First published online as a Review in Advance on March 29, 2006

# Dynamic Filaments of the Bacterial Cytoskeleton

Katharine A. Michie and Jan Löwe

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, UK; email: kmichie@mrc-lmb.cam.ac.uk, jyl@mrc-lmb.cam.ac.uk



Annu. Rev. Biochem.
2006. 75:467–92

The *Annual Review of Biochemistry* is online at
biochem.annualreviews.org

doi: 10.1146/
annurev.biochem.75.103004.142452



0066-4154/06/0707-
0467$20.00

## Key Words

FtsZ, MreB, ParM, tubulin, actin

## Abstract

Bacterial cells contain a variety of structural filamentous proteins necessary for the spatial regulation of cell shape, cell division, and chromosome segregation, analogous to the eukaryotic cytoskeletal proteins. The molecular mechanisms by which these proteins function are beginning to be revealed, and these proteins show numerous three-dimensional structural features and biochemical properties similar to those of eukaryotic actin and tubulin, revealing their evolutionary relationship. Recent technological advances have illuminated links between cell division and chromosome segregation, suggesting a higher complexity and organization of the bacterial cell than was previously thought.

## Contents



## INTRODUCTION

Research over the past two decades has uncovered the existence of a well-developed bacterial cytoskeleton. This revolution in the understanding of bacterial cell structure and dynamics has come about largely through the development of fluorescent labeling techniques for bacterial cells and the availability of complete genome sequences, together with structural and biochemical studies. We now know that proteinaceous filaments encircle and wind helically around the inside of the cell, providing intracellular organization and cytoskeletal functions reminiscent of those in eukaryotic cells. Although the discovery of a well-organized bacterial cytoskeleton caused much excitement, another surprise came when sequence analysis and/or the three-dimensional structural determination of many of the proteins involved revealed their homology to eukaryotic cytoskeletal proteins actin, tubulin, and those comprising intermediate filaments. The eukaryotic proteins perform a myriad of important functions, including establishing cell shape, providing mechanical strength, contributing to cell locomotion, assisting in the intracellular transport of organelles, as well as bringing about chromosome separation during mitosis and meiosis. It is becoming evident that the bacterial homologues have analogous or overlapping functions.

This review presents the current understanding of the most well-characterized bacterial cytoskeletal proteins with a focus on their biochemistry (for reviews on the cell biology of the bacterial cytoskeleton, see References 1–3). In particular, we focus on proteins whose assembly into dynamic filaments is regulated by cycles of nucleotide binding and hydrolysis. An intermediate filament homologue is also discussed; however, intermediate filaments are not dynamic and are not found in all eukaryotic cells—being required mainly for mechanical strength.

**Cytoskeleton:** the proteinaceous filaments that provide intracellular organization

**Protofilament:** a linear structural precursor assembled from protein that is able to assemble into a larger superstructure

## THE TUBULIN HOMOLOGUES

Tubulin is an indispensable eukaryotic cytoskeletal protein involved in many mechanical cellular processes (examples include chromosome segregation during mitosis and vesicular transport). Heterodimeric $\alpha\beta$-tubulin forms protofilaments that combine to make dynamic microtubules. Microtubules form "scaffolds" that motor proteins such as dynein and kinesin are able to track along.

### FtsZ Protein

The *ftsZ* gene (4) encodes a guanosine triphosphatase (GTPase) (5, 6) that is essential for cell division (7, 8). FtsZ was suggested to be a cytoskeletal protein (9) and was predicted to be a homologue of tubulin on the basis of a short sequence motif of seven amino acids (10–12). It is a highly conserved protein that is found in virtually all eubacteria and archaea (13), with a few exceptions (14–16). FtsZ is also present in some chloroplasts and mitochondria (17, 18).

**FtsZ in vivo.** FtsZ is the first protein known to localize to the mid-cell position prior to septum invagination during cell division,

remaining positioned as a ring structure (the Z ring) at the leading edge of the constricting division septum (9). Time-lapse images of cells expressing FtsZ-green fluorescent protein (GFP) fusions show the Z ring contracting while septum constriction occurs (19). Under some circumstances, FtsZ has been observed to form helical structures in cells (20–25), leading to the suggestion that the mature Z ring is a compressed helix (26).

FtsZ is an abundant protein [with estimates of between 3200 and possibly 15,000 molecules per *Escherichia coli* cell (27, 28) and 5000 for *Bacillus subtilis* (29)]. In vitro assembly of FtsZ and its homology to tubulin (discussed below) has lead to the idea that FtsZ assembles into linear polymers called protofilaments. A filament formed by end-to-end association of FtsZ monomers (40 Å in length) would require fewer than 500 monomers to span the circumference of a typical bacterial cell with a 1-μm diameter, but it is likely that the Z ring is more complex in structure than a single protofilament. Instead, the Z ring may contain several of these protofilaments associated (or bundled) together to form the Z-ring superstructure. Consistent with this, fluorescence recovery after photobleaching (FRAP) experiments indicate that an average of 30% of the total cellular FtsZ is assembled into the Z ring, more than enough to comprise a Z ring of several FtsZ filaments (30).

FtsZ displays a dynamic localization in that it exchanges between the Z ring and the cytoplasmic pool on a timescale of seconds (30, 31). Time-lapse movies of FtsZ assemblies in vivo have shown FtsZ rearranging from helical structures to rings and vice versa (21, 22, 26). However, as yet, there is no detailed information regarding the large-scale structure of FtsZ polymers in vivo, and this significantly limits our understanding of FtsZ action.

**FtsZ self-assembles in vitro.** FtsZ protofilaments have been assembled in vitro in a nucleotide-dependent manner (32, 33). Assembly with either nonhydrolyzable GMPCPP [guanylyl-(α,β)-methylene diphosphate] or with GDP indicates that nucleotide hydrolysis is not required for assembly (34, 35). Filaments of FtsZ have been studied by negative stain electron microscopy (EM), and a wide variety of polymer morphologies have been observed (including tubules, sheets, asters, straight and curved protofilaments, and minirings) (32, 34, 36–40). The wide range of conditions favoring FtsZ polymerization suggests that FtsZ could assemble unassisted into a polymer in vivo; however, the diversity of superstructures observed for FtsZ in vitro indicate that some (or all) of the filament types are probably artifactual. The question is which, if any, are representative of the in vivo situation. FtsZ polymers show highly dynamic and flexible behavior in vitro (41–43), and a recent analysis by atomic force microscopy (42) has shown that the dynamic FtsZ filaments continuously rearrange. Also, end-to-end joining of FtsZ filaments and depolymerization of FtsZ from within the middle of filaments has been observed in vitro (42).

**FRAP:** fluorescence recovery after photobleaching

There are a large number of in vitro FtsZ filament morphologies, but at low concentrations, FtsZ assembles into apparently single protofilaments. Coupled with the high abundance of FtsZ in the cell, it is thought that the in vivo form of FtsZ is likely to be composed of linear filaments of FtsZ laterally associated with a defined topology. Our attempt to understand the Z-ring structure in vivo is complicated by the presence of accessory and regulatory proteins. At least eight proteins (FtsA, ZipA, ZapA, EzrA, Noc, SlmA, MinC, and SulA) affect FtsZ assembly either by direct or indirect interactions in vivo. Some of these proteins are restricted to a limited number of organisms (for example ZipA is only present in the γ subdivision of the gram-negative bacteria) and are not well conserved, implying their mechanisms have evolved independently. These accessory proteins may inhibit Z-ring assembly, assist in the correct positioning of the Z ring, or directly effect the dynamics of the Z-ring structure. Very little is known about how these proteins exert their molecular effect on FtsZ [with the exception

**Lateral interaction:** any interaction between protofilaments

of SulA, whose mode of action is to titrate away monomeric FtsZ by binding to one of the polymerization interfaces (44)], and their biochemistry is beyond the scope of this review. (The reader is referred to References 45 and 46 for a review of these proteins.) Interestingly, current structural and sequence analysis of these proteins has failed to reveal homology to any of the eukaryotic tubulin accessory proteins. It is possible that the accessory proteins evolved after the evolutionary split of prokaryotes and eukaryotes: this is consistent with the highly extended evolutionary histories speculated for the large divergence in eukaryotic and bacterial actins and tubulins (47, 48).

**Subunit structure.** Although FtsZ's primary sequence identity to tubulin is low (10% to 18%), it is now generally accepted that FtsZ is a true prokaryotic homologue of tubulin because the three-dimensional tertiary structure revealed that the two proteins share the same fold (**Figure 1**) and both proteins assemble into remarkably similar protofilaments (49–52). FtsZ comprises two domains (52), reflecting thermal denaturation profiles of FtsZ from *Methanococcus jannaschii* and *E. coli*, which both show a clear two-step unfolding process, whereby the domains first separate from each other and then unfold completely (53, 54). It has been suggested that these two domains were derived from two separate proteins earlier in evolution because the N-terminal domain has a Rossmann fold similar to that of many ATPases, and the C-terminal domain is homologous to the family of chorismate mutase-like proteins (52). The N-terminal domain contains the central helix H7 and is essentially the nucleotide-binding domain. Compared with tubulin, this domain of FtsZ has an extra helix (called H0) that protrudes from the N terminus and shows high variability across FtsZ sequences (49). The C-terminal domain of FtsZ shows much less conservation than the N terminus when compared with tubulin. The C-terminal domain is also considerably shorter. In both proteins, this domain contains some residues important for GTP hydrolysis (51), which occurs during assembly into filaments (see below). It is interesting to note that FtsZ also has a conserved hydrophobic pocket similar to the taxol-binding pocket in tubulin at the interface of the N- and C-terminal domains, immediately adjacent to helix H7 and the active site. In tubulin, this binding pocket lies on the inside face of microtubules in a region thought to mediate lateral contacts (55). Filling this pocket with an as yet unknown natural accessory protein may help stabilize the filaments in the "straight," assembly-competent conformation (see below). It is thought that the natural binding partner for this pocket in tubulin might be a microtubule-associated protein (MAP) (56), although this is controversial (57). Regardless of the eukaryotic substrate, FtsZ has a homologous pocket, and whether proteins (such as Zap, ZipA, and FtsA) with functions analogous to MAPs that might stabilize FtsZ assembly bind in a similar fashion has yet to be determined.

**Filament structure.** Semicontinuous tubulin-like protofilaments of FtsZ have been successfully crystallized (52) (**Figure 1**, right), providing us with insight into how FtsZ may assemble into protofilaments. Not unexpectedly, the data suggest that FtsZ assembles in an orientation very similar to that observed for polymerized tubulin, with each FtsZ monomer maintaining head-to-tail interactions. These head-to-tail interactions are referred to as longitudinal contacts and are the basis of protofilament formation. All other interactions are referred to as lateral and function to bring protofilaments together. Lateral interactions may play important roles in Z-ring nucleation, assembly, regulation, and disassembly. Although the regions required for lateral tubulin-tubulin interactions within microtubules are known, the corresponding regions in FtsZ are quite different, with little conservation observed in the relatively short loop regions of FtsZ (51), which is consistent with the notion that FtsZ and tubulin do not

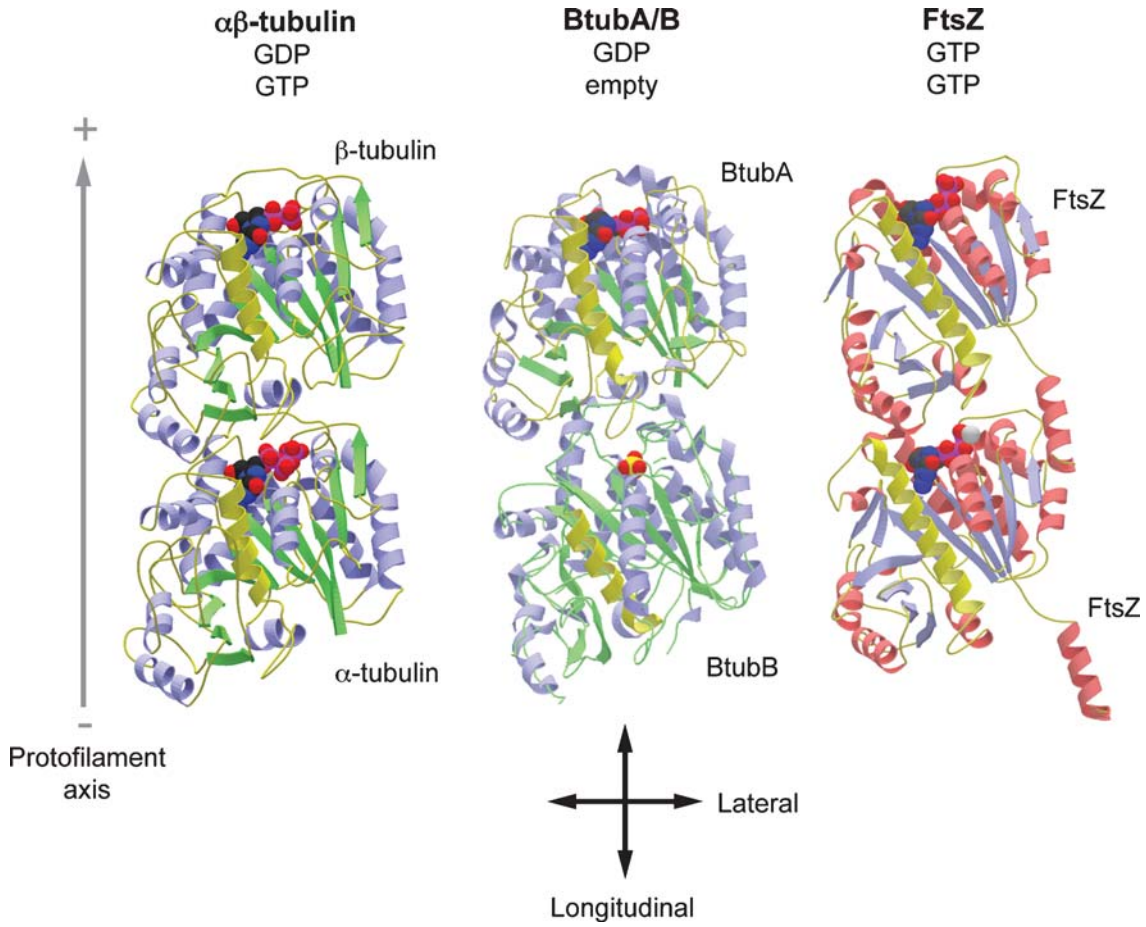


**Figure 1**

Structures of the α/β-tubulin heterodimer (*left*), the BtubA/BtubB heterodimer (*center*) and FtsZ dimer (*right*), showing the position of the nucleotide at the dimer interface, the conservation of fold, and the axis of protofilament extension (*up the page*). Lateral interactions between protofilaments could be formed at all or any of the interfaces perpendicular to the longitudinal axis of protofilament assembly. (*left*) The α/β-tubulin heterodimer observed in tubulin zinc sheets [Protein Data Bank (PDB) entry 1JFF] (172). (*center*) BtubA/BtubB heterodimer from *Prosthecobacter dejongeii* (PDB entry 2BTQ) (88). (*right*) FtsZ dimer obtained from nucleotide-free FtsZ from *Methanococcus jannaschii* soaked in MgGTP (PDB entry 1W5A) (52).

share similar lateral interactions or accessory proteins.

**Active site.** The GTPase active site is formed by the association of two FtsZ monomers, with the catalytic T7 loop [or synergy loop (58)] in the C-terminal domain of one monomer inserting into the nucleotide-binding pocket of the N-terminal domain of the adjacent molecule (**Figure 1**, right), thereby leading to association-dependent activation of the GTPase activity (51, 52, 59). Catalysis occurs by the polarization of a water molecule hydrogen bonded to two conserved aspartate side chains (*M. jannaschii* residues 235 and 238) within the T7 loop, promoting nucleophilic attack on the γ-phosphate and thus hydrolysis of GTP. A further

**Longitudinal interaction:** an interaction responsible for the formation of protofilaments

**Dynamic instability:** the switching of biological protein polymers between phases of steady elongation and rapid shortening

**Isodesmic assembly:** all intersubunit contacts are equivalent

contribution to the polarization of the γ-phosphate is provided by a magnesium ion coordinated by glutamine (*M. jannaschii* residue 75), several water molecules, and the α,β phosphates. Thus, GTP hydrolysis requires $Mg^{2+}$, consistent with in vitro observation (5, 6, 12).

**What can we learn about FtsZ from tubulin?** Although FtsZ is similar to tubulin in structure, there are some important differences between the two proteins. Microtubules are comprised of α- and β-tubulin, which form a tight α/β heterodimer in solution. The α/β heterodimer has a nonhydrolyzed, nonexchanging GTP bound at the dimer interface. In contrast, most bacteria have only one isoform of FtsZ (60–62), and thus each subunit interface is equivalent.

**Tubulin assembly characteristics.** Microtubules are assembled from α/β heterodimers joined end-to-end so that the α and β isoforms of tubulin alternate, with a second, hydrolyzable GTP molecule between each heterodimer subunit. After assembly, the nucleotide within the subunit cannot exchange, nor can subunits of tubulin within the filament exchange with the cytoplasmic supply. In addition to the longitudinal interactions between α/β heterodimers, extensive lateral associations between protofilaments further stabilize microtubule assembly, and a complete microtubule is comprised of 13 parallel protofilaments. Tubulin protofilaments have a distinct polarity because of the head-to-tail association of tubulin subunits, and owing to the alternating α and β forms of tubulin, β-tubulin is always present at one end (designated the plus end or fast-growing end), and α-tubulin is present at the other end (designated the minus end).

Microtubules exhibit dynamic instability, enabling them to disassemble rapidly in vivo. GTP hydrolysis drives the protofilaments toward a bent or curved polymeric state that is incompatible with the geometry of the microtubule wall. Kinetic stability of the microtubule is maintained by a GTP-bound cap that restrains and stabilizes the polymer in the straight conformation. If this cap is hydrolyzed, the tubulin filaments can adopt the curved or bent morphology, resulting in spontaneous disassembly of the filament. Thus, the state of the GTP cap determines how microtubules switch between states of rapid growth and rapid shrinkage (63).

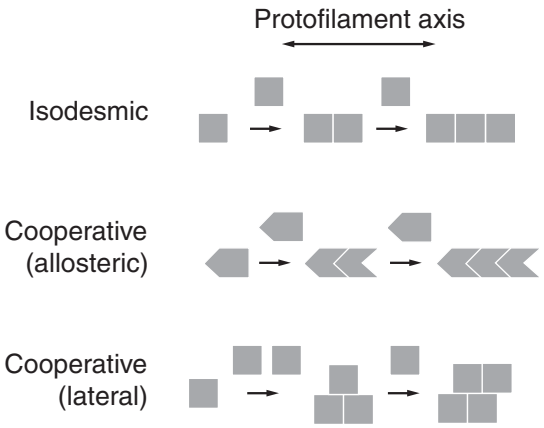


**Figure 2**

Schematic description of isodesmic and cooperative assembly mechanisms. The isodesmic model (*top*) assumes all subunit additions are equivalent, and thus the likelihood of assembly is directly proportional to the concentration of protein. Cooperative assembly (subdivided into allosteric and lateral) occurs when subunit additions are not equivalent. In the case of allosteric cooperative assembly (*center*), the nucleation of a dimer results in a conformational change within the dimer, which increases the affinity for the next subunit to bind. In the case of lateral cooperative assembly (*bottom*), assembly of more than two molecules is stabilized by a third molecule at a different interface; in the case of tubulin and FtsZ, this is known as a lateral protofilament interaction.

**Isodesmic versus cooperativity assembly.** Both actin and tubulin assemble via cooperative mechanisms in which nucleation is a rate limiting step (**Figure 2**). As a consequence, polymer assembly can be controlled by providing specific nucleation sites at a defined time and place in the cell. For an isodesmic assembly mechanism (**Figure 2**, top), polymer will rapidly assemble and disassemble at all places in the cell, and the cell must provide stabilization factors (to assemble the polymer into a defined structure) and topological information (to position it correctly). Research

into FtsZ assembly has so far failed to establish conclusively the kinetics and mechanism of FtsZ assembly. Significant factors limiting our understanding are the wide variation in FtsZ behavior under different experimental conditions and the consequent uncertainty about which of the observed behaviors may be relevant in vivo.

Isodesmic assembly occurs when linear multimers form in which each bond has an identical contact, and nucleation is just as favored as filament extension (**Figure 2**, top). Cooperative assembly occurs when multimers of protein only become stable after forming an unfavored but defined nucleus, wherein the bonds between the subunits are relatively weak and initiation is difficult. Once the unfavored nucleation step occurs, the filament can extend rapidly because subunits in the larger structure are stabilized by multiple bonds formed with other adjoining subunits (**Figure 2**, middle and bottom). There are three characteristics typical of cooperative assembly: a critical concentration for assembly, a lag in the assembly kinetics at low protein concentration, and a distribution of subunits at equilibrium into two distinct populations of monomers and very long polymers.

A cooperative assembly model for FtsZ protofilaments is now generally accepted for three reasons. First, both FtsZ assembly and GTPase activity have a critical concentration (64, 65). Second, FtsZ assembly shows an ~1 s lag of assembly even at very high protein concentrations, suggesting an "activation" step. This might be the time required to release GDP and bind GTP (66). Third, a size limit and relatively homogeneous protofilament length for FtsZ have been reported (67).

Results from analytical ultracentrifugation and scanning transmission electron microscopy suggest that the FtsZ protofilament is formed by a single chain of FtsZ monomers associated head to tail (68, 69). However, if the FtsZ molecules are rigid and if there is no communication between the two binding faces on a single monomer, then linear protofilament formation cannot be cooperative.

There are several models that attempt to reconcile the observed association into single protofilaments with the data showing cooperative assembly. It is possible that, initially, isodesmic assembly into single protofilaments occurs, followed by cooperative association of protofilaments (such that the cooperative kinetics overwhelm the effects of the isodesmic assembly), giving rise to an apparent overall cooperative assembly. However, this model is unlikely to be true because cooperative assembly is still observed at low concentrations when no bundling or grouping of protofilaments is detected (64). Mingorance et al. (42) have suggested that the isodesmic assembly of protofilaments is followed by a stabilizing cyclization event that they argue would show overall cooperative kinetics. They support this hypothesis with images of cyclic single protofilaments formed in vitro; however, the biological relevance of the observed small ring protofilaments is unclear.

Finally, if we abandon the assumption that FtsZ is a rigid molecule and that binding nucleotide or another subunit could induce a conformational change, then it is also possible that FtsZ exhibits cooperativity within a single protofilament. In this case, the initial dimerization process would cause a conformational change that increases the affinity of the next molecule to bind. Such a model could explain the observed cooperative kinetics reported for single protofilaments of FtsZ.

**Nucleotide exchange.** The structural data for the FtsZ filament strongly suggests that there are major differences in the solvent accessibility of the nucleotide pocket of FtsZ compared with tubulin; however, this data was obtained from crystals in which the nucleotide was soaked in and may not represent the bona fide nucleotide-bound state of the protofilament (52). Within tubulin protofilaments, the nucleotide-binding pockets are occluded, and nucleotide exchange is prohibited. This characteristic is essential for the dynamic

instability of tubulin polymers and provides the stabilizing GTP cap.

By contrast, the FtsZ nucleotide-binding pocket is partially exposed, potentially allowing nucleotide exchange, which could make nucleotide hydrolysis the rate-limiting step in filament disassembly (52, 70). This potential for nucleotide exchange may have important implications for FtsZ assembly and dynamics. If significant nucleotide exchange can occur, FtsZ filaments would not experience dynamic instability because the high ratio of GTP to GDP in the cell (71) would ensure that every molecule in the filament is in the GTP-bound state.

Data suggesting that FtsZ is unable to exchange nucleotides and has a GTP cap have been reported (41, 72), consistent with the tubulin paradigm of dynamic instability. However, other strong data suggest that nucleotide exchange does occur, thus excluding the possibility of dynamic instability (70, 73). Some attempts to determine the nucleotide state within filaments have suggested that the majority of FtsZ in protofilaments is bound to GTP (70, 73), although other data suggest the contrary (74). The conflicting data most probably arise from the different conditions used in the various assays, such as $Ca^{2+}$ concentrations (discussed below) and bundling states of the filaments that may sterically inhibit nucleotide exchange. In particular, the initial form of the soluble protein may be entirely monomeric in some investigations but include dimers and larger oligomers in other cases. Some mutants with reduced GTPase activity are able to support cell division, although with much slower dynamics (30, 75, 76), suggesting that either GTPase activity is modulated in vivo, or GTPase activity is much higher than required to support division. The observed rates of FtsZ assembly appear to differ greatly between different organisms (77), but these differences may also arise from varying experimental conditions.

The presence of $Ca^{2+}$ reduces FtsZ's GTPase activity in vitro (40, 43, 78) and increases bundling of the protofilaments (40, 43, 78, 79). $Ca^{2+}$ has also been reported to reduce the exchange of nucleotide (73). This effect on bundling observed with $Ca^{2+}$ may simply be an indirect effect of a decrease in GTPase activity. The decreased GTPase activity would lead to an increased stability of filaments. Long filaments should be present for a longer period of time, and this should result in conditions promoting lateral association. It is also possible that bundling may sterically inhibit nucleotide hydrolysis. Alternatively, increased bundling may be due to a more specific mechanism involving a currently unknown $Ca^{2+}$-binding site (40, 80).

**Conformational change.** It is thought that nucleotide hydrolysis brings about the disassembly of microtubules by a mechanism linked to a conformational change (81–83). In this hypothesis, the GDP-bound form of tubulin adopts a bent or curved form [observed experimentally (81)] that destabilizes lateral bonds in the microtubule, resulting in the peeling back of curved filaments from the end of a microtubule and the eventual disassembly of the filaments by heterodimer dissociation. Bending occurs at all interfaces in the tubulin protofilament (83), even between the two subunits of each heterodimer where the GTP is never hydrolyzed; thus bending need not be absolutely linked to nucleotide hydrolysis (see below).

Early work observed that FtsZ protofilament disassembly was concomitant with rapid nucleotide hydrolysis (33). FtsZ in the GTP-bound state predominantly forms straight protofilaments (32, 36), whereas GDP-bound FtsZ forms curved protofilaments (37, 65, 69, 84, 85). This observation has led to the suggestion that nucleotide hydrolysis causes a conformational change in the FtsZ filament that may be involved in converting the chemical energy of nucleotide hydrolysis into mechanical energy for constricting the Z ring (35). It has been predicted, on the basis of in silico modeling, that the T3 loop in the nucleotide-binding site will undergo a large

conformational change upon the GDP-to-GTP transition (86); however, no large conformational changes have been observed in the various cocrystal structures of FtsZ with GDP or GTP (52). It is important to note that FtsZ filaments formed in vitro do not always show consistent morphologies with the type of bound nucleotide (42). It has been suggested that the hydrolysis of GTP to GDP simply produces a repulsive electrostatic effect with the loss of the $\gamma$ phosphate and that this chemical repulsion is the mechanism behind disassembly (52, 68).

FtsZ function and disassembly in vivo may be regulated by the combination of a conformational change and an electrostatic effect. However, the oversimplified "spring" model in analogy to the GDP-depolymerization of tubulin is unlikely to reflect the real mechanism of FtsZ function in vivo. The filament energy formed by large superstructures (where the potential binding surfaces of the subunits are large) is very significant and can overcome almost all other molecular effects. For example, although FtsZ may prefer to adopt a bent conformation when bound to GDP in a free protofilament, the binding energy that accompanies favorable lateral association of protofilaments in a certain filamentous form of FtsZ might be large enough to restrain the FtsZ protofilament so that it is straight. Again, only elucidation of the in vivo superstructure of FtsZ will allow us to determine which of the behaviors observed in vitro is biologically relevant.

## BtubA/B

Two tubulin homologues (BtubA and BtubB) have been identified recently in the *Prosthecobacter* bacterial genus, and both show a closer relationship to eukaryotic tubulin than to FtsZ (87). BtubA is 31% to 35% identical and BtubB is 34% to 37% identical to $\alpha$- and $\beta$-tubulin, respectively but only 8% to 11% identical to FtsZ. These proteins do not exist in most bacterial species, and their low divergence from eukaryotic tubulin suggests that they are a product of a distant horizontal gene transfer (88). The cellular function of these two proteins is unknown; however, they assemble in vitro, and it has been speculated that BtubA/BtubB may contribute to the elongated spindle shape of *Prosthecobacter* (89).

The structure of BtubA bound to GTP closely resembles the structure of tubulin (**Figure 1**, middle), including the long loops responsible for lateral interactions in microtubules and the large helix-loop-helix domain (often referred to as the third C-terminal domain) that forms the outer surface of microtubules (88). The third C-terminal domain is absent from FtsZ (49). As in both tubulin and FtsZ, the N-terminal domain (which provides loops T1–T6 for nucleotide binding) is separated from the second domain by the central helix T7. Similarly, the second domain provides the T7 loop (which deviates substantially from both tubulin and FtsZ) that activates nucleotide hydrolysis in the protofilament when inserted into the active site of the adjacent subunit.

BtubA and BtubB form a weak heterodimer in vitro (88). A point of interest is that both BtubA and BtubB are able to refold in vitro without the help of chaperones, which is similar to some FtsZs and unlike normal tubulins (88). The crystal structure of BtubA/BtubB shows a tubulin-like heterodimer, with both intra- and interdimer bends evident. It is not possible to describe BtubA or BtubB as being analogous to either $\alpha$- or $\beta$-tubulin because both BtubA and BtubB have mixed characteristics of the two tubulin forms (88).

**In vitro self-assembly.** BtubA does not self-assemble in vitro (regardless of nucleotide presence), whereas a His-tagged BtubB (with any guanine nucleotide supplied) assembles into rings that appear to be one subunit thick (89). Interestingly, mixtures of BtubB and BtubA assemble into bundled linear protofilaments. Sontag et al. (89) observed bundles of BtubA/BtubB comprising 4–7 protofilaments

that were similar to double protofilaments as well as bundles of straight double protofilaments that twist, as shown by Schlieper et al. (88). It was not possible to determine the parallel/antiparallel arrangement of BtubA and BtubB protofilaments within these structures; however, the monomer repeat is 41.6 Å, a value that lies between the monomer spacings for FtsZ and tubulin, suggesting the filaments are similar (88). Filament bundles with hollow tubular profiles 40 nm in diameter (thicker than the 25 nm of microtubules) have also been observed (89). Analysis of BtubA/BtubB protofilaments indicates an equimolar ratio of each protein within the filaments, further supporting the idea that these proteins assemble in a fashion similar to αβ-tubulin whereby the BtubA/BtubB subunits alternate within the polymer (88, 89).

Little is known about the kinetics of BtubA/BtubB assembly, but critical concentrations for both GTPase activity and assembly have been observed, suggesting a cooperative assembly mechanism (89). Protofilament assembly is reversible; the filaments assemble relatively quickly and, over time, disassemble because of GTP consumption (88).

**The role of nucleotide hydrolysis.** BtubA and BtubB are able to bind one molecule of guanine nucleotide each (89); however, the rates of GTPase activity differ significantly between the two proteins (0.40 mol GTP per min per mol for BtubB and 0.13 mol GTP per min per mol for BtubA). When mixed together in equimolar amounts, the GTPase activity of the combined proteins is higher than that of either protein alone, suggesting the direct interaction of BtubA and BtubB (89).

## THE ACTIN HOMOLOGUES

Besides tubulin, actin is the other essential and ubiqutious eukaryotic cytoskeletal protein. Actin forms double-helical thin filaments composed of two strands. Actin filaments form the "tracks" that myosin (a motor protein) is able to move along.

### Actin

Actin is the prototypical member of a superfamily of ATPases that includes hexokinase and Hsp70. The actin family is very diverse in sequence and in function, showing a conserved fold related to ATPase activity. In a landmark paper in 1992, it was reported that the actin family also includes three bacterial proteins: ParM (StbA), MreB (and relatives), and FtsA. These proteins were identified as actin homologues (showing higher similarity to actin and Hsp70 than to the hexokinases) because they contain five conserved sequence motifs related to nucleotide binding and hydrolysis (90).

The actin fold is comprised of two large domains (named I and II). These two domains can be divided into two subdomains (A and B), and the larger subdomains (designated IA and IIA) share a common fold consisting of a five-strand ß-sheet surrounded by three α-helices. The two smaller subdomains (IB and IIB) show a wider variability in size and structure across the actin family, bestowing some of the properties unique to each protein. The two major domains of actin can rotate with respect to one another, and between the two domains lies a highly conserved ATP-binding pocket. Proteins of the actin family bind ATP, normally in association with $Mg^{2+}$ or $Ca^{2+}$, and coordinating Asp residues are important for nucleotide hydrolysis. Unlike Hsp70 and hexokinase, actin assembles in vivo into a dynamic helical polymer (called F-actin) with cooperative assembly characteristics. Within the filaments, actin assembles in a head-to-tail arrangement; thus, similar to tubulin, it has a distinct asymmetry. Actin shows structural changes upon polymerization (91) and exhibits the characteristic termed treadmilling. Treadmilling occurs when the two ends of a filament have different affinities for polymerization. New subunits assemble at the preferred end, and after nucleotide hydrolysis and phosphate release, subunits dissociate from the nonpreferred end thus leading to a flux of subunits though the filament. When the

rates of assembly and disassembly are equivalent, the filament maintains a constant length. Along with the rate of treadmilling, filament growth and shrinkage are controlled by the rates of monomer addition and dissociation. Actin dynamics are regulated by a range of accessory proteins that affect the assembly, disassembly, and rearrangement of actin filaments in vivo.

## ParM

ParM (previously StbA) is one of three components required for the correct partitioning of R1 low-copy number plasmids in *E. coli*. The two other components of the *par* system are *parC* and ParR. *parC* is a centromere-like sequence of DNA that contains the R1 *par* promoter sequence. ParR is a repressor protein that binds to the *parC* locus. ParM interacts with the ParR-*parC* complex (92) and apparently functions as a primitive mitosis-like spindle to move newly replicated plasmids to opposite poles of the cell (93).

The crystal structure of ParM confirmed the original sequence-based assignment of an actin fold (94), although ParM does show significant differences in loop, helix, and sheet arrangement within domains IB, IA, and IIB (**Figure 3**). The subdomain IB lacks a helix present in both MreB (see below) and actin. This subdomain also shows a longer loop (quite different from the equivalent Dnase I-binding loop of actin) as well as an unusual insertion of a strand from domain IA that is not observed for any other member of the actin family. Other differences include the replacement of a β-sheet with a helix-loop-helix

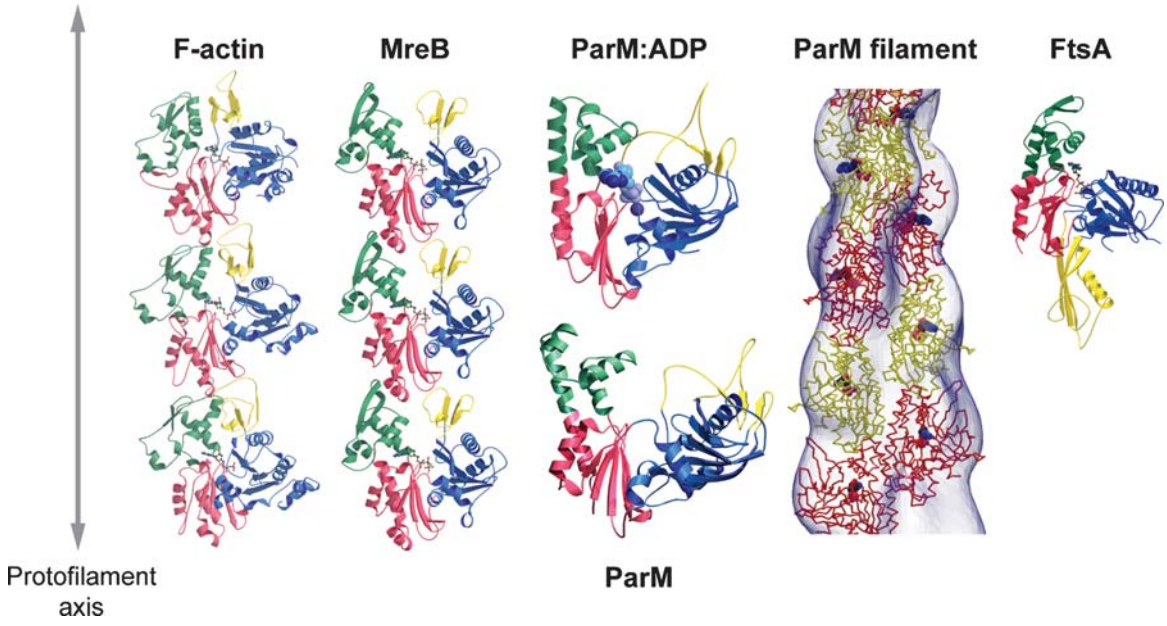


**Figure 3**

(*left*) Structures of F-actin filaments (PDB entry 1YAG) (91), (*second from the left*) MreB filaments from *Thermotoga maritima* (PDB entry 1JCE) (112), (*center*) ParM in the "open" and "closed" conformations, (*second from the right*) ParM filament (94), and (*right*) FtsA, showing the position of the nucleotide within the interdomain cleft, the conservation of fold, and the axis of the protofilament extension (*arrow*). (*center*, *top*) Note the closed conformation (PDB entry 1MWM) and (*bottom*) the open conformation of the apo and ADP-bound forms of ParM, both from *E. coli* plasmid R1 (94). The conformational change is predicted for all actin homologues. (*right*) AMPPNP-bound FtsA from *T. maritima* (PDB entry 1E4G) (136).

motif at the top of subunit IIB and the absence of a helix in subdomain IIA. These structural differences occur in the equivalent regions of actin that are involved in protofilament contacts. The three-dimensional structures of ADP-bound and apo forms of ParM revealed a $\sim 25^{\circ}$ conformational difference between domains I and II that closes the interdomain cleft (**Figure 3**). This conformational change is facilitated by the connecting helix H5 that acts as a mechanical hinge. Like actin, ParM is able to assemble into filaments, and the change in conformation is thought to be linked to the assembly characteristics of ParM filaments.

**ParM filaments.** In vitro, ParM self-assembles in the presence of ATP, ATPγS, AMPPNP, or ADP (93, 95), forming long double-helical filaments that gently twist with a crossover of 300 Å (shorter than the variable actin crossover that averages 360 Å). The helical nature of these filaments is thought to increase the overall strength of the filament and reduce the propensity to form stable lateral interactions, making these filaments less likely to bundle. Modeling ParM filaments using the crystal structure and three-dimensional reconstruction of ParM filaments from negative stain EM, coupled with our understanding of MreB and actin filaments, suggests that ParM assembles in a head-to-tail orientation (**Figure 3**) (94).

Kinetic analysis of ParM assembly in vitro also shows clear differences from actin. ParM filaments are believed to nucleate via a nucleation-condensation mechanism involving three monomers, as expected for a two-stranded helical polymer. ParM shows a 300-fold faster rate of nucleation to that of F-actin (95). Actin has a significant kinetic challenge to spontaneously nucleate, requiring specific nucleation factors that assist filament assembly in vivo. No such factors are apparently required for ParM. Although the in vitro rate of assembly ($5.3 \pm 1.3\ \mu M^{-1}s^{-1}$) of ParM filaments is similar to actin, ParM shows some interesting characteristics. ParM filaments display symmetrical, bidirectional polymerization, but actin assembles unidirectionally. The rate of assembly of ParM filaments in both directions is equal, whereas the disassembly of ParM filaments is unidirectional and catastrophic (shortening results in rapid, complete filament disassembly with a disassembly rate of $64 \pm 20\ s^{-1}$). This dynamic instability is also a feature of tubulin assembly but not actin. Interestingly, in vitro filaments of ParM assemble to a fairly constant length of 1.5 μm (95).

In vitro ParM shows cooperative ATPase activity (92). Disassembly of ParM filaments requires nucleotide hydrolysis (ADP-ParM filaments are extremely unstable with a critical concentration of ~100 μM compared to ~2 μM for ATP-ParM), and as a consequence of this, mutants deficient in ATPase activity show hyperstability, both in vitro and in vivo. The dynamic instability of ParM filaments is an integral part of ParM function, and ATPase mutants form stable filaments in vivo that are not dynamic and do not support plasmid partitioning (92, 93). It is assumed that ParM filament disassembly occurs via a mechanism similar to that postulated for F-actin: The energy released by nucleotide hydrolysis might invoke a large conformational change, which in turn weakens the intramolecular bonds between subunits, promoting filament disassembly. The conformational change seen in the two crystal structures of ParM containing ADP but without a nucleotide might represent such a mechanism (**Figure 3**) (94).

Finally, the spontaneous disassembly of ADP-ParM filaments differs from ADP-actin filaments, which requires severing factors, such as cofilin, to promote disassembly. Monomeric ADP-ParM subunits dissociate from filaments at a rate ~100 times faster than ADP-actin, and actin requires the nucleotide exchange factor profilin to achieve the same rate of ADP dissociation from monomers as ParM achieves alone. Recent work by Garner et al. (95) indicates that like actin, ParM filaments are stabilized by a cap of ATP-bound monomers.

**In vivo filaments—how does ParM really work?** Immunofluorescence microscopy revealed that ParM assembles into dynamic pole-to-pole axial filaments that are essential for plasmid partitioning (93). The intracellular expression of ParM produces ~15,000–18,000 molecules per cell (93). When assembled into filaments, this should be enough ParM to form a filament 15–20 times the cell length. So, it is certainly possible that the ParM filament is comprised of several parallel filaments.

Most *par* systems of plasmid partitioning only comprise three components. ParM is unable to assemble without the other components ParR and *parC* (93), and plasmid replication is required for ParM filament formation (96). The binding of ParM to ParR-*parC* is ATP dependent, and this stimulates ParM's ATPase activity. Møller-Jensen et al. (96) have observed plasmids attached to each end of the ParM filament. As the ParM filament extends in length, so does the distance between the plasmids. These data imply that ParM may provide the mechanical force required to push the plasmids to the poles of the cell, analogous to the mitotic apparatus in eukaryotic cells. It was proposed that ParR-parC complex functions as a nucleation point for ParM polymerization (96).

Garner et al. (95) propose that, at cellular concentrations of ParM, spontaneous nucleation and filament elongation would occur throughout the cell. Thus, rather than nucleation being a regulatory point in the mechanism, only filaments that manage to locate and bind to plasmid DNA (the ParR-*parC* interaction is known to stabilize ParM filaments below the steady-state critical concentration) would be protected from dynamic instability. Only when both ends are stabilized by binding to the ParR/plasmid complex, would segregation occur. It has been proposed that bidirectional elongation of ParM filaments stabilized by the interaction with the ParR-*parC* complex drives plasmid segregation.

Questions still remain unanswered. How is the ParM filament extended in vivo if the plasmids are bound to each end? It has been suggested that the DNA might be propelled by a treadmilling mechanism of ParM filaments, and the addition of new subunits to the ends of filaments may move the plasmid forward. It has also been speculated that in an unidentified mechanism ParM filaments might serve as a track on which motor proteins carry the plasmids to their destination. No potential motor proteins have been identified as yet. Another interesting issue is raised by the attachment of plasmids to each end of the ParM filament. As described above, ParM assembles into filaments with an inherent polarity. A mechanism whereby asymmetrical depolymerization occurs from a symmetrically assembled filament is difficult to reconcile. Either the two ends of the filament are chemically different, and the plasmid attachment sites are nonidentical, or the in vivo filament of ParM consists of filaments aligned in an antiparallel manner thus forming equivalent ends.

Finally, an axial filament that crosses the mid-cell site must be disassembled prior to cell division or somehow severed. In cells over expressing ParM with a deficient ATPase, hyperstable filaments form, and cell division is blocked. How is disassembly regulated? It is possible that once the plasmids meet the poles of the cell, they attach to the membrane or some kind of target, releasing the ParR-parC interaction and causing ParM filament to become unstable and disassemble.

## MreB

*MreB* is encoded in a cluster of genes involved in determining cell shape formation although its precise role(s) are still unknown (97–100). Some bacteria contain several related MreB-like genes. For example *B. subtilis* encodes MreB, Mbl, and MreBH, with each one showing a similar degree of sequence identity (101, 102). In *B. subtilis*, MreB appears to be required for the control of cell diameter (102, 103), and Formstone & Errington (98) suggest that MreB specifically functions to restrain cell diameter. Mbl in *B. subtilis*

is specifically involved in cell elongation and is required for the helical insertion of the peptidoglycan necessary for growth of some rod-shaped cells (104). In *Caulobacter crescentus*, MreB depletion causes abnormal lemon-shaped cell morphology (instead of the normal rod-shaped cell) with defects in cell wall integrity (105). Analysis of the conservation of MreB is complicated because the phylogenic assignment of these proteins is difficult owing to their similarities. It is interesting to note that those organisms that do not have MreB often have Mbl homologues, which may eventually turn out to be MreB (or vice versa).

Because MreB shows homology with actin and ParM, it was expected that MreB would form some kind of filament capable of mechanical work. On the basis of such an idea, hypothesis-driven research has implicated MreB in chromosome segregation for several organisms, including *B. subtilis* (101, 103, 106), *E. coli* (107), and *C. crescentus* (108), and data from the latter organism are the most convincing. MreB's involvement in chromosome segregation has been investigated with the aid of a small-molecule inhibitor called A22 (whose specific target is MreB) (108, 109). Treatment of *C. crescentus* cells with A22 causes a specific, rapid, and reversible disruption of MreB function. Studies involving the administration of A22 at specific times in the cell cycle revealed that MreB played an important role in the segregation of the origin-proximal loci of the *C. crescentus* chromosome. It appears that segregation requires at least two separate mechanisms, the first being an MreB-dependent separation, whereby a region near the origin is initially segregated, followed by a second mechanism that is independent of MreB, whereby the rest of the chromosome follows the origin (108, 110). In addition to a role in cell shape determination and chromosome segregation, MreB is also thought to mediate cell polarity in *C. crescentus* (111). Sequence analysis of MreB initially revealed similarities to FtsA (97), and MreB was predicted to have an ATPase fold similar to actin and hsp70 in the paper by Bork et al. (90).

**MreB structure.** The MreB crystal structure shows that MreB has a conserved actin fold comprising the two domains (I and II) with a nucleotide-binding site in the interdomain cleft between them (**Figure 3**) (112). Typical of other members of the actin family, the smaller domains, IB and IIB, show more diversity when compared to those of actin. These smaller domains, however, show the same topology as those of actin, suggesting a closer relationship of MreB to actin than Hsp70, FtsA, or hexokinase because the topology of these domains differs. Significant differences between MreB and actin are evident within the helix H8 loop. In actin, this region contains specific sequence insertions required for subunit-to-subunit interactions. These sequences are absent in MreB (112, 113).

**MreB filament.** All biochemical studies have been performed on MreB from *Thermotoga maritima* (a hyperthermophilic eubacterium) because MreB from most mesophilic organisms is difficult to handle. An advantage of this is that the data obtained about MreB are directly comparable. MreB assembles in vitro into straight and curved protofilaments in the presence of ATP (112, 114). Ring-like structures and filament bundling have also been reported; however, the biological implications of these structures have yet to be determined. Filamentous bundles of MreB formed in vitro have an increased rigidity, increasing the overall strength of the filaments, which may be very important to their function particularly if they are part of a mechanical apparatus (114).

No high-resolution data of the in vivo MreB filament are currently available, although crystals containing protofilaments of MreB have provided us with an atomic resolution insight into the self-association of MreB monomers (**Figure 3**). MreB assembles into filaments similar to that of F-actin, in that the subunit repeat, structure, and

subunit orientation are approximately the same (91, 112). However, F-actin shows axial rotation (or twist) within the filaments (F-actin filaments can be described as two twisted protofilaments), but only a small number of MreB filaments show a slight axial rotation (112).

The critical concentration for assembly of MreB (~3 nM) is much lower than the critical concentration of F-actin (~0.25 μM) (114, 115), implying that MreB has a higher affinity for other MreB monomers and MreB filaments than actin does for other actin monomers and filaments. This also suggests that MreB nucleation is a much more favorable process than actin nucleation, and it has been proposed that MreB polymerization occurs either without a nucleation phase or with an extremely short-lived nucleation step (114). Rapid nucleation is also suggested by the almost instantaneous assembly observed in the presence of ATP even at very low MreB concentration (114). Although actin requires accessory proteins to assist with in vivo assembly, the ease of MreB nucleation suggests that MreB may be more kinetically tuned to have less need for regulatory or accessory proteins to assist in filament assembly. Consistent with this, MreB shows faster polymerization rates than actin.

The role of nucleotide hydrolysis with respect to MreB assembly dynamics is poorly understood. Comparison of the MreB nucleotide-binding site with that of the ATP-bound actin indicates that most of the active-site residues are in the same position, with the exception of some of the residues that bind the γ-phosphate. Free phosphate is released in solutions of self-assembling MreB after ATP addition. A time lag between phosphate release and MreB polymerization is the basis for the suggestion that ATP hydrolysis might occur after MreB monomers incorporate into filaments (114). Currently, there is no data about the stability of MreB filaments in vitro, and the study of the disassembly of MreB filaments with respect to nucleotide hydrolysis should be very interesting.

**MreB in vivo.** In a variety of organisms, MreBs have been observed assembling into varied structures, i.e., rings in *Rhodobacter sphaeroides* (116), helical structures in *B. subtilis* (102, 117) and *E. coli* (107, 118), as well as bands and helical structures in *C. crescentus* (105). Time-lapse images and FRAP experiments have revealed that MreB and Mbl filaments are dynamic in vivo (101, 111, 117), although the biological significance for this is unknown.

**Molecular function.** How might MreB proteins function in vivo and what might be their roles? It has been speculated that the helical filaments of MreB-like proteins might provide positional information for the localization of the wall-synthesizing enzymes, the penicillin-binding proteins (PBPs), thereby controlling cell wall morphogenesis and thus cell shape (104, 105, 119). In the 1970s, experimental observations of *B. subtilis* mutants with helical cell morphology led to the suggestion that cell wall elongation occurs by helical peptidoglycan insertion (120). The suggestions that MreB-like proteins may distribute factors that affect the organization and mechanics of the cell wall and that the filament structures themselves may directly contribute to the mechanics of the cell wall (102, 104) provide a possible molecular mechanism for helical peptioglycan growth. Results from *C. crescentus* revealed MreB localizes in an FtsZ-dependent manner to the mid-cell position during cell division, leading to the proposal that MreB directs the switch from cell wall elongation to septum extension during division (105).

MreB's involvement in chromosome segregation may arise from an indirect function of MreB, or MreB might attach to an (as yet) unknown bacterial centromere (108, 119). Finally, it is possible that the functions of MreB proteins provide physical markers in the cell to which other essential processes are linked, thus giving rise to the apparent involvement in chromosome segregation, polarity determination, and cell shape

**WACA:** Walker A cytoskeletal ATPase

definition. Recently, it has been shown that MreC and MreD are linked to cell wall synthesis, and it has been proposed that the Mre proteins (MreB, Mbl, MreC and MreD) provide the link between intracellular organization and the extracellular cell wall synthetic machinery (121, 122). It may not be generally appreciated that filaments of MreB, MreC, MreD, and the peptidoglycan-synthesizing PBPs comprise a system vaguely analogous to the machinary used to position cellulose synthase in plants. This plant machinary utilizes cytosolic helical microtubules to provide information about where cellulose (rather than peptidoglycan in the case of MreB) is helically inserted on the outside of the cell (123).

## FtsA

FtsA was one of the first cell division proteins to be identified (124), and sequence analysis indicated that FtsA might belong to the actin superfamily (90). This caused some excitement because FtsA interacts directly with FtsZ (125–131). In vivo FtsA localizes to the Z ring and also to FtsZ helical structures (23) in an FtsZ-dependent manner (20, 23, 29). Its C terminus (containing a conserved amphipathic helix) has a role in interacting with FtsZ and in interacting with the membrane, suggesting that FtsA tethers the Z ring to the cell membrane (132, 133). Consistent with this, a defined FtsA/FtsZ ratio is required for normal cell division to occur (134, 135).

Structure determination of FtsA revealed an actin fold (less conserved than ParM or MreB) showing some differences particularly in the topology of the two small subdomains (136), with the small subdomain located on the opposite side of domain I when compared to actin (**Figure 3**) (136). This arrangement shows no homology with any known structure.

The actin superfamily is a diverse family united by a common ATPase domain, and self-assembly is an exception rather than the rule. FtsA's conserved ATPase fold, including a conserved catalytic ATP-binding pocket and its preferential ability to bind ATP suggest that an intrinsic part of FtsA's function is to hydrolyze ATP. However, large differences in its enzymatic activity have been observed. Although ATPase activity has been reported for *B. subtilis* FtsA (29), no activity has been detected for FtsA from *Streptococcus pneumoniae* (127). It is possible that ATP hydrolysis by FtsA is regulated in vivo by a conformational change evoked by some form of protein-protein interaction.

The observation that FtsA localizes to the Z ring in vivo, with its similarities to actin, indicates that FtsA itself might self-assemble into a polymer. Generally, attempts to study the self-assembly of FtsA from a variety of organisms has yielded largely negative results; however, very recently *S. pneumoniae* FtsA was observed to polymerize in vitro into bent and bundled long corkscrew-like helices, composed of paired protofilaments (127). The filaments were highly stable, requiring both adenosine nucleotide and magnesium for initial assembly, and showed no dynamic behavior (127). Because FtsA is unable to assemble into a detectable superstructure in vivo in the absence of FtsZ, it is possible that FtsA may require in vivo accessory proteins to assemble, explaining the different self-assembly potentials observed. The high stability of the FtsA filaments reported for *S. pneumoniae* FtsA hints at a more complicated in vivo scenario. Filaments that assemble spontaneously and that are extremely stable would need to be very carefully controlled by the cell. It is possible that with an external activator, FtsA filamentation or self-interaction, becomes reversible, as in the case of the Walker A cytoskeletal ATPase (WACA) family of proteins (see below). Very small changes in vivo may be significant in transforming a monomeric protein into a multimeric assembly. Alternatively, FtsA does not self-assemble but plays a role in tethering the Z ring to the membrane and in stabilizing the ring structure. This possibility is supported by the recent observation that a conserved amphipathic helix in the

C terminus of FtsA is essential for targeting FtsA to the membrane and to the Z ring (132).

## INTERMEDIATE FILAMENT HOMOLOGUE

Intermediate filaments are a class of cytoskeletal elements in eukaryotes that are often expressed tissue-specifically. They are comprised of five different filament structures formed from various forms of keratins, lamins, and other specialized proteins. Examples include filensin (which is found in the lens of the eye), the keratins that are expressed in epithelial cells, and the lamins (which are required for nuclear envelope integrity).

### Crescentin

Crescentin has been postulated to be a bacterial homologue of intermediate filament proteins (IFs) (137). Its amino acid sequence has a distinct seven-residue repeat that is predicted to form coiled-coil structures. Because of the dominating coiled-coil repeat, sequence comparisons are unreliable, but crescentin shares some important overall features with eukaryotic IF proteins. Analysis has revealed that the domain organization of crescentin is similar to animal IF proteins, suggesting that crescentin probably is a prokaryotic homologue of IFs.

Crescentin is required for determining the vibrioid or helical shape of *C. crescentus* cells. In vivo immunofluorescence microscopy and deconvolution analysis revealed that crescentin localizes as a continuous pole-to-pole helical filament along one side of the cell (137). In vitro purified crescentin is able to assemble into filaments with a width of about 10 nm. Remarkably, these filaments assemble spontaneously (in the absence of any energy source or cofactor) similar to IFs.

The correlation that crescentin is involved in determining cell shape and that it forms long filaments within the cell suggest that crescentin filaments assemble and somehow (directly or indirectly) associate with the cytoplasmic membrane specifically on one side of the cell. Furthermore, if the shape and helicity of the filament was somehow applied to the cell, a vibrioid or helical cell shape might be formed. In support of this, in stationary-phase cultures of *C. crescentus*, the cells become filamentous but also helical (138). Vibrioid cells are shorter than the helical pitch of the filament and are simply curved. In cells treated with cephalexin (which disrupts normal peptidoglycan synthesis), the intracellular localization of the crescentin filament was gradually disrupted. So, the function of crescentin is somehow linked to the biosynthesis of peptidoglycan, and it seems likely that its function must also be coordinated with the cell cycle. This poses an interesting question. Because crescentin does not require cofactors or nucleotide, and apparently assembles independently, how is this filament regulated? When a cell is dividing, how is the crescentin filament disassembled to allow daughter cell separation? It seems most likely that cofactors must be present to control crescentin assembly and disassembly.

**IF:** intermediate filament

## WALKER A CYTOSKELETAL ATPASE—A NEW FAMILY OF CYTOSKELETAL PROTEINS?

From the discussion above it is clear that prokaryotes possess a cytoskeleton composed of classical actin, tubulin and possibly intermediate filament-like proteins. However, the spatial organization of bacterial cells also relies on a further group of proteins that has no known direct counterpart in the cytoplasm of eukaryotes. We propose a new subclass of proteins, called the WACA proteins, which are required for the spatial regulation of chromosome partitioning and cell division. The WACA proteins belong to a large and functionally diverse family of ATPases that have a conserved deviant Walker A motif and dimerize in an ATP-dependent manner (139). Although these deviant Walker A proteins are structurally homologous, their

functions differ significantly, and it has been proposed that these proteins may be molecular switches (140). Recently, nitrogenase (an archetypal deviant Walker A ATPase) was shown to undergo conformational changes thought to control electron transfer processes. It was suggested that such conformational changes might be used to achieve directed motion, thus bestowing possible mechanical roles on the deviant Walker A ATPases (141). The WACA proteins are a specific subset of the deviant Walker A ATPases that have evolved the specialized function of forming ATP-induced, surface-dependent polymers (140), which might be considered as an additional component of the prokaryotic cytoskeleton.

The WACA family of proteins is comprised of MinD and the ParA/Soj plasmid and chromosome partitioning proteins, including SopA and ParF [reviewed by Hiraga (142)]. These proteins share extensive sequence homology and a similar three-dimensional structure (140, 143) (**Figure 4**). MinD is involved in the processes of Z-ring positioning during cell division, and ParA and Soj have roles in the processes of chromosome segregation, transcription, and organization of plasmids and chromosomes. All deviant Walker A proteins form dimers and are able to bind and hydrolyze ATP. The ATPase activity and dynamic behavior of the WACA proteins are regulated by the interaction of the WACA with an activation protein (144–146). MinD's ATPase activity is modulated by an interaction with MinE, ParB contains a small, N-terminal peptide known to activate ParA, and similarly Spo0J has a 20-amino acid N-terminal tail that activates Soj's ATPase (140, 147).

Typically the WACA proteins show dynamic behavior in vivo. They all have time-dependent localization patterns in the cell, with ParA alternating between nucleoids (148), MinD (from *E. coli*) oscillating from cell pole to cell pole (149), and Soj moving from pole to pole or nucleoid to nucleoid (150, 151). A notable difference between the WACA proteins is in the period of their oscillation. Although the Min system shows a regular fast oscillation of roughly a minute (152), both ParA and Soj show irregular,

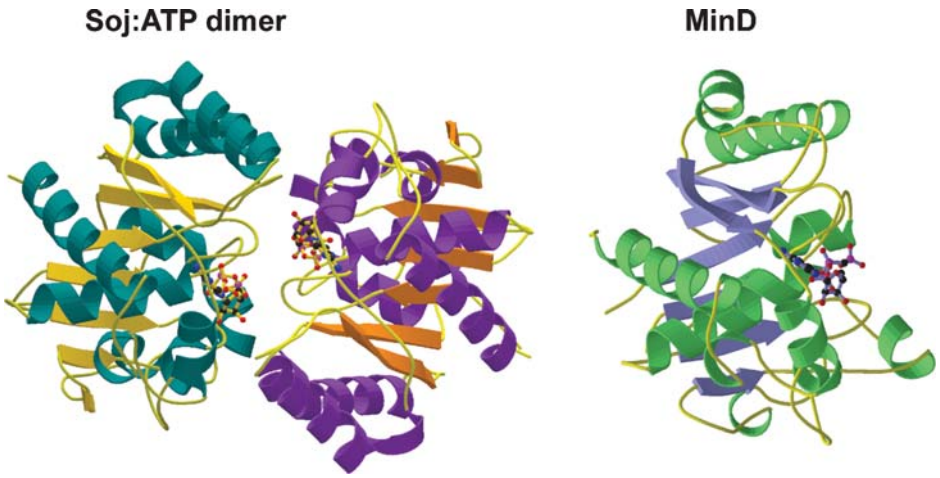


**Figure 4**

Structures of the Walker A cytoskeletal ATPase (WACA proteins). (*left*) Crystal structure of the MgATP-induced Soj (D44A mutant) dimer from *Thermus thermophilus* (PDB entry 2BEK) (140). (*right*) Structure of MinD (binding AMPPCP, a slowly hydrolyzable ATP analogue) from *Pyrococcus furiosus* (PDB entry code 1G3R) (143).

erratic "jumping" with periodicities of minutes, sometimes extending up to an hour (148, 150, 151).

The mechanism behind MinD oscillation is probably best understood. Discovered several years ago in *E. coli* (153), the MinCDE proteins help place the septum in the middle of the cell by inhibiting cell division at the cell poles. ATP-bound MinD is tethered to the membrane (154) by a C-terminal amphipathic helix that binds to the phospholipid bilayer (155, 156), similar to the amphipathic helix required for FtsA's membrane interaction (see below) (132). ATP-bound MinD interacts with MinC [the cell division inhibitor that directly inhibits FtsZ-ring formation (157)], recruiting MinC to the membrane. MinE is also able to bind to the ATP-bound MinD via a short activating peptide (at its N terminus). This interaction enhances MinD's ATPase turnover (158), and the ADP-bound MinD is released from the membrane. Thus, a self-organizing oscillating system is generated because MinD is more likely to rebind the membrane where there is no MinE and where there is already some MinD bound (on the other side of the cell). Thus, MinD oscillation is closely linked to ATP hydrolysis, and consistent with this, MinD mutants deficient in ATPase activity show a reduced oscillation rate (158). Several mathematical models of this system have been produced and faithfully reproduce properties observed in living cells (159–162). It is possible that related oscillatory mechanisms may be used by ParA (163) and Soj (164) to position plasmids and chromosome origins, respectively. It is interesting to note that MinD in *B. subtilis* has not been observed to oscillate but appears to be tethered to the cell poles by another protein called DivIVA. It is surprising that homologous proteins performing identical functions have such different mechanisms of action.

The WACA proteins show other interesting similarities consistent with cytoskeletal elements. Both MinD (118) and ParA (163) have been shown to assemble into dynamic helical structures in vivo. In the case of MinD, these helices were similar to but different in helical pitch and general spread along the length of the cell from those found for MreB (102), and it is thought that the MinD helices are independent of MreB filaments (118). The helical structures of ParA were also observed in the absence of MinD, indicating that ParA filaments are not simply interacting with MinD assemblies. The potential relationship of ParA with MreB has not been explored, but it is possible that the ParA structures are independent of other known helical filaments.

In vitro filaments of MinD have been observed; however, their formation is strongly dependent on the presence of both phospholipid and ATP (154, 165). Similarily, Soj assembly also has a dependence on ATP and DNA, and in their presence, Soj forms nucleoprotein filaments in a cooperative manner (140). Filaments have also been reported for ParF (a ParA homologue) with a requirement for ATP (166). The in vitro filaments described for Soj (140), MinD (165), and ParF (166) are not dynamic by themselves, but this simply reflects the fact that in contrast to FtsZ, the WACA subclass of proteins have specialized activator proteins that are required for ATP hydrolysis.

WACA proteins show an unusual characteristic whereby they bind to the entire surface area of their substrate. In vitro MinD coats phospholipid vesicles with high density; something that could be explained by surface-assisted polymerization (154), and Soj binds to DNA coating it completely (167). The process of surface-assisted polymerization can explain Soj/Spo0J oscillation along the nucleoid, similar to membrane binding of MinD and subsequent displacement by MinE, leading to pole-to-pole oscillation (146, 168).

An important unresolved question is how these proteins travel through the cell. Simple diffusion is one possibility; however, polymerization-depolymerization dynamics is another. Because there is not enough MinD in the cell to cover the whole membrane and helical structures have been

detected in vivo for the proteins, the second alternative seems more likely to us at the moment.

Dynamic behavior (fuelled by nucleotide turnover) seems to be generated by a set of two proteins (the WACA subclass and their ATPase-activating counterparts) in these positioning systems. In contrast, dynamic behavior is maintained by a single component in the case of FtsZ. However, it is interesting to note that it has been speculated that FtsZ protein arises from the fusion of a GTP-binding protein with its activator protein (52). Thus, in order to evolve, a dynamic system biology required two components: a nucleotide-binding protein and an activation domain; however, the two components need not be separate.

## SUMMARY POINTS

1. Bacterial cells contain a variety of dynamic filamentous proteins that bring about spatial and temporal organization analogous to the eukaryotic cytoskeleton.
2. Many of these proteins are related to actin and tubulin by an extended evolutionary history. These proteins retain the actin and tubulin protein fold, and they are able to form filaments in vitro and in vivo.
3. The tubulin homologues include FtsZ and a pair of cotranscribed proteins called BtubA and BtubB.
4. The actin homologues include ParM (a plasmid partitioning protein), MreB, and FtsA.
5. A single protein that forming filaments homologous to intermediate filaments has been identified in *C. crescentus* and is thought to regulate cell shape. Surprisingly, it has some sequence similarity and domain arrangement that are analogous to IFs but also assembles in the absence of a nucleotide or cofactors.
6. We propose that a subclass of the deviant Walker A ATPases [named Walker A cytoskeletal ATPases (WACAs)] have important, dynamic roles in organizing bacterial cells during cell division and plasmid/chromosome partitioning. These proteins should be categorized as a new class of bacterial cytoskeletal proteins.
7. Great leaps forward in our understanding of bacterial cellular organization have been facilitated by modern technologies (such as in vivo fluorescent tagging of proteins), and the discovery of the existence of cytoskeletal scaffolds has provided the means to speculate on the molecular mechanisms by which cell division, cell shape, and chromsome segregation are executed within bacterial cells. It seems likely that many of the mechanisms that have evolved in bacteria share similarities to the molecular processes that regulate cell shape, cell division, chromosome segregation, and possibly endo- and exocytosis in eukaryotes.
8. Currently, our understanding of the molecular processes behind cell division, cell shape determination, and plasmid/chromosome segregation is restricted by our inability to determine the in vivo forms of the superstructures, formed by the bacterial cytoskeletal proteins. It is expected that in time many accessory proteins with roles in assisting the assembly, disassembly, and regulation of such superstructures will be identified.

## FUTURE ISSUE TO BE RESOLVED

1. The first reports of dynamic filaments observed in bacteria caused quite a stir; however, with hindsight, the requirement for such in vivo assemblies is not surprising. It has always been perplexing that bacteria maintain their many and varied shapes in the absence of cytoskeletal elements, and the mechanisms involved in chromosome segregation and cytokinesis seemed hard to fathom without the existence of some form of scaffold or cytoskeletal organization. Various models have been proposed that can explain both chromosome segregation and cytokinesis in the absence of cytoskeletal elements (169, 170). Although bacteria clearly possess proteins that form cytoskeletal elements, the molecular mechanisms by which these cytoskeletal proteins may affect cellular processes are unknown, and much biochemical information is required to resolve this.
2. Why are dynamic assemblies a feature of the cytoskeleton? First, the dynamic nature of these filaments allows for their rapid reorganization. These dynamic structures have inherent flexibility, perfect for bringing about mechanical work and also for adapting to changing cell shape and size. Furthermore, dynamic instability greatly assists in the regulation of filament nucleation. Any filaments that might form without defined initiation directed by the cell will disassemble (171).
3. Biology has typically supplied accessory proteins to modulate the behavior of polymerizing proteins inside eukaryotic cells. In bacteria, the cytoskeletal proteins are apparently self-assembling proteins, and for most, no accessory proteins have been identified, with the exception of FtsZ. In the future, it is likely that many more accessory proteins will be identified that have roles in the specific nucleation, localization, disassembly, or stabilization of the various filaments. So far, all the bacterial proteins likely to be involved in accessory roles for the filamenting cell division, cell shape, and plasmid/chromosome partitioning proteins show no similarities to accessory proteins from eukaryotes.
4. Because many of these filamentous proteins are likely to assemble in vivo into structures with a thickness greater than a single molecule, the formation of lateral interactions between protofilaments and their regulation have important consequences for understanding filament assembly and function. Currently, little is known about any of the lateral interactions of any of the bacterial cytoskeletal proteins, and this limitation severely impedes our understanding the molecular mechanisms behind the processes of cell shape determination, cell division, and possibly chromosome segregation.
5. Identification of the mechanisms behind filament nucleation and assembly, coupled with an understanding of the in vivo filament form, should help us answer fundamental questions, such as how the asymmetry of the crescentin filament is established. Many of these cytoskeletal proteins assemble into helical structures in vivo; however, in vitro they form straight filaments. How is the helicity established?
6. Both actin and tubulin filaments show clear polarity that controls the direction of motor proteins and enables spatial organization in the eukaryotic cell. So far, there is little data indicating that any of these cytoskeletal homologues provide polarity in bacteria (except for MreB in *C. crescentus*). Although the proteins assemble in a

head-to-tail fashion and could provide polarity, little is known about any in vivo consequence of this. No motor proteins that might interact with the bacterial cytoskeleton have been identified.

7. Many of these cytoplasmic bacterial cytoskeletal proteins apparently convey structural direction to the peptidoglycan, thus adaptor proteins that traverse the membrane should exist to pass information from the cytoplasm to the peptioglycan synthesizing machinery in the periplasmic space. Recent data implicates MreC and MreD with these roles in the case of cell shape determination (122). It seems likely that similar molecular links will assist in the correct positioning and assembly of the septum synthesizing apparatus during cell division and also in the coupling of cell division with chromosome/plasmid segregation. These molecular links should reveal much about how the processes of cell shape, cytokinesis, and chromosome segregation are brought about.



## ACKNOWLEDGMENTS

K.A. Michie acknowledges support from UNESCO-L'Oreal and ESF/MRC EuroDYNA.

## LITERATURE CITED

1. Carballido-Lopez R, Errington J. 2003. *Trends Cell Biol.* 13:577–83
2. Errington J, Daniel RA, Scheffers DJ. 2003. *Microbiol. Mol. Biol. Rev.* 67:52–65
3. Møller-Jensen J, Löwe J. 2005. *Curr. Opin. Cell Biol.* 17:75–81
4. Lutkenhaus JF, Wolf-Watz H, Donachie WD. 1980. *J. Bacteriol.* 142:615–20
5. de Boer P, Crossley R, Rothfield L. 1992. *Nature* 359:254–56
6. RayChaudhuri D, Park JT. 1992. *Nature* 359:251–54
7. Beall B, Lutkenhaus J. 1991. *Genes Dev.* 5:447–55
8. Dai K, Lutkenhaus J. 1991. *J. Bacteriol.* 173:3500–6
9. Bi EF, Lutkenhaus J. 1991. *Nature* 354:161–64
10. Bermudes D, Hinkle G, Margulis L. 1994. *Microbiol. Rev.* 58:387–400
11. Erickson HP. 1995. *Cell* 80:367–70
12. Mukherjee A, Dai K, Lutkenhaus J. 1993. *Proc. Natl. Acad. Sci. USA* 90:1053–57
13. Margolin W, Wang R, Kumar M. 1996. *J. Bacteriol.* 178:1320–27
14. Glass JI, Lefkowitz EJ, Glass JS, Heiner CR, Chen EY, Cassell GH. 2000. *Nature* 407:757–62
15. Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, et al. 1998. *Science* 282:754–59
16. Kawarabayasi Y, Hino Y, Horikawa H, Yamazaki S, Haikawa Y, et al. 1999. *DNA Res.* 6:83–101
17. Beech PL, Nheu T, Schultz T, Herbert S, Lithgow T, et al. 2000. *Science* 287:1276–79
18. Osteryoung KW, Vierling E. 1995. *Nature* 376:473–74
19. Sun Q, Margolin W. 1998. *J. Bacteriol.* 180:2050–56
20. Addinall SG, Bi E, Lutkenhaus J. 1996. *J. Bacteriol.* 178:3877–84
21. Ben-Yehuda S, Losick R. 2002. *Cell* 109:257–66
22. Grantcharova N, Lustig U, Flardh K. 2005. *J. Bacteriol.* 187:3227–37
23. Ma X, Ehrhardt DW, Margolin W. 1996. *Proc. Natl. Acad. Sci. USA* 93:12998–3003

24. Mileykovskaya E, Sun Q, Margolin W, Dowhan W. 1998. *J. Bacteriol.* 180:4252–57
25. Stricker J, Erickson HP. 2003. *J. Bacteriol.* 185:4796–805
26. Thanedar S, Margolin W. 2004. *Curr. Biol.* 14:1167–73
27. Rueda S, Vicente M, Mingorance J. 2003. *J. Bacteriol.* 185:3344–51
28. Lu C, Stricker J, Erickson HP. 1998. *Cell Motil. Cytoskelet.* 40:71–86
29. Feucht A, Lucet I, Yudkin MD, Errington J. 2001. *Mol. Microbiol.* 40:115–25
30. Stricker J, Maddox P, Salmon ED, Erickson HP. 2002. *Proc. Natl. Acad. Sci. USA* 99:3171–75
31. Anderson DE, Gueiros-Filho FJ, Erickson HP. 2004. *J. Bacteriol.* 186:5775–81
32. Mukherjee A, Lutkenhaus J. 1994. *J. Bacteriol.* 176:2754–58
33. Mukherjee A, Lutkenhaus J. 1998. *EMBO J.* 17:462–69
34. Lowe J, Amos LA. 2000. *Biol. Chem.* 381:993–99
35. Lu C, Reedy M, Erickson HP. 2000. *J. Bacteriol.* 182:164–70
36. Bramhill D, Thompson CM. 1994. *Proc. Natl. Acad. Sci. USA* 91:5813–17
37. Erickson HP, Taylor DW, Taylor KA, Bramhill D. 1996. *Proc. Natl. Acad. Sci. USA* 93:519–23
38. Löwe J, Amos LA. 1999. *EMBO J.* 18:2364–71
39. Oliva MA, Huecas S, Palacios JM, Martin-Benito J, Valpuesta JM, Andreu JM. 2003. *J. Biol. Chem.* 278:33562–70
40. Yu XC, Margolin W. 1997. *EMBO J.* 16:5455–63
41. Chen Y, Erickson HP. 2005. *J. Biol. Chem.* 280:22549–54
42. Mingorance J, Tadros M, Vicente M, Gonzalez JM, Rivas G, Velez M. 2005. *J. Biol. Chem.* 280:20909–14
43. Mukherjee A, Lutkenhaus J. 1999. *J. Bacteriol.* 181:823–32
44. Cordell SC, Robinson EJ, Löwe J. 2003. *Proc. Natl. Acad. Sci. USA* 100:7889–94
45. Löwe J, van den Ent F, Amos LA. 2004. *Annu. Rev. Biophys. Biomol. Struct.* 33:177–98
46. Romberg L, Levin PA. 2003. *Annu. Rev. Microbiol.* 57:125–54
47. Doolittle RF. 1995. *Philos. Trans. R. Soc. London Ser. B* 349:235–40
48. Doolittle RF, York AL. 2002. *BioEssays* 24:293–96
49. Löwe J, Amos LA. 1998. *Nature* 391:203–6
50. Nogales E, Wolf SG, Downing KH. 1998. *Nature* 391:199–203
51. Nogales E, Downing KH, Amos LA, Löwe J. 1998. *Nat. Struct. Biol.* 5:451–58
52. Oliva MA, Cordell SC, Löwe J. 2004. *Nat. Struct. Mol. Biol.* 11:1243–50
53. Andreu JM, Oliva MA, Monasterio O. 2002. *J. Biol. Chem.* 277:43262–70
54. Santra MK, Panda D. 2003. *J. Biol. Chem.* 278:21336–43
55. Nogales E, Whittaker M, Milligan RA, Downing KH. 1999. *Cell* 96:79–88
56. Kar S, Fan J, Smith MJ, Goedert M, Amos LA. 2003. *EMBO J.* 22:70–77
57. Santarella RA, Skiniotis G, Goldie KN, Tittmann P, Gross H, et al. 2004. *J. Mol. Biol.* 339:539–53
58. Erickson HP. 1998. *Trends Cell Biol* 8:133–37
59. Scheffers DJ, de Wit JG, den Blaauwen T, Driessen AJ. 2002. *Biochemistry* 41:521–29
60. Faguy DM, Doolittle WF. 1998. *Curr. Biol.* 8:R338–41
61. Gilson PR, Beech PL. 2001. *Res. Microbiol.* 152:3–10
62. Margolin W, Long SR. 1994. *J. Bacteriol.* 176:2033–43
63. Desai A, Mitchison TJ. 1997. *Annu. Rev. Cell Dev. Biol.* 13:83–117
64. Caplan MR, Erickson HP. 2003. *J. Biol. Chem.* 278:13784–88
65. Huecas S, Andreu JM. 2004. *FEBS Lett.* 569:43–48
66. Chen Y, Bjornson K, Redick SD, Erickson HP. 2005. *Biophys. J.* 88:505–14

67. Gonzalez JM, Velez M, Jimenez M, Alfonso C, Schuck P, et al. 2005. *Proc. Natl. Acad. Sci. USA* 102:1895–900
68. Rivas G, Lopez A, Mingorance J, Ferrandiz MJ, Zorrilla S, et al. 2000. *J. Biol. Chem.* 275:11740–49
69. Romberg L, Simon M, Erickson HP. 2001. *J. Biol. Chem.* 276:11743–53
70. Romberg L, Mitchison TJ. 2004. *Biochemistry* 43:282–88
71. Neuhard J, Nygaard P. 1987. *Escherichia coli and Salmonella Typhimurium: Cellular and Molecular Biology*. Washington, DC: Am. Soc. Microbiol. Press
72. Scheffers DJ, den Blaauwen T, Driessen AJ. 2000. *Mol. Microbiol.* 35:1211–19
73. Mingorance J, Rueda S, Gomez-Puertas P, Valencia A, Vicente M. 2001. *Mol. Microbiol.* 41:83–91
74. Scheffers DJ, Driessen AJ. 2002. *Mol. Microbiol.* 43:1517–21
75. Lu C, Stricker J, Erickson HP. 2001. *BMC Microbiol.* 1:7
76. Phoenix P, Drapeau GR. 1988. *J. Bacteriol.* 170:4338–42
77. White EL, Ross LJ, Reynolds RC, Seitz LE, Moore GD, Borhani DW. 2000. *J. Bacteriol.* 182:4028–34
78. Marrington R, Small E, Rodger A, Dafforn TR, Addinall SG. 2004. *J. Biol. Chem.* 279:48821–29
79. Esue O, Tseng Y, Wirtz D. 2005. *Biochem. Biophys. Res. Commun.* 333:508–16
80. Santra MK, Beuria TK, Banerjee A, Panda D. 2004. *J. Biol. Chem.* 279:25959–65
81. Chretien D, Fuller S, Karsenti E. 1995. *J. Cell Biol.* 129:1311–28
82. Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, et al. 2004. *Nature* 428:198–202
83. Wang HW, Nogales E. 2005. *Nature* 435:911–15
84. Lu C, Erickson HP. 1999. *Cell. Struct. Funct.* 24:285–90
85. Huecas S, Andreu JM. 2003. *J. Biol. Chem.* 278:46146–54
86. Diaz JF, Kralicek A, Mingorance J, Palacios JM, Vicente M, Andreu JM. 2001. *J. Biol. Chem.* 276:17307–15
87. Jenkins C, Samudrala R, Anderson I, Hedlund BP, Petroni G, et al. 2002. *Proc. Natl. Acad. Sci. USA* 99:17049–54
88. Schlieper D, Oliva MA, Andreu JM, Löwe J. 2005. *Proc. Natl. Acad. Sci. USA* 102:9170–75
89. Sontag CA, Staley JT, Erickson HP. 2005. *J. Cell Biol.* 169:233–38
90. Bork P, Sander C, Valencia A. 1992. *Proc. Natl. Acad. Sci. USA* 89:7290–94
91. Holmes KC, Popp D, Gebhard W, Kabsch W. 1990. *Nature* 347:44–49
92. Jensen RB, Gerdes K. 1997. *J. Mol. Biol.* 269:505–13
93. Møller-Jensen J, Jensen RB, Lowe J, Gerdes K. 2002. *EMBO J.* 21:3119–27
94. van den Ent F, Møller-Jensen J, Amos LA, Gerdes K, Löwe J. 2002. *EMBO J.* 21:6935–43
95. Garner EC, Campbell CS, Mullins RD. 2004. *Science* 306:1021–25
96. Møller-Jensen J, Borch J, Dam M, Jensen RB, Roepstorff P, Gerdes K. 2003. *Mol. Cell* 12:1477–87
97. Doi M, Wachi M, Ishino F, Tomioka S, Ito M, et al. 1988. *J. Bacteriol.* 170:4619–24
98. Formstone A, Errington J. 2005. *Mol. Microbiol.* 55:1646–57
99. Levin PA, Margolis PS, Setlow P, Losick R, Sun D. 1992. *J. Bacteriol.* 174:6717–28
100. Varley AW, Stewart GC. 1992. *J. Bacteriol.* 174:6729–42
101. Soufo HJD, Graumann PL. 2004. *EMBO Rep.* 5:789–94
102. Jones LJ, Carballido-Lopez R, Errington J. 2001. *Cell* 104:913–22
103. Soufo HJD, Graumann PL. 2003. *Curr. Biol.* 13:1916–20
104. Daniel RA, Errington J. 2003. *Cell* 113:767–76
105. Figge RM, Divakaruni AV, Gober JW. 2004. *Mol. Microbiol.* 51:1321–32

106. Soufo HJD, Graumann PL. 2005. *BMC Cell Biol.* 6:10
107. Kruse T, Møller-Jensen J, Lobner-Olesen A, Gerdes K. 2003. *EMBO J.* 22:5283–92
108. Gitai Z, Dye NA, Reisenauer A, Wachi M, Shapiro L. 2005. *Cell* 120:329–41
109. Iwai N, Nagai K, Wachi M. 2002. *Biosci. Biotechnol. Biochem.* 66:2658–62
110. Bates D, Kleckner N. 2005. *Cell* 121:899–911
111. Gitai Z, Dye N, Shapiro L. 2004. *Proc. Natl. Acad. Sci. USA* 101:8643–48
112. van den Ent F, Amos LA, Lowe J. 2001. *Nature* 413:39–44
113. Galkin VE, VanLoock MS, Orlova A, Egelman EH. 2002. *Curr. Biol.* 12:570–75
114. Esue O, Cordero M, Wirtz D, Tseng Y. 2005. *J. Biol. Chem.* 280:2628–35
115. Nishida E, Sakai H. 1983. *J. Biochem.* 93:1011–20
116. Slovak PM, Wadhams GH, Armitage JP. 2005. *J. Bacteriol.* 187:54–64
117. Carballido-Lopez R, Errington J. 2003. *Dev. Cell* 4:19–28
118. Shih YL, Le T, Rothfield L. 2003. *Proc. Natl. Acad. Sci. USA* 100:7865–70
119. Kruse T, Gerdes K. 2005. *Trends Cell Biol.* 15:343–45
120. Mendelson NH. 1976. *Proc. Natl. Acad. Sci. USA* 73:1740–44
121. Kruse T, Bork-Jensen J, Gerdes K. 2005. *Mol. Microbiol.* 55:78–89
122. Leaver M, Errington J. 2005. *Mol. Microbiol.* 57:1196–209
123. Burk DH, Ye ZH. 2002. *Plant Cell* 14:2145–60
124. Donachie WD, Begg KJ, Lutkenhaus JF, Salmond GP, Martinez-Salas E, Vincente M. 1979. *J. Bacteriol.* 140:388–94
125. Descoteaux A, Drapeau GR. 1987. *J. Bacteriol.* 169:1938–42
126. Din N, Quardokus EM, Sackett MJ, Brun YV. 1998. *Mol. Microbiol.* 27:1051–63
127. Lara B, Rico AI, Petruzzelli S, Santona A, Dumas J, et al. 2005. *Mol. Microbiol.* 55:699–711
128. Ma X, Sun Q, Wang R, Singh G, Jonietz EL, Margolin W. 1997. *J. Bacteriol.* 179:6788–97
129. Ma X, Margolin W. 1999. *J. Bacteriol.* 181:7531–44
130. Wang X, Huang J, Mukherjee A, Cao C, Lutkenhaus J. 1997. *J. Bacteriol.* 179:5551–59
131. Yan K, Pearce KH, Payne DJ. 2000. *Biochem. Biophys. Res. Commun.* 270:387–92
132. Pichoff S, Lutkenhaus J. 2005. *Mol. Microbiol.* 55:1722–34
133. Pla J, Dopazo A, Vicente M. 1990. *J. Bacteriol.* 172:5097–102
134. Dai K, Lutkenhaus J. 1992. *J. Bacteriol.* 174:6145–51
135. Dewar SJ, Begg KJ, Donachie WD. 1992. *J. Bacteriol.* 174:6314–16
136. van den Ent F, Löwe J. 2000. *EMBO J.* 19:5300–7
137. Ausmees N, Kuhn JR, Jacobs-Wagner C. 2003. *Cell* 115:705–13
138. Wortinger MA, Quardokus EM, Brun YV. 1998. *Mol. Microbiol.* 29:963–73
139. Koonin EV. 1993. *J. Mol. Biol.* 229:1165–74
140. Leonard TA, Butler PJ, Löwe J. 2005. *EMBO J.* 24:270–82
141. Tezcan FA, Kaiser JT, Mustafi D, Walton MY, Howard JB, Rees DC. 2005. *Science* 309:1377–80
142. Hiraga S. 1992. *Annu. Rev. Biochem.* 61:283–306
143. Cordell SC, Löwe J. 2001. *FEBS Lett.* 492:160–65
144. Ma L, King GF, Rothfield L. 2004. *Mol. Microbiol.* 54:99–108
145. Zhou H, Schulze R, Cox S, Saez C, Hu Z, Lutkenhaus J. 2005. *J. Bacteriol.* 187:629–38
146. Leonard TA, Møller-Jensen J, Löwe J. 2005. *Philos. Trans. R. Soc. London Ser. B* 360:523–35
147. Radnedge L, Youngren B, Davis M, Austin S. 1998. *EMBO J.* 17:6076–85
148. Ebersbach G, Gerdes K. 2001. *Proc. Natl. Acad. Sci. USA* 98:15078–83
149. Raskin DM, de Boer PA. 1999. *J. Bacteriol.* 181:6419–24
150. Marston AL, Errington J. 1999. *Mol. Cell* 4:673–82
151. Quisel JD, Lin DC, Grossman AD. 1999. *Mol. Cell* 4:665–72

152. Raskin DM, de Boer PA. 1999. *Proc. Natl. Acad. Sci. USA* 96:4971–76
153. de Boer PA, Crossley RE, Rothfield LI. 1989. *Cell* 56:641–49
154. Hu Z, Gogol EP, Lutkenhaus J. 2002. *Proc. Natl. Acad. Sci. USA* 99:6761–66
155. Szeto TH, Rowland SL, Rothfield LI, King GF. 2002. *Proc. Natl. Acad. Sci. USA* 99:15693–98
156. Zhou H, Lutkenhaus J. 2003. *J. Bacteriol.* 185:4326–35
157. Hu Z, Mukherjee A, Pichoff S, Lutkenhaus J. 1999. *Proc. Natl. Acad. Sci. USA* 96:14819–24
158. Hu Z, Lutkenhaus J. 2001. *Mol. Cell* 7:1337–43
159. Howard M, Rutenberg AD, de Vet S. 2001. *Phys. Rev. Lett.* 87:278102
160. Kruse K. 2002. *Biophys. J.* 82:618–27
161. Meinhardt H, de Boer PA. 2001. *Proc. Natl. Acad. Sci. USA* 98:14202–7
162. Drew DA, Osborn MJ, Rothfield LI. 2005. *Proc. Natl. Acad. Sci. USA* 102:6114–18
163. Ebersbach G, Gerdes K. 2004. *Mol. Microbiol.* 52:385–98
164. Marsh JW, Taylor RK. 1999. *J. Bacteriol.* 181:1110–17
165. Suefuji K, Valluzzi R, RayChaudhuri D. 2002. *Proc. Natl. Acad. Sci. USA* 99:16776–81
166. Barilla D, Rosenberg MF, Nobbmann U, Hayes F. 2005. *EMBO J.* 24:1453–64
167. Leonard TA, Butler PJ, Löwe J. 2004. *Mol. Microbiol.* 53:419–32
168. Doubrovinski K, Howard M. 2005. *Proc. Natl. Acad. Sci. USA* 102:9808–13
169. Lemon KP, Grossman AD. 2000. *Mol. Cell* 6:1321–30
170. Woldringh CL, Mulder E, Huls PG, Vischer NOE. 1991. *Res. Microbiol.* 142:309–20
171. Mitchison T, Kirschner M. 1984. *Nature* 312:237–42
172. Löwe J, Li H, Downing KH, Nogales E. 2001. *J. Mol. Biol.* 313:1045–57