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Actin-based motility and cell-to-cell spread of bacterial pathogens

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Abstract

Subversion of the host actin cytoskeleton is a critical virulence mechanism used by a variety of intracellular bacterial pathogens during their infectious life cycles. These pathogens manipulate host actin to promote actin-based motility and coordinate motility with cell-to-cell spread. Growing evidence suggests that the tactics employed by pathogens are surprisingly diverse. Here, we review recent advances suggesting that bacterial surface proteins exhibit divergent biochemical mechanisms of actin polymerization and recruit distinct host protein networks to drive motility, and that bacteria deploy secreted effector proteins that alter host cell mechanotransduction pathways to enable spread. Further investigation into the divergent strategies used by bacterial pathogens to mobilize actin will reveal new insights into pathogenesis and cytoskeleton regulation.

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Annotated papers of interest:

*Law 2012: In this study, nexillin was found to regulate invasion and actin-based motility of *L. monocytogenes*, as well as host cell attachment of extracellular enteropathogenic *E. coli*.

*Madasu 2013: This study provided the first atomic-resolution structure of SFG rickettsiae Sca2 and revealed an unusual mechanism of mimicry of host formin proteins.

**Sarovich 2014: This study examined 24 years of human cases of Australian melioidosis and discovered that the disease type and severity were strongly correlated with a specific BimA variant, suggesting that BimA orthologs could differentially regulate *Burkholderia* spp. virulence.

**Fattouh 2015: In this study, the authors discovered that several host formins are specifically localized to *L. monocytogenes* protrusions, and act together with Rho signaling network, regulated the length of protrusions.

*Benanti Cell 2015: In this study, the authors discovered that two *Burkholderia* species polymerize actin tails by mimicking host Ena/VASP actin polymerases.

*Reed Curr Bio 2014: This study revealed the roles of the two SFG rickettsiae actin polymerization proteins, and showed each protein was independently needed during temporally-segregated phases of actin-based motility.

**Talman JCS 2014: In this study, the authors show that the ability of *L. monocytogenes* to elongate protrusions and promote spread relies on recycling of the distal actin filament network into the monomer pool to enable continued actin polymerization.

**Fukumatsu Cell Host Microbe 2012: The authors describe the surprising finding that *S. flexneri* target tricellular junctions to promote spread, and rely on a non-canonical method of clathrin-mediated trafficking to promote of protrusion engulfment.

*Bishai Cell Microbio 2013: In this study, the authors show that myosin X is needed for the formation of long protrusions by *S. flexneri*, and propose the intriguing model that myosin X provides some of the force needed to push the protrusion into neighboring cells.

**Dragoi I&I 2015: Using live cell imaging, this study elegantly describes *S. flexneri* protrusion dynamics and is the first to identify vacuole-like protrusions (VLPs).

Leung I&I 2013: This study along with [45] demonstrated that InlC is a critical *L. monocytogenes* virulence factor that promotes cell-to-cell spread through its ability to inhibit the host protein Tuba.

*Lamason Cell 2016: Using live cell imaging, this study provides the first description of *Rickettsia parkeri* cell-to-cell spread and shows that the secreted bacterial protein Sca4 promotes protrusion engulfment by reducing intercellular junctional tension.

Introduction

In eukaryotic cells, the actin cytoskeleton regulates a variety of functions. For example, it provides structural integrity at cell-cell junctions to maintain tissue integrity, and dynamically reorganizes to promote the formation of membrane extensions or invaginations during cell migration and intracellular trafficking [1-3]. Due to its importance in these diverse cellular processes, the actin cytoskeleton is also a critical target of intracellular bacterial pathogens. Many pathogens hijack actin at different steps of their life cycle, and investigating these processes has revealed new ways in which host cells regulate actin cytoskeleton dynamics in uninfected settings [4].

In this review, we will discuss recent advances in our understanding of the molecular mechanisms by which intracellular bacterial pathogens exploit actin. We will focus on pathogens within four genera, including *Listeria monocytogenes*, *Shigella flexneri*, *Burkholderia* spp. in the pseudomallei group, and spotted fever group (SFG) *Rickettsia* spp. These bacteria are evolutionarily diverse - *Listeria* spp. are Gram-positive firmicutes, whereas the others are Gram-negative alphaproteobacteria (*Rickettsia* spp.), betaproteobacteria (*Burkholderia* spp.) or gammaproteobacteria (*Shigella* spp.). They are also transmitted by different routes, and cause a spectrum of diseases including listeriosis (*L. monocytogenes*), shigellosis (*S. flexneri*), meliodosis (*B. pseudomallei*), glanders in equines (*B. mallei*), and spotted fever- and eschar-associated rickettsioses (*Rickettsia* spp.) [5]. Despite their overall diversity, these pathogens share a common mechanism of infection. In particular, they invade non-phagocytic cells and escape the phagosome into the cytosol where they polymerize actin filaments to generate actin “comet” tails on their surface to drive movement. Actin-based motility propels the bacteria through the cytosol and enables spread into neighboring cells (Figure 1) [6-8].

We will focus on two themes that have emerged recently. The first is that, despite common features of infection, recent work has revealed surprising differences in the molecular mechanisms of actin-based motility. Older work showed a critical role for the host Arp2/3 complex and its nucleation promoting factors (NPFs) in actin assembly [9,10], but we are now learning that diverse biochemical mechanisms of actin polymerization are used by pathogens, resulting in divergent actin filament organization and parameters of motility. We are also learning that various host proteins regulate bacterial motility. The second emerging theme is that the parameters and mechanisms of spread are also quite diverse between pathogens, with differential dependence on actin-based motility and distinct ways of remodeling the actin cytoskeletal network at cell-cell junctions. Though more work is needed to fully elucidate the molecular mechanisms and key players involved in motility and spread, we are beginning to understand that these are dynamic and complicated processes coordinated by a network of host and bacterial factors.

Diverse biochemical mechanisms of actin-based motility

Once inside host cells, the pathogens highlighted in this review polymerize actin on their surface to rocket through the cytoplasm, leaving in their wake actin comet tails. Early work showed that several bacterial species hijack the host Arp2/3 complex to polymerize actin

tails consisting of branched filament networks, leading to motility characterized by curved or meandering paths (Figure 2) [9,11]. At the molecular level, the bacterial surface proteins ActA from *L. monocytogenes*, BimA from *B. thailandensis* (BtBimA) and RickA from SFG rickettsiae mimic host nucleation promoting factors (NPFs) to activate the Arp2/3 complex [12-17]. In contrast, *S. flexneri* IcsA (also called VirG) recruits the host NPF N-WASP to the bacterial pole to activate Arp2/3 [18,19]. These early studies supported the idea that the Arp2/3 complex was crucial for pathogen motility, and many assumed this mechanism was conserved across all species.

It is now becoming clear, however, that hijacking the Arp2/3 complex is not the only pathway bacteria use to polymerize actin. Recent work has uncovered that actin assembly by different pathogens occurs by vastly different biochemical mechanisms, resulting in differences in the types of actin networks generated and the parameters of motility exhibited (Figure 2). For example, in addition to RickA, SFG *rickettsiae* also express Sca2, a mimic of host formin proteins, which directly nucleates actin and promotes filament elongation by processively associating with the barbed end of an actin filament and preventing host capping protein activity [20,21]. In contrast, BimA proteins from *B. pseudomallei* (BpBimA) and *B. mallei* (BmBimA) mimic host Ena/VASP actin polymerases in their ability to promote filament elongation and bundling [22]. Actin tails formed by Sca2- and BpBimA/BmBimA-mediated actin assembly are composed of long bundled actin filaments, leading to straighter paths of motility (Figure 2a) [11,22]. The existence of bacterial formin and Ena/VASP mimics underscores the surprising variety of actin-based motility mechanisms bacteria have evolved by coopting diverse host actin assembly strategies.

It remains unclear, though, how the evolution of distinct actin polymerization mechanisms influences virulence. Interestingly, BimA from the non-pathogenic *Burkholderia* species *B. thailandensis* uses an Arp2/3-dependent polymerization pathway, whereas BimA from the pathogenic species *B. pseudomallei* and *B. mallei* use an Ena/VASP mimic (Figure 2b), suggesting these differences could contribute to virulence. Moreover, studies of Australian *B. pseudomallei* strains that express either BpBimA or BmBimA revealed that there is an association between BmBimA expression and neurological melioidosis, and BpBimA and pneumonia [23], again suggesting a correlation between differences in actin assembly and virulence. It remains to be determined whether and how the mechanism of actin assembly influences virulence for these and other pathogens.

Temporally regulated deployment of distinct actin polymerizing proteins

Most pathogens that undergo actin-based motility use a single actin polymerizing protein throughout the course of infection. In contrast, SFG rickettsiae express two different actin assembly proteins, RickA and Sca2 [20,24] (with the exception *R. peacockii*, in which the *rickA* and *sca2* genes are mutated). However, the respective contributions of these proteins to motility had remained uncertain. Recently, these proteins were found to regulate temporally-segregated phases of motility for SFG species *R. parkeri* (Figures 1 and 2b) [11]. Early in infection (<2 hpi), *R. parkeri* expresses RickA on one bacterial pole, and polymerizes short and curved Arp2/3-dependent actin tails [11,15,16], as described above. Later in infection, polar localization of RickA is lost in favor of Sca2 polar localization on

the bacterial surface, resulting in the polymerization of long and straight actin tails (Figure 2) [11]. Although a *rickA* mutant shows a modest defect in cell-to-cell spread, Sca2 is required for efficient spread *in vitro* [11]. Moreover, Sca2 is important for virulence of the SFG species *R. rickettsii* in a guinea pig model of infection [25].

It is still unclear how or why SFG rickettsiae evolved two phases of motility. This may adapt the pathogen to different hosts (arthropod versus mammalian) or different cell types within a single host [26]. Determining how these host environments influence RickA- and Sca2-dependent motility, how the two motility phases are temporally regulated, and how this contributes to disease will be important for understanding SFG pathogenesis.

Host factors that regulate motility

Additional work has focused on how actin polymerization and actin-based motility are regulated by host proteins (Figure 2b). So far, two main classes of host proteins have been identified: actin-binding proteins and the signaling proteins that regulate them. Previous work had shown that host actin binding proteins including the Arp2/3 complex, cofilin, and capping protein are crucial for reconstituting actin-based motility driven by ActA and/or IcsA, and that VASP, α-actinin and profilin played a stimulatory role [27]. Some recent work has focused on the Arp2/3 complex itself, which is a protein complex consisting of seven subunits that were all previously considered to be important for complex activity [28,29]. However, genome-wide RNAi screening revealed *L. monocytogenes* motility may not require the p16 subunit of the Arp2/3 complex [30], which is surprising since p16 was previously thought to be important for Arp2/3 integrity [28]. p16 is still recruited to actin tails [30], suggesting it may nevertheless regulate Arp2/3 activity. Additional actin-binding proteins have also been recently implicated in *L. monocytogenes* motility, including nexillin and lamellipodin (Lpd) (Figure 1b). siRNA-mediated depletion of nexillin results in irregularly shaped actin tails, possibly due to defects in nexillin-mediated actin filament crosslinking and stabilization of the actin network [31]. Lpd depletion or overexpression differentially influences the percentage of motile *L. monocytogenes* and their velocity, with subsequent effects on cell-to-cell spread [32]. These effects may be mediated through Lpd's interaction with VASP on the bacterial surface [32], in agreement with VASP's previously-discovered role in regulating ActA-mediated polymerization [33-35]. As with *L. monocytogenes* and *S. flexneri*, *R. parkeri* Sca2-mediated actin-based motility also requires a core set of host actin-binding proteins, which includes profilin, fimbrin/T-plastin, capping protein and cofilin [36].

Host protein kinases and signaling effectors also modulate actin polymerization by bacterial pathogens. The serine/threonine kinase casein kinase 2 beta (CK2-β) phosphorylates the *L. monocytogenes* ActA protein and promotes its association with the Arp2/3 complex [37]. In contrast, Bruton's tyrosine kinase (Btk) and Abelson tyrosine kinase (Abl) phosphorylate N-WASP to promote N-WASP activity and/or recruitment to the surface of *S. flexneri* [38,39]. In addition, *S. flexneri* recruits the host protein Toca-1 (transducer of Cdc42-depdenent actin assembly) to relieve N-WASP autoinhibition and initiate actin polymerization on the bacterial surface [40].

These recent studies reveal that bacteria capable of actin-based motility subvert a variety of host factors to promote movement. However, we still do not know how all of these factors are recruited or their precise mechanism of action. Moreover, we don't know whether each pathogen hijacks a unique profile of cytoskeletal and regulatory proteins that may reflect distinct modes of actin-based movement. It is also possible that the differential availability of host proteins in different organisms, cell types or even within sub-regions of the cell could regulate the parameters of bacterial motility and cell-to-cell spread. What is clear, though, is that studying how motile bacteria hijack the actin cytoskeleton will reveal new pathogenic strategies and new ways in which the actin cytoskeleton itself is regulated, even in uninfected contexts.

Bacterial cell-to-cell spread

A key role of actin-based motility for *L. monocytogenes*, *S. flexneri* and SFG rickettsiae is to enable spread to neighboring cells. Early studies focusing on cell-to-cell spread of *S. flexneri* and *L. monocytogenes* revealed that motile bacteria propel themselves into the plasma membrane to induce long protrusions that extend into a neighboring cell (Figure 1). These protrusions are eventually engulfed into double-membrane vesicles within the receiving cell. This is followed by vesicle lysis, which releases bacteria into the cytosol to continue another cycle of growth and spread [7]. *Burkholderia* spp. have evolved a different strategy for spread that involves inducing host cell-cell fusion (Figure 1) [6], which will not be discussed further here. In the sections below, we will review the diverse strategies bacteria use to manipulate the actin cytoskeleton to promote protrusion initiation, establish protrusion morphology and enable protrusion engulfment and resolution during spread.

Initiating protrusions

Previous work showed that the propulsive force from actin-based motility is sufficient to promote protrusion formation for *E. coli* expressing *S. flexneri* IcsA [41], suggesting that the same is true for all pathogens that exhibit actin-based motility. Although actin-based motility is needed for bacteria to spread [6,11,18,19,25,42], it is important to consider that, *in vivo*, spread occurs in tissues where cells are tightly linked to one another via intercellular junctional complexes and the underlying actomyosin network (Figure 3a). As junctions form and mature, this mechanical coupling promotes junction tension and stabilization [2,3,43]. Stiff host cell junctions may resist protrusion initiation, suggesting that, in addition to using the force from actin-based motility, pathogens may need to remodel the dense actin-rich networks at the cell cortex for protrusion initiation.

In support of this notion, *L. monocytogenes* protrusion initiation frequency is influenced by the age of the cell monolayer, suggesting that junction maturation is critical for limiting spread and may be difficult to overcome using only the force of actin-based motility [44]. Furthermore, *L. monocytogenes* protrusion initiation is enhanced by the secreted effector internalin C (InlC), which disrupts protein-protein interactions involving the scaffolding protein Tuba at tight junctions [45] (Figure 3b). Normally, Tuba promotes apical junction stiffness by recruiting Cdc42 and activating N-WASP-mediated actin polymerization at the cell cortex [46]. InlC disrupts Tuba-mediated tension, which has been proposed to promote

protrusion initiation [45] and enhance virulence in mice [47]. Recent work showed that InlC also disrupts the interaction between Tuba and the host membrane-trafficking protein Sec31A [48]. It was proposed that this might regulate spread by regulating the trafficking of tension-regulating proteins to the plasma membrane, although this awaits further investigation.

S. flexneri may also remodel the actin networks at junctions and target specific junctional regions to promote protrusion formation (Figure 3). For example, *S. flexneri* spread is enhanced by host Diaphanous (Dia) related formins [49], and inhibited by Ena/VASP family members VASP and EVL [50]. These factors do not influence bacterial motility in the cytosol [49,51], suggesting they specifically influence actin networks at the cortex and intercellular junctions. It is also intriguing that *S. flexneri* predominantly initiates protrusion formation at tricellular junctions in epithelial cells, and that initiation requires the tight junction protein tricellulin [52]. In contrast, *L. monocytogenes* displays no known spatial preference in the protrusion initiation site. It is unclear why tricellular junctions are targeted by *S. flexneri*, but recent work showed that tricellulin regulates junctional tension through Tuba and Cdc42 [53]. Moreover, tight junctions are hubs for endosomal recycling pathways that enable junctional remodeling under different stimulatory conditions [54]. How tension is utilized in this process is still unclear, but the fact that both *L. monocytogenes* and *S. flexneri* manipulate tension to promote protrusion formation highlights the importance of overcoming this critical host barrier to initiating spread.

Although *L. monocyto*genes and *S. flexneri* may remodel the cell cortex to affect tension, they nevertheless require the force from actin-based motility for protrusion initiation. In contrast, we recently discovered that *R. parkeri* typically ceases actin-based motility before initiating protrusion formation (Figures 1 and 3b) [55]. This is surprising given the role of actin-based motility in protrusion initiation by other pathogens, and the importance of motility for SFG rickettsiae spread [11,25]. It seems likely, then, that actin-based motility is necessary to position *R. parkeri* near cell junctions, but that other force-generating mechanisms promote protrusion initiation (Figure 3b). Nothing more is known about how SFG rickettsiae initiate protrusions, and future work will be needed to investigate the host and bacterial proteins involved.

Establishing Protrusion Morphology

Recent work suggests that the size and shape of membrane protrusions that form during cell-to-cell spread is influenced by actin-polymerization dynamics, motor proteins and host membrane-cytoskeletal linkers. Interestingly, each bacterial species has evolved a distinct mechanism for manipulating these processes and proteins, resulting in dissimilar protrusion morphologies.

L. monocytogenes protrusions are long (≤ 20 μm) and undergo stages of elongation followed by “fitful” movement before the protrusion resolves into a vesicle [44,55]. Recent work demonstrated that this behavior of bacteria in protrusions might be due to the dynamics of the actin network within the protrusion. In particular, local actin recycling is observed within *L. monocytogenes* protrusions and may be needed for sustained pushing forces against the

membrane to promote protrusion elongation (Figure 3b) [56]. The cytoskeletal network generated by *L. monocytogenes* also changes as bacteria move into protrusions. Near the bacterium, short, branched filaments colocalize with the Arp2/3 complex, followed by longer bundled filaments toward the distal end of the protrusion (Figure 3b) [56-58]. This distal actin network may be polymerized by host Diaphanous-related formins (Dia1-3), which were recently discovered to localize to *L. monocytogenes* protrusions (Figure 3b) [58]. Actin networks may also be tethered to the host cell plasma membrane via the membrane-cytoskeletal linker protein Ezrin (Figure 3b), which was shown to regulate *L. monocytogenes* protrusion formation, morphology and spread [59]. The dynamics, organization and cortical linkages of the actin network within *L. monocytogenes* protrusions are likely to be key contributors to bacterial movement within protrusions and the formation of an elongated protrusion morphology.

S. flexneri also induces long (≤ 30 μm) protrusions [60]. However, the actin network in these protrusions is thought to collapse after protrusion initiation and elongation, resulting in vacuole-like protrusions (VLPs) characterized by thin membranous stalks tethered to a membrane-enclosed bacterium (Figure 3b) [61]. VLPs are enriched in phosphotyrosine residues and their formation is dependent on the class II phosphatidylinositol-3 kinase PIK3C2A, which generates phosphatidylinositol-3-phosphate (PI(3)P) at the protrusion membrane (Figure 3b) [61,62]. The same was not observed for *L. monocytogenes* protrusions [61], suggesting that *S. flexneri* may modulate specific host signaling pathways to dismantle the actin network during VLP formation. Interestingly, the length and thickness of *S. flexneri* protrusions is also regulated by the motor protein myosin X [60]. Myosin X localizes within protrusions and is thought to facilitate protrusion elongation by linking the host membrane to the bacterium-associated cytoskeleton and providing some of the force needed for membrane distension (Figure 3b) [60]. This model might help explain how *Shigella*-induced VLPs extend into neighboring cells in the absence of the extensive internal actin networks seen in *L. monocytogenes* protrusions. Interestingly, myosin X also promotes *L. monocytogenes* spread, suggesting it could be a conserved target of bacterial pathogens [60]. Other myosin motors and regulators have been implicated in *S. flexneri* spread, but they do not appear to influence cytosolic motility or protrusion formation and morphology, suggesting a potential role in protrusion resolution [63,64]. Overall, it seems that the machinery necessary for establishing *S. flexneri* protrusions may rely on contributions from host signaling pathways, altered actin polymerization forces and myosin-mediated contraction of the actin network.

The *Rickettsia* species *R. parkeri* is an outlier with regard to protrusion morphology, as it forms very short protrusions (2-3 μm) (Figures 1 and 3b) [55]. These tinier protrusions may require an entirely different set of host and bacterial factors to establish their morphology. However, no proteins have yet been found that control protrusion size, and thus identifying such factors is needed to better understand how protrusion size is modulated.

Protrusion resolution and vesicle escape

Protrusions containing bacteria are eventually resolved into double-membrane vesicles in the recipient cell. This is followed by vesicle lysis and then escape to the cytosol [7,8]. Very

little is known, however, about how the morphologically distinct protrusions formed by different pathogens are engulfed into receiving cells. Nevertheless, we have begun to identify a diverse group of host and bacterial factors involved in this process.

Recent work in *R. parkeri* demonstrated that reducing vinculin-mediated intercellular tension facilitates protrusion resolution [55]. Vinculin is an actin-binding protein that resides at adherens junctions (as well as at focal adhesions) and strengthens junctions by crosslinking the actomyosin network to E-cadherin complexes [65]. The *R. parkeri* secreted effector surface cell antigen 4 (Sca4) inhibits vinculin in the donor cell by interfering with the interaction between vinculin and α-catenin, and reduces vinculin-dependent tension (Figure 3b). This suggests that differences in actomyosin tension between donor and recipient cells might enhance membrane flexibility to promote protrusion engulfment [55]. Interestingly, Sca4 does not regulate protrusion initiation, in contrast with *L. monocytogenes* InlC, which was proposed to promote protrusion formation by reducing tension (Figure 3b) [45]. This suggests pathogens have evolved distinct mechanisms of reducing host cell tension to control different steps of spread.

Although the mechanisms of *L. monocytogenes* and *S. flexneri* protrusion resolution have remained more enigmatic, several proteins may regulate this step. For example, the host serine/threonine kinases CSKN1A1 and STK11 promote resolution of *L. monocytogenes* [66] and *S. flexneri* [62] protrusions, respectively. As mentioned above, *S. flexneri* protrusion resolution is also dependent on PIK3C2A-mediated VLP formation [61]. Finally, the *L. monocytogenes* metalloprotease (Mpl) may promote protrusion resolution by regulating ActA processing and localization on the bacterial surface [67]. Future work will be needed to better understand these mechanisms and to elucidate how they are all coordinated during protrusion resolution.

Host membrane trafficking pathways have also been found to regulate protrusion resolution. *S. flexneri* requires a non-canonical clathrin mediated endocytic pathway for resolution [52]. Furthermore, host cell dynamin is required for engulfment (Figure 3b) [52], suggesting that this scission protein may specifically recognize the narrow VLP stalks to promote vesicle resolution. Drug-mediated inhibition of clathrin-mediated endocytosis did not inhibit *L. monocytogenes* spread, however, suggesting *L. monocytogenes* may use an alternate trafficking pathway [52]. More work is needed to define the trafficking pathways involved in protrusion resolution for different pathogens and better understand how host cell trafficking pathways recognize these large and morphologically distinct cargos.

Conclusions and future directions

In recent years, we have witnessed the key discoveries that intracytosolic bacterial pathogens exhibit a surprising diversity of actin assembly pathways that drive actin-based motility, as well as divergent actin-regulated pathways that enable cell-to-cell spread. Despite these developments, we still don’t fully understand the processes of motility and spread at the mechanistic level, or how the mechanisms compare between pathogens. Moreover, the diversity of spread mechanisms extends well beyond what we covered in this review, and also includes those involving ejectosomes (*M. marinum* [68]), trogocytosis (*Francisella*

tularensis [69]), efferocytosis (*L. monocytogenes* [70]) and cell-cell fusion (*Burkholderia* [6]). Thus, it is clear that bacterial pathogens have evolved many ways to manipulate the host cell machinery to promote their life cycle. Additionally, emerging evidence suggests that pathogen-induced changes to the host cytoskeleton can be sensed by the innate immune system, and that the cytoskeleton may play an important role in cellular defense pathways [71,72]. Future advances will require that we identify the full network of host and bacterial factors that interact during infection, determine how cytoskeleton perturbation may be sensed and/or used in innate immunity, and pursue mechanistic analyses of the function of individual players. In the end, these studies will reveal important mechanisms of pathogenesis as well as new regulatory mechanisms of the host actin cytoskeleton.

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Highlights

- Intracellular bacterial pathogens use different strategies for actin-based motility
- Diverse networks of host and bacterial proteins coordinate motility
- Pathogens also exhibit species-specific differences in cell-to-cell spread
- Spread involves forces generated by actin-based motility and/or relaxation of tension

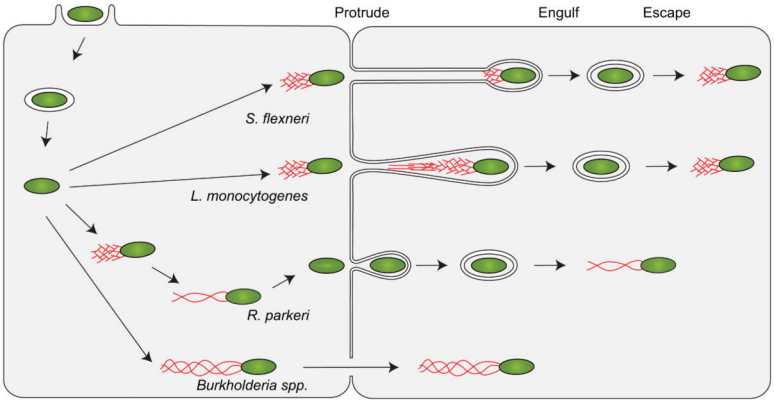

Figure 1. Life cycles of intracellular bacterial pathogens that harness actin-based motility to enable cell-to-cell spread

The cartoon depicts the intracellular life cycles of the pathogens discussed in this review. After invading bacteria are phagocytosed and escape the phagosome, they enter the host cell cytosol, where they polymerize actin using distinct mechanisms and undergo actin-based motility, forming actin comet tails with different filament organizations. *R. parkeri*, a representative of the SFG of *Rickettsia* spp., undergo two temporally segregated and biochemically-distinct phases of actin-based motility, as depicted. All of these pathogens also undergo diverse pathways of cell-to-cell spread via protrusion- and vesicle-mediated transfer (for *S. flexneri*, *L. monocytogenes*, and *Rickettsia* spp.), or direct cell-cell fusion (for *Burkholderia* spp). Actin, red; bacteria, green.

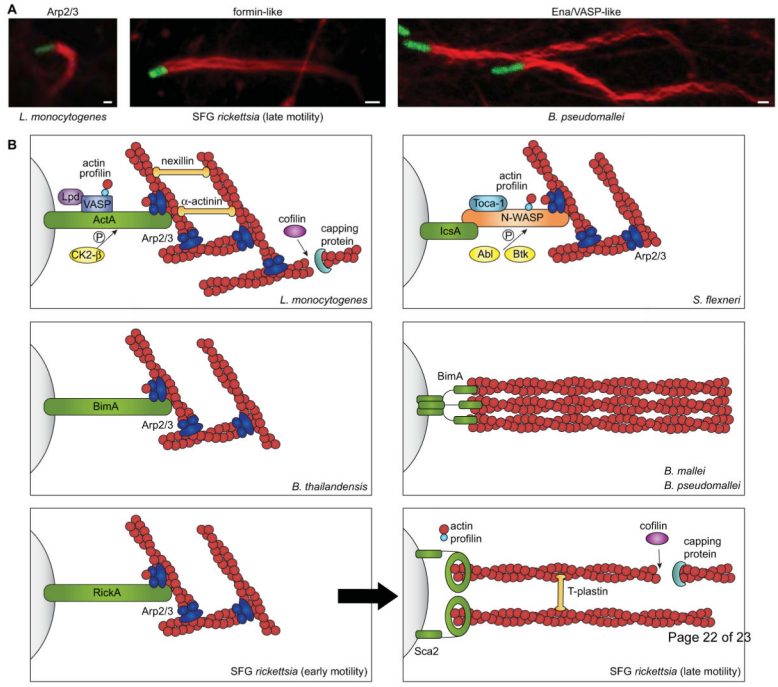

Figure 2. Actin-based motility is regulated by diverse molecular mechanisms

(A) Images of different bacterial pathogens and their associated actin tails in infected host cells. Each image corresponds to one of the three types of host actin polymerization pathways hijacked or mimicked for actin-based motility (Arp2/3, formin-like and Ena/VASP-like). Actin is labeled with phalloidin, red; bacteria, green. Scale bar, 1 μm. (B) A closer look at the molecular mechanisms of actin polymerization at the bacterial surface highlights the impressive coordination between host and bacterial proteins, and reveals critical differences between pathogens. In addition to the bacterial surface proteins that promote actin polymerization (in green), many host proteins regulate the nucleation (Arp2/3 complex, N-WASP, Toca-1), elongation (Eva/VASP, profilin, lamellipodin (Lpd)), protein phosphorylation (CK2-β, Abl, Btk), actin crosslinking (nexillin, α-actinin, T-plastin) and actin dynamics and recycling (capping protein, cofilin). For some pathogens, though, host protein regulators await discovery.

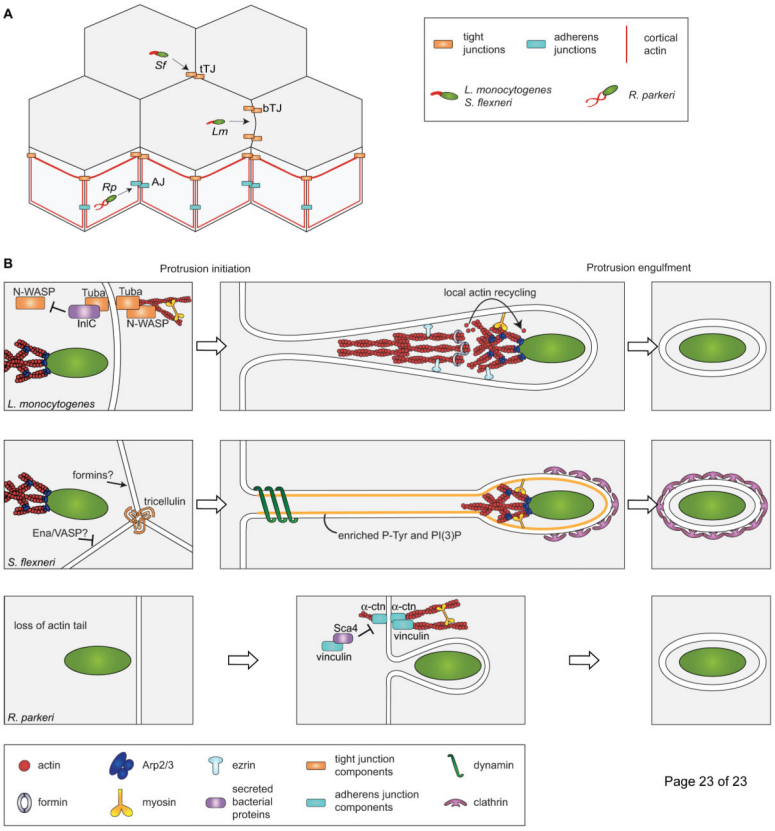

Figure 3. Distinct strategies of bacterial cell-to-cell spread

(A) Host cells in tissues are mechanically coupled together via actin cytoskeletal networks that link together adhesive junctional complexes. Bacteria have evolved diverse strategies to manipulate different junctions to promote spread by either reducing bicellular tight junction (bTJ) or adherens junction (AJ) tension, or spreading primarily at tricellular tight junctions (tTJ). (B) A closer look at the specific stages of spread, with particular emphasis on protrusion initiation, morphology and engulfment. *L. monocytogenes* secrete InlC into the host cell where it inhibits the Tuba:N-WASP interaction and reduces tension to promote protrusion initiation. Once in a protrusion, the combination of spatially-segregated actin polymerization strategies, along with host membrane-anchoring and motor proteins, help drive the extension of long protrusions into the neighboring cell. In polarized epithelia, *S. flexneri* protrude predominantly at tricellular junctions. Protrusion initiation may also be regulated by formin- or Ena/VASP-mediated changes to the cortical actin network. Once *S. flexneri* enter protrusions, the actin network collapses, resulting in VLPs that are enriched in phosphotyrosine and PI(3)P and that are extended into neighboring cells with assistance of myosins. Non-canonical clathrin-mediated endocytosis then promotes engulfment of the protrusion into the neighboring cell. *R. parkeri* are distinct from the others in that they lose their actin tails before spreading, and generate short protrusions. *R. parkeri* protrusion engulfment is regulated by the secreted bacterial protein Sca4, which may promote host plasma membrane flexibility by interfering with the vinculin:α-catenin interaction and relaxing vinculin-dependent intercellular tension.