

## The Bacterial Cytoskeleton

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Until little more than a decade ago, the cytoskeleton was considered to be a hallmark of eukaryotic cells, setting them apart from their simple bacterial ancestors. Researchers arrived at this view primarily on the basis of conventional light and electron microscopy studies, which painted the bacterium as an 'amorphous bag of enzymes and DNA', devoid of any internal organisation or structure. How quickly times change. Thanks largely to the development of green fluorescent protein (GFP) fusion technology and immunofluorescence microscopy (IFM) methods for bacteria, we now know that the bacterial cell is highly organised at the level of protein localisation and does indeed possess a *bona fide* cytoskeleton.

Bacterial homologues have been identified for all of the major groups of eukaryotic cytoskeletal proteins, namely the actin, tubulin and intermediate filament groups. These homologues not only share sequence and structural similarity with their eukaryotic counterparts, they exhibit all the characteristics of genuine cytoskeletal proteins: (i) they form ordered filamentous structures within the cell, which can be highly dynamic or virtually static, (ii) they are capable of self-assembly *in vitro* into large polymeric filaments, (iii) they can bind and hydrolyse nucleotides (ATP or GTP),

and couple the nucleotide hydrolysis cycle with polymer dynamics, and (iv) they function in a range of important cellular processes, including cell division, chromosome segregation, cell shape maintenance and the establishment of cell polarity. In addition, bacteria possess a number of genuine cytoskeletal proteins that have no homology with eukaryotic cytoskeletal elements.

In this Showcase on Research article, we focus on the two most well studied bacterial cytoskeletal proteins, the tubulin homologue FtsZ and the actin homologue MreB, and discuss the wealth of emerging data regarding their cellular localisation and dynamics, functions and mechanisms of action. Other major players in the bacterial cytoskeleton are summarised in **Table 1**.

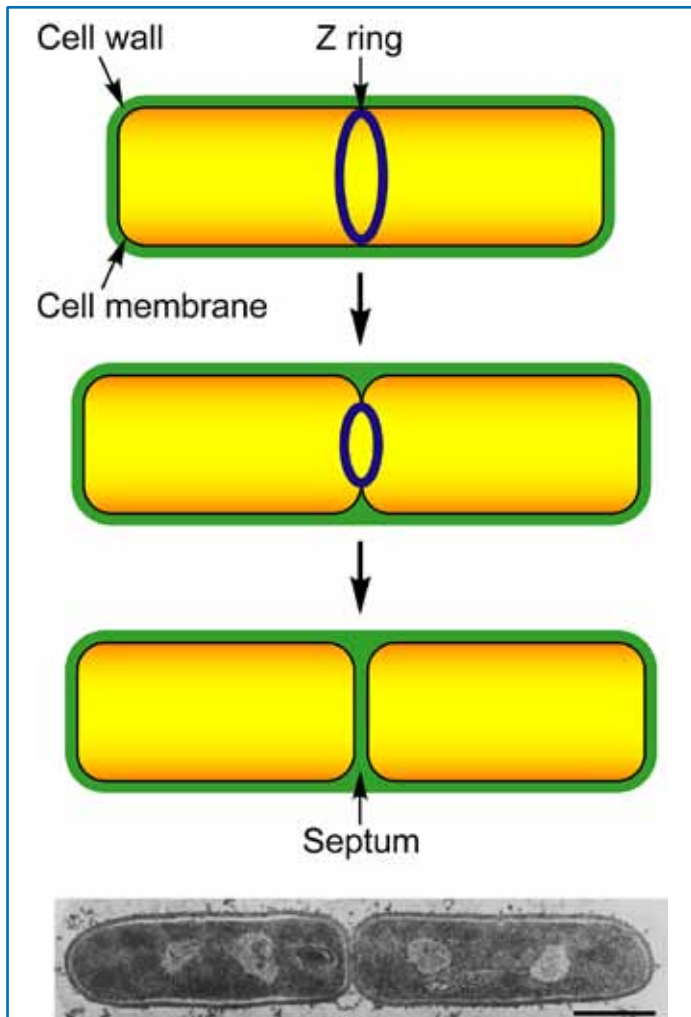
### A Brief History of FtsZ

The *ftsZ* gene was first identified in 1980 as part of a genetic screen for genes involved in the process of cell division in bacteria (1). Bacterial cell division occurs through the coordinated ingrowth of the cell envelope layers (the cell membrane(s) and cell wall) to form a septum that splits the progenitor cell into two independent compartments (**Fig. 1**). Cells containing mutations in *ftsZ* were found to be

**Table 1. Major proteins of the bacterial cytoskeleton.**

Protein	Eukaryotic homologue	Properties/ functions
FtsZ	Tubulin	Forms a contractile ring structure <i>in vivo</i> that mediates cell division.
BtubA/ BtubB	Tubulin	Present in only a small subset of bacterial species. Thought to have been acquired by horizontal gene transfer from a eukaryotic parent. Very similar to tubulin in structure and biochemical properties. <i>In vivo</i> function is unclear.
MreB	Actin	Forms a helical cytoskeletal element that functions in a number of processes including cell morphogenesis and chromosome segregation.
ParM	Actin	Assembles into actin-like helical filaments <i>in vitro</i> . Plays a key role in plasmid segregation by forming dynamic filamentous structures that physically push plasmid molecules to opposite sides of the cell.
MamK	Actin	Present in magnetotactic bacteria (those with the ability to sense and respond to magnetic fields). Forms a cytoskeletal element that coordinates the intracellular organisation of magnetosomes (membranous structures containing magnetically active iron crystals).
Crescentin	Intermediate filament proteins	Resembles eukaryotic intermediate filament proteins in its ability to polymerise into long filaments <i>in vitro</i> in the absence of nucleotides or other cofactors. Acts as a determinant of cell curvature in the crescent-shaped bacterium <i>Caulobacter crescentus</i> .
MinD	None	Displays no homology to eukaryotic cytoskeletal proteins. Assembles into short double-stranded filaments in the presence of ATP <i>in vitro</i> . Forms an extended helical structure underneath the bacterial cell membrane that plays an important role in the selection of the division site.
ParA	None	Exhibits sequence homology and biochemical similarity to MinD. Functions in plasmid segregation.

unable to form a septum, and simply grew larger and larger before eventually dying (1). For this reason, FtsZ is essential for bacterial division and survival (2). The physiological importance of FtsZ is further exemplified by the fact that it is extremely well conserved, being found in virtually every species of bacteria and archae (3). Intriguingly, FtsZ homologues are also present in plant chloroplasts and in the mitochondria of some lower eukaryotes, where they are required for organelle division (4).



**Fig. 1. Bacterial cell division and the Z ring.**

In a typical bacterial cell, the cytoplasm (shown in yellow) is enclosed by a cell membrane (black line) and a rigid, shape-defining cell wall (green). Some bacteria also possess a second outer membrane (not shown). Bacteria come in many shapes and sizes, and in this example a rod-shaped cell is depicted (rod-shaped bacteria are traditionally the most well studied). Cell division in bacteria occurs through the invagination of the cell membrane(s) and cell wall, which ultimately produces a septum that splits the cell into two independent daughter compartments. This process depends on the bacterial tubulin homologue FtsZ, which assembles into a 'Z ring' structure (blue) at the nascent division site prior to septum formation. During the invagination of the cell envelope, the Z ring constricts and maintains a position at the leading edge of the developing septum. In the lower part of the figure, an electron micrograph is presented depicting a bacterial cell, of the species *Bacillus subtilis*, at the completion of the division process. The scale bar represents 1  $\mu\text{m}$ .

In 1991, a landmark study revealed that the FtsZ protein localises to the nascent division site prior to septum formation in the model organism *Escherichia coli*, where it forms a ring-like structure termed the Z ring (5). The Z ring was found to extend around the circumference of the rod-shaped cell, associated with the inner face of the cytoplasmic membrane, and to constrict at the leading edge of the developing septum during cytokinesis (Fig. 1). This was one of the first reports of a protein localising to a specific subcellular address in bacteria. The existence of the Z ring has since been confirmed in many bacterial species using GFP and immunofluorescence techniques to label FtsZ under the fluorescence light microscope (reviewed in ref 4).

Over the course of the 1990s, several key findings revealed the Z ring to be a true cytoskeletal element and the FtsZ protein a structural and functional homologue of eukaryotic tubulin. Although tubulin and FtsZ exhibit only limited amino acid identity (<10%; see ref 6), the discovery of a seven residue 'tubulin signature motif' in the primary FtsZ sequence provided the first clue that the two proteins may share an evolutionary relationship (7). This idea was further supported by similarities in the biochemical properties of FtsZ and tubulin: both are GTPases and reversibly assemble into linear, unbranched polymer strands (protofilaments) *in vitro* in a GTP-dependent manner (8, 9). In the cell, Z ring formation was shown to be the earliest detectable event in the division process. Moreover, it was discovered that FtsZ does not depend on the presence of any other known division proteins to form a Z ring, suggesting that the ring *in vivo* is composed primarily of FtsZ homo-polymers (10). In 1998, the simultaneous publication of the crystal structures of FtsZ and tubulin revealed that the two proteins share a very similar overall fold (Fig. 2), confirming their homology beyond any doubt (11, 12).

#### Structural Differences Between FtsZ and Tubulin

Tubulin is well known for its ability to self-assemble into dynamic tubular superstructures, or microtubules, both *in vivo* and *in vitro*. Microtubules are required for a number of important processes in the eukaryotic cell, including chromosome segregation and organelle transport. The microtubule structure consists of 13 tubulin protofilaments, which are bound together to form a hollow tubular array via interactions between the lateral protofilament surfaces (13).

Importantly, protofilaments formed by FtsZ closely resemble those found in microtubules (14). FtsZ and tubulin polymerise through an equivalent head-to-tail interaction of protein subunits to produce linear, unbranched protofilament strands (15). However, despite these similarities, FtsZ protofilaments do not assemble into microtubule-like structures either *in vitro* or in the cell. This is due to the fact that the FtsZ monomer lacks certain key loops that in tubulin are involved in mediating the lateral contacts between protofilaments (13). Rather than forming microtubules, FtsZ has been shown to assemble into a wide variety of different polymer structures *in vitro*, including protofilament bundles and sheets (14, 16). In each case, the type of structure formed depends intimately on the experimental conditions used. For this reason, it is currently unclear whether any of the FtsZ structures seen *in vitro* actually exist under physiological conditions.

The first insight into the fine structure of the Z ring *in vivo* has recently been provided with the use of a relatively new technique known as electron cryotomography (ECT). ECT employs a rapid freezing (cryofixation) approach for sample preparation, which preserves the natural organisation of the cell much more accurately than traditional electron microscopy methods. ECT revealed the Z ring of the bacterium *Caulobacter crescentus* to be made up of just a few short FtsZ filaments, arranged somewhat erratically around the division site (17). This confirms that the intracellular organisation of FtsZ is vastly different to that of tubulin, which probably reflects the fact that the two proteins have evolved to perform very different functions.

### Functions of the Z Ring

Being the first event in the bacterial cell division process, the formation of the Z ring establishes both when and where the cell will divide. It is imperative that the division septum ultimately forms at the correct time and place, so as to accurately partition the genetic material into the daughter cells. For this reason, Z ring assembly is tightly regulated by a number of spatial and temporal mechanisms (reviewed in ref 4).

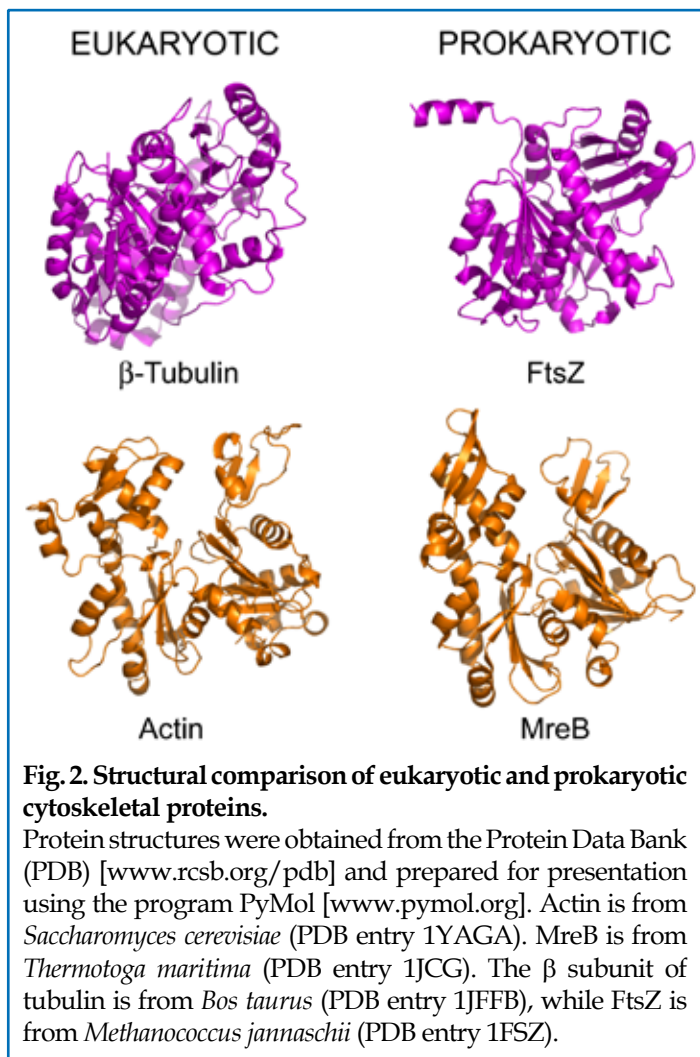
Once formed, the Z ring is thought to perform two main functions. First, it acts as a molecular scaffold to recruit at least 10 different proteins to the site of division, which are required for the formation of a mature septum. All known cell division proteins depend on FtsZ for their localisation to the division site, and several localise through direct interactions with FtsZ (10).

The Z ring is also thought to play an active role during the invagination of the division septum, generating a pinching force on the cell membrane. Recently, Erickson and colleagues showed that when purified FtsZ was incorporated into lipid vesicles *in vitro*, it could assemble into Z rings and produce visible constrictions in the vesicle membrane in the presence of GTP, without the addition of other proteins (18). This work shows that, like tubulin, FtsZ is able to use the energy derived from nucleotide hydrolysis to generate mechanical force. The mechanism of force generation, however, differs considerably from that of the tubulin protein. FtsZ does not exhibit dynamic instability, a phenomenon in which microtubules switch between phases of steady polymer growth and rapid disassembly, enabling them to facilitate, for example, the movement of chromosomes (16). Instead, evidence suggests that FtsZ generates force through a change in protofilament conformation, from straight to curved, upon GTP hydrolysis (17). Since FtsZ polymers are tethered to the cell membrane, this conformational change could exert an inward pinching force on the membrane during septum formation.

### FtsZ Helices and the Mechanism of Z Ring Assembly

Over the last few years, technological and methodological advances in fluorescence microscopy have revealed that FtsZ not only forms ring structures *in vivo*, but also assembles into dynamic helices (4, 19). These helices can extend across the entire bacterial cell, and unlike the Z ring, are present throughout the whole cell cycle (Fig. 3). Recent work in our laboratory, using time-lapse microscopy to track the localisation of FtsZ in growing cells of the bacterium

*Bacillus subtilis*, has shown that the helical FtsZ cytoskeleton actually plays a key role in the formation of the Z ring (19). In newborn *B. subtilis* cells, FtsZ moves randomly within the extended helical pattern. However, at a later stage of the cell cycle, the majority of FtsZ molecules within the helix are redistributed towards the future site of division at the cell centre. At the appropriate time, these FtsZ molecules are then remodelled from a helical conformation into the final Z ring structure. This work overturns the previously held view that the Z ring is assembled directly from FtsZ monomers at the cell centre via polymerisation from a specific site on the membrane (4).



**Fig. 2. Structural comparison of eukaryotic and prokaryotic cytoskeletal proteins.**

Protein structures were obtained from the Protein Data Bank (PDB) [www.rcsb.org/pdb] and prepared for presentation using the program PyMol [www.pymol.org]. Actin is from *Saccharomyces cerevisiae* (PDB entry 1YAGA). MreB is from *Thermotoga maritima* (PDB entry 1JCG). The  $\beta$  subunit of tubulin is from *Bos taurus* (PDB entry 1JFFB), while FtsZ is from *Methanococcus jannaschii* (PDB entry 1FSZ).

### More Cytoskeletal Helices: the Bacterial Actin Homologue MreB

Another important component of the bacterial cytoskeleton is the protein MreB. Like FtsZ, MreB was first identified over 20 years ago, but was not confirmed as a genuine cytoskeletal component until much later. In 2001, the publication of the MreB crystal structure revealed a strikingly similar fold to that of eukaryotic actin (Fig. 2), despite the fact that the two proteins share only ~15% amino acid sequence identity (20). In the same year, a landmark study by Errington and co-workers showed that MreB assembles into extended helical structures underneath the cell membrane in *B. subtilis*, verifying the existence of an actin-like MreB cytoskeleton in bacteria (21). Similar helical MreB structures have since been observed in various other species using

immunofluorescence and GFP labelling techniques (see Fig. 3; reviewed in ref 22). Interestingly, these studies have also revealed that the helical MreB cytoskeleton is highly flexible and dynamic *in vivo*, and is able to undergo gross changes in overall morphology and cellular distribution over the course of the cell cycle (23). In fact, it has been demonstrated in some species that MreB molecules from within the helix can be remodelled into ring-like structures at the nascent division site around the time of septum formation (Fig. 3; see ref 24). This remodelling depends on the presence of FtsZ, suggesting that the MreB and FtsZ cytoskeletons may somehow be coordinated in time and space, and that MreB could play a role in cell division.

### The Biochemistry of MreB

*In vitro*, MreB has been shown to self-assemble into long filamentous structures, each composed of two side-by-side linear polymers (20). In contrast to the characteristic helical double-stranded filaments of eukaryotic actin, these MreB structures are completely straight (20). Curiously, MreB can use ATP and GTP equally well for polymerisation, again differing from actin self-assembly, which only occurs in the presence of ATP (20, 25). MreB filaments have also been shown to exhibit a higher structural rigidity than those of actin (25). As for FtsZ/tubulin, these structural differences between MreB and actin probably reflect the vast differences in cellular functions performed by each protein.

### MreB and the Maintenance of Cell Shape

The MreB cytoskeleton functions in a diverse range of cellular processes in bacteria, including chromosome segregation and the establishment of cell polarity (23). The most well-known, and perhaps most significant role of MreB, however, is in cell shape control in rod-shaped bacterial species. The importance of this role is exemplified by two distinct observations. First, genes coding for MreB have been shown to be present in almost all rod-shaped

species, but usually absent in round (cocoid) bacteria (26). Second, depletion of the MreB protein in rod-shaped organisms leads to a rapid loss of cell shape control, with cells adopting a spherical morphology (22).

Cell shape in bacteria is defined by the rigid cell wall. On current evidence, it appears that the helical MreB cytoskeleton is able to regulate cell shape by acting as a scaffold for the recruitment and positioning of enzymes involved in the synthesis of new cell wall material (peptidoglycan; reviewed in ref 22). In this way, MreB could direct the insertion of new wall material during rod-shaped bacterial growth, allowing the cell to elongate while maintaining its characteristic cylindrical morphology.

### Conclusion and Perspectives

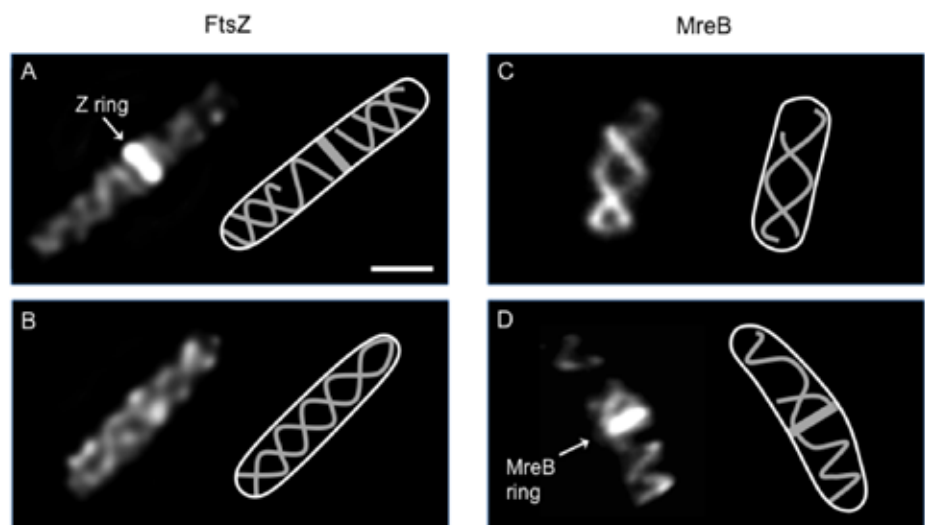
In little more than 10 years, a plethora of information has emerged on the complex inner workings of the bacterial cell. As we continue to uncover more about the organisation and function of bacterial cytoskeletal proteins, yet more proteins are identified that form dynamic filaments *in vivo* or regulate cytoskeletal activity. The explosion of research in this area can largely be attributed to the development of fluorescence methods for visualising protein localisation in bacteria. These techniques have revolutionised the way in which bacterial cell biology is studied. The recent emergence of electron cryotomography and super-resolution fluorescence microscopy methods (see ref 27) promises to further invigorate the field, providing details on cytoskeletal organisation at an ultrastructural level and shedding insight into the mechanisms of action of the component proteins *in vivo*.

It is clear that while bacterial cytoskeletal proteins share much in common with their eukaryotic homologues, profound structural and functional differences do exist. Further research will no doubt help us to understand how the cytoskeleton arose in early life forms, and how it evolved across all the domains of life.

**Fig. 3. Intracellular localisation of FtsZ and MreB in bacteria.**

FtsZ images (A and B) show fixed cells of the bacterium *Bacillus subtilis* labelled with a fluorescent antibody against the FtsZ protein. Images of MreB (C and D) are from live *Escherichia coli* cells expressing a GFP-MreB fusion. Cartoons show an approximate outline of each cell (white line), and a trace of the protein localisation (grey). **A.** A *B. subtilis* cell containing a Z ring at the cell centre. The Z ring appears as a sharp band of fluorescence in the two dimensional image. A fainter, helical-like background staining can also be seen. **B.** A cell captured in the early stages of the cell cycle, prior to Z ring formation. An extended helical pattern is observed throughout the cell. **C.** MreB helices in *E. coli*. **D.** An *E. coli* cell containing a ring-like MreB structure in addition to an extended helical pattern.

The scale bar represents 1  $\mu\text{m}$  for all images. Panels A and B have been adapted from ref 19, while C and D are from ref 28 (copyright 2007, National Academy of Sciences, USA).



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