating the switch between intracellular microtubule transport and actin-based motility at the plasma membrane. Moreover, they show that a viral protein B5R is required to activate Src and induce A36R protein phosphorylation and the signaling cascade that leads to Arp2/3 activation.

85. Frischknecht F, Moreau V, Rottger S, Gonfloni S, Reckmann I, Superti-Furga G, Way M: **Actin-based motility of vaccinia virus mimics receptor tyrosine kinase signalling**. *Nature* 1999, **401**:926-929.

86. Moreau V, Frischknecht F, Reckmann I, Vincentelli R, Rabut G, Stewart D, Way M: **A complex of N-WASP and WIP integrates signalling cascades that lead to actin polymerization**. *Nat Cell Biol* 2000, **2**:441-448.

87. Scaplehorn N, Holmstrom A, Moreau V, Frischknecht F, Reckmann I, Way M: **Grb2 and Nck act cooperatively to promote actin-based motility of vaccinia virus**. *Curr Biol* 2002, **12**:740-745.

88. Komano J, Miyauchi K, Matsuda Z, Yamamoto N: **Inhibiting the arp2/3 complex limits infection of both intracellular mature vaccinia virus and primate lentiviruses**. *Mol Biol Cell* 2004, **15**:5197-5207.

89. Ward BM, Moss B: **Vaccinia virus A36R membrane protein provides a direct link between intracellular enveloped virions and the microtubule motor kinesin**. *J Virol* 2004, **78**:2486-2493.

90. Rosenshine I, Ruschkowski S, Stein M, Reinscheid DJ, Mills SD, Finlay BB: **A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation**. *EMBO J* 1996, **15**:2613-2624.

91. Campellone KG, Leong JM: **Tails of two Tirs: actin pedestal formation by enteropathogenic *E. coli* and enterohemorrhagic *E. coli* O157:H7**. *Curr Opin Microbiol* 2003, **6**:82-90.

92. Bougneres L, Girardin SE, Weed SA, Karginov AV, Olivo-Marin JC, Parsons JT, Sansonetti PJ, Van Nhieu GT: **Cortactin and Crk cooperate to trigger actin polymerization during *Shigella* invasion of epithelial cells**. *J Cell Biol* 2004, **166**:225-235.