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MOLECULES OF THE BACTERIAL CYTOSKELETON

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■ **Abstract** The structural elucidation of clear but distant homologs of actin and tubulin in bacteria and GFP labeling of these proteins promises to reinvigorate the field of prokaryotic cell biology. FtsZ (the tubulin homolog) and MreB/ParM (the actin homologs) are indispensable for cellular tasks that require the cell to accurately position molecules, similar to the function of the eukaryotic cytoskeleton. FtsZ is the organizing molecule of bacterial cell division and forms a filamentous ring around the middle of the cell. Many molecules, including MinCDE, SulA, ZipA, and FtsA, assist with this process directly. Recently, genes much more similar to tubulin than to FtsZ have been identified in Verrucomicrobia. MreB forms helices underneath the inner membrane and probably defines the shape of the cell by positioning transmembrane and periplasmic cell wall–synthesizing enzymes. Currently, no interacting proteins are known for MreB and its relatives that help these proteins polymerize or depolymerize at certain times and places inside the cell. It is anticipated that MreB-interacting proteins exist in analogy to the large number of actin binding proteins in eukaryotes. ParM (a plasmid-borne actin homolog) is directly involved in pushing certain single-copy plasmids to the opposite poles by ParR/*parC*-assisted polymerization into double-helical filaments, much like the filaments formed by actin, F-actin. Mollicutes seem to have developed special systems for cell shape determination and motility, such as the fibril protein in *Spiroplasma*.

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INTRODUCTION

Bacteria possess clear homologs of actin and tubulin (1, 2, 37, 157). FtsZ is the bacterial tubulin homolog, and MreB and ParM are bacterial actin homologs. These proteins carry out functions similar to their counterparts in eukaryotic cells, and the structures of their filaments as well as of the monomers closely resemble actin and tubulin. Interestingly, no other component of the "bacterial cytoskeleton" has yet been found to be similar to a eukaryotic homolog, highlighting the evolutionary distance between these organisms as well as the fact that the last common ancestor of the cytoskeleton was probably very simple. A number of proteins modulate the function of these bacterial filamentous proteins, stressing the need for many auxiliary proteins to tame the power of polymerizing proteins inside a cell, just as in eukaryotes. The lack of a wider conservation of the overall architecture of the cytoskeletons between bacteria and eukaryotes is probably also the reason why it took so long to discover filamentous proteins organizing the cytosol in bacteria. Another important reason has been the lack of positional information for bacterial macromolecules, mainly due to the small size of the cells. In fact, discovery of the FtsZ ring in *Escherichia coli* using immunofluorescence was one of the first reports showing the localization of a protein in bacteria (11). There are many proteins, nucleic acids, and cell wall components localized to certain parts of the bacterial cell, these having been investigated primarily by using GFP fusion proteins [first accomplished by Ma et al. (99)]. There are also proteins that move at a rapid rate [first demonstrated by Raskin & de Boer (130)], although it is unclear whether this is by an active transport mechanism or modulated diffusion. Cell shape is another example of a phenomenon that requires accurate placement of a large number of cellular components [first shown to be related to the bacterial cytoskeleton by Jones et al. (73)]. An exciting recent development is the finding of actin-like proteins in plasmid segregation and the filamentous nature of chromosome segregation proteins, possibly providing the first glimpse of a mechanism responsible for DNA segregation in bacteria (109). Some of the above observations rely definitely, others probably, on the bacterial cytoskeleton, just as almost anything in eukaryotes that we describe with the term cell biology.

Here, we describe the filamentous components FtsZ, MreB, ParM, MinD, and fibril protein, and, if known, their directly interacting modulators and what is known about them at the functional, molecular, and atomic level.

FtsZ: TUBULIN-LIKE FILAMENTS

FtsZ, a 37- to 43-kDa protein, is almost ubiquitous in bacteria, including archaea, and binds and hydrolyzes GTP. It was discovered in 1993–1995 that FtsZ was an excellent candidate for a bacterial tubulin homolog (32, 112), on the basis of the existence of the tubulin signature motif in the sequence of FtsZ and on the ability of FtsZ to polymerize. FtsZ is the key player in bacterial cell division and forms a filamentous ring around midcell that constricts during division (11). FtsZ is the first constitutive (and known) component of the bacterial septum, and a number of other proteins depend on it for localization. In *E. coli*, the septum assembles in sequential order: FtsZ, FtsA/ZipA, FtsK, FtsQ, FtsW, FtsI, and FtsN (37, 97, 135). Whereas FtsK, FtsQ, FtsW, FtsI, and FtsN have separate functions, FtsA and ZipA interact with FtsZ and modulate its activity. FtsZ shows weak sequence similarity to tubulin, most notably in the tubulin signature motif involved in GTP binding, and can be polymerized into protofilaments and protofilament-derived higher-order structures under many conditions (14, 113). It was noted that the protofilament spacing of approximately 42 Å is close to the 40 Å spacing in microtubules (36). The three-dimensional structure of FtsZ (89) indeed showed it to be surprisingly similar to the structure of tubulin derived from Zn-induced sheets by electron crystallography (121) (Figure 1, *left*).

Both proteins are composed of two clear domains (119) with the N-terminal domain, including core helix H7, making all contacts to the nucleotide. Despite the similarities, the two proteins differ at the C terminus, where FtsZ lacks tubulin's two long α helices. The tubulin structure, which was solved in its polymerized state, provided a unique insight into the mechanism of polymerization-dependent GTPase activation: A loop, T7 (119), or synergy loop (33) inserts into the nucleotide binding pocket of the next subunit in the protofilament and activates hydrolysis. FtsZ has a similar loop in the same position, and it was predicted then that FtsZ might use the same mechanism to provide energy for filament disassembly [microtubule dynamic instability (106)] shortly after polymerization (114, 145, 169). Later, experimental proof that FtsZ polymerizes into the same protofilaments as tubulin was obtained using calcium-induced sheets of FtsZ and low-resolution three-dimensional electron microscopic image reconstruction together with the crystal structure (90) (Figure 2).

Not surprisingly, the existence of the T7-dependent mechanism of GTPase activation in tubulin and FtsZ has only been confirmed experimentally using mutagenesis of FtsZ (115, 139, 140), as recombinant tubulin is still difficult to obtain. The current lack of atomic resolution data for the FtsZ protofilament makes it impossible to understand in detail how T7 activates GTPase activity. In addition, the conformational changes that are expected to accompany nucleotide binding and hydrolysis have been elusive. Cocrystal structures of FtsZ in the GDP and GTP state have not shown any significant changes (J. Löwe, unpublished data). Fairly large alterations in the structure of FtsZ (and tubulin) are expected from significant

changes in the curvature of protofilaments trapped in the GTP or GDP state (93). This work shows that GTP-bound FtsZ protofilaments are straight, whereas the GDP form produces curved filaments, helices, and rings, again in close analogy to tubulin. The conformational event is triggered by phosphate release, as hydrolysis in the filament is almost instantaneous and the straight polymer still contains the γ-phosphate (105, 141). Why hasn't it been possible to crystallize FtsZ in different conformations? Probably because the active site is only complete with at least two FtsZ molecules in a protofilament arrangement, and the conformational change may need all these interactions to be in place. Also, two protofilaments may form a tight dimer (90, 123). However, it has been predicted by molecular dynamics that loop T3 undergoes a large movement upon GDP-to-GTP transition (26). To make progress on this critical issue, we believe that atomic information for at least an FtsZ dimer is needed. Mutants that are deficient in protofilament formation on one side (active site side or T7, top/bottom in Figure 1, center) may be a step in this direction (95, 146).

Apart from the T7 and active site surfaces that are occupied by the protofilament contact when FtsZ is polymerized, in principle four more surfaces can be used with which other proteins may interact (Figure 2, *front/back/left/right*). Two of these are probably occupied by lateral contacts between FtsZ protofilaments. If, as predicted, the in vivo polymer of FtsZ is a face-to-face dimer (123), then only one surface on each protofilament is occupied by the self-interaction, leaving three sides unoccupied. Tubulin protofilaments assemble into microtubules, and the regions on tubulin that are responsible for the necessary interactions have been identified (120). The M-loop facilitates most interactions and is much shorter and different in FtsZ. Despite much effort, microtubule-like structures have never been obtained with FtsZ in vitro, and it is likely that FtsZ higher-order structures are different from microtubules. In fact, we have currently no idea what the in vivo arrangement of FtsZ protofilaments is, apart from simple calculations of the number of molecules in a cell and the length of the filament needed to form a ring. Without providing all arguments, it is possible that the FtsZ ring consists of only a double filament made from two FtsZ protofilaments. Estimates of the number of FtsZ monomers per *E. coli* cell vary between 3200 (136) and 15,000 (94), providing enough protein for many protofilaments going around the middle of the cell. However, only about 30% of cellular FtsZ is assembled into the ring (147). Some FtsZ protofilaments have been observed as clear dimers (90, 123) and kinetic evidence points in the same direction (15), although these authors come to more or less the opposite conclusion. A structure with twofold symmetry would also fit well with the overall twofold symmetry of a dividing cell, a theme that seems to be common to a number of cell division proteins and was first postulated by King et al. (78). In summary, we simply do not know what kind of lateral interactions FtsZ utilizes in the cell and many different ideas have been put forward (35, 91, 93). To start making progress on this critical issue, site-directed mutagenesis and in vivo imaging have been used (146).

FtsZ in Chloroplasts and Mitochondria

FtsZ has been conserved in at least some organisms when certain bacteria became part of eukaryotic cells. Chloroplasts are derived from cyanobacteria and mitochondria are thought to have originated from α-proteobacteria. The plastid division machinery contains FtsZ that forms a ring similar to the Z-ring in bacteria (161) in addition to several plastid division rings of unknown composition (124, 132). Mitochondria were first thought to lack FtsZ. However, FtsZ has recently been reported in the chromophyte alga *Mallomonas splendens* (5) and the red alga *Cyanidioschyzon merolae* (150). The most interesting finding is that apparently all organelles in eukaryotes require cytosolic dynamin to divide (3, 43, 107). This provides evidence for a possible functional connection between FtsZ in prokaryotic division, chloroplasts and some mitochondria, and the essential role that dynamin plays in cytokinesis (74, 151). FtsZ and dynamin may play similar roles in prokaryotic and eukaryotic cytokinesis (34). In plastid division, however, the dynamin ring sits on the outside of the dividing organelle, not on the inside of the membrane as does FtsZ. Furthermore, the GTPase domain of dynamin is of the Ras-type GTPase fold (117), in contrast to FtsZ, which belongs to the Rossmann folds (119).

FtsA and ZipA: Attachment or Modulation of the Z-Ring?

FtsA, a 47-kDa protein, directly interacts with the C terminus of FtsZ (52, 100, 168). Apart from FtsZ, FtsA is the only septum protein without a membrane anchor. Somewhat surprisingly, the interaction site overlaps with that of ZipA on FtsZ (see below), and the proteins seem to have similar, almost exchangeable functions, as demonstrated recently by a gain-of-function point mutation in FtsA that removes the requirement for ZipA completely (45). FtsA binds ATP (138, 159), but it has not been possible to demonstrate hydrolysis yet, most likely because of a missing binding partner or substrate. Not much is known about its function, but FtsA also has a role in sporulation in *Bacillus subtilis*, and these roles can be separated using mutations (77). It seems that FtsA and ZipA are needed for the assembly of downstream components, not so much for forming the Z-ring itself (50, 128). FtsA and possibly also ZipA are stoichiometric components of the septum, as the ratio of FtsA:FtsZ has to be well maintained to support division (6, 23) and stays the same throughout the cell cycle (136) with about 100–1000 FtsA molecules per cell (136, 163) and an FtsZ:FtsA ratio of about 5:1 in both *E. coli* and *B. subtilis*. As predicted by Bork et al. (13), FtsA belongs to the actin family of proteins. The crystal structure of FtsA from *Thermotoga maritima* shows the typical two-domain architecture of actin (159) with subdomain 1B shifted by one β sheet, moving it to the other side of the molecule (Figure 3). It has therefore been renamed subdomain 1C. No published reports of FtsA polymerization are available. No experimental information is available on the FtsZ interaction site in FtsA.

Although FtsA and ZipA seem to have overlapping roles and also a similar interaction site on FtsZ that mostly involves the last dozen residues (100), they share nothing in terms of sequence or even architecture. ZipA (*E. coli*: 37 kDa) is anchored at the septum via a single N-terminal transmembrane helix followed by a charged domain, a mostly unfolded 8- to 20-nm-long P/Q-rich domain (122), and the globular FtsZ binding domain, whose structure is known (110, 111). The crystal structure of the C-terminal domain of ZipA has been solved as a complex with the last 17 amino acids of FtsZ (110) that are absent/disordered in all available FtsZ crystal structures and probably only become ordered upon ZipA binding (Figure 4).

Little is known about the exact function of ZipA. Genetic evidence points toward a stabilization of the Z-ring, a function that overlaps with FtsA (128). In vitro and sequence data suggest some similarities to microtubule-associated proteins (MAPs) in the presumably unstructured acidic domain, and similar bundling and polymerization enhancing functions (131).

ZapA: FtsZ Polymerizing Factor

ZapA (YshA) was discovered while screening for FtsZ polymerization enhancers in a MinD overexpression background, inhibiting division through blocking FtsZ ring formation (49). ZapA is a small protein of about 10 kDa, containing roughly 50% coiled-coil. It binds to the FtsZ polymer and, by an unknown mechanism, renders FtsZ filaments more stable and enhances bundling, a property believed to be connected to its dimeric or tetrameric nature. The interaction is stoichiometric, meaning that ZapA binds to the polymer and does not simply assist with polymer formation (49, 88). ZapA is widely distributed in bacterial species, although it is not essential in *B. subtilis* (but see EzrA below). The crystal structure of ZapA from *P. aeruginosa* shows it to be a tetramer (Figure 5) with four globular domains at opposite ends of the coiled-coil stalk (88). The molecule is about 100 Å long. It is not known which parts interact with FtsZ or how ZapA stabilizes the polymer.

EzrA: Destabilizing FtsZ Assembly in *Bacillus subtilis*

EzrA, a 62-kDa protein from *B. subtilis* and a few other related species, was discovered by screening for extragenic suppressors of a temperature-sensitive FtsZ GFP fusion protein (83). EzrA is anchored to the inner membrane via a single N-terminal transmembrane helix and has at least four large coiled-coil segments. It is not known whether it acts directly or indirectly on FtsZ. EzrA is a negative regulator of FtsZ ring formation (83). A loss-of-function mutation in EzrA induces polar FtsZ rings, similar to GTPase-defective FtsZ mutants, leading to the conclusion that EzrA regulates FtsZ polymer stability (84). The same mutation also suppresses the filamenting effect of MinCD overexpression. That the filament dynamics of FtsZ are tightly regulated in *B. subtilis* is also highlighted by the fact that the loss of both ZapA (promoting assembly) and EzrA (negatively affecting assembly)

leads to a severe block of division, whereas the loss of one of them has no effect (49). EzrA colocalizes with FtsZ in the middle of the cell (83) and also during the ring-to-helix transition during sporulation (7). No biochemical information is available at the moment.

SulA: SOS–Induced Cell Division Inhibitor

sulA and *sulB* (FtsZ) were discovered in the search for suppressors of *lon* mutants (44) and are by far the genes longest known in the field. SulA directly binds to FtsZ (10, 56, 72) and is activated by the lexA promoter, which in turn is activated by DNA-damage-induced RecA (87). The pathway is called the SOS response, and many genes, most of them involved in DNA repair, depend on the lexA promoter (38). SulA, a 19-kDa protein, is a dimer and binds two FtsZ molecules on each open dimer side (21) (Figure 6).

The SulA fold is related to a fragment of RecA, but this seems accidental and probably only shows how nature reuses successful designs for new purposes. SulA does not bind nucleotide. SulA binds to the T7-side of FtsZ and completely blocks the protofilament interface on that side of FtsZ using only one stretch of residues (residues 99–125 in *P. aeruginosa* SulA). The other protofilament interface on FtsZ is free (Figure 6) and contains GTP or GDP in different crystals. It is believed that SulA occupies free T7-interfaces of FtsZ and renders these molecules incapable of polymerizing. Given enough SulA, eventually all FtsZ will be inactive, the Z-ring will disappear, and division will halt. This only works because the Z-ring inside the cell is remarkably dynamic with a half-life of about 30 s or less (147; H.P. Erickson, personal communication), as measured using FRAP. FtsZ monomers will constantly come out of the polymer and SulA will bind to them with high affinity. Finally, Lon protease is needed to remove SulA proteolytically to resume division. SulA is not widely distributed among bacterial genomes. Recently, it has been reported that an unrelated protein, YneA, in *B. subtilis* expressed during the SOS response is directly or more likely indirectly responsible for Z-ring removal and cell division inhibition (76).

Septum Site Selection: Inhibiting Z-Ring Formation with the MinCDE System

In undisturbed cells, Z-rings form with great positional accuracy at the middle of the cell [*E. coli* standard deviation: 2.6% (170), *B. subtilis*: 6.7% (104)]. Two mechanisms that can explain at least part of the precision of septum site placement have been proposed. Although nucleoid exclusion was described more than a decade ago (116), little mechanistic progress has been made. The position of newly formed septa is influenced by the position of the nucleoid in strains with perturbed DNA segregation or replication. However, in certain cases septa can be formed so that they divide (“guillotine”) nucleoids (17). It has been suggested that it is the local concentration of the DNA that determines the localization of the septum rather than a checkpoint mechanism. It is currently not known how

the link between DNA segregation and division is made or which proteins are involved.

The other known mechanism for septum site selection involves three proteins in *E. coli*: MinC, MinD, and MinE (25). This system has recently attracted a lot of attention because it was the first example of oscillating proteins in bacteria (130). MinC is a homodimer made from two 26-kDa subunits (19, 63) (Figure 7). Each subunit contains an N-terminal affector domain that has been suggested to directly inhibit FtsZ polymerization (10, 66, 127), although the effect might be more indirect (71).

MinD, a 30-kDa protein (Figure 8) belonging to a large family of small ATPases [SIMIBI (82)], binds to MinC and phospholipid bilayers in the presence of ATP, most probably while dimerizing (65). MinD self-interacts to form larger polymers (61, 148). ATP hydrolysis induces a conformational change in MinD that causes tubulation of lipid vesicles coated by MinD (61). The binding of MinD to membranes is facilitated by a transient C-terminal helix that inserts into the hydrophobic core of the bilayer (65, 149, 171). The mechanism of membrane binding is similar to that observed for the endocytosis protein epsin and its relatives (40). The ATPase activity of MinD is increased by binding to phospholipids and MinE (Figure 9), the third component of the Min system (61, 64, 81). MinE displaces MinD from the membrane.

How do these three proteins, MinC, MinD, and MinE, work together to find the middle of the cell? Significant progress on this question was made when a report showed that MinD oscillates from pole to pole in *E. coli* every 30 to 50 s (130). It was later shown that MinC also oscillates from pole to pole because it binds to MinD (62, 129). MinE forms a ring-like structure that oscillates between the two three-quarter positions with a similar frequency (41, 51). Combining this knowledge with the biochemical data mentioned above, the following model of septum site selection emerges: MinC inhibits FtsZ polymerization and travels through the cell bound to MinD. MinD binds to the membrane at one pole in a cooperative manner (polymerizing), ensuring that all MinCD ends up at the same pole region. MinE increases the ATP hydrolysis rate of MinD and eventually displaces it from the membrane. The only place for MinCD to assemble is the other pole, where the same event occurs about 30 to 50 s later. MinE follows this oscillation with the same frequency but a slightly different phase and does not quite reach the poles. FtsZ ring formation is possible where the concentration of MinC is lowest, i.e., in the middle of the cell. Apart from finely balanced affinities of the proteins for each other (and for itself, in the case of MinD), a certain ATP hydrolysis rate for MinD, and the concentrations of the proteins, nothing else has to be assumed in computer simulations (60, 79, 103) to reproduce the oscillations of these proteins. In this model, preferential binding of MinD at the poles is a consequence of the rod-shape of the *E. coli* cell, as MinD reassembles furthest away from the old site (18). MinE mutants that are affected in their ability to stimulate MinD also show altered oscillation frequencies (64). In *B. subtilis*, MinE is replaced by DivIVA (31), which seems statically placed at the poles, recruiting the MinCD inhibitor

of Z-ring formation permanently (75). Therefore the mechanism of preventing FtsZ polymerization in *B. subtilis* seems different from that in *E. coli*, and it might also indicate that the main function of the Min system is to prevent FtsZ polymerization at old septa (poles). Instead of forming diffuse patches at the poles, MinD is organized into helices on the inside of the inner membrane (143). This has opened up a new exciting possibility for the mechanism of movement of MinD. The MinCDE model described above does not make any assumptions about the way MinCD travels through the cell. Possibilities include an active, motor-like mechanism or passive diffusion. As MreB (see below) forms filamentous, helical tracks on the inside of the inner membrane, it is tempting to propose that MinD [and indeed its relatives in DNA segregation that also oscillates ParA (30) and Soj (101)] are moving on, polymerizing on, or are guided in some way by MreB tracks inside the cell. Although MreB helices have a different pitch, Shih et al. (143) did not look at MinD and MreB placement in the same cell; an active transport mechanism based on MreB is certainly a possibility (47). Despite the beauty of the Min system, however, another equally accurate mechanism seems to exist in *B. subtilis* (104).

SpoIIE: Sporulation-Specific Morphogen Interacting Directly with FtsZ

SpoIIE is a 92-kDa protein localized in the inner membrane of *B. subtilis* with a clear three-domain architecture: an N-terminal transmembrane domain with 10 putative transmembrane helices, a cytoplasmic central domain interacting with FtsZ (96), and a C-terminal PP2C protein phosphatase domain. The principal function of SpoIIE seems to be the indirect activation of the first spore-specific sigma factor, sigmaF, dependent on the formation of an asymmetric septum (28), since SpoIIE mutants exist that uncouple this important link (39, 57). Furthermore, the recent description of a helical FtsZ ring intermediate during the transition to asymmetrical division has revealed a possible role of SpoIIE in directing this transition (7).

TWO TUBULIN GENES IN VERRUCOMICROBIA

Epixenosomes, epibiontic organisms living on the protozoon ciliate *Euplotidium itoi*, contain tubules that show striking similarity to microtubules (9, 133). These organisms belong to the Verrucomicrobia (126). Recently, the genome of *Prosthecobacter dejongeii* was sequenced and found to contain two genes closely related to tubulin (68). Strikingly, the two genes, *btubA* and *btubB*, are part of an operon and share about 35% sequence identity with tubulins (compared with the $<$20% for FtsZ). No in vitro data are yet available investigating whether these proteins form bona fide microtubules or whether BtubA and BtubB form a heterodimer similar to α- and β-tubulin, although some evidence from investigating active site

residues would appear to speak against that possibility (68). Also unresolved are the questions of what microtubules and tubulin do in these organisms and what the evolutionary relationship between tubulin, Btubs, and FtsZ is. We believe it is not inconceivable that these organisms acquired the *btub* genes by horizontal gene transfer from their eukaryotic hosts, although the genome sequenced is actually from a freshwater organism, which might reflect a recent change in lifestyle. Another possibility is that the tubulin-like genes represent the "real" tubulin ancestor. Even if that is the case, the evidence of mechanistic and structural similarity between tubulin and FtsZ is so strong that the verrucomicrobial genes would take the place of intermediates between the basic FtsZ building block and tubulin. It would, of course, have far reaching consequences for the origin of the eukaryotic cell.

ACTIN-LIKE FILAMENTS

In a landmark publication in 1992 it was predicted by comparing the surprisingly similar structures of actin, hexokinase, and Hsp70 ATPase domain that several bacterial proteins should adopt the same fold (13): DnaK (Hsp70), FtsA, ParM (StbA), and MreB. The prediction is essentially correct and at least two of these proteins clearly function by forming actin-like (proto)filaments in the bacterial cell.

MreB: Cytosolic Filaments for Shape Determination

MreB has been known for a long time to affect cell shape (162). Its known relationship to actin (13) inspired an immunofluorescence study of MreB and Mbl (MreB-like) in *B. subtilis* showing that these proteins form dynamic, helical filaments on the inside of the cytosolic membrane (73). Mutant forms of these genes affect cell shape. Mbl is responsible for guiding the machinery for cylindrical growth, and Mbl filaments have been shown to be dynamic with no apparent polarity and with a half-life of 8 min (16). MreB-like genes are exclusively found in noncocci cell types, although they are not required to maintain all nonspherical shapes. Another rod-determining mechanism seems to exist in *Corynebacterium glutamicum*, as shown using labeled vancomycin to indicate localization of active peptidoglycan synthesis (24). Encouraged by the predicted structural similarity to actin and the clear functional parallels (shape-determination function of actin and MreB-like proteins), we (158) were able to solve the crystal structure of MreB from *T. maritima* (Figure 10). The protein was found to polymerize easily under mild conditions with a strict requirement for ATP (158). Electron microscopy of the polymerized product revealed straight and curved filaments and small two-dimensional sheets. Diffraction analysis of these polymers showed a longitudinal repeat of about 51 Å. Close inspection of the crystal packing in the three-dimensional crystals revealed that the crystals contain a protofilament of MreB with exactly the same subunit spacing of 51 Å. Obviously, the protein had

crystallized in its polymeric form and this enabled a direct comparison of the polymer to the polymeric form of actin, F-actin: The completely straight protofilament of MreB (158) is closely related to one strand of F-actin (59).

The similarities between MreB-like proteins and actin therefore extend from a weak sequence similarity, a similar cellular role in shape determination, to closely related structures of the monomer and most importantly the polymer, as proven directly by visualizing the atomic structure of the filament by high-resolution X-ray crystallography. Despite these findings the unexpectedly low sequence conservation for these proteins (and ParM, see below) has raised some questions about the evolutionary relationship (27). Sequences of actins in eukaryotes are extremely well conserved and it seems difficult to explain why a central filament-forming protein in the bacterial and eukaryotic cytoskeletons has such divergent sequences despite sharing so many common features. This could be caused by a combination of early divergence (last common ancestor of eukaryotes and prokaryotes) and the fact that the only properties these proteins have to retain are nucleotide binding and hydrolysis coupled to polymerization and interactions with other proteins. Because not one interacting protein has yet been found to be common to eukaryotes and prokaryotes, both systems have had complete freedom to develop into different sequences. This is in contrast to metabolic enzymes, for example, which are usually part of conserved pathways, connected by small-molecule ligands, which severely restricts the number of possible mutations.

It is expected that factors will be identified that modulate filament formation, dynamics, and disassembly, just as for actin, tubulin, and FtsZ. Currently, no such factors are known.

ParM: Plasmid Segregation by an F-Actin-Like Filament

Perhaps the most surprising role of filament-forming proteins inside bacteria is the partitioning system of certain low-copy plasmids, including *E. coli* plasmid R1 (108). Single-copy plasmid stability is accomplished with only three elements: ParM, ParR, and the *cis*-acting DNA element *parC* (46). ParR binds to 10 direct repeats of *parC* and forms a nucleoprotein complex that is thought to pair the plasmids prior to segregation (70). ParR binding to DNA increases its affinity for ParM and induces ParM polymerization. The filaments formed are thought to push the plasmids to the pole regions, one copy per daughter cell (109). Bork et al. (13) have also shown ParM (StbA) to be the fourth actin-like protein in bacteria (after Hsp70, FtsA, and MreB), and its crystal structure indeed shows it to be closely related to actin (160) (Figure 11).

Two different crystal forms in the empty and ADP-bound state are the first to demonstrate the dramatic conformational change upon nucleotide binding for any actin-related molecule. This large conformational shift is required (in contrast to FtsZ) because in the protofilament of actin and MreB the active site is not formed by two adjacent molecules and polymerization-dependent ATPase activation cannot be accomplished by residues interacting directly with the nucleotide. Instead,

upon nucleotide binding, the molecule dramatically changes its shape, forming the protofilament interface between subdomains IB and IIB (Figure 11). Once nucleotide is bound, all residues required for hydrolysis are in place. This is in contrast to FtsZ, in which two subunits are required to form a complete active site (Figure 2). There are some surprising differences between the structures of actin, MreB, and ParM, especially in subdomain 1B, which is most variable for all actin-like proteins. Despite this, ParM polymerizes into double-helical filaments that are similar to F-actin (Figure 12).

As no host factors have been found so far, it is assumed that these three components are all that is required for pushing the plasmids apart. Plasmids need to be paired, polymerization has to be initiated, filaments have to be elongated while being attached to the plasmid, and filaments may have to be depolymerized. These functions are the direct equivalent of mitosis in eukaryotes, and it is exciting to mention that ParR has some weak sequence homology to other segregation proteins of the ParB family (J. Löwe, unpublished data) and chromosomally encoded DNA segregation proteins of similar function (*parC* binding). The best-characterized system in this context is Spo0J from *B. subtilis* and 8–10 copies of *cis*-acting *parS* elements situated around 20% of the *oriC* region (86). The similarity between these proteins might indicate an underlying symmetry between these systems and help provide answers to an old question: How are chromosomes segregated in bacteria? Other systems for chromosome segregation seem to exist, as highlighted by the recent discovery of a large, centromere-like structure on the *B. subtilis* chromosome (167) and a protein, RacA, that is involved in tethering the fast-moving end of the chromosome to the pole during segregation (8).

OTHER FILAMENT SYSTEMS

Fibril Protein

Mollicutes (*Mycoplasma*, *Spiroplasma*, and *Acholeplasma*) are the smallest free-living bacteria. They lack a cell wall, have a defined shape, and glide (*Mycoplasma*) or swim (*Spiroplasma*) despite not having flagella (152). An internal cytoskeletal ribbon may be responsible for *Spiroplasma*'s ability to swim (154). The fibrils from *Spiroplasma* were isolated a long time ago (165) and probably consist of a single protein, fibril protein, that comprises 515 amino acids (166). The protein consists of two domains, an N-terminal domain closely related to phosphorylases and a C-terminal domain of unknown function and origin, separated by a low-complexity linker. No biochemical data are currently available. The protein is thought to arrange into protofilaments as a tetramer, and seven protofilaments make up the ribbon that can be seen in sectioned cells (153–155). Several properties distinguish it from other cytoskeletal proteins: The fibril protein has been found only in *Spiroplasma* so far. The ribbon-like structure is extraordinarily stable. The system is probably more related to bacterial flagella than to other, more dynamic cytoskeletal structures that use nucleotide hydrolysis-regulated polymerization and

depolymerization mechanisms. Also, GFP labeling or immunofluorescence experiments are needed to formally prove the identity of the filament-forming protein in the cells. For GFP labeling, new genetic tools in mollicutes must be developed for that purpose. The gliding motility of *Mycoplasma* (134) is interesting, as this organism lacks any genes for type IV pili or flagella and its ultrastructure shows a high degree of intracellular organization (54, 144), including filaments. The mechanism of *Mycoplasma* gliding still awaits discovery.

EF-Tu

It has been speculated that the bacterial elongation factor (EF)-Tu has a dual role, acting as a "cytoskeletal web" as well as aiding in translation (102). This is mostly based on the ability of the protein to form filaments in vitro under certain favorable conditions (4, 22, 55), the fact that overexpression of EF-Tu mutants induces *E. coli* to lose shape and lyse (102), its high intracellular concentration of up to 5–10% of soluble protein, and its apparent association with the membrane. Unfortunately, GFP-aided in vivo localization has not been performed, although this would be straightforward to do in *E. coli*. The GDP- and GTP-bound forms polymerize equally well, raising questions about the disassembly of the formed polymer, as the cell has little control over polymerization and depolymerization.

THE FUTURE

Now that the existence of actin- and tubulin-like filaments in bacteria is beyond doubt, we need to shift our focus to the mechanistic details of how these proteins function, how they are organized using other factors, and how exactly these proteins are arranged in the bacterial cell.

For FtsZ, the most pressing question in this context is how the ring "constricts." Unfortunately, it is difficult to envisage how this could be answered without detailed knowledge of the in vivo FtsZ polymer. For that purpose, we need better electron microscopic images of the septum at different stages of the cell cycle. It might be advantageous to study this process in wall-less bacteria, since the role of the cell wall–synthesizing machinery in constriction can be excluded. As the most basic constituents of the cytoskeleton are conserved between prokaryotes and eukaryotes, it seems reasonable to assume that certain mechanistic aspects of their function are retained. For example, we may expect certain details of cytokinesis to be similar in eukaryotes and prokaryotes, although in one system actin forms a ring and in the other FtsZ (tubulin). However, tubulin is involved in cytokinesis in eukaryotes (125) and the actin ring might only function to constrict until the septum is about as small as it is at the beginning in prokaryotes. In this context, two other polymerizing GTPases in eukaryotes must be investigated: dynamin and septin. The eukaryotic cytoskeleton is unthinkable without the mechano-chemical motor proteins myosin, kinesin, and dynein. It is not impossible to imagine that motor

proteins exist in one form or the other in bacteria as well. We now have knowledge of tracks and dynamically moving proteins and DNA in bacteria. Bacterial motor proteins could be related to kinesin and myosin (80) or belong to the AAA family of proteins such as dynein (156). The alternative is that all dynamic movements related to FtsZ, MreB, and ParM are caused by careful regulation of filament seeding, growth, and depolymerization. At least the *parC*/ParR/M system is simple enough to test this possibility in vitro. Even in the presence of motor proteins, intracellular filaments need a great deal of regulation and the proteins responsible have started to appear for FtsZ. We expect an analogous set of proteins to emerge for MreB, and the screening methods used for finding FtsZ-modulating proteins can be used in a similar way for MreB. As for FtsZ and tubulin, no clear homologs of actin binding proteins can be identified in bacteria by sequence comparisons.

A number of unexpected findings over the past two decades have shown that the view of the prokaryotic cell as a single, simple, and unstructured entity with a single circular piece of DNA is overly simplistic. It is therefore anticipated that bacterial physiology will play a much greater role in the future. Some bacteria contain linear chromosomes and plasmids (58) and have mitosis-like DNA movements (69, 85, 118, 164). Vesicles in prokaryotes have been described (48), a nucleus has been found in *Gemmata obscuriglobus* (42), and some bacteria are magnetotactic (12) with small, membrane-enclosed intracellular magnetite crystals that function like a compass needle (142). Multicellular organization has been described for many prokaryotic organisms [for example in Myxobacteria (29)]. Ultimately, molecular approaches will be required to understand the relationships of these systems with those in eukaryotes. It will be most exciting to see how these phenomena relate to the bacterial cytoskeleton.

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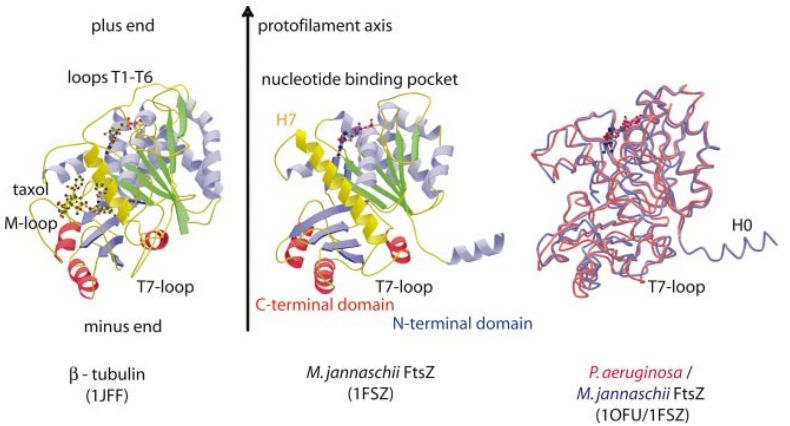

Figure 1 Structures of β-tubulin (92) (*left*) and FtsZ from *Methanococcus jannaschii* (89) (*center*). Both proteins share the same two-domain fold with an N-terminal nucleotide binding domain showing a Rossmann fold and a C-terminal domain (119). The Rossmann fold produces six loops on one side of the β sheet; these loops are involved in binding the nucleotide (T1–T6). Loop T7 on the other side of the molecule is involved in nucleotide hydrolysis when tubulin and FtsZ are polymerized. RMS deviation for 268 (80% of FtsZ) aligned Cα atoms: 2.8 Å; sequence identity: 16%. Right: Superposition of FtsZ from *Pseudomonas aeruginosa* (*red*) and *M. jannaschii* (*blue*). Positional RMS deviation over 298 (97% of *Pa* FtsZ) Cα atoms: 1.3 Å; sequence identity: 44%. PDB entry codes are in parentheses.

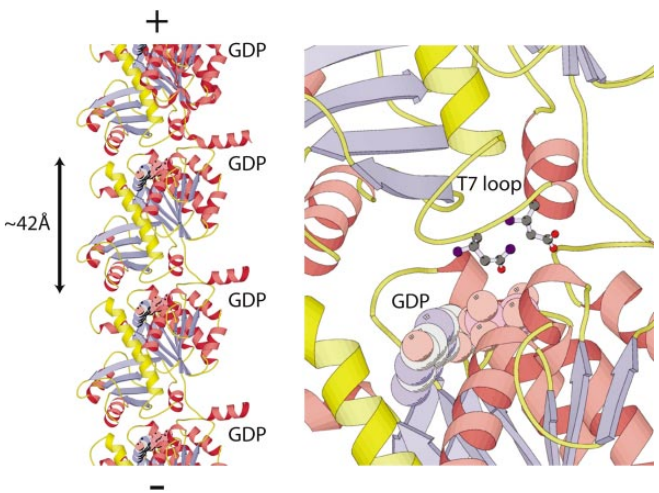

Figure 2 FtsZ protofilament (90). FtsZ forms a one-dimensional chain of molecules with the nucleotide bound between two subunits. Residues in loop T7 contact the nucleotide directly and are required for hydrolysis. The intersubunit spacing along the filament (~42 Å) is similar to that in a tubulin protofilament (40 Å).

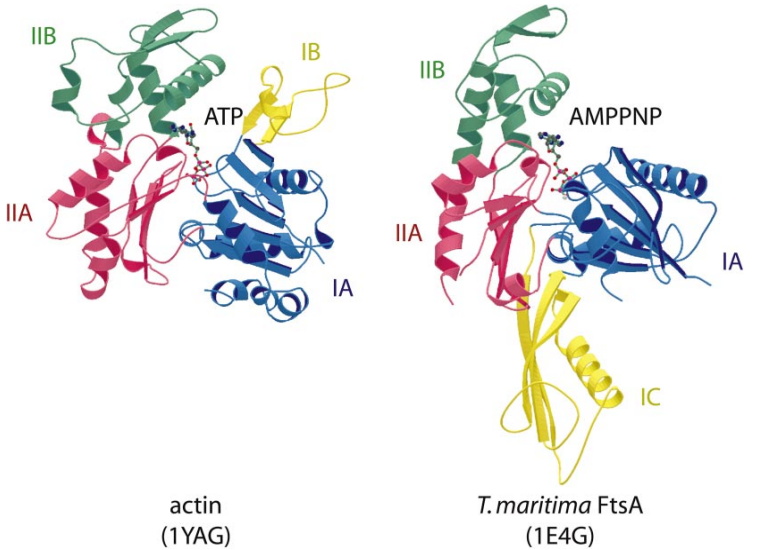

Figure 3 Crystal structure of FtsA from *T. maritima* (159). Left: Yeast actin showing the two-domain structure that can be subdivided into four subdomains. Nucleotide binds in a cleft between the two domains. Right: FtsA showing subdomain 1C on the opposite side of the molecule. Positional RMS deviation over 229 (62% of FtsA) Cα atoms: 2.7 Å; sequence identity: 12%. PDB entry codes are in parentheses.

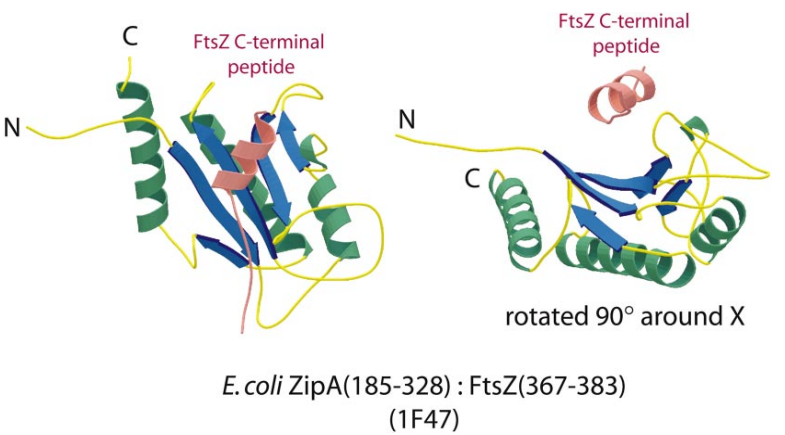

Figure 4 Crystal structure of the *E. coli* ZipA C-terminal domain in complex with an FtsZ C-terminal peptide (110). The FtsZ peptide binds in a cleft above the central β sheet. PDB entry code is in parentheses.

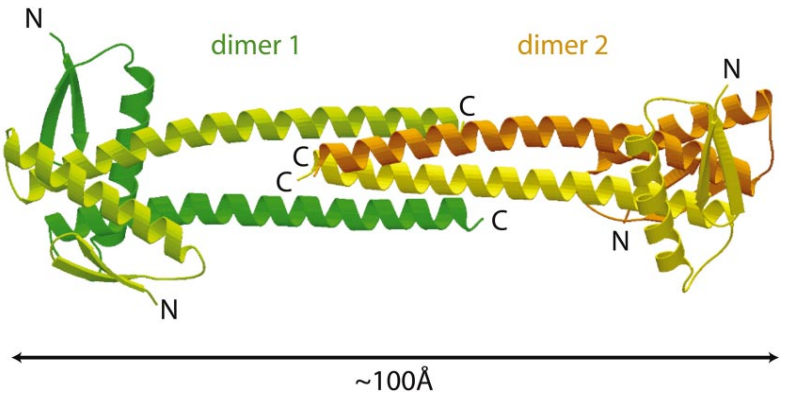

Figure 5 Crystal structure of ZapA from *P. aeruginosa* (88). Four molecules form a bone-like structure about 100 Å long.

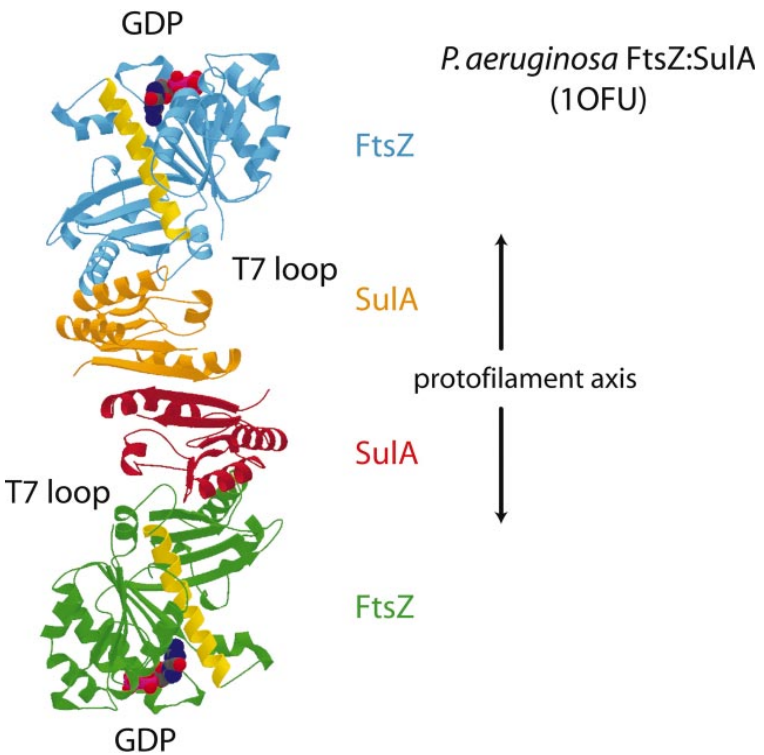

Figure 6 Crystal structure of *P. aeruginosa* FtsZ inhibited by SulA (21). SulA is a dimer and binds to two FtsZ molecules via their T7 protofilament interface, thus blocking filament formation. The overall complex is dimeric and the T1–T6 (nucleotide binding) surfaces on FtsZ are free. PDB entry code is 1OFU.

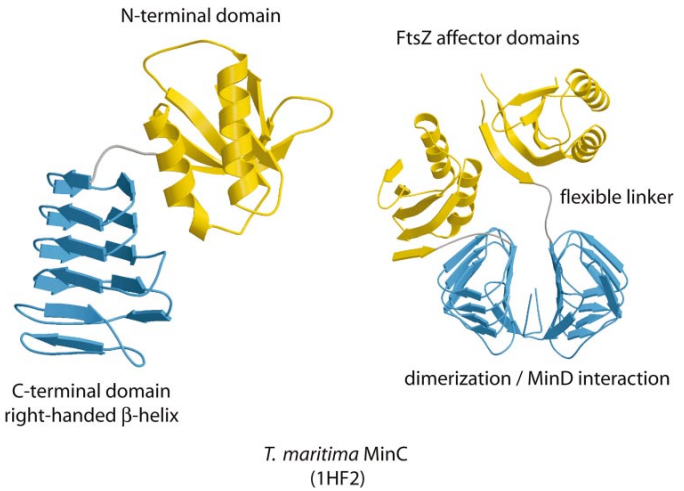

Figure 7 Crystal structure of MinC from *T. maritima* (19). The protein is composed of two domains, with the N-terminal domain affecting FtsZ polymerization and the C-terminal domain inducing dimer formation. Both domains are connected by a flexible linker, and the C-terminal domain binds MinD. The C-terminal domain is a right-handed β helix fold with one of the three surfaces of the triangle facilitating dimer formation. PDB entry code is 1HF2.

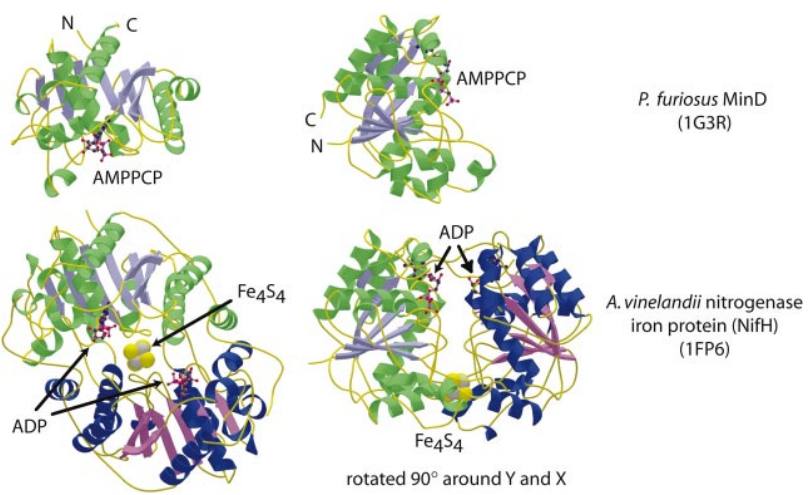

Figure 8 Crystal structure of MinD from *Pyrococcus furiosus* and in comparison to nitrogenase iron protein (NifH) (53). Three crystal structures of MinD (20, 53, 137) show MinD to be a monomer; however, most members of the P-loop-containing class of proteins, to which MinD belongs (82), form dimers with the nucleotide binding site shared between two monomers. Invitro, MinD has been demonstrated to dimerize (67). RMS deviation over 209 (88% of MinD) Cα atoms: 2.2 Å; sequence identity: 20%. PDB entry codes are in parentheses.

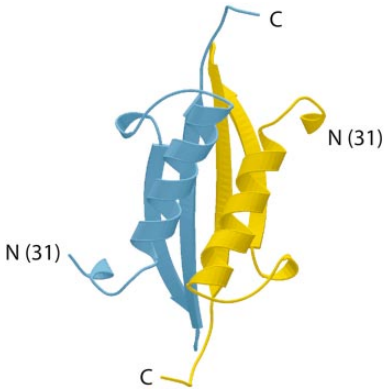

Figure 9 NMR-derived structure of the C-terminal topological specificity domain of MinE, forming a tight dimer (78). MinD interacts with the N-terminal domain of MinE (98). PDB entry codes are in parentheses.

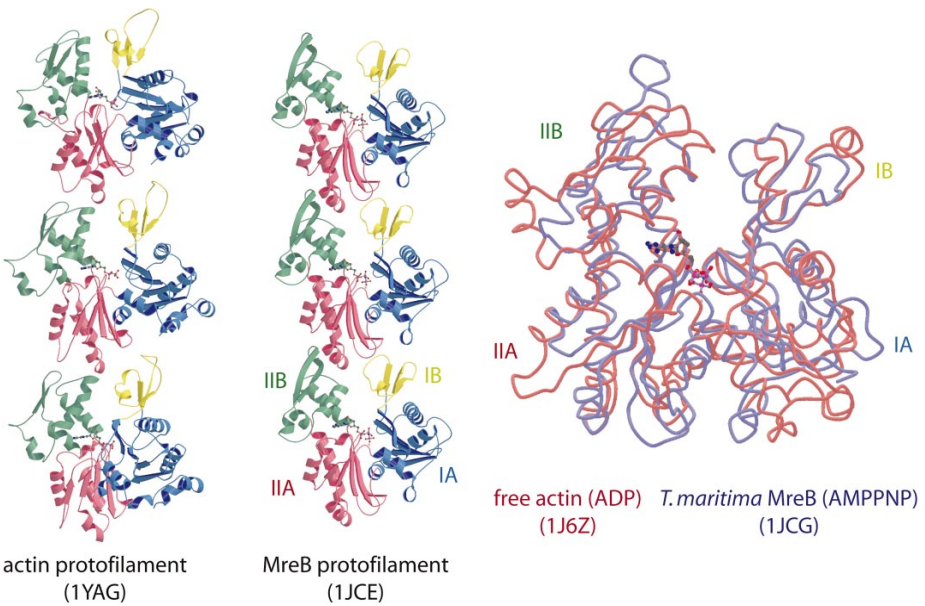

Figure 10 Crystal structure of MreB from *T. maritima* (158). Left: The MreB crystals contain protofilaments that are similar to one strand (protofilament) of F-actin (59). The tip of subdomain IIA inserts into the cleft formed by subdomains IB and IIB of the next subunit. No direct contact is made with the nucleotide of the next subunit. Right: Superposition of uncomplexed actin and MreB. Despite having very low sequence identity, the two proteins have essentially the same fold. RMS deviation over 264 (79% of MreB) Cα atoms: 2.9 Å; sequence identity: 16%. PDB entry codes are in parentheses.

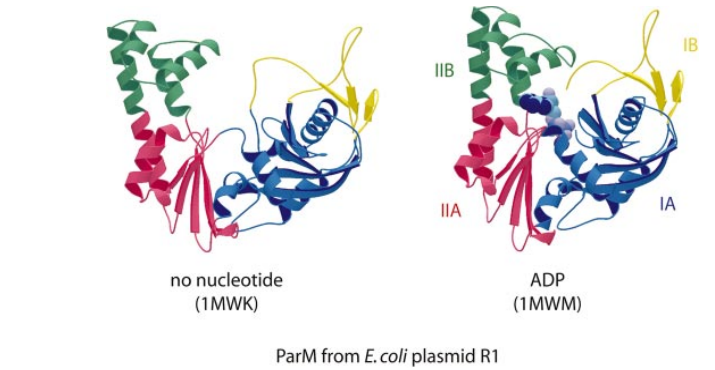

Figure 11 Crystal structure of ParM from plasmid R1 in the absence of nucleotide and in the presence of ADP (160). A large conformational change moving domains I and II relative to each other around a hinge is induced upon nucleotide binding. It is believed that only the closed conformation is capable of polymerization. When compared with yeast actin (1YAG, not shown), ParM:ADP superimposes with 235 (74% of ParM) Cα atoms, positional RMS deviation: 2.5 Å. PDB entry codes are in parentheses.

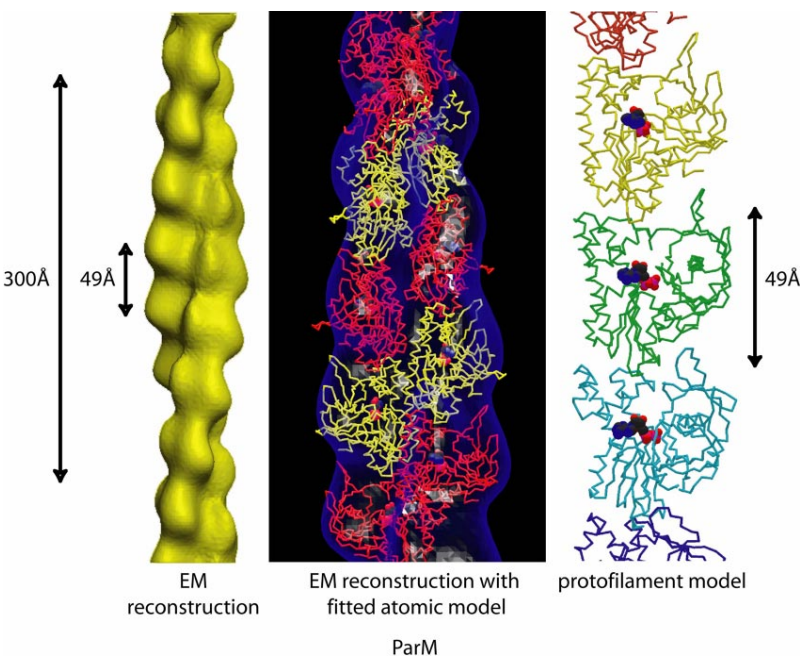

Figure 12 ParM double-helical filaments (160). Left: Helical reconstruction of a single ParM filament. Note the helical parameters that are close to F-actin (F-actin repeat: 54 Å; crossover distance: 360 Å). Middle: Fitting of the ParM:ADP crystal structure into the EM reconstruction on the left. Right: The putative protofilament arrangement is similar to MreB and actin.