Bacterial Actins and Their Interactors

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Abstract Bacterial actins polymerize in the presence of nucleotide (preferably ATP), form a common arrangement of monomeric interfaces within a protofilament, and undergo ATP hydrolysis-dependent change in stability of the filament—all of which contribute to performing their respective functions. The relative stability of the filament in the ADP-bound form compared to that of ATP and the rate of addition of monomers at the two ends decide the filament dynamics. One of the major differences between eukaryotic actin and bacterial actins is the variety in protofilament arrangements and dynamics exhibited by the latter. The filament structure and the polymerization dynamics enable them to perform various functions such as shape determination in rod-shaped bacteria (MreB), cell division (FtsA), plasmid segregation (ParM family of actin-like proteins), and organelle positioning (MamK). Though the architecture and dynamics of a few representative filaments have been studied, information on the effect of interacting partners on bacterial actin filament dynamics is not very well known. The chapter reviews some of the structural and functional aspects of bacterial actins, with special focus on the effect that interacting partners exert on the dynamics of bacterial actins, and how these assist them to carry out the functions within the bacterial cell.

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1 Family of Bacterial Actins

Actin-like proteins that perform functions similar to the eukaryotic actin cytoskeleton began to be experimentally characterized about 15 years ago. For example, MreB, the chromosomally encoded bacterial actin, which is one of the major shape-determining factors in rod-shaped bacteria, was observed to form filaments within the bacterial cell, similar to actin (Jones et al. 2001). Structure determination of MreB indeed confirmed the striking similarities with the fold of the eukaryotic actin (van den Ent et al. 2002). Subsequently, many other bacterial actin-like proteins have been characterized both functionally and structurally. The prominent examples are those involved in shape determination (MreB; Jones et al. 2001; van den Ent et al. 2001), plasmid segregation (ParM; Møller-Jensen et al. 2002), organelle positioning (MamK; Komeili et al. 2006), and cell division (FtsA; van den Ent and Löwe 2000; Szwedziak et al. 2012). Furthermore, a large number of bacterial actins, termed as ALPs or actin-like proteins have been identified in a variety of bacterial genomes, most of which are predicted to be involved in plasmid segregation (Derman et al. 2009). Phylogenetic trees depicting representatives of actin family proteins show their sequence and functional divergence (Derman et al. 2009; Draper et al. 2011; Ingerson-Mahar and Gitai 2012; Ozyamak et al. 2013b).

This chapter describes the structure of bacterial actins, and their comparison with the structure of the eukaryotic actin, both as monomers and filaments. This is followed by how the variation in filament dynamics effects the cytoskeletal functions performed by the bacterial actins. Common features between the eukaryotic and bacterial actins in the context of interactors that affect filament dynamics are discussed.

1.1 Structure of Bacterial Actins

The members of the bacterial actin families share a common fold (Bork et al. 1992) and form polymeric assemblies that assist them in their function. This section describes a brief structural comparison between various actins with respect to their monomeric fold, arrangement in protofilaments and assembly of functional filaments. There are many recent reviews on the comparison of the various bacterial actins, and hence the readers are requested to refer to them for more details (Ingerson-Mahar and Gitai 2012; Ozyamak et al. 2013b; Eun et al. 2015). The

recent novel additions to the family of actins include the four-stranded tubular structure of *Bt*ParM (Jiang et al. 2016) and crenactin from the archaea *Pyrobaculum calidifontis* (Ettema et al. 2011; Lindås et al. 2014; Izoré et al. 2014; Braun et al. 2015). Though closer to eukaryotic actin in sequence and structure, crenactin possibly forms an interesting assembly of a single protofilament, hitherto not observed in any of the actin family filaments (Izoré et al. 2014; Braun et al. 2015). Since the chapter deals with bacterial actins and the function of crenactin is not known yet, further discussion on crenactin is not included here.

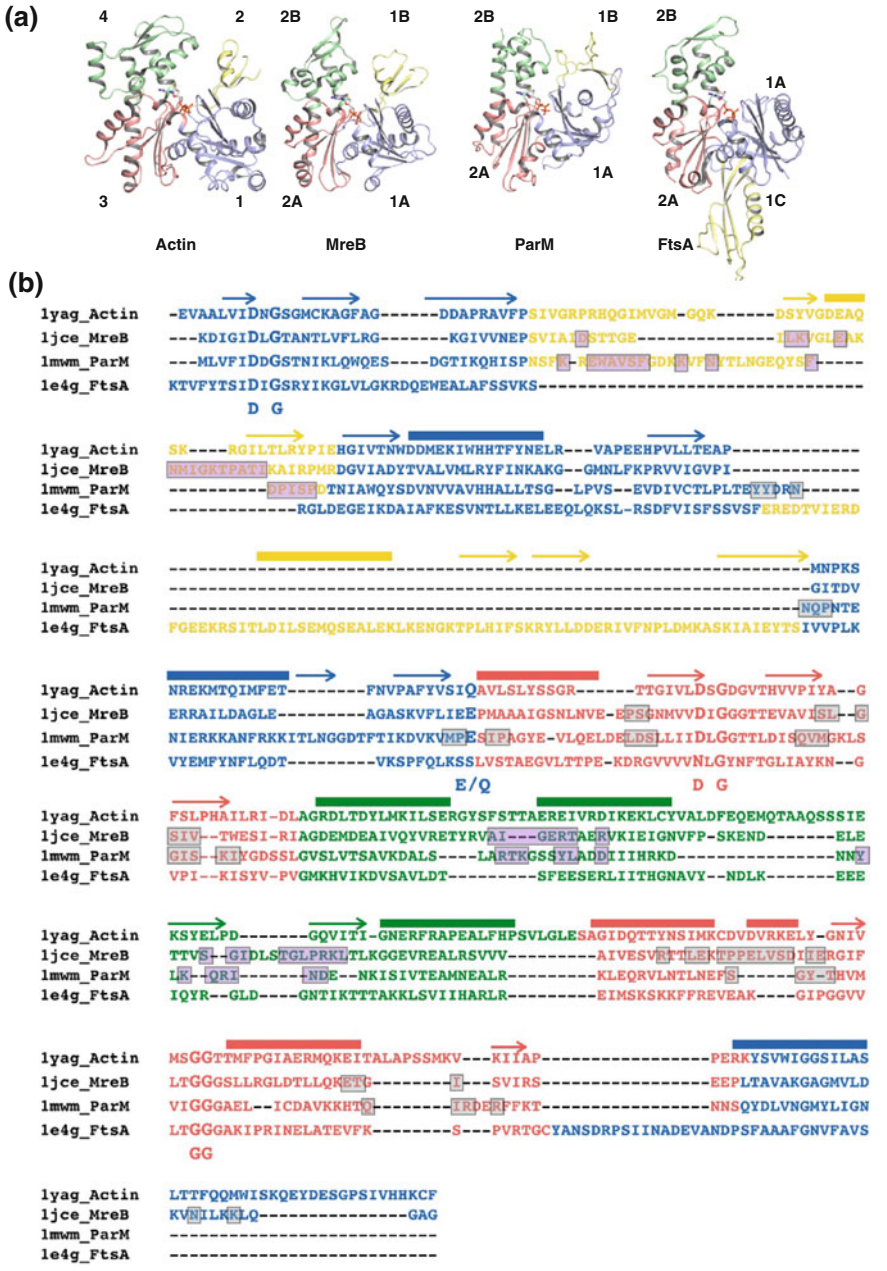
1.1.1 Monomer

All the major members of the bacterial actin families share the actin fold (Bork et al. 1992), comprising four subdomains (Fig. 1a), either termed as 1, 2, 3, and 4 (as in eukaryotic actin nomenclature) or as 1A, 1B, 2A, and 2B (according to the bacterial actin nomenclature) respectively. The largest variability among the four subdomains is observed in subdomain 2 (or 1B). Subdomain 2 (1B) is comparatively shorter in sequence in ParM. An exception is FtsA, in which subdomain 2 forms a domain-swapped arrangement (termed as subdomain 1C) and is present as an insertion within domain 1 (1A) in sequence (Fig. 1a, b; van den Ent and Löwe 2000; Szwedziak et al. 2012).

1.1.2 Protofilament

In addition to the actin fold, a common feature that unifies the bacterial actins is the assembly of their monomers into filamentous oligomeric structures. All the representative members of the bacterial actin families have an arrangement of monomers similar to that of actin within their protofilaments (Fig. 2a). In all protofilament architectures, monomers are oriented such that the interface is formed between subdomains 2 (1B) and 4 (2B) of the bottom monomer and subdomains 1 (1A) and 3 (2A) on the top monomer, respectively (Fig. 2a, residues at the interface are highlighted in the sequence alignment in Fig. 1b). The domain-swap in FtsA also allows for an actin-like protofilament formation, with the subdomain 1C occupying the position corresponding to subdomain 2 (1B) within a protofilament (Fig. 2a).

Though there is negligible sequence conservation among intra-protofilament interface residues of the different actin family proteins, the positions of the residues at the protofilament interface remain conserved (Fig. 1b). For example, Fig. 1b highlights that residues in the corresponding secondary structure elements of the subdomains form the interface for ParM and MreB. The same is true also for actin and other protofilaments in the family such as psk41 ParM (Popp et al. 2010), pB171 ParM (Rivera et al. 2011), Alp12 (Popp et al. 2012), AlfA (Polka et al. 2014), BtParM (Jiang et al. 2016), and MamK (Ozyamak et al. 2013a). The extent of the interface depends on insertions or deletions present within these sequences



(Fig. 1b). The variation in the sequences at the interface contributes towards changes in the helical parameters of the actin-like filaments (see Ozyamak et al. 2013b for figure and helical parameter comparison).

◀ **Fig. 1** The actin fold. **a** Crystal structures of the representative members of the actin family (Actin, MreB, FtsA and ParM) with the subdomains labeled and *color coded*. PDB IDs: Actin—1YAG, MreB—1JCE, FtsA—1 E4G, ParM—4A62. **b** A structure-based sequence alignment of the actin, MreB, ParM and FtsA obtained using ProMALS3D (Pei et al. 2008). The various subdomains are *color-coded* in the sequence alignment according to (**a**), and the secondary structure elements marked using *arrows* for β-strands and rectangles for α-helices. The residues at the intra-protofilament interface for MreB and ParM are highlighted in *gray* and *pink-shaded boxes* respectively for the *top* and *bottom* monomers in a protofilament. The interface residues were identified using the PDB-ePISA webserver (http://www.ebi.ac.uk/pdbe/pisa/) for the PDB IDs 1JCE (MreB) and 5AEY (ParM)

1.1.3 Filament Architecture

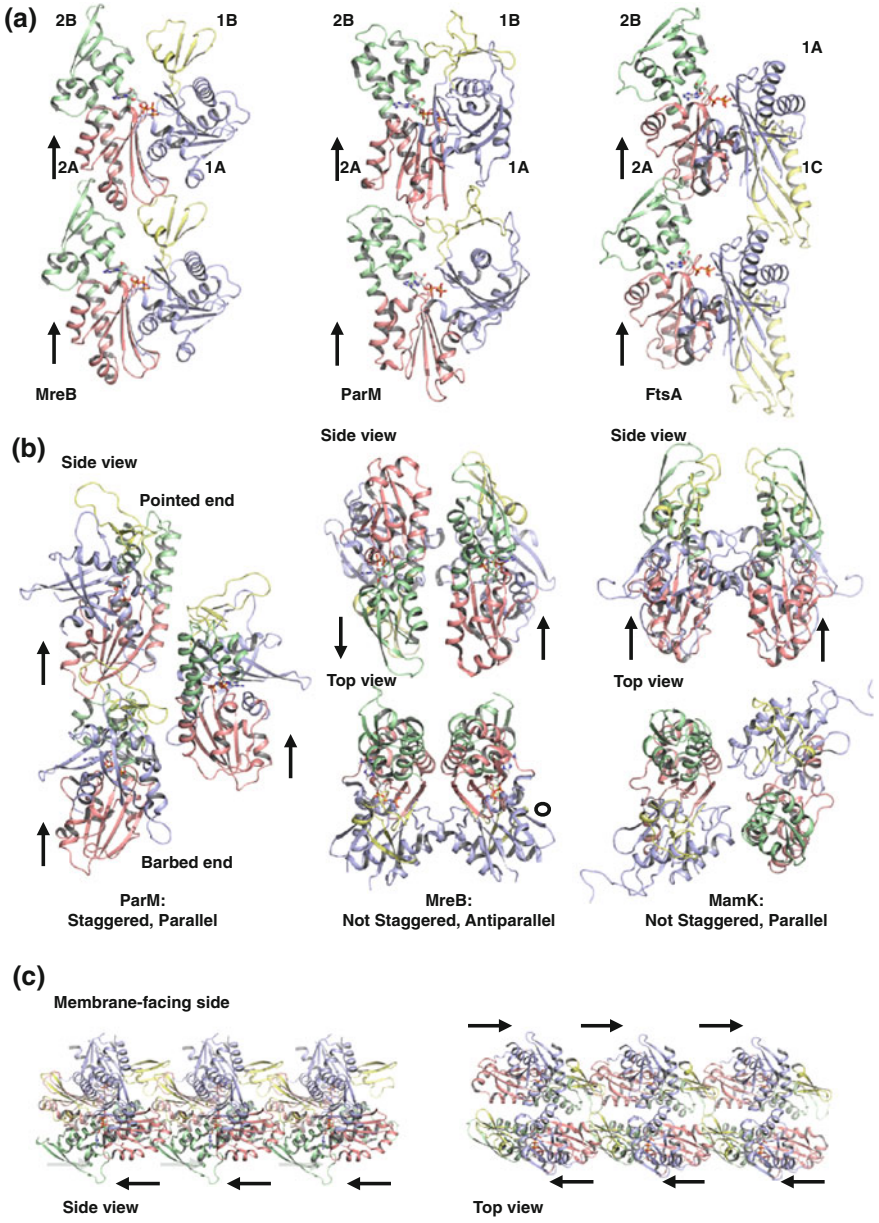
Eukaryotic actin can be described as a two-stranded filament with the two protofilaments coming together in a right-handed twist. Since the protofilaments are arranged in a parallel fashion, there is a polarity for the filament, the two ends termed as pointed end and barbed end, respectively (Fig. 2b). This is conserved across actins of all eukaryotic organisms. In contrast, in bacterial actins, the protofilaments come together in different ways to form a functional filament (Ozyamak et al. 2013b).

Antiparallel arrangement of MreB

For MreB, there are in vitro observations and in vivo crosslinking studies suggesting that the two protofilaments come together in an antiparallel fashion (Fig. 2b, van den Ent et al. 2014). This arrangement results in the orientation of the membrane-binding residues towards the membrane in all the monomeric subunits of the filament (Fig. 2c; Salje et al. 2011). This facilitates interaction with RodZ and other proteins of the cell wall synthesis machinery that are located within the cell membrane (van den Ent et al. 2010; see Sect. 2.1).

Bacterial actins in plasmid segregation

The two-stranded filament, similar to eukaryotic actin but with varying helical parameters, is the most common assembly of protofilaments that has been characterized till now. Some examples of bacterial actins performing plasmid segregation that form two-stranded filaments are *E. coli* R1 plasmid ParM (van den Ent et al. 2002; Bharat et al. 2015), pB171 ParM (Rivera et al. 2011), AlfA (Polka et al. 2009), psk41 ParM (Popp et al. 2010), etc. In these filaments, the two protofilaments occur in a staggered and parallel arrangement (Fig. 2b), and hence these have a structural polarity (two structurally dissimilar ends) similar to eukaryotic actin filaments, though the helical parameters including handedness and the twist differ. The existence of structural polarity implies that the adaptor protein that links the bacterial actins to the plasmid DNA can potentially interact only at one end of the filament. Hence, to achieve plasmid segregation, there should be an antiparallel arrangement of structurally polar filaments. In *E. coli* R1 plasmid ParM, it has been demonstrated that ParR*C* complex binds only to the barbed end of the double-stranded actin-like filament, and two such filaments can come together in an



antiparallel manner to achieve plasmid segregation (Gayathri et al. 2012). Variations of this exist in the different bacterial actins involved in plasmid segregation. AlfA also forms filaments composed of two protofilaments in a parallel, staggered arrangement (Polka et al. 2014). During plasmid segregation, bundles of filaments in mixed orientation may be present (Polka et al. 2014).

◀ **Fig. 2** Filament architecture of bacterial actins. **a** Two adjacent monomers within a protofilament for MreB, ParM and FtsA highlight the similarity in subdomain arrangement (PDB IDs: MreB—1JCE, ParM—5AEY, FtsA—4A2B). The *arrows point* in the direction from subdomains 2A–2B. **b** Monomers in staggered versus unstaggered and parallel versus antiparallel arrangements in ParM, MreB and MamK filaments. Two perpendicular views (*side* and *top views*) are shown for MreB and MamK to illustrate the parallel and antiparallel orientations. The *arrows point* in the direction from subdomains 2A–2B, *closed* and *open circle* denotes an *arrow* pointing outside and inside of the plane respectively. PDB IDs: MreB—1JCE, MamK—fitted model in the EM reconstruction (Ozyamak et al. 2013a; http://faculty.washington.edu/jkoll/structures.html) **c** Antiparallel protofilaments of MreB (PDB ID: 4CZJ). The N-terminal residues and the loop between residues 97–104 (not modeled in the crystal structure) of subdomain 1A face the membrane-binding side. Two views of the filament arrangement are shown for illustrating the antiparallel nature. *Light gray arrows* represent the direction of the monomers behind the plane

In addition to two-stranded filament architecture, novel assemblies of four-strand and tubular structures have also been recently characterized for the Alp family of bacterial actins involved in plasmid segregation. In Alp12, the protofilament arrangement itself consists of parallel and antiparallel orientations, forming a four-stranded filament of novel arrangement with mixed orientation within it (Popp et al. 2012). Newly identified filament architecture of *Bt*ParM from *Bacillus thuringensis* demonstrates the formation of a double-stranded filament consisting of two protofilaments in antiparallel orientation in the presence of nucleotide, with an elaborate twist (Jiang et al. 2016). In the presence of *Bt*ParR, two such double-stranded filaments come together to form a tubular structure made of four protofilaments. The four-stranded tubular structure thus formed consists of alternate protofilaments in parallel and antiparallel orientations.

The reasons for the variability of protofilament organization, especially as observed among bacterial actins in the same function of plasmid segregation, are intriguing. ParM, AlfA, pB171, Alp12, and *Bt*ParM are all involved in plasmid segregation. However, the protofilament arrangement in these bacterial actins range from variation of two-stranded filaments as in ParM and AlfA, four-stranded filaments as in Alp12, and tubular architecture of four-stranded filaments as in *Bt*ParM. The variability might be the resultant of regulation of filament dynamics by binding partners. The thickness of the filament might have evolved related to the load (size of the plasmid that acts as the cargo), the mechanism of chromosome segregation and cell division in the particular bacterium, etc. These are factors that remain to be investigated.

Parallel and unstaggered arrangement in MamK

In MamK, the bacterial actin involved in magnetosome formation and positioning in magnetotactic bacteria, the two protofilaments are oriented in parallel, but do not possess a stagger as present in actin or other ParM filaments (Fig. 2b). This arrangement has been attributed to an insertion in the inter-protofilament interface in MamK structures (Ozyamak et al. 2013a). The functional relevance of such an architecture compared to a staggered configuration is unknown. The stagger of monomers between protofilaments can have implications with respect to rate of

growth of the filaments, as the presence of a stagger can contribute towards an added interface for an incoming monomer.

FtsA

Among the bacterial actins, very less is known about the filament architecture of FtsA. Protofilament of FtsA has been observed in its crystal structure (Fig. 2a), however the further assembly of its protofilaments that form the functional state is not known. FtsA interacts closely with the tubulin cytoskeleton, namely the FtsZ protein, and contributes to ring constriction in bacteria leading to cell division.

The next section discusses the effect of filament architecture on dynamics of assembly of bacterial actins.

1.2 Dynamics of Filament Assembly

An important feature of a cytoskeletal filament such as actin or tubulin, which undergoes nucleotide dependent polymerization, is filament dynamics. Filament dynamics refers to the characteristics of filament assembly and disassembly. Dynamics exhibited by actin-like and tubulin-like filaments is the result of the relative stabilities between the NDP versus NTP conformations within the filament. Typical filament dynamics behavior of actin and tubulin family proteins includes treadmilling and dynamic instability, respectively. Similar to the variety of protofilament architectures observed, the filament dynamics of the bacterial actins also tend to vary (Garner et al. 2004, 2007; Popp et al. 2010; Gayathri et al. 2012; Polka et al. 2014).

1.2.1 MreB

The protofilament architecture of MreB and in vivo studies suggest that MreB forms an antiparallel arrangement of protofilaments (van den Ent et al. 2014). This implies that the assembly of MreB filaments is structurally nonpolar, in contrast to the eukaryotic actin filaments that exhibit structural and kinetic polarity. Short MreB filaments have been observed to rotate circumferentially perpendicular to the longitudinal axes of rod-shaped cells (Garner et al. 2011; Domínguez-Escobar et al. 2011; van Teeffelen et al. 2011). The role of filament dynamics in this process, if any, has not been confirmed so far. The rotation seems to be dictated by the cell wall synthesis machinery, as suggested by studies characterizing the role of RodZ and DapI in MreB circumferential movement (Morgenstein et al. 2015; Reuff et al. 2014). It has also been proposed that DapI can bind to unpolymerised MreB, and thus initiates the assembly of the peptidoglycan machinery on DapI, when MreB attaches to the membrane upon polymerization (Reuff et al. 2014). Another study showed that MreB filaments dissociate from the cell membrane on depletion of cell

wall precursors, and hence the filament dynamics depend on the availability of the precursors (Schirner et al. 2014). There have been some earlier studies in *Caulobacter crescentus*, which suggest that MreB filaments treadmill (Kim et al. 2006). With the current idea of antiparallel arrangement of MreB filaments and the concept of circumferential motion of MreB driven by peptidoglycan synthesis, what contributes to the kinetic polarity of MreB filaments is unknown.

1.2.2 Bacterial Actins in Plasmid Segregation

Actin-like proteins in plasmid segregation are most well studied with respect to filament dynamics, since many of the plasmid segregation systems, consisting of three components, have been reconstituted in vitro. Hence, filament dynamics have been characterized both in vitro and in vivo for these. Similar to the filament architecture, the filament dynamics also vary between the different bacterial actins. ParM exhibits dynamic instability (Garner et al. 2004). ParM assembles into filaments in the presence of ATP without the requirement of a nucleating factor, and disassembles once ATP is hydrolysed. ParM filaments are stabilized in the presence of ParR*C* complex (formed by the adaptor protein ParR that links ParM to the centromeric sequence *parC*) (Garner et al. 2007). ParR*C* complex also functions towards increasing the rate of growth of filaments from one end, thus modulating filament dynamics (Gayathri et al. 2012). AlfA filaments are stable and tend to bundle in the absence of AlfB (Polka et al. 2009). In the presence of AlfB and *parN*, AlfA filaments exhibit treadmilling (Polka et al. 2014). The dynamics of filaments such as Alp7A has been looked at within the bacterial cell (Derman et al. 2009). BtParM forms tubular four-stranded filaments in the presence of BtParR (Jiang et al. 2016).

To summarize the observed trends, the adaptor-DNA complex modulates the filament dynamics in plasmid partitioning systems, such that proper segregation through directional movement of plasmids towards opposite ends is achieved after the plasmid DNA is captured at the structurally polar filament ends.

1.2.3 MamK

In vitro characterization of polymerization dynamics using an untagged version of MamK protein showed that MamK polymerizes in the presence of ATP and not ADP (Ozyamak et al. 2013a). Filament disassembly is triggered once ATP is hydrolysed, as observed from measurements using light scattering and ATP hydrolysis. MamK filaments are more stable compared to other bacterial actins such as ParM due to bundling dependent on salt concentration. Single molecule fluorescent studies to observe growth differences between the two ends have not yet been performed for MamK. An interesting study in the filament dynamics of MamK involves the characterization of polymerization properties along with the

MamK-like protein present in a magnetosome island (Abreu et al. 2014). More details on MamK dynamics in the presence of interactors are reviewed in a later section (Sect. 2.3).

1.2.4 FtsA

FtsA filaments have been observed along with FtsZ as a sandwich between the cell membrane and FtsZ filament in cryotomography of *Caulobacter* cells (Szwedziak et al. 2014). The same study also observed FtsA and FtsZ filaments in liposomes. Swirling movement of fluorescently labeled FtsZ was observed in an in vitro reconstitution of FtsZ and FtsA filaments on a lipid layer (Loose and Mitchison 2014). The filament formation requires the presence of either ADP or ATP, and nucleotide hydrolysis is not a requirement. ATP hydrolysis activity has not been detected yet for FtsA (Lara et al. 2005). It is probable that FtsA does not hydrolyse ATP especially since the canonical catalytic residues of the actin fold are absent (Fig. 1b). FtsA filaments act cooperatively along with FtsZ to assemble the FtsZ ring to the cell membrane and to cause ring constriction. One of the mechanisms of ring constriction proposes that the mismatch in the filament dimensions between FtsA ($\sim$5 nm repeat distance) and FtsZ filaments ($\sim$4 nm repeat distance) contributes towards the generation of curvature required for constriction (Szwedziak et al. 2014).

With the above brief introduction to the structure, function, and dynamics of the prominent members of the bacterial actin families, I now move on to the various interacting proteins that have been characterized for bacterial actins. Eukaryotic actins are very well conserved, and one of the reasons proposed for its high conservation is the wide variety of interacting proteins that regulate its polymerization (Gunning et al. 2015). One of the distinguishing features between prokaryotes and eukaryotes was proposed to be the absence of interacting proteins to the bacterial cytoskeletal family that are similar to nucleators and molecular motors that walk along the tracks (Theriot 2013). The next section gives an overview of the various interacting proteins of bacterial actins that affect their dynamics and the functional significance of these interactions. However, the molecular basis of the correlation between dynamics and function of many interactors of the bacterial cytoskeleton is not known yet.

2 Interactors of Bacterial Actins

A large number of proteins that interact with the eukaryotic actin cytoskeleton are known. Eukaryotic actins require accessory proteins that assist in nucleation, elongation, etc. Formins, profilin, cofilin, Spire, fascin, etc., are some of them (Chesarone and Goode 2009; Campellone and Welch 2010; Dominguez and Holmes 2011; Breiher 2013). Bacterial pathogens very often hijack the host actin cytoskeleton with the help of nucleating factors that enable formation of actin

filaments that aid their entry or movement within the host cell (Bugalhão et al. 2015). They can also manipulate the cytoskeleton through controlling the GTPases that regulate the cytoskeleton. In bacteria, the information about interactors and how they modulate the bacterial cytoskeleton dynamics is limited. The current section reviews some of the available information on the bacterial cytoskeleton in this regard.

2.1 Interactors of MreB

MreB is the chromosomally encoded bacterial actin responsible for the rod shape in bacteria (Jones et al. 2001). The rotation of MreB filaments within the cell was proposed to be the result of the action of the cell wall biosynthesis machinery (Garner et al. 2011; Domínguez-Escobar et al. 2011; van Teeffelen et al. 2011). MreB is located near to the cell membrane, and it directly interacts with the cell membrane through an amphipathic helix at the N-terminus (in Gram-negative bacteria), or in combination with a hydrophobic loop (in Gram-positive bacteria) (Salje et al. 2011). The direct interaction of MreB with the membrane also brings it into proximity for interactions with other components of the cell wall elongation machinery such as MreC, MreD, RodZ, and the penicillin binding proteins PBP-1a and PBP2.

In addition to cell-shape determination, MreB also plays a role in various functions such as chromosome segregation (Kruse et al. 2003; Gitai et al. 2005), cell motility, positioning of molecules involved in pili formation (e.g., *Pseudomonas*, Cowles and Gitai 2010) and motility complexes (e.g., *Myxococcus xanthus*, Mauriello et al. 2010). MreB has also been shown to directly interact with FtsZ (Fenton and Gerdes 2013). Most of these functions of MreB have been implicated based on experiments carried out with a small molecule inhibitor of MreB named A22 (Iwai et al. 2002). However, a direct interaction and a molecular mechanism have been shown for very few proteins. The following subsections give details about the proteins that are known to interact with MreB.

2.1.1 RodZ

MreB is linked to the motion of the cell wall synthesis machinery through contacts with RodZ, which has also a periplasmic domain (Alyahya et al. 2009; Bendezú et al. 2009). Interaction between the cytoplasmic domain of RodZ and MreB has been characterized biochemically and structurally (van den Ent et al. 2010). Since the interface is away from the membrane-binding face of the monomer or the filament, RodZ binds MreB in both the monomeric and filament form, and facilitates membrane binding of the polymerized MreB. This is the only crystal structure published till date of an interactor in complex with MreB (Fig. 3a; PDB ID: 2WUS).

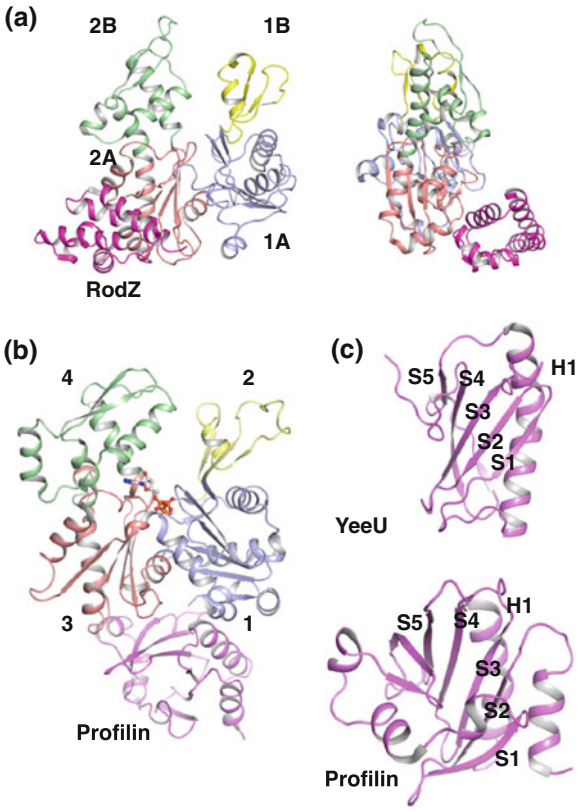


Fig. 3 Interacting proteins of MreB **a** Crystal structure of MreB complexed with RodZ. Two perpendicular side views are shown to highlight the interaction with subdomain 2A. (PDB ID: 2WUS) **b** Crystal structure of actin complexed with profilin (PDB ID: 2BTF) **c** Structural comparison of YeeU (PDB ID: 2H28) with profilin. The corresponding secondary structure elements between YeeU and profilin in DALI (Holm and Rosenström 2010) superposition are labeled

2.1.2 MbiA

A filament disruptor of MreB is MbiA in *Caulobacter crescentus* (Yakhnina and Gitai 2012). The function of MbiA is not known. It was identified as a protein that colocalises with MreB. Overexpression studies of MbiA showed that it disrupts MreB filaments, producing an effect very similar to that of A22, the small molecule inhibitor specific to MreB.

2.1.3 YeeU and YeeV

YeeU and YeeV form a toxin–antitoxin (TA) system in *E. coli* (Brown and Shaw 2003). The TA pair interacts with both MreB and FtsZ cytoskeletons in bacteria (Tan et al. 2011). YeeV acts to disrupt or destabilize both MreB and FtsZ filaments, resulting in the formation of lemon-shaped cells. Hence, YeeV has been renamed as CbtA (Cytoskeleton Binding Toxin A). In contrast, the corresponding antitoxin of

YeeV, YeeU functions as an antitoxin by exerting an antagonist effect on the cytoskeleton (Masuda et al. 2012). YeeU also interacts with both FtsZ and MreB. It causes bundling of the cytoskeletal filaments, thereby preventing their disassembly. The effects of YeeU makes the filaments insensitive to other inhibitors such as A22, M265 of MreB, and SulA, MinC, etc., of FtsZ. YeeU has been consequently named as CbeA (Cytoskeleton Bundling Enhancer A). The action of these could be similar to the actin severing and bundling proteins, such as cofilin and fascin, though sequence or fold similarities have not been detected. Structural comparison using DALI server (Holmes and Rosenström 2010) shows that the crystal structure of YeeU (Arbing et al. 2010; PDB ID: 2H28) has structural similarities to proteins of the profilin fold (Fig. 3b). The interaction site on the actin monomer or filament has to be identified for such comparisons to obtain mechanistic insights.

2.1.4 Interaction Partners in *Myxococcus xanthus* Motility

Motility in *Myxococcus xanthus* and the frequent reversals between its leading pole and the lagging pole involve movement of motor complexes such as AglQRS (equivalent to the MotA and MotB complexes of flagellar motor; Nan et al. 2011, 2013) and AglZ (Mignot et al. 2007) and regulatory components namely the small GTPase MglA and its GTPase activating protein MglB (Nan et al. 2015). These complexes move in a helical track along the body of the organism. Since the components of the motor complexes extend till the substratum and facilitate the movement of the organism, these have been termed as focal adhesion-like complexes, drawing parallels from the eukaryotic cell crawling (Mignot et al. 2005, 2007; Nan and Zusman 2016). The helical track has been hypothesized to be MreB based on the disruption of movement upon treatment with A22 (Mauriello et al. 2010; Nan and Zusman 2016). Interaction of *Myxococcus xanthus* MreB with AglZ, one of the cytoplasmic components of the focal adhesion-like complex, has been demonstrated based on pull-down assays (Mauriello et al. 2010). A recent in vitro experiment involving *Myxococcus* MreB and MglA, a small Ras-like GTPase involved in spatial positioning of motility complexes during reversals show that MglA interacts with MreB (Treurner-Lange et al. 2015; Nan et al. 2015). The interaction between MreB and the small Ras-like GTPase homologue in spatial positioning of motility complexes requires to be understood at a molecular level in order to delineate the role of the cytoskeleton in the process. The effect, if any, of the interaction on the filament dynamics of MreB will also be interesting to understand. Many other components in *Myxococcus xanthus* motility such as FrzCD, a part of the chemosensory pathway, also interacts with MreB either directly or indirectly through unknown mediator proteins (Mauriello et al. 2009).

2.1.5 Filament Interactors of MreB

EF-Tu

In addition to its role in translation, EF-Tu has been shown to form a helical localization near the cell membrane, similar to MreB localization. The filaments formed by EF-Tu were observed to be static as opposed to the dynamic filaments of MreB, and the two proteins were shown to interact in vivo and in vitro (Defeu Soufo et al. 2010). An in vitro study characterizing the filament dynamics of MreB using light scattering and fluorescence microscopy showed that the polymerized content of MreB is accelerated in the presence of EF-Tu (Defeu Soufo et al. 2015). Further studies on the significance of this interaction and its role in cell shape determination, and the molecular mechanism behind the modulation of MreB filament dynamics by EF-Tu have to be carried out. Interestingly, an interaction between the eukaryotic orthologue of EF-Tu, EF-1A and the eukaryotic actin has also been observed (Yang et al. 1990; Gross and Kinzy 2005).

Spiroplasma Fibril

An example of plausible interfilament interaction between bacterial cytoskeletal filaments is the interaction between Fib, a novel bacterial cytoskeletal protein in the helical cell wall-less bacterium, *Spiroplasma* and MreB (Kürner et al. 2005). There are five different MreBs in *Spiroplasma* and the role of these MreBs in shape determination and motility has not been established yet (Ku et al. 2014). Based on electron cryotomography images of *Spiroplasma*, and the spacing between the layer lines, the interacting filaments have been hypothesized to be Fib and MreB (Kürner et al. 2005). The role of filament dynamics of MreB, if any, in the kinking motion of *Spiroplasma* (Shaevitz et al. 2005) is yet to be established.

2.2 *Filament Stabilizers and Nucleators of Bacterial Actins in Plasmid Segregation*

Examples of interactors that modulate filament dynamics are known for bacterial actins involved in plasmid segregation. Among the three components of the plasmid segregation system, the adaptor protein and its complex with the plasmid DNA formed at the centromeric sequence influences the dynamics of the actin-like cytoskeleton (Garner et al. 2007; Gayathri et al. 2012; Polka et al. 2014).

Structural information on the interaction between the bacterial actin and the adaptor protein has been characterized for the ParMR*C* system (Gayathri et al. 2012). The crystal structure of ParM complexed with interacting C-terminal peptide of ParR provided a description of the interaction between a bacterial actin and a modulator of filament dynamics. Structural information of the interacting complex (PDB ID: 4A62), along with the ParR*C* ring structure and a high-resolution filament

reconstruction provided mechanistic insights into the stabilization of ParM filaments by ParR*C* (Gayathri et al. 2012). The dimensions of the ParR*C* ring matches with the short pitch helix dimensions of the ParM filament, and hence provides an explanation of how ParR*C* could accelerate the growth of ParM filaments at one end only. The mechanism of elongation stimulated by ParR*C* has been proposed to be analogous to the formin-assisted elongation of actin filaments (Gayathri et al. 2012). The interaction between ParM and ParR also reveals the striking similarity with the interaction of actin polymerization modulators such as Spire, formin, cofilin, twinfilin, etc., which all interact through an amphipathic helix recognizing the cleft between subdomains 1 and 3 of actin (Fig. 4). Thus the interactors of bacterial actin filaments also appear to utilize a similar strategic location on the actin fold for regulating filament dynamics and comes within the purview of the unifying hypothesis put forward for eukaryotic actin-binding proteins (Dominguez 2004; Dominguez and Holmes 2011).

The molecular basis of how other adaptor proteins and DNA complexes effect changes in their respective filament dynamics and enables plasmid segregation is yet to be elucidated. Though the filaments are of the same fold, the interacting proteins need not necessarily belong to the same fold. The number and arrangement of the repetitive sequences in the centromeric sequences (iterons) that are the binding sites of the adaptor protein also differ resulting in different geometries of the adaptor-DNA complexes. This corroborates with the different helical geometries observed for the corresponding actin-like filaments. The variety of plasmid segregation mechanisms based on actin-like filaments with varied filament architecture ensures that there are a large number of possible ways of manipulation of the dynamics of actin-like filaments. This will also ensure that a particular filament motor associated with the plasmid segregation machinery exclusively segregates the related plasmid.

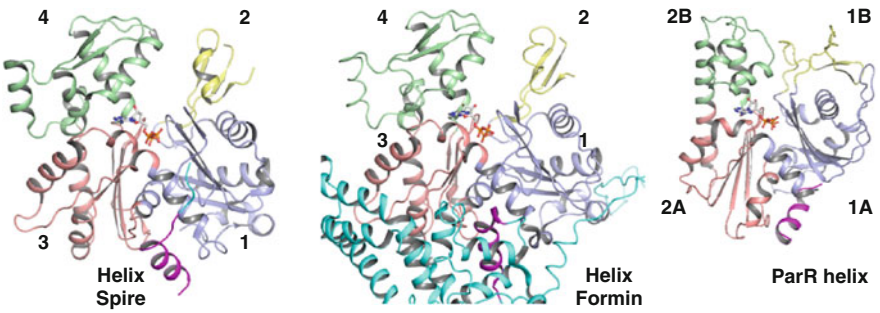


Fig. 4 ParR and the actin-binding proteins Spire and formin interact with the actin fold at a pocket formed between subdomains 1A and 2A (subdomains 1 and 3 for actin). The interacting helix is highlighted in pink and the rest of Spire and formin is shown in cyan. (PDB IDs: Actin-Spire—3MMV, actin-formin—1Y64, ParM-ParR—4A62)

E. coli R1 ParM is a two-stranded staggered filament and the organization of ParR on *parC* iterons forms a helical structure that matches the filament geometry. Similarly, it is probable that the short oligomeric structures of BtParR direct the formation of the tubular structure of BtParM. This can be hypothesized because the four-stranded structure is formed only in the presence of BtParR (Jiang et al. 2016). AlfB, the adaptor protein of AlfA is also capable of nucleating AlfA filaments on one end and destabilizing the other end (Polka et al. 2014). The mechanism of regulation of AlfA dynamics by AlfB is also not known. These are probable examples of how the adaptor protein-DNA complex assemblies can nucleate actin filaments of varied architecture.

2.3 *Interactors of MamK*

MamK, the bacterial actin involved in magnetosome positioning, has a few potential interactors present on the magnetosome genomic island (Draper et al. 2011; Pan et al. 2012). Potential interactors of MamK were identified based on screening for candidate genes encoded by the magnetosome genomic island. Interactors that have been identified till date are MamJ and the MamJ-like protein LimJ (Draper et al. 2011). Both of them affect the dynamics of MamK filaments in vivo. Filament turnover of MamK-GFP filaments in vivo were observed only in the presence of MamJ and LimJ expression. Destabilization of the MamK filaments within the cell by MamJ and LimJ is required for the proper formation of the magnetosome cluster. It is not yet established if their interaction with MamK is direct or mediated through other proteins.

Another interesting example of an interacting protein of MamK is Amb0994, a protein similar to a methyl-accepting chemotaxis protein (MCP), with a cytoplasmic localization in the magnetotactic bacteria *Magnetospirillum* (Philippe and Wu 2010). The protein localizes at the tip of MamK, towards the poles. MamK filaments sense the change in the magnetosome alignment in response to the magnetic field, which in turn transmits the signal to the MCP-like protein. This signals to the rest of the machinery equivalent to the chemotaxis pathway and results in directed movement of the bacterium. Cytoskeletal filaments in eukaryotic organisms also respond to a magnetic field, as observed in sockeye salmon and honey bee (Mann et al. 1988; Hsu et al. 2007). In these, torque linked to magnetic crystal clusters result in the opening of ion channels through signaling mediated by the cytoskeleton.

In the magnetosome islet outside the genomic island, another protein similar to MamK, called MamK-like has been found encoded in the bacterium, *Magnetospirallum magneticum*. Interplay between MamK-like and MamK in magnetosome positioning has been characterized (Abreu et al. 2014). It has been observed in vivo that both MamK and MamK-like can coexist in filaments in vivo. The presence of an alanine corresponding to the conserved catalytic glutamate of

actins raised a suspicion that MamK-like may not perform ATP hydrolysis and might result in more stable MamK filaments if they were capable of copolymerization. However, studies showed that MamK-like also possessed ATP hydrolysis activity, though slightly weaker than MamK. The presence of MamK and MamK-like monomers within the same filaments or interactions between MamK-like and MamK filaments might act as a novel means of regulation of MamK filament dynamics.

A molecular mechanism behind any of the interacting partners with MamK has currently not been characterized.

2.4 *Interactors of FtsA*

As a component of the divisome, FtsA, is a bacterial actin that connects the FtsZ filaments in the divisome ring with the cell membrane and the other divisome components in the membrane. Filaments of FtsA have been observed in vivo. The C-terminal tail of FtsZ binds to FtsA at the domain 2B (Fig. 5; Szwedziak et al. 2012). More than other interactors that affect FtsA dynamics, FtsA seems to be a major player in affecting FtsZ dynamics and curvature, thereby assisting ring constriction. The combined action of FtsA and FtsZ is an example of an interaction between two different cytoskeletal filaments, which contribute towards performing the cytoskeletal function of ring constriction. It is interesting to note here that the dynamics of FtsA may not play a role in this process, since there is no requirement of ATP hydrolysis and FtsA does not appear to hydrolyse ATP. The architecture of FtsA monomer with the swapped 1C domain might also imply that the regulatory region of the actin fold between domains 1 and 3 is not accessible to external factors.

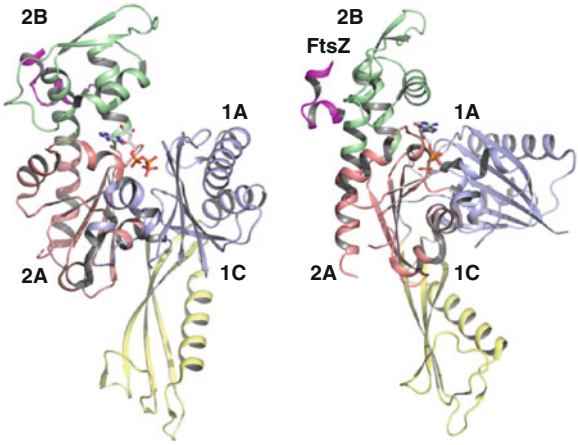


Fig. 5 Interaction between FtsA and FtsZ C-terminal tail. Two side views are shown to highlight the interaction of FtsZ with subdomain 2B. (PDB ID: 4A2A)

3 Comparison Between Interacting Proteins of Eukaryotic and Bacterial Actins

The existence of multiple types of actin filaments for multiple functions (structural diversity) of bacterial actins ensures that these are available for carrying out the various functions and hence the requirement for a large number of regulatory components is avoided in a bacterial system. The variability in a common scaffold (e.g., actin fold) results in the formation of a variety of filament structures ranging from single-stranded filaments to tubules.

An interesting feature of cytoskeletal filaments is the assembly of filaments into higher order structures such as cytoskeletal organization in muscle, spindle formation, formation of cilia, or flagella in eukaryotes. Such an organization is brought about by orientational arrangement of cytoskeletal filaments aided by molecular motors such as myosin, dynein, and kinesin (Theriot 2013). In bacteria, in the absence of molecular motors equivalent to myosin, kinesin, and dynein, a definite arrangement of cytoskeletal filaments has not been characterized yet. The discovery of a directional arrangement of ParM filaments to form a bipolar spindle demonstrates that such arrangements are possible without the presence of molecular motors, if the surface characteristics of the filaments allow for complementarity and the interaction energetics permits interfilament sliding (Gayathri et al. 2012).

Another function carried out by molecular motors in eukaryotes is transport of cargo. Analogous spatial positioning in bacteria appears to be effected by the dynamics of the filament itself. Hence, the interactors that modulate the filament dynamics assist in spatial positioning, as exemplified by the bacterial actin-like filaments in plasmid partitioning.

The study of the bacterial actin family demonstrates that diversification according to the functional necessity seems to be the path chosen for carrying out the various functions in bacteria. In contrast, in eukaryotic systems, the various functional requirements are executed by the regulatory components that interact with the actin cytoskeleton, while the cytoskeleton itself is highly conserved. Analogues of many cytoskeletal interactors and functions carried out by the eukaryotic cytoskeletal components appear to be present also in the bacterial cytoskeletal systems. However, the absence of sequence or structural similarity precludes the search of such equivalents by existing similarity-based computational search methods. Hence one of the approaches to identify eukaryotic analogues in prokaryotic systems is to perform searches based on functional similarity. This can be achieved through understanding the molecular mechanism of fundamental processes such as spatial positioning, chromosome segregation, cell division, etc., which are the functions carried out by the cytoskeleton in a typical eukaryotic scenario. This will help us to obtain profound insights on the evolution of the unity and diversity in cytoskeletal systems across bacteria, archaea, and eukaryotes.

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