# 2
# Molecular Components of the Bacterial Cytoskeleton

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**Abstract** It is only relatively recently that a prokaryotic cytoskeleton akin to that in eukaryotes has been identified, revealing a much higher order of cellular complexity than was previously thought. The proteins that form these bacterial cytoskeletal elements not only carry out similar roles to their eukaryotic counterparts, but they also have related protein folds, suggesting an ancient evolutionary relationship and

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the conservation of fundamental mechanisms. This chapter will introduce to the reader what is known at the molecular level regarding the proteins that comprise eubacterial and, in some cases, archaeal cytoskeletal elements.

## 2.1 Introduction

### *2.1.1 What Is a Cytoskeleton?*

In eukaryotes, the definition of the cytoskeleton has come to encompass several types of filamentous structures within the cell, some of which are dynamic structures, whereas others are more stable. Each of these filament types is largely composed of a single protein component that can assemble into polymers in vivo and also in vitro (a schematic showing the way these types of proteins assemble into filaments is shown in Fig. 2.1). The systems of these filaments contribute significantly to cellular organisation and are responsible for determining and maintaining cell shape (as well as contributing to mechanical strength); for the movement of molecules, vesicles, and organelles; and for cell division.

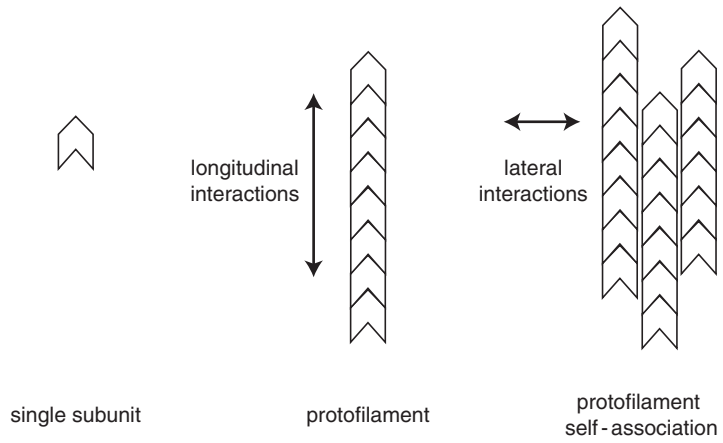


**Fig. 2.1** The assembly of protein monomers into polymeric structures. Schematic description of polymerisation of cytoskeletal proteins. A single protein subunit is depicted on the *left*, and protofilament assembly is represented in the *middle*, showing the axis of longitudinal filament extension. On the *right*, one type of protofilament self-association (sheet formation) and the axis of lateral interactions is shown. Note that protofilament self-association may occur in many different ways, including the antiparallel alignment of filaments. Lateral interactions may also arise in all directions perpendicular to the axis of filament extension to form bundles, tubes, and asters. Accessory proteins may also mediate lateral interactions between protofilaments

In eukaryotes, a cytoskeletal filament is constructed from a protein belonging to one of the three cytoskeletal protein superfamilies: actin, tubulin (which form dynamic actin filaments and microtubules, respectively), and intermediate filaments (IFs), including the keratins, lamins, and other specialised proteins that form more static filaments.

### *2.1.2 The Cytoskeleton in Bacteria*

Bacteria have historically proven problematic to the cell biologist studying cellular organisation, mainly because of the generally small size of their cells. Their tiny dimensions stretch the resolution of optical microscopes to the limit, and, for decades, bacteria were thought not to possess cytoskeletal elements. Only a few internal structures had been observed in bacterial cells, and these were apparently organism specific and obscure.

However, ideas regarding prokaryotic cellular organisation began to change after important results were reported in 1991. Using immunoelectron microscopy, Bi and Lutkenhaus discovered that a well-conserved protein (called FtsZ) that was linked to cell division localised with a unique pattern at the mid-cell site before any observable septum invagination in *Escherichia coli*. Furthermore, during cell

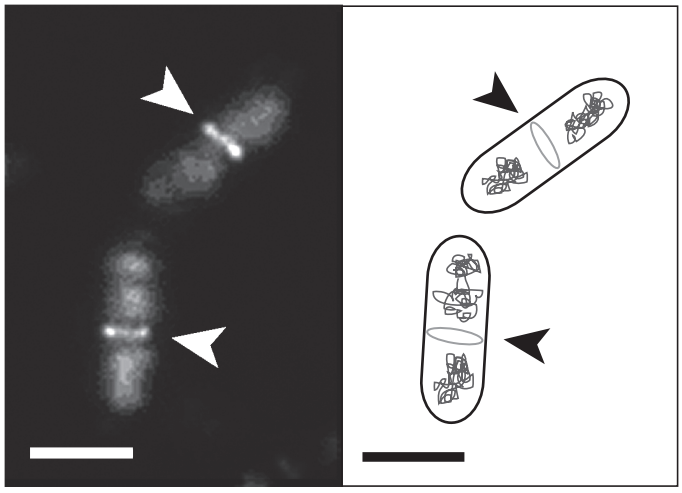

**Fig. 2.2** FtsZ assembles into a structure at the middle of the cell in most eubacteria and archaea. *Left*, subcellular localisation of FtsZ and in DNA *Bacillus subtilis* cells as visualised by an overlay of immunofluorescently labelled FtsZ and DAPI-stained DNA. FtsZ localisation is indicated by the *arrows*. The *right* schematic depicts the cell membrane (*oval outline*), the position of the Z ring (shown by the *gray ring* structure denoted by the *arrows*), and DNA (represented by the *twisted lines*) close to the poles of the cell. Scale bar represents 1 μm

division, FtsZ remained at the leading edge of the enclosing septum, with a pattern consistent with a constricting ring structure (Bi and Lutkenhaus 1991). This was one of the first observations of a highly organised intracellular structure that assembles within bacterial cells. Since then, FtsZ rings have been observed in many bacteria (Fig. 2.2) and are thought to form the basic cytoskeletal structure underpinning the division apparatus.

The progress of our understanding of bacterial cell organisation has been accelerated by advances in optical microscopy methods, including the use of green fluorescent protein (GFP) fusion technologies, time-lapse imaging, and deconvolution analysis. More recently, the development of cryo-electron tomography techniques promises to reveal even greater detail (Lucic et al. 2005; Briegel et al. 2006).

### *2.1.3 Bacteria May Have Many Families of Cytoskeletal Proteins*

We now know that bacteria have considerable intracellular organisation, with several cytoskeletal elements, including the cell division apparatus, also called the divisome or septasome. In fact, all three of the known eukaryotic cytoskeletal proteins (actin, tubulin, and IFs) have counterparts in eubacteria that form filamentous structures with cytoskeletal roles. In this chapter, the prokaryotic cytoskeletal proteins are discussed, with a focus on the biophysical and biochemical qualities of these proteins, which include a tubulin homologue called FtsZ; two specialist tubulin homologues (BtubA and BtubB) found in *Prosthecobacter* species; a range of bacterial actin-like proteins, including MreB, which helps maintain cell shape in many rod-shaped bacteria; some actin-like specialist elements, such as ParM and MamK filaments; and a single IF-like protein represented by crescentin, which is involved in determining cell shape in *Caulobacter crescentus*. Recently, a potential new class of cytoskeletal proteins called the WACA proteins, for *W*alker *A* *c*ytoskeletal *A*TPase, has been described in bacteria, with yet-unidentified counterparts in eukaryotes. The protein members of this family include ParA, MinD, Soj, and MipZ.

## 2.2 The Tubulin Superfamily

In this section, the bacterial tubulin-like proteins are described. To introduce the reader to these types of proteins, the first section covers the molecular characteristics and some interesting biochemical properties of eukaryotic tubulin, followed by a summary of what is known regarding FtsZ. A brief description of the proteins linked to cell division follows. Finally, two specialist tubulin-like proteins (BtubA and BtubB) are briefly discussed.

### *2.2.1 Eukaryotic Tubulin*

Eukaryotic cells express several tubulin proteins, including α-, β-, γ-, δ-, and ε-tubulin. The best-characterised tubulins are α- and β-tubulin, which are the main components of microtubules. Microtubules are essential cytoskeletal elements that assemble in all eukaryotes, and are required for many intracellular transport events and for cell division. For example, microtubules form the mitotic spindle that provides the framework for separation of daughter chromatids toward opposite cell poles during mitosis.

Microtubules are generally comprised of 13 filaments. Each filament is made up of longitudinally end-to-end-associated heterodimers of αβ-tubulin that laterally associate into a tube-like structure (see Fig. 2.1 for a schematic representation of protein polymer formation). The core structures of α- and β-tubulin are composed of two β-sheets surrounded by α-helices (Nogales et al. 1998b), making up two functional domains. The N-terminal of the two domains has a Rossmann fold similar to that of many ATPases, and it contains a GTP binding site. The C-terminal domain is structurally homologous to the family of chorismate mutase-like proteins, and carries some of the catalytic residues for GTP hydrolysis (Nogales et al. 1998a).

The tubulin proteins are GTPases, with the active site formed at the interface between subunits, using essential amino acid residues from both subunits. Thus, GTPase activity only occurs when two or more subunits associate, where the N-terminal domain of one subunit provides the nucleotide-binding site and the C-terminal domain of another subunit provides the "T7 loop" that has the residues responsible for nucleotide hydrolysis.

Microtubules are assembled from αβ-tubulin heterodimers joined end-to-end so that α and β subunits of tubulin alternate, with GTP binding pockets between each (See Fig. 2.3 for the arrangement of α and β subunits of tubulin within a filament). This arrangement results in a distinct polarity, with β-tubulin always present at one end (designated the plus end) and α-tubulin at the other end (called the minus end).

Microtubules exhibit dynamic instability, whereby the filaments may grow or shrink rapidly. This characteristic arises from three biochemical features. First, subunit exchange within the filament does not occur and the filaments can only assemble and disassemble from the ends. Second, the nucleotide-binding pocket between monomers of tubulin is occluded and nucleotide exchange is prohibited within the filament. Third, it has been proposed that GTP hydrolysis induces a destabilising conformational change within the filament, causing a bent or curved morphology. The GDP "bent" form of the filament is unstable and, if unrestrained, the filament disassembles rapidly. However, at the end of the microtubule, a GTP cap can stabilise the filament, restraining the filaments in the "straight" conformation, enabling the filaments to grow. If the GTP-cap is hydrolysed to GDP, the filaments are free to spontaneously disassemble. Thus, the state of this cap has a dramatic effect on whether microtubules grow or shrink (Desai and Mitchison 1997).

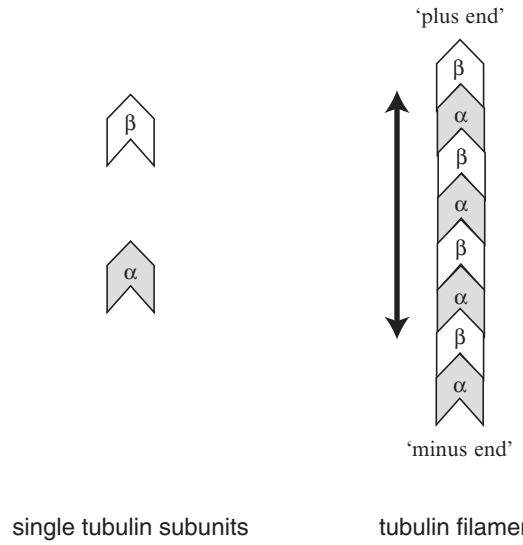


**Fig. 2.3** Assembly of α and β subunits of tubulin within a single protofilament. Note that all of the subunits align in the same orientation along the length of the protein, but alternate

### *2.2.2 FtsZ*

In bacteria, the first known step in cell division is the localisation of FtsZ into a ring structure (the Z ring) at the nascent division site (Bi and Lutkenhaus 1991). FtsZ is one of a number of proteins (see Sect. 2.2.3 below) that are involved in cell division. Many of these cell division proteins were identified by the isolation of conditional mutants that have filamentous cellular phenotypes when grown at a nonpermissive temperature. Thus, many of the cell division proteins have been termed *fts*, for *f*ilamentous *t*emperature *s*ensitive proteins (Rothfield et al. 1999). Z ring formation is followed by the localisation of a series of other cytoplasmic and membrane-bound proteins that also play a part in cell division. It is the final structure of all these proteins built up around the Z ring that is the apparatus that carries out cell division that is referred to as the "divisome" or "septasome."

Much of the research focusing on the molecular processes in bacterial cell division and the roles of the divisome has been centred on understanding the function of FtsZ. This is for several reasons. FtsZ is the first protein known to localise to the future division site and it is essential for the division process. In addition, FtsZ is extremely well conserved among most bacteria and archaea, suggesting that it performs a fundamental biological role. FtsZ is thought to be absent only from the Crenarchaeota (Kawarabayasi et al. 1999; Vaughan et al. 2004), *Ureaplasma urealyticum* (Glass et al. 2000), a *Pirellula* species (Glockner et al. 2003), and the *Chlamydiaceae* family (Stephens et al. 1998; Brown and Rockey 2000; Read et al. 2000). Finally, consistent

with the endosymbiont model for the origins of chloroplasts and mitochondria, FtsZ is also found in these organelles in some eukaryotes (Osteryoung and Vierling 1995; Beech et al. 2000), where it retains a conserved function in organelle division (Osteryoung and McAndrew 2001; Vitha et al. 2001).

In the early 1990s, a conserved tubulin motif was identified within the FtsZ sequence (Mukherjee et al. 1993; Bermudes et al. 1994), leading to the idea of an ancient evolutionary relationship between FtsZ and tubulin. This was surprising because the proteins only share an average of 10 to 18% sequence identity in amino acid sequence alignments (de Pereda et al. 1996). However, in 1998, the debate was settled when the three-dimensional protein structure of FtsZ was reported and FtsZ was shown to have the same two-domain fold as tubulin, with a conserved GTPase binding pocket in the N-terminal domain, and the residues responsible for GTP hydrolysis residing on a flexible loop (called the T7 loop) in the C-terminal domain (Löwe and Amos 1998; Nogales et al. 1998a).

Consistent with its relationship to tubulin, FtsZ assembles in vivo into a highly ordered structure, although its precise molecular architecture is undefined. It is only very recently that structures suggested to be FtsZ filaments have been observed by cryo-electron tomography, although more information is required to confirm this interpretation (Briegel et al. 2006). The Z-ring structure is extremely dynamic and, in vivo, it is remodelled or rebuilt continually with FtsZ subunits exchanging between the ring and the cytoplasm in a timeframe of seconds, as observed by *f*luoresence *r*ecovery *a*fter *p*hotobleaching (FRAP) experiments (Stricker et al. 2002; Anderson et al. 2004). There are also multiple reports of moving ring and helical structures assembled from FtsZ in vivo, as observed by time-lapse live-cell microscopic imaging of FtsZ–GFP fusions in both wild-type and mutant bacterial strains (Ben-Yehuda and Losick 2002; Thanedar and Margolin 2004; Grantcharova et al. 2005; Michie et al. 2006). The dynamic behaviour of the Z ring suggests that it is a flexible and adaptable structure.

In vitro, FtsZ self-assembles into protofilaments (protofilaments are linear chains of associating molecules). Several types of protofilaments have been observed, including rings and straight and curved forms. FtsZ protofilaments also assemble into higher-order structures, including sheets, tubules, asters, and bundles. All of these reported assemblies form under a wide range of conditions and in almost all known nucleotide-bound states, including the GTP-, GDP-, and the nonhydrolysable GMPCPP-bound forms (GMPCPP: guanylyl-($\alpha$,$\beta$)-methylene diphosphate) (Bramhill and Thompson 1994; Mukherjee and Lutkenhaus 1994; Erickson et al. 1996; Yu and Margolin 1997; Mukherjee and Lutkenhaus 1998; Löwe and Amos 1999; Löwe and Amos 2000; Lu et al. 2000; Oliva et al. 2003). The role of GTP hydrolysis in FtsZ function is still debated, and in vivo FtsZ mutants with reduced GTPase activity are able to support cell division (Phoenix and Drapeau 1988; Lu et al. 2001; Stricker et al. 2002), suggesting that either the intrinsic GTPase activity of FtsZ is modulated or it is much higher than required to support division.

The sheer number of different polymer morphologies and the wide range of conditions that support FtsZ self-assembly argue in favour of a model whereby

FtsZ assembles in vivo unassisted by specific proteins. However, we do not know which in vitro polymer form of FtsZ most closely resembles the native form, and the situation is further complicated by the large number of proteins that are known to interact with FtsZ either directly or indirectly during assembly of the divisome, which could alter the biochemical properties of FtsZ. Fortunately, structural determination at the atomic level of protofilaments formed by FtsZ in vitro has provided insight into the likely arrangement of individual FtsZ subunits within the polymer (Oliva et al. 2004). Monomers of FtsZ within protofilaments assemble in a head-to-tail arrangement similar to those observed for αβ-tubulin within microtubules. In a protofilament, each FtsZ monomer maintains longitudinal contacts with an almost identically arranged FtsZ molecule (with a 10° tilt) above and below (Oliva et al. 2004), such that all the molecules are aligned in the same orientation. Thus, the main interactions that form the basis of single protofilament formation are longitudinal interactions between FtsZ molecules. It seems likely that this will be the case in vivo. Sandwiched between two associating FtsZ molecules is a nucleotide-binding pocket, contributed by one molecule of FtsZ, and a catalytic loop for GTP hydrolysis that is contributed by an adjacent FtsZ molecule in the protofilament. The catalytic loop contains two conserved aspartate residues and a glutamine residue that coordinates a magnesium ion. This arrangement precipitates a special characteristic whereby nucleotide hydrolysis cannot occur unless FtsZ self-associates, and, thus, GTPase activity reflects FtsZ–FtsZ interaction (Nogales et al. 1998b; Scheffers and Driessen 2002; Oliva et al. 2004).

The nucleotide-binding pocket of FtsZ shows important differences to the nucleotide-binding pocket of tubulin. The dynamic instability of tubulin assembly is critically linked to the fact that the nucleotide-binding pockets of tubulin are occluded in the microtubule-assembled form, and nucleotide exchange is prohibited. In contrast, the nucleotide-binding pocket of FtsZ is likely to be solvent accessible, which would more readily allow nucleotide exchange (Oliva et al. 2004). This characteristic could critically affect the dynamic behaviour of FtsZ filaments. Analysis by atomic force microscopy has shown that FtsZ filaments continuously rearrange in vitro (Mingorance et al. 2005). End-to-end joining of FtsZ filaments and depolymerisation of FtsZ from within the middle of filaments have been observed in vitro, further suggesting that nucleotide exchange occurs at internal sites at least in a single protofilament (Mingorance et al. 2005).

Whether the Z ring is one protofilament thick or comprised of a bundle of filaments laterally associated has not been experimentally determined, but it is generally thought that the Z ring is comprised of several to many filaments, and the concentration of FtsZ in the cell is sufficiently high (3000–15000 FtsZ molecules per cell) to wind around the cellular circumference many times (Lu et al. 1998; Feucht et al. 2001; Rueda et al. 2003). Using data collected in the FRAP experiments described previously, it was estimated that approximately 30% of the cellular FtsZ seems to be present in the Z-ring structure at any one time (Stricker et al. 2002), which is consistent with the multifilament model for Z rings.

If the Z ring were comprised of multistranded FtsZ filaments, then lateral interactions between adjacent protofilaments would probably be important for the

assembly of the Z ring and for maintaining its stability. Indeed, in the case of tubulin, lateral interactions between protofilaments are essential for forming microtubules and are also important for tubulin's interactions with accessory proteins. It is likely that FtsZ lateral interactions are also important for interacting with other proteins and also with the cell membrane. Although several proteins are known to interact directly with FtsZ, including FtsA, ZapA, and MinC, little is known regarding the molecular nature of these interactions, and further investigation into the binding surfaces of FtsZ and its partner proteins should be very illuminating.

Finally, it is worth noting that several bacterial and archaeal genomes encode multiple *ftsZ* genes. This may provide some clues regarding the evolutionary history of the tubulin superfamily. In addition, *ftsZ* genes were recently discovered in plasmids of various *Bacillus* species (Scholle et al. 2003; Tang et al. 2006; Tinsley and Khan 2006). This is surprising because all of these organisms carry a chromosomal copy of FtsZ and are viable without these plasmids. This raises the question of what role the plasmid-encoded FtsZ plays. Preliminary research indicates that, at least for one such plasmid gene, it is required for the survival of the plasmid in its host, and, thus, thought to be involved in the replication and/or segregation of the plasmid (Tinsley and Khan 2006). Such a function would be more reminiscent of the function of tubulin than FtsZ.

### *2.2.3 Proteins of the Divisome*

The proteins involved in cell division can generally be divided into two classes: those probably performing a direct role in the function of the divisome, i.e., building the division septum, and those responsible for regulating the divisome, i.e., determining when and where the Z ring assembles (see Chap. 1). To give the reader an appreciation for the complicated nature of the structure that assembles with complete dependence on the FtsZ cytoskeletal structure, the following section very briefly summarises divisome assembly and regulation (for a detailed description, see Margolin 2005; Møller-Jensen and Löwe 2005; Harry et al. 2006; Shih and Rothfield 2006; Lutkenhaus 2007).

After FtsZ localisation, a large number of proteins assemble at the mid-cell site. These proteins include the cytoplasmic proteins FtsA, ZipA, ZapA, SepF, EzrA, and the transmembrane proteins FtsK, FtsQ/DivIB, FtsB/DivIC, FtsL, FtsW, FtsI/ PBP 2B/ PBP3, and FtsN. In *E. coli*, protein assembly at mid-cell occurs largely in hierarchical steps and is dependent on FtsZ assembly. The cytoplasmic proteins assemble first, followed by membrane-bound proteins. However, the localisation orders of homologous proteins vary between species, and some of the membrane-bound proteins that assemble late in the localisation hierarchy are interdependent on each other for assembly and are thought to form a complex outside of the divisome (Daniel et al. 1998; Daniel and Errington 2000; Robson et al. 2002; Buddelmeijer and Beckwith 2004). Some of the later-assembling proteins, such as FtsI/PBP2B, are known to be involved in cell wall (peptidoglycan) biosynthesis

(Yanouri et al. 1993), whereas others, such as FtsK in *E. coli*, are involved in coordinating chromosome segregation with cell division by facilitating the complete separation of chromosomes into the newly forming cells created by the division septum (Liu et al. 1998).

The divisome needs cues that allow it to assemble at the correct time and place in the cell. A number of proteins are known to interact with FtsZ in a manner presumed to regulate its localisation. With the exception of SulA, the molecular details of how these proteins exert control over FtsZ are unknown. SulA is a cell division inhibitor that is expressed as a part of the *E. coli* SOS response to DNA damage. SulA functions by binding to the FtsZ polymerisation interface and titrating away monomeric FtsZ, thus, inhibiting Z-ring assembly (Cordell et al. 2003).

The second best-characterised proteins known to affect Z-ring assembly are the Min proteins (for a detailed review, see Lutkenhaus 2007). The Min proteins form a part of a system that, in rod-shaped bacteria, is responsible for the inhibition of inappropriate assembly of the divisome near the poles of the cell. MinC is the component of the system that interacts directly with FtsZ to inhibit Z-ring assembly, although the nature of the MinC–FtsZ interaction has yet to be defined (Bi and Lutkenhaus 1990; de Boer et al. 1990; Hu et al. 1999). The subcellular localisation of MinC at the cell poles is established by the other Min proteins, thereby, only inhibiting cell division at the cell poles (de Boer et al. 1989; Fu et al. 2001; Hale et al. 2001; Shih et al. 2002).

Other proteins, including EzrA, SlmA, ZapA, Noc, and MipZ, have stabilising or destabilising roles, probably by interacting directly with FtsZ, yet the mechanisms by which they do this are largely unknown. Both ZapA (Gueiros-Filho and Losick 2002) and MipZ (Thanbichler and Shapiro 2006b) are thought to stabilise the Z ring. In contrast, EzrA (Levin et al. 1999), SlmA (Bernhardt and de Boer 2005), and Noc (Wu and Errington 2004) are thought to destabilise the Z ring. Whether these proteins work at the level of FtsZ polymerisation, at the level of protofilament bundling, or by some other indirect mechanism remains to be investigated.

### *2.2.4 BtubA and BtubB*

FtsZ is not the only tubulin-like protein in prokaryotes. The bacterial genus *Prosthecobacter* expresses two unusual tubulin homologues called BtubA and BtubB (Jenkins et al. 2002). These proteins do not exist in other bacterial species, suggesting that they probably assemble into a specialist cytoskeletal element. Both proteins show a closer relationship to eukaryotic tubulin than to FtsZ, although the crystal structures of BtubA and BtubB revealed that each protein has mixed characteristics of α- and β-tubulin and cannot be assigned to either α- or β-tubulin (Schlieper et al. 2005). The function of BtubA and BtubB is unknown, however, they self-assemble in vitro, assuming a filamentous form similar to both αβ-tubulin and FtsZ. Their low divergence from eukaryotic tubulin suggests that they might be products of horizontal gene transfer events (Schlieper et al. 2005).

## 2.3 The Actin-Like Superfamily

The actin family of ATPases is very diverse in sequence and function. The members of this family share a conserved ATPase fold and a conserved set of sequence motifs involved in nucleotide binding. Not all members of this family are classified as cytoskeletal proteins, because they do not all share the ability to polymerise, nor do they all have roles in defining the shape of the cell. Within prokaryotes, there exist a number of actin-like proteins, most of which were first identified in a sequence homology search based on the catalytic core shared by actin, hexokinase, and the hsp70 proteins (Bork et al. 1992). The actin-like proteins in prokaryotes include MreB, MreB-like proteins (Mbl and MreBH), ParM (StbA), FtsA, ActA, DnaK (hsp70), and the hexokinases. Of this group, DnaK (hsp70) and the hexokinases are not cytoskeletal proteins, and these proteins will, thus, not be discussed further. The case for FtsA is currently ambiguous and is discussed briefly in Section 2.3.5. More recently, another protein called MamK was identified with similarity to actin and MreB (Komeili et al. 2006). MamK is apparently a specialised protein only found in magnetotactic organisms.

MreB, ParM, and MamK are thought to have important roles in cell shape determination, plasmid segregation, and the assembly of specialist cytoskeletal elements. These proteins are described in detail in the sections after a brief description of actin.

### *2.3.1 Eukaryotic Actin*

Actin is a highly abundant protein, found in almost all eukaryotic cells. It forms a dynamic network that various motor proteins (which transport molecules, vesicles, and organelles) use to track around the cell. In some cell types, actin is largely responsible for determining the shape of the cell and enabling cell locomotion. For example, actin filaments are directly involved in the formation of pseudopodia, central to the mechanism enabling the locomotion of amoeba. Actin, together with myosin, forms a core part of the machinery that enables contraction in muscle cells.

The two domains, named I and II, that comprise actin can be divided into two subdomains: A and B. The larger two of these, designated IA and IIA, comprise a five-strand β-sheet enclosed by three α-helices. IB and IIB, the two smaller subdomains, show variation in both size and structure across the actin family and impart some of the distinct properties of each protein. Between the two domains lies a highly conserved ATP-binding pocket containing essential aspartate residues that, together with either $Mg^{2+}$ or $Ca^{2+}$, bind and hydrolyse ATP, which is central to disassembly of F-actin filaments.

Actin shows cooperative assembly kinetics, with a slow nucleation step requiring nucleation factors in vivo to stimulate and control actin filament assembly. The actin subunits themselves assemble in a head-to-tail arrangement, forming a dynamic

helical polymer known as F-actin (similar to the linear filaments depicted in Fig. 2.1). After polymerisation, each actin subunit undergoes structural changes (Holmes et al. 1990), facilitated in part by rotation of domains I and II with respect to each other. Because of the head-to-tail arrangement of the monomers within the filament, actin has a distinct asymmetry, and the ends of the filaments have different biochemical qualities. Actin displays treadmilling behaviour that occurs because the asymmetrical ends of the actin filament have different affinities for polymerisation. Actin subunits preferentially assemble at one end (called the barbed end), and, after ATP hydrolysis and phosphate release, subunits dissociate from the nonpreferred end, called the "pointed end" (Korn et al. 1987). This leads to a net movement of subunits through the filament. Filaments maintain constant length only when assembly and disassembly rates are equivalent. Thus, the filament length (growth and shrinkage) and the rate that actin subunits pass along the filament while treadmilling are controlled by the rates of monomer addition and dissociation. Many accessory proteins that affect the assembly, disassembly, and rearrangement of actin filaments in vivo have been identified in eukaryotes (Schmidt and Hall 1998).

### *2.3.2 MreB*

For a long time, a cluster of genes (known as *mre* for murein cluster e) was known to be important for determining the cell shape in many of the more complex-shaped bacteria, including the rod-shaped *Bacillus subtilis* and *E. coli*, as well as the differentiating *C. crescentus*. These genes are also present in some mollicutes and archaea, but are largely absent from coccoid bacteria, and are also absent from some rod-shaped bacteria (Daniel and Errington 2003). The *mre* cluster contains the *mreB*, *mreC*, and *mreD* genes. Some organisms have several *mreB*-related genes, such as *B. subtilis*, which also has *mrebl* (*mreB*-like) and *mrebh* (*mreB* homologue). *mreB* seems to be essential because mutation or depletion of MreB and MreB-like proteins results in severe defects in normal cell morphology, normally resulting in cell death (Varley and Stewart 1992; Figge et al. 2004; Kruse et al. 2005). A common feature of *mreB* mutants and strains depleted of MreB is abnormalities in cell size, particularly cell width (Jones et al. 2001). *mbl* mutant strains of *B. subtilis* display distorted twisted and bent morphologies (Abhayawardhane and Stewart 1995). These phenotypes are strongly suggestive of the disruption of key cytoskeletal structures.

Supportive of a cytoskeletal role, in vivo, MreB and the other MreB-like proteins form a helical filament close to the cytoplasmic face of the cell membrane (Jones et al. 2001). It is now thought from a number of experiments that the MreB family of proteins play essential roles in localising cell wall synthesis machinery along the length of the lateral cell wall. Initial experiments performed in *B. subtilis* using a fluorescent derivative of vancomycin that binds to newly synthesised peptidoglycan revealed that new peptidoglycan is inserted in a helical pattern along the

length of the cell. This pattern of vancomycin localisation was dependent on the MreB-like protein, Mbl (Daniel and Errington 2003), although these results have been questioned (Tiyanont et al. 2006). Experiments testing the localisation dependencies of a component of the peptidoglycan synthesis machinery (penicillin-binding proteins [PBP]-2) in *C. crescentus* demonstrated that localisation was dependent on MreB (Figge et al. 2004) and that the cell wall hydrolase enzyme, LytE, in *B. subtilis* has a localisation pattern dependent on MreBH (Carballido-Lopez et al. 2006).

Data suggesting that MreB has roles in the segregation of DNA (Kruse et al. 2003; Soufo and Graumann 2003) has caused great controversy and currently remains a point of contention (Hu et al.; Gitai et al. 2004; Formstone and Errington 2005; Gitai et al. 2005; Kruse et al. 2006; Defeu Soufo and Graumann 2006).

The three-dimensional atomic structure of MreB, solved by X-ray crystallography, revealed that MreB shares the same fold with actin (van den Ent et al. 2001). Fortunately, MreB crystallised in a polymerised form, providing insight into the likely arrangement of MreB self-association within protofilaments. Both MreB and actin show very similar protein–protein contacts with similar subunit repeats being 51 Å and 55 Å in MreB and F-actin, respectively. They also show similar orientations, with both proteins forming two-stranded filaments (van den Ent et al. 2001). One notable difference is in the amount of rotation occurring within these double filaments; MreB shows very little twist (or axial rotation) in comparison with F-actin, where the filaments twist around one another.

In vivo, MreB and MreB-like filaments are highly dynamic. Time-lapse imaging of fluorescently labelled MreB in *E. coli* and *C. crescentus* and of MreB, Mbl, and MreBH in *B. subtilis* have revealed a variety of helical localisation patterns, with different filament pitches adopted within a population of cells. This most likely relates to the observation that the localisation of these proteins changes dynamically throughout the cell cycle. For example, in synchronised *C. crescentus* cells, MreB has been observed to localise as a helical structure along the length of the cell that collapses into a single band at mid-cell at a time coinciding with Z-ring formation (this was shown to be dependent on Z-ring assembly). During the progression of cell division, the MreB "band" then expands out again until it stretches along the length of both daughter cells (Gitai et al. 2004). FRAP experiments with Mbl in *B. subtilis* have shown that the Mbl filaments are continuously remodelled, with a half-life of approximately 8 min. Surprisingly, this technique also revealed that, unlike F-actin, there is no apparent polarity in Mbl filaments with Mbl turnover occuring along the length of the filaments (Carballido-Lopez and Errington 2003). The number of Mbl molecules in the cell is approximately 10-fold higher than what would be required to assemble a single helical protofilament, assuming the Mbl filaments were similar to MreB filaments (Jones et al. 2001), and it has been suggested that Mbl might assemble into short protofilaments that are able to bundle together without a uniform polarity into a multistranded "cable" (Carballido-Lopez and Errington 2003). The dynamic nature of such a cable might then arise from the continuous replacement with the cytoplasmic supply of Mbl subunits dissociating from the ends of each short protofilament within the bundle (Carballido-Lopez and

Errington 2003). An important piece of the puzzle finally came together when it was revealed that single MreB molecules within the MreB cable exhibit treadmilling behaviour in vivo in *C. crescentus*, whereas the overall cable shows no polarity (Kim et al. 2006). Thus, it seems that the MreB family of proteins assemble into short protofilaments, each of which is capable of treadmilling while assembled into a bundled cable that shows no overall polarity.

In vitro studies of MreB filaments have identified straight and curved protofilaments as well as small ring-like structures (with circumferences too small to span the diameter of the cell), and bundles of filaments. As in the case for the filament morphologies observed for FtsZ, the biological relevance of such structures has not been determined and some of them are probably artefactual. It is interesting to note that bundled filaments of MreB show significant increases in rigidity, which might be very important if the MreB cables in vivo carry out a mechanical role. It is also possible that auxiliary proteins regulate the assembly of MreB in vivo, either by mediating MreB–MreB interactions, or MreB–membrane interactions.

Although MreB filament formation is nucleotide dependent (as is the case for F-actin), with ATP and GTP inducing filament formation (van den Ent et al. 2001), the assembly dynamics for MreB are much faster than those observed for F-actin (Esue et al. 2005). MreB has a critical concentration for assembly (~3 nM), 100-fold lower than actin, and, unlike actin, MreB does not have a pronounced nucleation step (Esue et al. 2005).

In eukaryotes, actin filaments serve as "tracks" for use by motor proteins. In addition, a large number of proteins are known to interact with actin in a regulatory context. It was anticipated that similar factors would be identified for MreB. However, despite much searching, no candidate motor proteins that interact with MreB have yet been identified in prokaryotes.

### *2.3.3 ParM*

ParM (also called StbA) is a specialist cytoskeletal element, because it is only required for the correct partition of the R1 low-copy number plasmids in *E. coli*, and, as such, is not essential for normal cell function.

R1 plasmids are actively partitioned by the *par* system, comprised of three components that are sufficient to move plasmids to opposite ends of the cell rapidly after replication (Jensen and Gerdes 1999). The *par* system is comprised of ParM and two other components (ParR and *parC*). In vivo, ParM assembles into dynamic filaments that orient along the length of the cell (Møller-Jensen et al. 2002). Dual-labelling immunofluorescence microscopy experiments demonstrated that the R1 plasmids localise to opposite ends of the ParM filament structures (Møller-Jensen et al. 2003). The association of the ParM filament with R1 plasmids requires ParR. The ParR protein cooperatively binds to the cis-acting centromere-like *parC* DNA sequence encoded on the R1 plasmid (Jensen and Gerdes 1997). Thus, ParM was suggested (Møller-Jensen et al. 2002), and recently shown in vitro (Garner et al.

2007), to function like a rudimentary mitotic spindle, moving the newly replicated plasmids toward opposite poles of the cell.

ParM was known to have similarities to the actin family from early sequence analysis (Bork et al. 1992). When the structure was determined in 2002, it was obvious that the core ATPase domains of actin were conserved, but differences arise in the domains IA, IB, and IIB in the form of strand insertions, absent helices, and extended loops (van den Ent et al. 2002). Although ParM filaments are structurally similar to those of actin, being two-stranded, and winding helically around each other, differences arise in the helical repeats of the filaments, with a full turn occurring every 300 Å in ParM filaments, whereas the actin repeat occurs every 360 Å (van den Ent et al. 2002). Further comparison of ParM with actin reveals that the largest regions of difference correlate with the interaction faces of actin for protofilament contacts, suggesting that ParM might exhibit differences in assembly.

Indeed, ParM has three biochemical properties distinguishing it significantly from actin. First, ParM shows rapid self-assembly, with a nucleation rate 300 times faster than actin, and does not require special nucleation factors to assemble in vivo like actin does (Garner et al. 2004). Second, whereas actin assembles unidirectionally, ParM filaments assemble bidirectionally in a symmetrical fashion, but disassemble unidirectionally (Garner et al. 2004). The filaments themselves are able to self-assemble with a dependence on $Mg^{2+}$ and either ATP, ADP, or either of the nonhydrolysable ATP analogues ATP-γ-S or AMPPNP, although the ADP form is extremely unstable (Møller-Jensen et al. 2002; Garner et al. 2004). Third, ParM filaments exhibit dynamic instability and are able to switch between periods of rapid growth and catastrophic disassembly, whereas actin exhibits a more steady-state tread-milling behaviour (Garner et al. 2004).

Although the molecular arrangement of the ParM filament in vivo is not precisely known, ParM is highly expressed (~15000–18000 molecules per cell), suggesting that either the functional in vivo ParM filament is likely to be comprised of more than one subunit in thickness (Møller-Jensen et al. 2002), or that the high concentration of protein is required to promote filament nucleation and polymerisation. In vitro, the three components of the Par system are able to self-assemble into a machine capable of providing mechanical force suitable for plasmid partition (Garner et al. 2007). ParM filaments were observed to self-assemble into filaments, nucleating at ParR/*parC* complexes. The ParM filaments exhibited dynamic instability, growing and shrinking rapidly but only from free-ends not bound to ParR/*parC* complexes, suggesting that the ParR/*parC* complex functions to stabilise ParM filaments. When ParM filaments were bivalently bound to ParR/*parC* complexes at each end, a stable "spindle" was formed, protected from catastrophic disassembly. This "spindle" was able to push apart ParR/*parC* complexes by the addition of new ParM subunits into the ParM filament. Surprisingly, the addition of ParM monomers occurred at the ends of the filaments and not in the middle of the filaments, leaving an unresolved question regarding the mechanism by which ParM extends the ends of the filaments while the ParR/*parC* complex is attached.

Although ParM is restricted to some low-copy plasmids in *E. coli*, another actin-like protein, called AlfA, has recently been shown to be required for the segregation of a low-copy number plasmid in *B. subtilis*. AlfA assembles in vivo into long filaments with no apparent polarity and its ATPase activity is linked to plasmid partitioning (Becker et al. 2006). Sequence analysis revealed that AlfA lies between MreB and ParM on a phylogenetic tree, suggesting that variations of polymerising actin-like proteins might be a common theme for low-copy plasmid segregation across bacteria.

### *2.3.4 MamK*

Magnetotactic bacteria are a diverse group of aquatic bacteria that are able to orient themselves within (geo)magnetic fields. It is thought that this ability helps in the search for favourable habitats. To perform this, these bacteria have special organelles called magnetosomes that are aligned within the cell by a customised cytoskeletal element to form a structure that has a function akin to a compass needle. The magnetosomes are formed by the invagination of the inner cell membrane (Komeili et al. 2006) around particles of magnetite ($Fe_3O_4$) or greigite ($Fe_3S_4$) (Bazylinski and Frankel 2004), and the mechanisms that orchestrate their formation are still being elucidated. Of relevance to this chapter, however, are the proteins that arrange the magnetosomes into a linear structure. Gene mutation studies have identified two proteins, MamK and MamJ, that are essential for the correct assembly of the magnetosomes into a linear structure (Komeili et al. 2006). Our current understanding of these proteins suggests that MamK is the filament-forming cytoskeletal protein, whereas MamJ is probably an adapter protein that tethers the magnetosomes to the MamK filament (Scheffel et al. 2006). MamK shares sequence homology with both MreB and ParM, and all the known MamK proteins cluster into a distinct family of bacterial actin-related proteins that probably have phylogenetic and functional properties differing significantly from both ParM and MreB.

Using cryo-electron tomography, Komeili et al. (2006) observed filaments with a thickness of 6 nm running parallel to and closely associated with the magnetosomes. In a strain in which the *mamK* gene was deleted the magnetosomes lost their linear organisation and no comparable filaments were observed. Supplementing this strain with GFP–MamK restored magnetosome localisation and caused the reappearance of filaments in some cells, suggesting that MamK may be the cytoskeletal protein responsible for aligning the magnetosomes. Immunogold labelling experiments by Pradel et al. (2006) revealed that MamK is indeed a part of the filament, but, most importantly, they demonstrated that GFP–MamK from *Magnetospirillum magneticum* self-assembles into filaments spontaneously in *E. coli* in the absence of any other gene from *M. magneticum*. This finding suggests that MamK could exist as an independent self-assembling cytoskeletal element (Pradel et al. 2006). Coexpression in *E. coli* of fluorescently labelled MreB from *E. coli* and MamK from *M. magneticum* revealed that both

proteins have independent localisation patterns and coexist as independent structures. Little is known regarding the biochemistry of MamK, and experiments directed at investigating MamK assembly have not yet clearly resolved how MamK filaments might nucleate, or whether ATP binding and hydrolysis are required for assembly (Pradel et al. 2006).

### *2.3.5 Other Actin-Like Proteins: Ta0583 and FtsA*

Archaeal actin-like genes are highly divergent and scattered sporadically across the different orders, suggesting that they do not form fundamental functions in these organisms, but have more specialised roles, and have probably been acquired by lateral gene transfer. One such protein of unknown function, Ta0583, is encoded by *Thermoplasma acidophilum*. Sequence analysis of Ta0583 places it approximately equidistant from all members of the actin family, although it has a slightly higher similarity to ParM than either MreB or actin. As predicted from its primary sequence, the crystal structure of Ta0583 revealed an actin-like fold with conservation of all subdomains (Roeben et al. 2006). Although biochemical analysis revealed that Ta0583 is an active ATPase, polymerisation assays failed to observe Ta0583 self-assembly. Interestingly, addition of 5% glycerol to a solution of recombinant Ta0583 resulted in the formation of crystalline sheets of protein with a longitudinal repeat of 51 Å (Roeben et al. 2006), which is the same as the repeat reported for MreB (van den Ent et al. 2001). However, the similarities in repeat distance are only circumstantial evidence of a cytoskeletal function and the protein sheets might not be biologically relevant, because there is no in vivo data supporting filament formation. Also, the levels of Ta0583 in vivo were found to be low—less than 0.04% of total cellular protein (Roeben et al. 2006), unlike the high expression levels of other actin-like proteins that form filaments in vivo.

The actin-like protein FtsA, which interacts directly with FtsZ, linking the Z ring to the membrane, might also be debated to be a cytoskeletal protein. Although attempts to induce FtsA self-assembly in vitro have largely failed, FtsA from *Streptococcus pneumoniae* self-assembles in the absence of nucleotide into very stable corkscrew-like filaments (Lara et al. 2005). In a strain of *E. coli* carrying a mutation in the *ftsA* gene that causes a deletion of the membrane targeting sequence, long cytoplasmic filaments have been observed (Gayda et al. 1992). These filaments were suggested to have formed from aberrant FtsA. Further fluorescent labelling of similar *ftsA* mutants revealed localisation in the shape of a rod along the length of the cell, consistent with the cytoplasmic filaments being comprised, at least in part, of mutant FtsA (Pichoff and Lutkenhaus 2005). The composition and biological relevance of these filaments has yet to be shown, and FtsA is generally considered as an accessory protein with important regulatory roles in cell division. It is possible, however, that in vivo FtsA undergoes some form of polymerisation that may be surface assisted (similar to the WACA proteins discussed in section 5) by the membrane or by a protein such as FtsZ.

## 2.4 Proteins from the IF Family

### *2.4.1 IFs in Eukaryotes*

Intermediate filaments, so named because they form filaments with a diameter between that of F-actin and microtubules, are abundant, very stable filaments that have roles in providing mechanical support in a wide range of eukaryotic cell types. They have no roles in cell motility, they are unable to undergo treadmilling, and there are no known motor proteins that use IFs for tracking. Instead, IFs tend to take on structural roles that are more permanent, such as mediating cell–cell and cell–matrix contacts, but they do exhibit some dynamic features (Helfand et al. 2004).

IFs are comprised of proteins that are extremely α-helical. The proteins in this generic family have conserved structural features, being comprised of a central coiled–coil motif with varied N and C termini. IF proteins are highly divergent in sequence and vary considerably in molecular weight. In addition, no characteristic conserved sequence motifs that have facilitated the identification of tubulin and actin homologues have been identified in IF proteins. Instead, IF proteins are comprised of large regions of coiled coil, which is common in many other proteins.

Why might bacteria possess IF proteins? Both eubacteria and archaea are capable of assuming a wide range of shapes (for a review on bacterial cell shape, see Young 2006). Precisely how they do this is unknown, and filaments formed by IF proteins might provide the cell with structural restraints suitable for establishing cell morphology, as happens in eukaryotic cells. To date, there has only been one bacterial protein ascribed to the IF family, crescentin.

### *2.4.2 Crescentin*

A clue to how cell shape is controlled has been obtained from *C. crescentus* during a screen for insertion mutants that affect cell morphology. *C. crescentus* is a vibrioid-shaped bacterium that was found to form rod-shaped cells on the disruption of the *creS* gene (Ausmees et al. 2003). The gene product of *creS* is crescentin, which is thought to be at least a functional homologue of IFs in eukaryotes (Ausmees et al. 2003).

Crescentin has an amino acid sequence comprised of heptad repeats (a heptad repeat is a structural motif of seven amino-acids with hydrophobic residues occuring every one and four amino acids and polar residues at all other positions)—consistent with coiled–coil structures and the IF family of proteins. However, as discussed, the coiled–coil repeat is a poor criterion to judge homology, and the IF proteins do not have any known enzymatic or nucleotide-binding capability clearly marking their role.

However, there are several reasons why crescentin is thought to be related to IFs. Firstly, crescentin is able to assemble in vitro into filaments under conditions very

similar to those required for IF assembly. These filaments are stable and do not require nucleotide or cofactors. Furthermore, the crescentin filaments have physical dimensions very similar to IF filaments (Ausmees et al. 2003).

More support comes from in vivo data, in which crescentin is observed to form a filament with a long helical turn that localises to the concave side of the cell. Interestingly, *C. crescentus* cells, when left for a long time in stationary culture, exhibit a helical twist morphology with a similar pitch to the twist observed in the crescentin filaments formed in vitro. Unfortunately, the molecular three-dimensional structures of IF proteins have proven remarkably hard to solve and currently there is no atomic–structural data known for crescentin or for IF proteins, making it difficult to resolve whether these proteins share similar protein folds.

## 2.5 A Fourth Cytoskeletal Family: The Walker A Cytoskeletal ATPases

Bacteria express proteins belonging to a family of deviant Walker A ATPases that can be described as a fourth family of cytoskeletal elements that have no known counterpart of eukaryotic cytoskeletal proteins. These proteins (the Walker A cytoskeletal ATPases [WACA] proteins) are widely distributed, and most bacteria encode one or more members of the family, which includes ParA, MinD, Soj, SopA ParF, IncC, and probably MipZ. The WACA family is characterised by a high conservation of primary sequence (including a deviant Walker A motif), a conserved three-dimensional structure, and ATP-dependent dimer formation. A conserved characteristic of this family is their mutual ATPase stimulation brought about by a companion "activation" protein. WACA proteins exhibit increased ATPAse activity when their activation proteins are present. For example, MinD is modulated by MinE, ParB activates both ParA (Radnedge et al. 1998) and MipZ (Thanbichler and Shapiro 2006a), and the short N-terminal tail of SpoOJ activates Soj (Radnedge et al. 1996; Leonard et al. 2005). Although these proteins share overall sequence and structural homology, their biological roles differ, being involved in DNA segregation, plasmid partitioning, and the positioning and timing of Z ring assembly. It has been proposed that they may be molecular switches (Leonard et al. 2005), yet the molecular mechanisms by which they function remain largely unknown.

Of the WACA members that have been examined, all have the ability to polymerise in vitro, suggesting that these proteins might form cytoskeletal elements. However, the nature of the polymers formed by these proteins is unusual, with the proteins apparently binding to the entire surface of their substrate by some form of surface-assisted polymerisation. For example, MinD in vitro binds phospholipids vesicles with high density (Hu et al. 2002), and Soj completely coats DNA (Leonard et al. 2004; Leonard et al. 2005). Further evidence for the cytoskeletal nature of these proteins comes from in vivo localisation patterns, which are varied across the group but include helices, gradients, and discrete patches (Hu and Lutkenhaus 1999; Marston and Errington 1999; Raskin and de Boer 1999; Shih et al. 2003).

Interestingly, these localisation patterns show dynamic and time-dependent behaviour, in which the pattern changes over time. As examples, Soj localisation alternates erratically as discrete patches between nucleoids and around the same nucleoid (Marston and Errington 1999). MinD oscillates between the cell poles, moving from one end of the cell to the other in *E. coli* (Hu and Lutkenhaus 1999; Raskin and de Boer 1999), but, in *B. subtilis*, MinD forms a fixed gradient, with the highest concentration spreading from the poles (Marston et al. 1998). The periodicities of altered localisation patterns also vary significantly. The time between Soj movements ranges from minutes to up to 1 hour (Marston and Errington 1999), whereas MinD oscillations occur rapidly and rhythmically, taking approximately a minute to move over the entire length of the cell (Hu and Lutkenhaus 1999; Raskin and de Boer 1999).

In general, the precise roles of this class of proteins and the molecular mechanisms behind these roles are poorly understood. Many questions remain unanswered, such as how these proteins are able to dynamically change their localisation patterns. Nor is it clear in many cases why their localisation patterns change.

## 2.6 Other Cytoskeletal Elements

Over the years, many obscure filamentous structures have been observed in bacteria and archaea (Hixon and Searcy 1993; Izard et al. 1999). For example, the highly expressed elongation factor GTPase EF-Tu protein (Beck et al. 1978; Schilstra, 1984) has been reported to form a "cytoskeletal web" within the cell (Mayer 2006). This idea is controversial, and further characterisation is required to determine the role, if any, of EF-Tu as a cytoskeletal protein (Vollmer 2006; Nanninga 1998; Löwe et al. 2004).

Although some of the obscure filaments previously observed may represent specialist cytoskeletal systems unique to a specific organism (such as MamK), and others that have been reported may be observations of structures we are already familiar with, i.e., FtsZ, MreB and related helices, and crescentin, it is also possible that there are further cytoskeletal elements that are ubiquitous and have yet to be identified.

Recent information suggests that a dynamin homologue (called *b*acterial *d*ynamin-*l*ike *p*rotein [BDLP]) is present in many bacteria (Low and Löwe 2006). Although dynamin is not considered a cytoskeletal element in eukaryotes, dynamin (and BDLP) self-assembles into regular structures in the presence of lipid (it tubulates membranes) in vitro. The role of BDLP in bacteria is unknown, however, dynamin-like proteins present in mitochondria and chloroplasts of many eukaryotes have essential roles in organelle division. Dynamin division rings similar to Z rings assemble at the division plane during organelle division. Because chloroplasts and mitochondria have endosymbiotic bacterial origins and the division of these organelles bears some resemblance to prokaryotic cell division, it is tempting to

speculate that BDLP might assemble into a cytoskeletal element to assist with division in bacteria.

## 2.7 Conclusions—The Future

The discovery of cytoskeletal elements in bacteria has changed the way biologists think about bacteria. The old paradigm of the "bag of enzymes" bacterial cell is being left to rest, while the new era of microbiology is revealing highly ordered and complex structures that regulate essential biological processes in prokaryotes.

Many of the cytoskeletal proteins have overlapping roles with those of eukaryotes based on ancient phylogenetic relationships. However, it is fascinating that, across the domains of life, some of these cytoskeletal proteins seem to have switched biological functions during evolution. For example, the tubulin homologue FtsZ has a central role in bacterial cell division, whereas actin contributes a similar role in eukaryotes. Likewise, tubulin provides the scaffold for mitosis in eukaryotes, whereas actin homologues mediate plasmid segregation mechanisms in prokaryotes.

The conserved properties of cytoskeletal proteins reveal the basic characteristics for minimal self-assembling mechanical systems. Understanding how the minimal system works is important if we plan to make practical use of such systems. For instance, the development of complex engineered systems that require structural order might be successful if we understand the fundamental properties of the self-ordering and self-assembling cytoskeletal systems. From a medical perspective, the specific design of antibacterial drugs targeting cytoskeletal proteins will rely on a detailed knowledge of the important similarities and differences between the cytoskeletal proteins of the bacterial pathogen and eukaryotic host.

An increasing amount of research is focused on identifying agents that disrupt the normal function of the bacterial cytoskeleton. Such agents would be useful not only as tools to facilitate our understanding of cytoskeletal systems, but also as potential therapeutic antimicrobial agents, and, with the increased incidence of antibiotic-resistant bacteria, the need to develop novel antibacterial agents is becoming more pressing.

Existing antimicrobial agents target a relatively narrow range of cellular functions—largely being those involved in cell wall, protein, and DNA synthesis. The bacterial cytoskeletal elements provide more targets that might be exploited for antimicrobial development because the cytoskeletal components are essential for cell viability and the proteins within these systems are often highly conserved across bacteria while remaining significantly different to eukaryotic systems, allowing for the development of selective and specific agents.

Because the discovery of the prokaryotic cytoskeleton has been relatively recent, the development of agents inhibiting cytoskeletal function is still in its infancy, however there are already promising leads. Currently, FtsZ has been the most intensively targeted protein, with a range of methods used to identify lead compounds (Paradis-Bleau et al. 2004; Margalit et al. 2004; White et al. 2002;

Stokes et al. 2005). To a lesser extent, MreB, ZipA, and FtsA have also been targeted (Gitai et al. 2005; Iwai et al. 2002; Kenny et al. 2003; Paradis-Bleau et al. 2005; Sutherland et al. 2003) and it is likely that many more of the proteins involved in the prokaryotic cytoskeleton will eventually provide useful avenues for developing antimicrobial agents.

Currently, we still know very little regarding the in vivo nature of the macromolecular arrangement of the cytoskeletal filaments. The molecular mechanisms that regulate these cytoskeletal structures are also not yet discernible and, thus, much basic research into the bacterial cytoskeleton is required. With advances in electron tomography of filaments within bacterial cells, and further cell biology experiments, most of the cytoskeletal proteins will eventually be characterised at the molecular and macromolecular level.

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