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Minireview

MINIREVIEW

Actin-based motility of bacterial pathogens: mechanistic diversity and its impact on virulence

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One sentence summary: This minireview explores the roles of bacterial pathogen actin-based motility in survival and cell–cell spread, the diverse spectrum of motility mechanisms, and the impact of this mechanistic diversity on pathogenicity and virulence.
Editor: Nicholas Carbonetti

ABSTRACT

A diverse spectrum of intracellular bacterial pathogens that inhabit the cytosol have evolved the ability to polymerize actin on their surface to power intracellular actin-based motility (ABM). These include species of *Listeria*, *Burkholderia* and *Rickettsia*, as well as *Shigella* and *Mycobacteria*. Here, we provide an overview of the roles of bacterial ABM in survival and virulence. Moreover, we survey the molecular mechanisms of actin polymerization in host cells and describe how bacterial pathogens mimic or harness the full diversity of these mechanisms for ABM. Finally, we present ABM through a new lens by comparing motility mechanisms between related species of *Listeria*, *Burkholderia* and *Rickettsia*. Through these comparisons, we hope to illuminate how exploitation of different actin polymerization mechanisms influences ABM as well as pathogenicity and virulence in humans and other animals.

Keywords: bacterial pathogen; *Listeria*; *Burkholderia*; *Rickettsia*; cytoskeleton; actin-based motility

INTRODUCTION

Intracellular bacterial pathogens remodel and exploit the host cell environment to support their survival and growth. A common target of bacterial pathogens is the host cell actin cytoskeleton, which is a dynamic system of filaments that is central to shape determination, movement, phagocytosis and intracellular trafficking. Because of its importance in these processes, the actin cytoskeleton is manipulated by many bacterial pathogens at multiple stages of infection (Fig. 1) (reviewed in Haglund and Welch 2011). For example, most intracellular bacterial pathogens mobilize actin during invasion (reviewed in Carabeo 2011). Following invasion, some pathogens remain within a membrane-bound compartment and target actin to subvert membrane trafficking (Haglund and Welch 2011). Perhaps the most striking mobilization occurs when bacteria that escape into the cytosol polymerize actin on their surface and use filament assembly to power intracellular actin-based motility (ABM), generating actin comet tails that trail the moving bacteria (reviewed in Haglund and Welch 2011; Welch and Way 2013; Truong, Copeland and Brumell 2014) (Fig. 1). Since the discovery of ABM (Bernardini *et al*. 1989; Tilney and Portnoy 1989), this phenomenon has captured the attention of researchers in microbiology and cell biology, and studying this process has led to important advances in both fields.

Several genera of bacterial pathogens contain species that are capable of ABM (Table 1). These include *Shigella* and *Mycobacteria*, for which only one species undergoes ABM, as well as *Listeria*, *Burkholderia* and *Rickettsia*, for which there are two or more species that undergo ABM. Interestingly, different bacterial genera, and even different species within a single genus, have evolved distinct molecular strategies to assemble actin and power motility by mimicking and/or recruiting host actin assembly factors. Understanding these diverse mechanisms can reveal how differences in motility influence pathogenicity.

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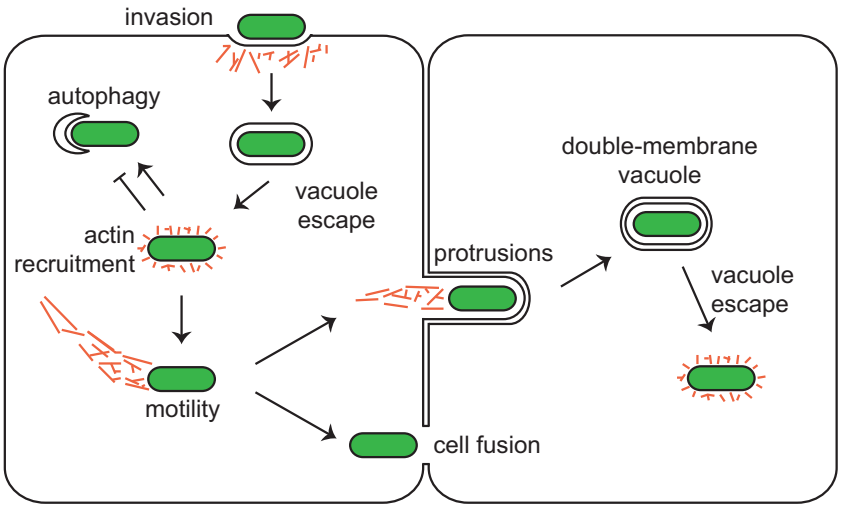

Figure 1. Actin assembly and ABM play several roles in infection. Actin (orange) is mobilized during multiple stages of infection by intracellular pathogens (green). Actin facilitates bacterial invasion of the host cell. For bacterial pathogens that escape the vacuole into the cytosol, the recruitment of actin and/or actin-interacting proteins on the bacterial surface can influence its susceptibility to autophagy. Moreover, actin assembly powers bacterial ABM through the host cytosol, enabling bacteria to reach the cell periphery, where they enter into protrusions that are engulfed by neighboring cells or promote cell–cell fusion.

Table 1. Bacterial pathogens that undergo ABM.

Genus	Subgroup	Species	Disease	ABM protein
Listeria	n/a	*L. monocytogenes*	Listeriosis (human)	ActA
	n/a	*L. ivanovii*	Listeriosis (ruminant)	ActA
	n/a	*L. seeligeri*	Non-pathogenic	ActA
Burkholderia	n/a	*B. pseudomallei*	Melioidosis (human)	BimA
	n/a	*B. mallei*	Glanders (equine)	BimA
	n/a	*B. thailandensis*	Non-pathogenic	BimA
Rickettsia	SFG	*R. rickettsii*, *R. conorii*, *R. parkeri*, others	Spotted fever and eschar-associated rickettsioses	RickA, Sca2
	TRG	*R. felis*, others	Flea-borne typhus	RickA, Sca2
	TG	*R. prowazekii*, *R. typhi*	Epidemic typhus, murine tyhpus	Sca2 (truncated in *R. prowazekii*)
	AG	*R. bellii*, others	Non-pathogenic	RickA, Sca2
Shigella	n/a	*S. flexneri*	Diarrhea in humans	IcsA
Mycobacterium	n/a	*M. marinum*	Skin lesions	Unknown

In this minireview, we provide an overview of the roles of bacterial ABM in cell–cell spread, autophagy avoidance and virulence. Moreover, we survey host and bacterial actin-polymerizing molecules and the distinct molecular mechanisms by which they harness actin. Finally, we present ABM through a new lens, comparing motility mechanisms between related species of *Listeria*, *Burkholderia* and *Rickettsia*. We intend to open a window into the evolution of ABM mechanisms, the roles of ABM in adapting bacteria to the intracellular environment, and how differences in motility may influence pathogenicity and virulence in humans and other animals.

ROLES OF ABM IN SURVIVAL AND VIRULENCE

ABM influences multiple stages of bacterial infection in the host. One key function of ABM is to promote cell–cell spread (Fig. 1), as mutants that fail to polymerize actin are defective in spread (Bernardini *et al.* 1989; Domann *et al.* 1992; Kocks *et al.* 1992; Kleba *et al.* 2010; French *et al.* 2011; Reed *et al.* 2014). Motility facilitates bacterial movement to the host plasma membrane, where *Listeria*, *Rickettsia* and *Shigella* spp. enter into protrusions in the plasma membrane of the donor cell that can be engulfed by a receiving cell (Tilney and Portnoy 1989; Kadurugamuwa *et al.* 1991; Gouin *et al.* 1999; Van Kirk, Hayes and Heinzen 2000; also reviewed in Ireton 2013). In contrast, *Burkholderia* spp. induce fusion of infected cells with uninfected neighbors to form multinucleated giant cells (MNGCs) (Kespichayawattana *et al.* 2000; French *et al.* 2011). The ability of these bacteria to spread directly between host cells or fuse cells without leaving the cell allows them to evade immune defenses that are active in the extracellular environment.

A second and emerging role for pathogen actin assembly and ABM is the manipulation of pathways that target bacteria for destruction via autophagy (reviewed in Mostowy and Shenoy 2015) (Fig. 1). For *Listeria monocytogenes*, *actA* mutants are more susceptible to autophagy (Birmingham *et al.* 2007; Yoshikawa *et al.* 2009), and ActA is proposed to recruit host actin assembly proteins to protect bacteria from recognition by the autophagy machinery (Yoshikawa *et al.* 2009). In contrast, *Shigella flexneri* IcsA stimulates autophagy (Ogawa *et al.* 2005), and actin

assembly by *S. flexneri* promotes the formation of cages of septin proteins around the bacteria that are important for recruiting the autophagy machinery (Mostowy *et al.* 2010, 2011). However, *S. flexneri* also counteracts the autophagy machinery using the type III secreted effector IcsB (Ogawa *et al.* 2005), which acts together with actin regulators (Baxt and Goldberg 2014) to antagonize the recruitment of autophagy proteins. Thus, the role of actin assembly proteins and ABM in modulating autophagy is complicated and may differ between pathogens. Moreover, whether and how actin recruitment by other pathogens such as *Rickettsia*, *Burkholderia* and *Mycobacteria* affects autophagy remains to be determined.

The roles of ABM in bacterial infection have been revealed by identifying and studying mutations in the genes encoding actin assembly proteins (Table 1). For example, the *actA* gene in *L. monocytogenes* is necessary for ABM (Domann *et al.* 1992; Kocks *et al.* 1992), and the corresponding gene in *L. ivanovii* fulfills the same role (Kreft, Dumbsky and Theiss 1995). ActA is also an important virulence factor for *L. monocytogenes*, as an *actA* deletion mutation exhibits a 10^3-fold increased LD_{50} in mice (Brundage *et al.* 1993), a 30-fold increased LD_{50} in zebrafish (Levraud *et al.* 2009) as well as reduced maternal-fetal transmission in pregnant guinea pigs (Bakardjiev, Stacy and Portnoy 2005) and mice (Le Monnier *et al.* 2007) (the role of ActA in virulence is also reviewed in Travier and Lecuit 2014). The *bimA* gene in *Burkholderia pseudomallei* (Stevens *et al.* 2005b), *B. mallei* (Schell *et al.* 2007) and *B. thailandensis* (French *et al.* 2011) is similarly required for actin polymerization. Surprisingly, a *B. mallei bimA* mutant has an identical LD_{50} to wild type upon intraperitoneal injection of Syrian hamsters (Schell *et al.* 2007), and it remains unclear whether *bimA* is a virulence factor during other routes of infection or for other *Burkholderia* species. *Rickettsia* have two genes that are required for different temporal phases of ABM—*rickA* is required for early-phase ABM of *Rickettsia parkeri* (Reed *et al.* 2014) and *sca2* is required for late-phase ABM of *R. rickettsii* (Kleba *et al.* 2010) and *R. parkeri* (Reed *et al.* 2014). Sca2 is required for virulence of *R. rickettsii*, as a *sca2* mutant fails to induce fever in guinea pigs (Kleba *et al.* 2010), but the role of RickA in virulence remains unclear as there are no published studies using a *rickA* mutant in an animal model. For *S. flexneri*, the *icsA* gene is required and sufficient for actin assembly (Bernardini *et al.* 1989; Goldberg and Theriot 1995), and necessary for virulence in macaque monkeys (Sansonetti *et al.* 1991). No genes required for *Mycobacterium marinum* ABM have yet been identified.

To fully understand the function of ABM proteins in virulence in animal models, we must also take into account alternative roles for these proteins. For example, *L. monocytogenes* ActA is also required for bacterial aggregation and biofilm formation, and ActA-dependent aggregation enhances bacterial persistence within the mouse intestine and shedding in the feces (Travier *et al.* 2013). Moreover, *R. conorii* Sca2 can enable host cell invasion (Cardwell and Martinez 2009), although the role of this activity in animals has not been investigated. Future studies will reveal the mechanistic details of how pathogen ABM proteins harness actin for cell–cell spread and autophagy manipulation, and how their roles of actin mobilization and their alternative roles contribute to virulence and disease.

BACTERIAL MOTILITY FACTORS MIMIC EUKARYOTIC HOST ACTIN NUCLEATORS

The bacterial actin assembly proteins mentioned above—including ActA, BimA, RickA, Sca2 and IcsA—act by recruiting and/or mimicking distinct families of host cell actin-polymerizing proteins (reviewed in Haglund and Welch 2011; Bugalhão, Mota and Franco 2015) (Fig. 2). Some of these host proteins function by accelerating or bypassing the rate-limiting nucleation step of actin assembly, which is the formation of a nucleus consisting of three or more actin monomers (monomeric actin is also called globular or G-actin) (Fig. 2A). Others promote the subsequent elongation of actin filaments (F-actin) at the faster-growing barbed (or plus) ends, and often inhibit the activity of capping proteins that would otherwise terminate the assembly process. During or after assembly, additional proteins further organize F-actin into branched, cross-linked or bundled arrays. Host actin nucleating and elongating factors are divided into several types (reviewed in Campellone and Welch 2010; Firat-Karalar and Welch 2011) that are mentioned below together with their bacterial mimics or binding partners.

One key actin-nucleating factor is the seven-subunit Arp2/3 complex (Fig. 2B). It works together with host proteins called nucleation promoting factors (NPFs) that contain a conserved WCA domain consisting of a G-actin-binding WASP-homology 2 (WH2 or W) sequence(s), and Arp2/3-binding central (C) and acidic (A) sequences. Upon activation by NPFs, the Arp2/3 complex binds to the side of a pre-existing filament and initiates the formation of a new filament that elongates to form a Y-branch (reviewed in Rotty, Wu and Bear 2013). The bacterial actin assembly proteins ActA from *Listeria* species (Welch *et al.* 1998; Skoble, Portnoy and Welch 2000; Boujemaa-Paterski *et al.* 2001; Zalevsky, Grigorova and Mullins 2001), BimA from *Burkholderia thailandensis* (Sitthidet *et al.* 2010; Benanti, Nguyen and Welch 2015) and RickA from *Rickettsia* species (Gouin *et al.* 2004; Jeng *et al.* 2004) all contain WCA-like domains that mimic those of host NPFs to activate the Arp2/3 complex (Fig. 2C) (also reviewed in Welch and Way 2013). In contrast, the *Shigella flexneri* IcsA protein recruits and activates the host NPF N-WASP (Egile *et al.* 1999). Thus, mimicry or recruitment of NPFs is a common pathogenic strategy to enable ABM.

A second family of host actin-polymerizing proteins is the formins, which promote both nucleation and processive elongation of actin filaments (Fig. 2B). Formins have a conserved formin homology 2 (FH2) domain that forms a homodimeric ring (Xu *et al.* 2004), which nucleates new actin filaments and then remains associated with the growing barbed ends to accelerate elongation and inhibit capping (reviewed in Paul and Pollard 2009; Chesarone, DuPage and Goode 2010). Formins also have a proline-rich formin homology 1 (FH1) domain that binds to the G-actin-binding protein profilin, and in some cases WH2-like sequences that bind to G-actin, both of which supply a pool of actin to fuel elongation (Paul and Pollard 2009; Chesarone, DuPage and Goode 2010). The bacterial actin assembly protein Sca2 from the spotted fever group (SFG) *Rickettsia* species *Rickettsia parkeri* and *R. conorii* functionally mimics host formins in its ability to enhance nucleation, promote profilin-dependent elongation and inhibit capping (Fig. 2C) (Haglund *et al.* 2010; Madasu *et al.* 2013). The Sca2 N-terminal domain is proposed to interact with the C-terminal domain to enable the formin-like activities (Haglund *et al.* 2010; Madasu *et al.* 2013), although the N-terminal domain is structurally distinct from the formin FH2 domain (Madasu *et al.* 2013). Sca2 also has WH2 and proline-rich sequences that participate in filament assembly (Haglund *et al.* 2010; Madasu *et al.* 2013). Therefore, although Sca2 from SFG *Rickettsia* species is a functional mimic of host formins, it is unclear whether it acts by a similar or distinct molecular mechanism. Moreover, both *Listeria monocytogenes* and *S. flexneri* use host diaphanous-related formins to facilitate protrusion formation during

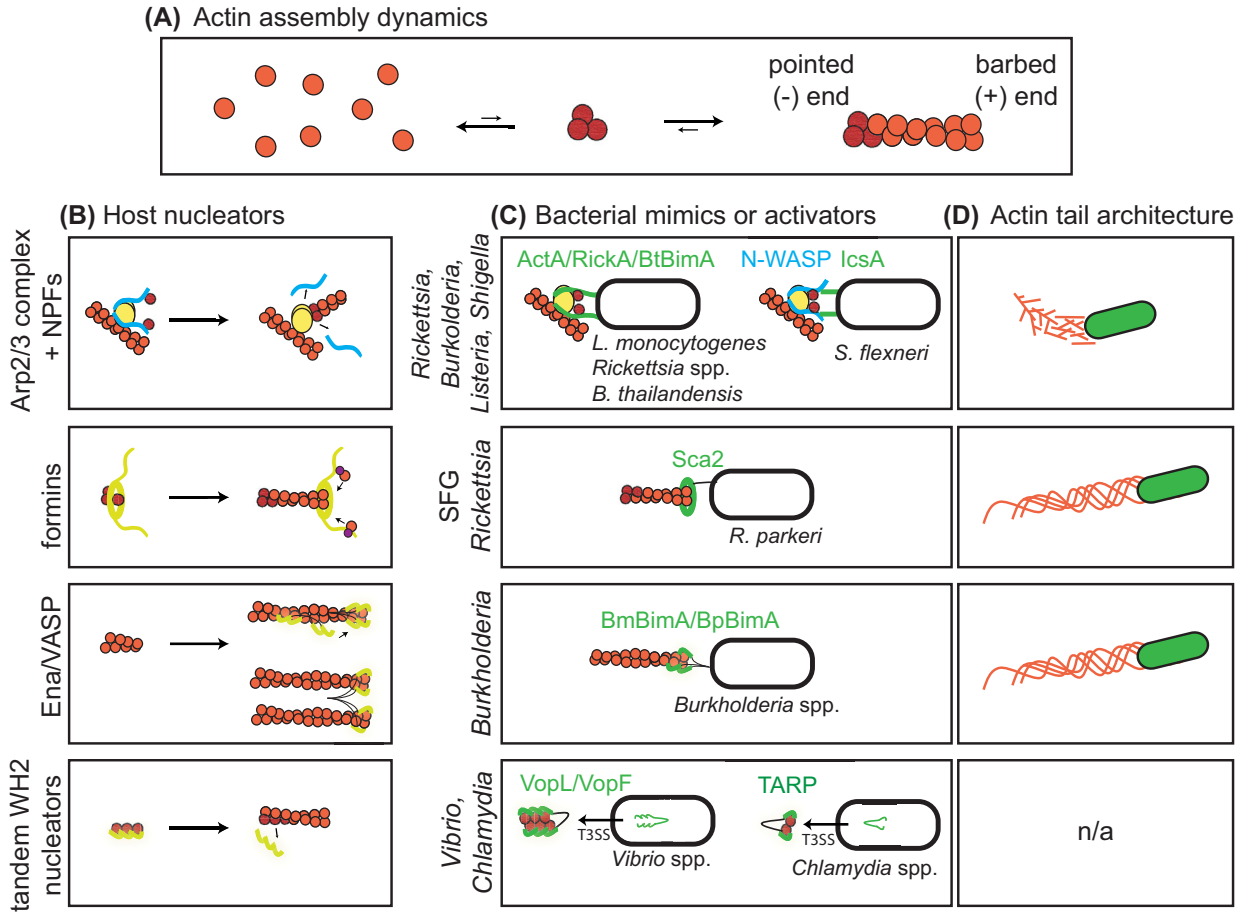

Figure 2. Eukaryotic actin nucleators and their bacterial mimics. (**A**) Spontaneous nucleation of actin (orange) involves the formation of a trimer (red), which is kinetically unfavorable. Once a trimer forms, the filament can elongate (or shrink) at both the barbed (+) end or pointed (–) end, although elongation (and shrinking) is faster at the barbed end. (**B**) Four types of host proteins promote actin nucleation and/or elongation, including the Arp2/3 complex (yellow) and NPFs (blue), formins (yellow) and profilin (magenta), Ena/VASP proteins (yellow), and tandem-WH2-based nucleators (yellow). (**C**) Bacterial proteins (green) mimic or recruit each different class of host actin-polymerizing proteins (yellow and blue). (**D**) Representation of actin filament organization in actin tails (orange) corresponding to the bacterial genera and/or species indicated in (C).

cell–cell spread (Heindl *et al.* 2010; Fattouh *et al.* 2015), suggesting a role for formins in ABM within membrane protrusions at the cell periphery.

A third type of host actin assembly factors is the Ena/VASP family. These multifunctional proteins accelerate barbed-end elongation, antagonize capping proteins and promote filament bundling (Barzik *et al.* 2005; Hansen and Mullins 2010; Winkelman *et al.* 2014) (Fig. 2B). Ena/VASP proteins contain a conserved Ena/VASP homology 1 (EVH1) domain that binds to proline-rich sequences in interacting partners to enable localization, a central proline-rich domain that binds to profilin as well as Ena/VASP homology 2 (EVH2) sequences that bind to F-actin and G-actin (reviewed in Bear and Gertler 2009). Their tetrameric structure is also important for the interaction with actin filament ends (Bachmann *et al.* 1999; Hansen and Mullins 2010). The bacterial actin assembly BimA proteins of the *Burkholderia* species *B. mallei* and *B. pseudomallei* were recently shown to be mimics of Ena/VASP proteins that can promote elongation, antagonize capping protein and bundle F-actin, as well as nucleate actin (Benanti, Nguyen and Welch 2015) (Fig. 2C). These BimA proteins contain one or more WH2 motifs (Stevens *et al.* 2005a; Sitthidet *et al.* 2010, 2011) that may be similar to sequences in the EVH2 domain and mediate interactions with G-actin and/or F-actin (Benanti, Nguyen and Welch 2015). Moreover, BimA oligomerization into a trimeric structure is central to actin assembly activity, as is Ena/VASP oligomerization into a tetramer. It remains unclear, however, the precise molecular mechanism by which these BimA proteins mimic the activity of Ena/VASP proteins.

Finally, a fourth type of host actin assembly protein is the tandem-WH2-based nucleators (Fig. 2B). While bacterial mimics of this nucleator family have been identified, none are known to be involved in ABM. However, we include a brief discussion of them in this review to illustrate the full range of diversity observed in bacterial mimics of actin assembly factors. Tandem-WH2-based nucleator proteins have up to four WH2 motifs that bind to G-actin to facilitate nucleation and are also implicated in regulating elongation (reviewed in Carlier *et al.* 2011; Dominguez 2016). Bacterial mimics of tandem WH2 nucleators include two secreted effector proteins from *Vibrio* species, VopF from *Vibrio cholerae* (Tam *et al.* 2007; Pernier *et al.* 2013; Avvaru, Pernier and Carlier 2015) and VopL from *V. parahaemolyticus* (Liverman *et al.* 2007; Namgoong *et al.* 2011; Yu *et al.* 2011; Zahm *et al.* 2013), as well as TARP from *Chlamydia* species (Jewett *et al.* 2006, 2010; Jiwani *et al.* 2013) (Fig. 2C). Because none of these proteins are implicated in pathogen ABM, they will not be discussed further.

From the examples presented above, it is clear that pathogens have evolved to mimic or recruit all of the major

types of host actin nucleation and elongation proteins. Moreover, pathogen mimics of all such host proteins, except the tandem-WH2-based nucleators, have been shown to participate in ABM, and it is possible that tandem-WH2-based mediators of ABM will be discovered. What remains unclear is how mimicry or recruitment of different host actin assembly proteins affects ABM parameters, adapts pathogens to their particular environmental niche and affects virulence in animals.

ABM PROTEINS—ORTHOLOGS, ADAPTATION AND VIRULENCE

Answers to the question of why pathogens have evolved proteins that mimic different host actin assembly factors may come from exploring the unexpected observation that bacterial ABM proteins from related species often exhibit divergent sequences and mechanisms of actin assembly. In this section, we consider sequence and mechanistic differences in orthologs of ABM proteins from selected bacterial genera, and explore how these differences may affect ABM parameters and disease.

Listeria species ActA

The genus *Listeria* comprises a group of eight Gram-positive soil saprotrophs (Bakker *et al.* 2010b). Six species are non-pathogenic, including *Listeria innocua*, *L. welshimeri*, *L. seeligeri*, *L. marthii*, *L. rocourtiae* and *L. grayi*. Two species are facultative pathogens—*L. monocytogenes* is a pathogen of animals including humans, and *L. ivanovii* is primarily a pathogen of ruminants. Consistent with an important role of ABM in pathogenicity, the pathogenic species *L. monocytogenes* and *L. ivanovii* are also the only two observed to undergo ABM (Chakraborty *et al.* 1995; Gouin *et al.* 1995; Kreft, Dumbsky and Theiss 1995).

The *Listeria actA* gene, which is required for ABM, encodes the actin polymerization protein ActA, a transmembrane protein exposed on the bacterial surface. The gene is contained within the *prfA* virulence gene cluster (Vazquez-Boland *et al.* 1992), which was suggested by population genetics analysis to be present in the most common recent ancestor of *Listeria* genus (Bakker *et al.* 2010a). This cluster was lost in the most recent common ancestors of *L. welshimeri* and *L. marthii*, and was lost during the evolution of most *L. innocua* strains (Bakker *et al.* 2010a), consistent with the failure to observe ABM for these species. The *prfA* cluster is retained in *L. monocytogenes* and *L. ivanovii*, which undergo ABM, as well as in *L. seeligeri*, for which ABM has not been observed (Gouin, Mengaud and Cossart 1994) (Fig. 3A). It has been suggested, though, that *L. seeligeri* exhibits low expression of virulence genes (Gouin, Mengaud and Cossart 1994), hinting that low *actA* expression may contribute to the failure to observe ABM, and perhaps to a loss of virulence.

Interestingly, the amino acid sequence of ActA differs considerably between orthologs from *L. monocytogenes* (LmActA), *L. ivanovii* (LiActA) and *L. seeligeri* (LsActA) (Gouin *et al.* 1995; Kreft, Dumbsky and Theiss 1995; Gerstel *et al.* 1996; Müller *et al.* 2010), with only roughly 20% identity across orthologs. However, all three orthologs retain common sequence features and overall domain organization (Fig. 3B). These include an N-terminal WCA-like domain similar to that of host NPFs, which in LmActA is required for actin nucleation and Y-branch formation with the Arp2/3 complex *in vitro* (Skoble, Portnoy and Welch 2000; Boujemaa-Paterski *et al.* 2001) and ABM in cells (Lasa *et al.* 1997; Pistor *et al.* 2000; Skoble, Portnoy and Welch 2000). Sequence features also include putative casein kinase 2 (CK2) recognition motifs (Kreft, Dumbsky and Theiss 1995), and phosphorylation by host CK2 is required for efficient ABM and cell–cell spread of *L. monocytogenes* (Chong *et al.* 2009). ActA also contains

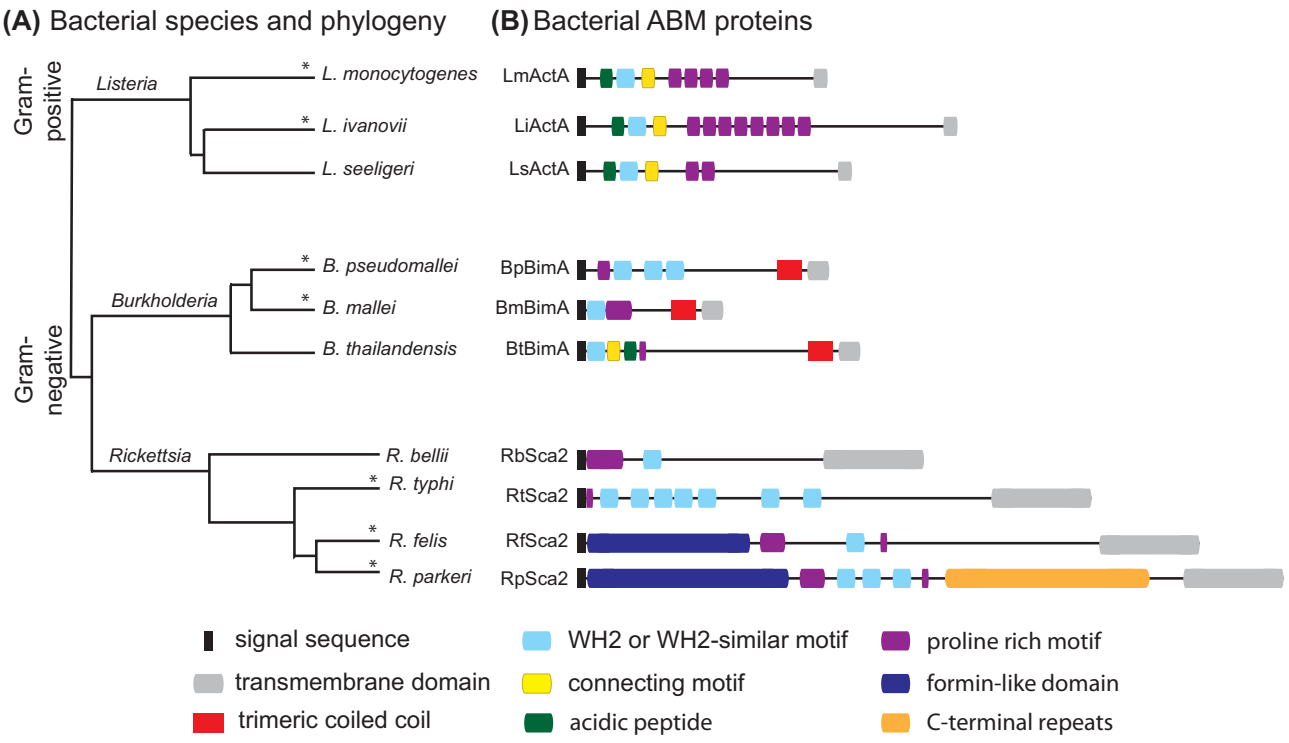

Figure 3. Species differences in actin assembly proteins. (**A**) Select species within the genera *Listeria*, *Burkholderia* and *Rickettsia* are represented according to their evolutionary relationships as determined by molecular phylogenetic analyses (branch lengths shown do not indicate evolutionary distances). Asterisk (*) denotes pathogenic species. (**B**) Schematic representations of bacterial ABM proteins are grouped to show domain comparisons between orthologs (see domain key below).

3–8 central proline-rich motifs (numbers differ between orthologs) that bind to and recruit host Ena/VASP proteins (Chakraborty *et al*. 1995; Pistor *et al*. 1995; Gerstel *et al*. 1996). These are dispensable for ABM but are required for rapid movement (Smith, Theriot and Portnoy 1996; Laurent *et al*. 1999; Loisel *et al*. 1999; Auerbuch *et al*. 2003) and enhanced directional persistence (Auerbuch *et al*. 2003). The common sequence features in ActA orthologs suggest that they all promote actin nucleation and Y-branching with the Arp2/3 complex and recruit Ena/VASP proteins to promote elongation, thus enabling cooperation between nucleation and elongation factors (reviewed in Chesarone and Goode 2009).

Although all three ActA orthologs likely function by the same molecular mechanism, their significant sequence variability may represent an adaptation of each *Listeria* species to different hosts or cell types. Because only LmActA has been extensively studied, we do not know how differences in ActA sequence, in particular within the WCA and PR motif regions, influences actin assembly *in vitro*, ABM in different cell types, or colonization and virulence in different animal models. Elucidating the impact of different orthologs will require replacing the *actA* gene in one species with orthologs from another species, which has only been done in a single study to show that *L. ivanovii actA* can restore ABM in an *L. monocytogenes actA* mutant strain (Gouin *et al*. 1995). Differences in ActA expression may also contribute to differences in ABM, as was suggested for *L. seeligeri* (Gouin, Mengaud and Cossart 1994). Thus, it remains to be determined how ActA sequence and expression may adapt different species to their environment or affect virulence and disease.

Burkholderia species BimA

Burkholderia is a genus consisting of more than 40 species of Gram-negative bacteria, most of which are saprophytes. Species in the *pseudomallei* group are the only ones shown to undergo ABM in host cells (Kespichayawattana *et al*. 2000; Stevens *et al*. 2005a). This group includes the human pathogen *Burkholderia pseudomallei*, which causes melioidosis (Cheng and Currie 2005), and the animal pathogen *B. mallei*, which causes glanders in equines but can also cause disease in humans (Khan *et al*. 2013). A third species, *B. thailandensis*, is not pathogenic to humans or other mammals, but is virulent in *Drosophila melanogaster*, suggesting that it may be a pathogen of other animal hosts (Pilátová and Dionne 2012). Within the *pseudomallei* group, *B. mallei* appears to be a clonal descendant of *B. pseudomallei* that has undergone genome reduction, whereas *B. thailandensis* and *B. pseudomallei* are closely related (Nierman *et al*. 2004; Kim *et al*. 2005).

The *bimA* gene, which encodes the trimeric autotransporter and actin polymerization protein BimA, is retained in all members of the *pseudomallei* group, but is absent in other *Burkholderia* species (Fig. 3A). Like other autotransporter proteins, BimA is composed of a C-terminal membrane-anchored autotransporter domain through which the remainder of the protein, or N-terminal passenger domain, is secreted and exposed on the bacterial surface (reviewed in Dautin and Bernstein 2007). The sequence of the BimA passenger domain from *B. pseudomallei* (BpBimA), *B. mallei* (BmBimA) and *B. thailandensis* (BtBimA) is generally highly conserved within each species (Sitthidet *et al*. 2008), except for BpBimA from a subgroup consisting of 12% of *B. pseudomallei* strains isolated in Australia, which is nearly identical to BmBimA (Sitthidet *et al*. 2008). However, BimA differs significantly between species, with only 25%–29% identity between orthologs in the passenger domain. In contrast to *Listeria* ActA, BimA orthologs have distinct sequence features and overall organization (Fig. 3B). BtBimA contains an N-terminal WCA domain similar to that in host NPFs and activates the host Arp2/3 complex to promote nucleation and Y-branch formation (Stevens *et al*. 2005a; Sitthidet *et al*. 2010; Benanti, Nguyen and Welch 2015). In contrast, BpBimA and BmBimA lack the Arp2/3-binding CA sequences and instead contain one (Bm) or three (Bp) WH2 motifs (Stevens *et al*. 2005a; Benanti, Nguyen and Welch 2015). These WH2 motifs are crucial for BpBimA and BmBimA activities *in vitro*, which include nucleating actin, binding to barbed ends, promoting elongation, removing capping protein and bundling F-actin, all similar to the activities of host Ena/VASP proteins (Benanti, Nguyen and Welch 2015). Thus, surprisingly, BimA orthologs mimic entirely different classes of host actin-polymerizing proteins, with BtBimA mimicking host NPFs for the Arp2/3 complex, and BpBimA/BmBimA mimicking the Ena/VASP family.

Interestingly, differences in the actin assembly mechanisms of BimA orthologs have been shown to translate into differences in ABM parameters and virulence characteristics. Experiments to discern the functional differences between BimA orthologs began with the successful complementation of the ABM defect of a *B. pseudomallei bimA* mutant by expression of BtBimA or BmBimA (Stevens *et al*. 2005a). Later experiments, in which *B. thailandensis* BimA was replaced with each BimA ortholog, showed that expression of each ortholog causes distinctive ABM parameters and actin filament organization in comet tails (Benanti, Nguyen and Welch 2015) (Fig. 2D). For example, bacteria expressing BtBimA follow more curved trajectories and assemble a dense network of F-actin. In contrast, bacteria expressing BmBimA or BpBimA move in straighter trajectories and assemble bundled F-actin strands. Moreover, bacteria expressing BmBimA are less frequently associated with actin tails, and less efficient at forming plaques in host cell monolayers and inducing MNGC formation. In a hint that BimA ortholog differences may also influence virulence, studies of Australian *B. pseudomallei* strains that express either BpBimA or BmBimA revealed that there is a significant association between expression of BmBimA and neurological melioidosis, and between expression of BpBimA and pneumonia (Sarovich *et al*. 2014). Future work using cell culture and animal models, as well as additional epidemiological studies, will reveal whether and how differences in BimA sequence and/or expression influence bacterial adaptation to particular cell types or hosts, and impacts virulence in animals and humans.

Rickettsia species RickA and Sca2

The genus *Rickettsia* encompasses more than 30 species, all of which are obligate intracellular endosymbionts or parasites. *Rickettsia* typically lives in arthropod vectors (ticks, fleas, lice and mites). They can be transmitted to mammalian hosts by arthropod bites (reviewed in Azad and Beard 1998; Uchiyama 2012), where they primarily infect endothelial cells, and can also infect macrophages, dendritic cells and other cell types (reviewed in Walker and Ismail 2008). The genus is divided into four groups that differ in host range and in the type and severity of disease caused (if any) (Gillespie *et al*. 2007, 2008). Members of the SFG, which include *Rickettsia rickettsii*, *R. conorii* and *R. parkeri*, can cause spotted fever disease of varying severity in humans and other mammals (Uchiyama 2012). Within the typhus group (TG), *R. prowazekii* causes epidemic typhus, whereas *R. typhi* causes murine typhus (Uchiyama 2012). The transitional group (TRG) species *R. felis* infects mammals, causing a flea-borne

rickettsiosis, and is considered an emerging human pathogen (Brown and Macaluso 2016). Ancestral group (AG) members such as *R. bellii* are arthropod endosymbionts and are not considered pathogenic. Species within all four groups have been suggested to undergo ABM.

Unlike other bacterial pathogens that undergo ABM, *Rickettsia* has genes encoding two different actin assembly proteins: RickA and Sca2. The *rickA* gene is present in SFG, TRG and AG species, but is absent in TG species (McLeod *et al.* 2004; Walker and Yu 2005). The amino acid sequence of RickA is generally well conserved, with greater than 45% identity between species. All RickA orthologs contain a C-terminal WCA domain similar to that in host NPFs. RickA proteins from the SFG species *R. conorii* and *R. rickettsii* were shown to activate the host Arp2/3 complex to promote nucleation and Y-branch formation *in vitro* (Gouin *et al.* 2004; Jeng *et al.* 2004), suggesting the same is true for other orthologs. In the SFG species *R. parkeri*, RickA was found to mediate an early phase of ABM (occurring 15–60 min post infection) that is characterized by slow and meandering movement and the formation of short and curved actin tails (Reed *et al.* 2014) (Fig. 2D). One function of early motility appears to be in cell–cell spread, as a *rickA::tn* mutant forms smaller foci of infection in monolayers of host cells (Reed *et al.* 2014). Although the importance of RickA in infection of arthropods and mammals remains unclear, the high degree of conservation of RickA sequence in SFG, TRG and AG species suggests that any role in infection is conserved across *Rickettsia* species. The absence of RickA in TG species is consistent with the fact that they grow to high numbers in host cells and then promote cell lysis and release to enable spread, which may bypass the need for ABM to promote spread by protrusion formation and engulfment.

In contrast with *rickA*, the *sca2* gene, encoding the autotransporter actin assembly protein Sca2, is present in most *Rickettsia* species. However, *sca2* is interrupted in *R. peacockii* and *R. canadensis*, and is truncated in *R. prowazekii*, in keeping with the failure to observe ABM for these species (Heinzen *et al.* 1993; Baldridge *et al.* 2005; Simser *et al.* 2005; Haglund *et al.* 2010). The amino acid sequence of Sca2 is well conserved within SFG species, with >70% identity throughout the passenger domain, particularly in the N-terminal and C-terminal domains that are necessary for its ability to mimic host formins (Haglund *et al.* 2010; Cardwell and Martinez 2012; Madasu *et al.* 2013), and additionally in the central WH2 and proline-rich sequences that participate in actin assembly. Sca2 from TRG species *R. felis* retains the N-terminal domain of SFG Sca2 (Fig. 3B), but with less sequence conservation, suggesting that it may also be a functional mimic of formins. However, Sca2 orthologs from the TG species *R. typhi* and the AG species *R. bellii* are very divergent in that they lack the N-terminal and C-terminal sequences conserved in SFG and TRG species, although they still contain putative WH2 motifs (Fig. 3B) (Haglund *et al.* 2010; Madasu *et al.* 2013). This suggests that TG and AG Sca2 orthologs do not mimic host formins, but may instead mimic a different class of host actin-polymerizing proteins, perhaps the tandem WH2 based or Ena/VASP family.

Differences in the actin assembly mechanisms of Sca2 orthologs may translate into differences in ABM parameters. In the SFG species *R. parkeri*, Sca2 mediates a late phase of ABM (occurring >8–24 h post infection) that is characterized by fast and directionally persistent movement, and the formation of long comet tails consisting of F-actin bundles (Reed *et al.* 2014) (Fig. 2D). Similar ABM properties and F-actin organization are observed for other SFG species (Heinzen *et al.* 1993; Gouin *et al.* 1999; Van Kirk, Hayes and Heinzen 2000), consistent with the high-sequence conservation of SFG Sca2. Although Sca2 from the TRG species *R. felis* is more divergent, the properties of *R. felis* ABM have not been described beyond that it appears to form actin tails (Ogata *et al.* 2005). Interestingly, the AG species *R. bellii*, which expresses a divergent Sca2, can nevertheless assemble long comet tails of bundled F-actin (Oliver *et al.* 2014). On the other hand, the TG species *R. typhi*, which also expresses a divergent Sca2, only infrequently forms actin tails that are shorter than those formed by other species (Teysseire, Chiche-Portiche and Raoult 1992; Heinzen *et al.* 1993; Van Kirk, Hayes and Heinzen 2000). Thus, although differences in Sca2 appear to be correlated with differences in ABM, more work is needed to better characterize the activity of Sca2 from diverse *Rickettsia* species to determine the relationship between the molecular mechanisms of actin assembly and specific ABM properties. Unfortunately, such work may be hindered by the relative difficulty of genetic manipulations in *Rickettsia* species.

Because Sca2-driven ABM is central to virulence for the SFG species *R. rickettsii* (Kleba *et al.* 2010), it is reasonable to speculate that differences in ABM characteristics between *Rickettsia* species will impact virulence. However, the role of ABM in virulence is complicated. The most recent common ancestor of the *Rickettsia* genus was capable of ABM based on the fact that the AG species *R. bellii* contains both *rickA* and *sca2* genes and undergoes ABM (Oliver *et al.* 2014). However, *R. bellii* is primarily considered a tick endosymbiont and not a pathogen, suggesting that ABM evolved as an adaptation to survival within an arthropod or other host, rather than for virulence humans or other mammals. During the evolution of virulent species, Sca2 both gained and lost functionality. In the TRG and SFG species, Sca2 acquired the N-terminal and C-terminal domains that mimic host formin proteins, and this functionality may be linked to virulence. However, in the TG species *R. typhi*, Sca2 activity may be reduced because ABM is infrequent (Teysseire, Chiche-Portiche and Raoult 1992; Heinzen *et al.* 1993; Van Kirk, Hayes and Heinzen 2000), and in *R. prowazekii* Sca2 is truncated and this species does not undergo ABM. Thus, although ABM mechanisms may play a crucial role in survival and influence virulence for some *Rickettsia* species, some species have discarded ABM entirely, and in all cases other factors certainly contribute to pathogenicity (Clark *et al.* 2015).

CONCLUDING REMARKS

The ability to undergo ABM has evolved independently in diverse genera of bacterial pathogens. ABM arose as an adaptation to bacterial life inside the cytosol of diverse eukaryotic host cells, and has been co-opted as an essential virulence strategy during infection of humans and other animals. This process enables cell–cell spread during infection, and may also affect intracellular survival by modulating host autophagy pathways. In recent years, it has become clear that bacterial pathogens have evolved to mimic or usurp the full spectrum of host actin polymerization molecules, including the Arp2/3 complex and NPFs, formins, Ena/VASP proteins and tandem-WH2-based factors. Perhaps more surprisingly, even closely related pathogens can mimic entirely different actin polymerization pathways, suggesting that there is considerable plasticity with regard to ABM mechanisms. The evolution of diversity in ABM proteins sequences and mechanisms of action may be driven by selection for specific ABM characteristics, as well as by selection for alternative roles of ABM proteins in other processes such as invasion or autophagy avoidance. Emerging information suggests that differences in ABM mechanisms may also impact the

ability of related species to colonize host organisms, and/or cause disease in humans and animals, perhaps by adapting pathogens to infect different cell types. Future investigations into the diversity of pathogen actin assembly mechanisms, and their contribution to colonization and virulence in different hosts and cell types, will illuminate the evolutionary history of ABM and further our understanding of the selective pressures that influence this important interface between intracellular pathogens and the cytoskeleton of their eukaryotic hosts.

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