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REVIEW

The dynamic nature of the bacterial cytoskeleton

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Abstract Three of the four well-established bacterial cytoskeletal systems—the MreB, MinCDE, and FtsZ systems—undergo a variety of short-range and long-range dynamic behaviors. These include the cellular reorganization of the cytoskeletal elements, in which the proteins redistribute from a predominantly helical pole-to-pole pattern into annular structures near midcell. Despite their apparent similarity, these dramatic redistributional events in the three systems are in large part independent of each other. In addition, some of the cytoskeletal structures undergo oscillatory behavior in which the helical elements move repetitively back-and-forth between the two ends of the cell. The details and mechanisms underlying these dynamic cellular events are just now being revealed by fluorescence microscopy of intact cells, fluorescence photobleaching recovery studies, single molecule tracking techniques, and in vitro studies of the purified proteins.

Keywords Bacterial cytoskeleton · MreB · FtsZ · Min · FRAP · Single molecule

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Introduction

Eukaryotic and prokaryotic cells both contain internal cytoskeletal structures, defined as extended filamentous elements that show long-range order within the cell and are based on proteins that are capable of self-assembly into extended polymeric filaments. The fact that eukaryotic cells contain internal cytoskeletons has been known for almost half a century, but the fact that bacterial cells also contain cytoskeletal structures was not appreciated until the present decade. Because of this, much more is known about the eukaryotic cytoskeleton.

Eukaryotic cytoskeletal structures fall into three groups, based on the type of protein that forms the basic polymeric scaffold. These include: (1) actin-based networks, (2) tubulin-based elements that are organized into microtubules, and (3) intermediate filament structures that are based on one or more intermediate filament-type proteins. It is now well established that both actin- and tubulin-based cytoskeletal elements are highly dynamic structures, both at molecular and microscopic levels. As discussed below, recent studies have revealed that many bacterial cytoskeletal structures also exhibit a variety of interesting and often unique dynamic behavior.

Most of what we know about the bacterial cytoskeleton comes from study of rod-shaped organisms, especially *Bacillus subtilis*, *Escherichia coli*, and *Caulobacter crescentus*. At least four types of cytoskeletal structures are present in these cells. These include structures based on prokaryotic homologs of the eukaryotic actin, tubulin, and intermediate filament proteins (e.g., the MreB family of bacterial actins, the tubulin homolog FtsZ, and the intermediate filament protein homolog Crescentin). In addition, bacteria contain a fourth cytoskeletal system based on the MinD protein, which has no obvious homologs in animal

cells and appears unique to bacteria and to plastids of plant cells, which are thought to have originated as bacterial symbionts. In bacterial cells, each of the four proteins is organized into extended filamentous structures that will be described in more detail later. The proteins have also been shown to self-assemble into filamentous polymers in vitro. The molecular organization of the filamentous cellular structures has not been firmly established. It is likely that the extended structures in all four groups are composed of shorter polymeric segments held together directly or indirectly by inter-polymer interactions. Beyond this, little is known about the detailed molecular architecture of the cytoskeletal structures.

Interestingly, the bacterial actin (MreB), FtsZ, and MinD-based cytoskeletal structures resemble each other in their overall cellular organization. In all cases, the proteins, together with associated proteins, are associated with the inner surface of the cytoplasmic membrane and wind helically around the cell between the two poles. In addition, the systems can also assemble into annular structures. Despite these similarities, the different cytoskeletal systems can each assemble in the absence of the others and, therefore, represent independent systems within the cell. Several other proteins are organized in similar helical structures within bacterial cells, but these have generally not yet been shown to self-assemble into filamentous polymers, and their possible relationship to the known cytoskeletal systems has not been defined.

For further information about the bacterial cytoskeletal systems, the reader is referred to several recent review articles [1–5].

Experimental techniques

Fluorescence microscopy

The ability to follow changes in protein localization patterns in living cells has greatly advanced our understanding of cytoskeletal dynamics in bacterial cells. Most studies have used fluorescence microscopy of cells expressing endogenously synthesized cytoskeletal proteins labeled with fluorescent moieties, such as green fluorescent protein (Gfp) and its derivatives, to examine the cellular organization of different cytoskeletal elements. The ability to examine entire cells has provided extensive information about the long-range organization of the cytoskeletal structures and their dynamic behavior during the cell cycle.

Fluorescence recovery after photobleaching (FRAP)

FRAP experiments provide indirect information about protein movements and fluxes within intact cells. FRAP experiments are performed by rapidly photobleaching a subsection of a cell or of a fluorescently labeled cytoskeletal structure, followed by time-lapse imaging of the photobleached region to monitor the rate and extent of recovery of fluorescence [6]. The rates of recovery provide information on the rates of movement of the molecules and, by inference, the effective viscosity of the medium. Significant recovery of fluorescence in the bleached region can reflect either molecular exchange processes or physical movement of intact protofilaments.

In theory, FRAP experiments can distinguish between molecular exchange phenomena and filament movement. If fluorescence recovery is mainly driven by molecular exchange, the rates and extent of fluorescence recovery should be uniform across the bleached region. On the other hand, if fluorescence recovery is mainly due to physical movements of filaments, then the center of the photobleached region is expected to recover more slowly than the edge. In practice, however, it is extremely difficult in bacterial cells to determine whether there is a difference in recovery rates within the bleached region. Because of the small size of a bacterial cell, only a short segment is photobleached in FRAP experiments to ensure that a sufficient pool of unbleached molecules is available to feed the recovery process. The length of the bleached segment is only 3–4 times the theoretical spatial resolution of a fluorescence microscope, making it very difficult to resolve the center of the bleached segment from the edge.

Neither standard fluorescence microscopy nor FRAP approaches can define the molecular events that are responsible for the macrophenomena that are observed. The reason for this is the small size of bacterial cells ($\sim 2.5 \times 0.5$ μm for *E. coli*), which is of the same order of magnitude as the spatial resolution of the optical microscope (~ 0.3 μm).

Single molecule imaging and tracking techniques provide a way to circumvent this limitation. Single molecule methods can determine the positions of individual molecules at spatial resolutions well below the diffraction limit. This is accomplished by mathematical computation of the centroid position of fluorescence emitted from a single molecule, based on the Gaussian distribution of the emitted fluorescence. This approach has no theoretical upper limit, and is limited only by the signal-to-noise ratio of the image [7]. In vitro single molecule imaging studies have shown that a protein molecule can be localized with an accuracy of ~ 1 nm [8]. It is possible to carry out centroid-based single molecule imaging studies of labeled proteins within living bacterial cells with a resolution ≤ 30 nm (J.Y., unpublished results).

Different strategies have been used to exploit the high resolution of centroid tracking to image single molecules.

One strategy is to photobleach the entire cell until only one or a few isolated fluorescent protein molecules are left [9]. However, the most useful single molecule approach is photoactivation localization microscopy (PALM) [10]. The PALM technique uses photoactivatable fluorescent protein tags (PAFPs) [11] in genetically engineered chimeric proteins. PAFP molecules, such as Dendra2, EosFP, or PAGfp, typically emit fluorescence at a short wave length (e.g., green for Dendra2) in their nascent form, but can be irreversibly converted into a long wave length-emission form (e.g., red for Dendra2) by excitation with blue or UV light. Because there is little spectral bleed-through, single molecule detection can be carried out in the presence of a high level of unactivated molecules. A short low intensity pulse is used to photoactivate the PAFP-labeled proteins. Because the activation is stochastic, each excitation pulse activates only one or a few molecules per cell. Individual molecules can thus be imaged and their movements followed before the next excitation pulse.

To study their dynamic behavior, the spatial trajectories of the molecules are followed in time until the signal disappears, due to either turnover or photobleaching. By repetitively carrying out the activation and tracking sequence, a very large number of individual protein molecules can be imaged and studied using automated methods. Photobleaching that occurs during the acquisition period is corrected for by first determining the photobleaching rate using, for example, fixed cells.

The single molecule imaging approach is especially suited for study of molecules within extended filamentous polymeric structures, such as cytoskeletal elements. Labeling of individual molecules within filamentous structures generates fiduciary marks that can be directly monitored to determine whether the polymer is stationary or moving, and to follow the position and the details of the movement within the cell. In addition, the pattern of signal disappearance gives information about protein assembly dynamics and subunit turnover.

In addition to establishing topological patterns of movement, single molecule mobility characteristics can help distinguish between different protein states. At usual exposure times (typically 0.1–1 s), only membrane-associated and/or filamentous molecules will give useful images in single molecule imaging experiments because of their relatively slow movement (e.g., the diffusion constant of an integral membrane protein is $\sim 0.01\ \mu m^2/s$ [12]). In contrast, the diffusion of monomeric or oligomeric cytosolic proteins is much more rapid (e.g., the diffusion constant of cytosolic Gfp is $\sim 8\ \mu m^2/s$ [13]). Because of this, molecular movements during image acquisition of cytosolic molecules leads to loss of resolution of individual molecules.

The MinCDE cytoskeletal system

The MinCDE system is a membrane-associated cytoskeletal system that is normally responsible for placement of the bacterial division site at its normal midcell location in a number of bacterial species. The MinCDE proteins have no apparent homology to any eukaryotic cytoskeletal proteins, and this system was the first uniquely bacterial cytoskeletal system to be described. The Min system provides the most dramatic example of dynamic behavior of a prokaryotic cytoskeletal system.

The Min system ensures that septation occurs at midcell by preventing septation at all other sites along the length of the cell (reviewed in [14]). This is accomplished by a division inhibitor protein, MinC, which blocks septation by preventing formation of stable FtsZ rings (see "FtsZ cytoskeletal structures"). This prevents assembly of the cell division machinery at membrane sites in the vicinity of MinC. The MinC division inhibitor is given topological specificity by the action of the MinD protein and, in some species such as *E. coli*, proteins such as MinE. These site-specificity proteins prevent the MinC division inhibitor from localizing at midcell while permitting it to prevent septation at other cellular locations. This restricts septation to the midcell site [1].

The Min proteins of *E. coli* are organized as filamentous, membrane-associated cytoskeletal elements that coil around the cell from pole-to-pole (Fig. 1) [15, 16]. A similar long-range helical cytoskeletal organization is characteristic of the bacterial actin homolog MreB and its associated proteins and, under certain conditions, of the bacterial tubulin homolog FtsZ. However, the helical MinCDE elements can assemble in the absence of the MreB or FtsZ cytoskeletal structures and therefore represent an independent class of prokaryotic cytoskeletal element [17].

MinD appears to provide the basic framework of the MinCDE helical structures. In vitro studies have shown that MinD polymerizes into extended filamentous structures on the surface of lipid vesicles in a reaction that requires ATP-binding [18, 19]. The association of the MinD polymers with the vesicle surface is mediated by a MinD carboxyterminal amphipathic helix [20–22]. The MinD–membrane interaction is believed to mediate the association of the cytoskeletal MinCDE structures with the inner surface of the cytoplasmic membrane. The other two proteins of the Min system, MinC and MinE, associate with the polymeric MinD structure via MinE- and MinC-binding sites on the surface of the MinD protein [23]. The molecules that compose this cytoskeletal system undergo repetitive changes in their long-range organization during the normal division cycle.

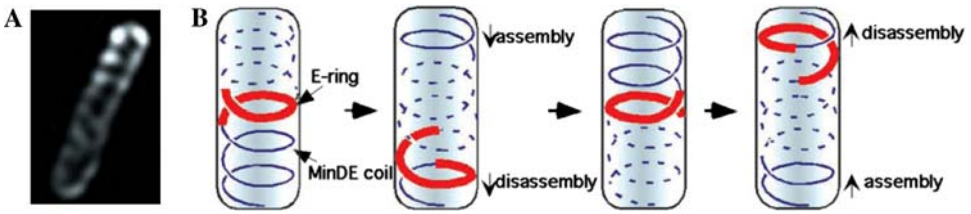

Fig. 1 Dynamic behavior of MinCDE system. **a** Fluorescence micrograph of Yfp-MinD showing coiled helical structure extending along the cell cylinder with high concentration of Yfp-MinD in coils at one end of the cell ("polar zone"). **b** Distribution of MinDE at different stages of an oscillation cycle. *Blue lines* represent MinDE coiled structures within the polar zones (*solid line*) and extending to the opposite pole (*dashed line*). *Red line* represents MinE ring. (Reprinted from [15], with permission of the publisher)

Most work on the dynamic behavior of the Min proteins has come from studies in *E. coli*. Prior to the discovery of the cytoskeletal nature of the Min system, it was observed that the Min proteins were predominantly localized within polar zones at one or other end of the rod-shaped cell [24, 25]. It was then discovered [24] that the polar zones repetitively disappeared from one end of the cell and reappeared at the opposite cell pole. This leads to oscillation of the polar zones between the two poles with a periodicity of approximately 1–2 min. The oscillatory events occur many times during each division cycle (20–30 min for cells growing in rich media).

The pole-to-pole oscillation of the Min proteins reflects their redistribution within the underlying helical Min cytoskeletal elements [15], as shown diagrammatically in Fig. 1b. The cycle is initiated by the accumulation of MinC, MinD, and MinE within the coils at one end of the helical structure, forming the polar MinCDE zones. The polar zones then appear to grow vectorially toward midcell, along the helical framework of the underlying MinCDE structure. When the growing polar zone approaches midcell, a new structure (the MinE-ring) appears at its leading edge (Fig. 1b). The MinE ring (E-ring) is formed by assembly of MinE molecules into the helical loops just ahead of the growing polar zones. The E-ring blocks extension of the MinCDE polar zone across midcell, providing a "stop-growth" mechanism. This is followed by retraction of the MinCDE coiled structure and MinE ring back to the original pole. Oscillation occurs by repetition of the same sequence of events alternately at the two cell poles. These observations have led to the suggestion that the underlying helical MinCDE framework might act as a guide or template for assembly of new polar zones during the oscillatory cycles.

By preventing extension of the MinCDE polar zones across midcell, the E ring ensures that MinC is excluded from the desired midcell site. Because of the rapid oscillations of the MinCDE polar zones, the time-averaged concentration of the MinC division inhibitor is maintained at high levels at other sites along the length of the cell. As a result, septation is limited to the midcell site.

In vitro studies, primarily by Lutkenhaus and his collaborators (reviewed in [14]), have provided the following biochemical explanation for these events: (1) binding of ATP leads to the membrane association and polymerization of MinD, beginning at the cell pole and this leads to formation of the MinD polar zone at one end of the cell and its vectorial growth toward midcell; (2) ATP-binding increases the affinity of MinD for MinC and MinE, leading to their association with the membrane-associated polymeric MinD filaments; (3) when the MinD filaments approach midcell, MinE molecules assemble into the MinE ring which blocks further growth of the polar zones; it is not known what triggers E-ring assembly although it may be related to the decrease in cytosolic MinD concentration that results from accumulation of MinD molecules within the polar zones; (4) the high concentration of MinE in the E-ring activates the latent MinD ATPase activity, thereby converting MinD-ATP to MinD-ADP at the midcell end of the polar zone, and this leads to MinD depolymerization, release from the membrane, and dissociation of MinD-associated MinC and MinE; as a result, the polar zone, with its attached MinE ring retracts back to the pole; (5) the released MinD molecules are now available to initiate the process at the opposite end of the cell, thereby perpetuating the oscillatory cycle.

Two models have been proposed to explain why the events are initiated at the cell poles. In both models, the high local concentration of MinE in the E-ring promotes release of membrane-associated MinD (see above) and also inhibits the membrane reassociation of MinD in the vicinity of the E-ring. In the first model, the cell poles contain specific MinD-binding sites that act as initiation sites for MinD polymerization, thereby explaining why assembly of the polar zones begins at the cell pole [26]. In this model, MinD molecules released during retraction of the polar zones cannot reattach to the same poles because the binding sites are still occupied or because there is a delay before the sites again become competent. The next cycle of MinD polymerization is thus limited to the unoccupied binding sites at the opposite pole. In the second model, there are no MinD-specific polar binding sites and

oscillation occurs based only on the diffusion properties and membrane-affinity properties of MinD, and on the interactions of MinD with MinE [27–31].

Mathematical models based on both schemes generate oscillation cycles that are consistent with the experimental observations [26–31]. However, recent studies have shown that periodic waves of MinD are spontaneously formed when MinD, MinE, and ATP are added to supported lipid bilayers, in the absence of other proteins [32]. Within this pattern, a high concentration of MinE accumulates at the edge of each MinD wave, reminiscent of the MinE rings that assemble at the edge of the polar zones in vivo. Although the wave-like behavior does not completely reproduce the cellular behavior of the system, it shows that dynamic MinD structures with associated MinE bands can form spontaneously on the surface of membrane bilayer in the absence of specific membrane-binding sites.

Although many details remain to be defined, the MinCDE system represents the most dramatic example of dynamic long-range reorganization of a prokaryotic cytoskeletal system.

The MreB-associated cytoskeleton

MreB is a prokaryotic actin homolog that is part of a cytoskeletal system present in almost all rod-shaped bacteria. The MreB-associated cytoskeleton is required for maintenance of rod shape [1, 33, 34] and for establishment of cell polarity [1, 35, 36]. It is also possible, although not firmly established, that MreB plays a role in chromosome segregation [37–41]. Many bacteria possess a single actin-like MreB protein (e.g., *E. coli*, *C. crescentus*), whereas others contain two or more MreB paralogs (e.g., *Thermotoga maritima*, *B. subtilis*).

MreB is organized into membrane-associated filamentous structure that coil around the long axis of the cell (Fig. 2a). In vitro studies have shown that MreB self-assembles into filamentous polymeric structures in the presence of ATP [42, 43] making it likely that extended MreB polymers form the framework of the filamentous helical cytoskeletal structures. As described below, the MreB-associated cytoskeleton undergoes cell cycle-related changes in organization, in which the extended helical structures reorganize into transverse ring structures that are associated with the future division site (Fig. 2b) [15, 44]. The MreB helical elements and ring structures have been identified in *E. coli* [15, 44], *B. subtilis* [45, 46], *C. crescentus* [47, 48], and *Rhodobacter sphaeroides* [49], and they likely are characteristic of most rod-shaped bacteria. Interestingly, the actin cytoskeleton of eukaryotic cells also forms ring structures at the cell division site but, as discussed below, the structural organization and functional characteristics of the prokaryotic and eukaryotic annular structures are quite different. Although most attention has been paid to the actin homolog MreB, other proteins involved in cell shape determination, including MreC, MreD, Pbp2, and RodA in *E. coli* [50] and the three MreB paralogs of *B. subtilis* [51], are also associated with the MreB cytoskeletal structures.

Remodeling of the MreB-associated cytoskeletal structures has been defined by fluorescence studies of cells that express Gfp- or Yfp-labeled MreB (Fig. 2a). In synchronized predivisional cells of *C. crescentus*, the MreB helical structures are replaced by MreB rings near the future plane of division in predivisional cells. By the time division is complete, the rings disappear and MreB spirals reappear along the entire length of the daughter cells (see [48]: Fig. 1c). In *E. coli* cells, MreB rings appear at midcell in the longest cells in the population, which represent cells late in the cell cycle [50]. This is consistent with the synchronized cell studies of *Caulobacter*. MreB ring structures at midcell are also present in elongating cells of *R. sphaeroides* [49]. The *Rhodobacter* rings remain at midcell in predivisional cells and relocate to new positions in the daughter cells before the completion of septation.

Time-lapse studies in *E. coli* have shown that the single MreB rings are later transformed into paired rings (doublets) (Fig. 2b) that flank the FtsZ cytokinetic ring at the future division site. It has been suggested that the doublet structures observed in *Caulobacter* cells are artifacts of Gfp-MreB overexpression [48]. This is clearly not the case in *E. coli*, where immunofluorescence studies of chromosomally encoded MreB also showed the doublet structures [44].

Subsequent to their formation, the two doublet MreB rings in *E. coli* move apart (Fig. 2b–d) in a process that is accompanied by disappearance of the segment of the

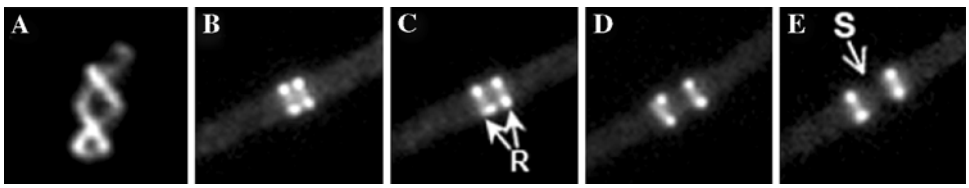

Fig. 2 Yfp-MreB helical cytoskeleton (**a**) and cytoskeletal doublet rings (**b**). The progressive movement apart of the rings is shown in panels **b–d**. (Reprinted from [44], with permission of the publisher)

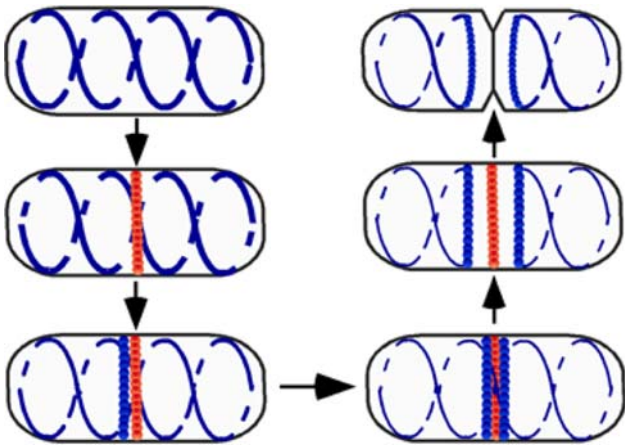

Fig. 3 Division and segregation of the MreB cytoskeleton during the *E. coli* cell cycle (**a**) and proposed model for the role of the cytoskeletal rings in the interruption and resealing of the MreB cytoskeletal helices that leads to duplication of the MreB helical structure (**b**). MreB in *blue*, FtsZ in *red*. (Reprinted from [44], with permission of the publisher)

underlying helical structure that lies between the rings (Fig. 3) [44]. The new septum is formed between the paired MreB rings [44] (Fig. 2d, e). The MreB ring structures then disappear and the helical pole-to-pole structures are reformed as the cells enter the next cell cycle (Fig. 3). These observations led to the suggestion that the MreB rings may be specialized structures involved in the division and segregation of the bacterial cytoskeleton during the cell cycle [44]. The bacterial MreB rings are not located within the septum during the course of septal ingrowth (Fig. 2d) [44, 49]. This distinguishes them from the actin contractile rings of eukaryotic cells that are located at the leading edge of the invaginating septum and are thought to play a direct role in eukaryotic septal constriction.

The MreB cytoskeletal rings are formed after assembly of the FtsZ cytokinetic ring that acts as a scaffold for assembly of the septasomal cell division machinery. The formation of MreB cytoskeletal rings is dependent on the presence of Z-ring [44]. Consistent with these findings, the newly formed cytoskeletal rings are located adjacent to the Z-ring [44, 50]. These observations suggest that the Z-ring provides a signal that triggers assembly of the cytoskeletal ring. This could involve direct interaction of the Z-ring with the cytoskeletal proteins or with an intermediary assembly protein, or could reflect local effects of the Z-ring on membrane or murein structure that promote cytoskeletal ring assembly.

Assembly of the protein components of the cytoskeletal ring is accompanied by a marked decrease in the concentration of the proteins within the lengthwise helical cytoskeletal structures [44, 50]. Three mechanisms can be considered to explain this transformation from helical to annular organization: (1) molecules may be released from the helical elements and move through the cytoplasm and/or along the cytoplasmic membrane to the site of ring assembly near midcell; (2) the midcell ring may be assembled by deposition of newly synthesized protein molecules, presumably accompanied by turnover of the components of the pre-existing helical elements; and (3) individual molecules or protein complexes from the coiled structures may translate along the helical track to the site of ring assembly. Techniques such as single molecule imaging (described below) will be needed to distinguish between these possibilities.

In addition to the long-range cytoskeletal reorganizations described above, short-range rotational movements of coils within the helical framework have been observed on a time scale of $\sim$1 min in time-lapse studies of *B. subtilis* cells expressing Gfp-MreB [52]. Fluorescence photobleaching (FRAP) and single molecule imaging experiments have begun to provide further information about the dynamic behavior of the MreB-associated cytoskeleton. In *B. subtilis*, FRAP studies of Gfp-MreB labeled cells showed recovery of fluorescence following photobleaching of labeled MreB filaments [53]. The results indicated that MreB cytoskeletal filaments are dynamic structures but are significantly more stable than FtsZ rings, which appear to turn over in a time frame of seconds (see below). It is not known whether the fluorescence recovery reflected exchange of individual molecules or physical movement of protofilaments within the helical MreB elements.

The dynamic behavior of MreB filaments was confirmed in studies in which labeled MreB was expressed in the fission yeast *Schizosaccharomyces pombe*. Interestingly, MreB assembled into long filaments in the *S. pombe* cells, although the structures were not organized as coiled structures as they are in bacterial cells [54]. Since *S. pombe* cells are much larger than bacteria, it was possible to photobleach a much longer segment of the filament and monitor fluorescence recovery in different portions of the bleached region. This showed that the fluorescence recovery was uniform across the bleached region, thereby indicating that the movement of fluorescence into the bleached area was either due to molecular exchange or filament turnover instead of physical movement of the filamentous elements (see "Experimental techniques", above). It is not known whether this also applies to the MreB cytoskeletal elements within bacterial cells.

Single molecule tracking experiments have provided direct evidence that MreB elements actually are mobile when present in the helical cytoskeletal structures of bacterial cells. PALM experiments ("Experimental techniques", above) in *C. crescentus* showed that individual EYFP-MreB molecules move directionally at a rate of $\sim$6.0 nm/s [9]. Interestingly the movements of MreB

molecules were usually oriented along the short axis of the cell cylinder, which prompted the authors to suggest that the molecules were moving along the helical tracks of the MreB cytoskeleton. The implications of this for the molecular organization and macroscopic behavior of the helical structures remain to be defined. It is not known whether this phenomenon is specific to *C. crescentus* or is a general characteristic of MreB cytoskeletal structures.

Despite considerable progress in our understanding of the MreB cytoskeleton, many unanswered questions still remain, especially related to the long- and short-range dynamic behavior of the structures. For example, how do the MreB helical structures grow during cell elongation and how do they divide later in the cell cycle? Do MreB molecules move processively along the helical track? Do molecules move in and out of the polymers, thus continuously exchanging with the cytoplasmic fraction? Are the MreB cytoskeletal rings at midcell formed from protein molecules or protofilaments that originate from the longitudinal helical structures? What is the mechanism involved in the movement apart of the MreB cytoskeletal rings prior to cytokinesis? Single molecule tracking techniques have the potential to provide important new information on these and other questions.

FtsZ cytoskeletal structures

FtsZ is a ubiquitous bacterial protein that is a structural homolog of eukaryotic tubulin (reviewed in [1]). Although it does not form microtubular structures, FtsZ resembles tubulin in its three-dimensional structure, its ability to polymerize unidirectionally into linear protofilaments in the presence of GTP, and in its organization as ordered filamentous structures within the cells [55].

During the bacterial cell cycle, FtsZ assembles into an annular structure (the 'Z-ring') at midcell. Other proteins subsequently associate with the Z-ring to form the complex cell division machinery that catalyzes septal invagination [56]. The Z-ring remains at the leading edge of the ingrowing septum until septation is complete, when the ring disassembles.

In addition to the Z-ring, FtsZ can sometimes be visualized in helical structures coiled around the length of the cell (Fig. 4a). These structures are reminiscent of the MreB and Min helical cytoskeletal elements described in the preceding sections of this review. In *B. subtilis*, the FtsZ helical structure appears to be an intermediate in assembly of the midcell Z-ring [57, 58] (Fig. 4a, b). It is not known whether individual FtsZ subunits leave the coils to reassemble into the Z-ring, or whether the ring itself may be part of the helical framework. It is possible, for example, that the Z-ring could be formed by compression of the

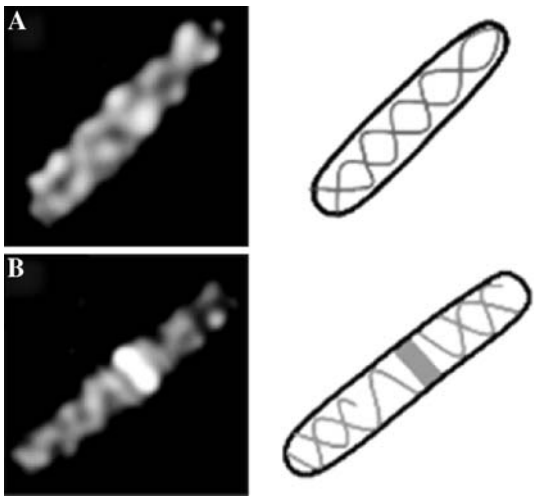

Fig. 4 Two-dimensional images of FtsZ structures in *B. subtilis* cells stained with anti-FtsZ antibody. **a** Cell with an FtsZ helix, but no Z ring. **b** Cell with a Z-ring and FtsZ helix. A cartoon for each cell is shown depicting the interpreted location of FtsZ within the cell. (Reprinted from [57], with permission of the publisher)

normally extended helical FtsZ structure. The FtsZ helical structure also appears to be an intermediate in generation of the polar Z-ring that is formed during *B. subtilis* sporogenesis. This was shown by Ben-Yehuda and Losick in the first demonstration that FtsZ helical structures may be involved in Z-ring biogenesis [59].

FtsZ helical structures are also present in *E. coli* cells [60] although their relationship to the midcell Z-rings is less clear than in *B. subtilis.* Interestingly, the FtsZ helical structures sometimes oscillate between the two ends of the *E. coli* cell or across distances of <8–9 μm in filamentous cells on a time scale similar to the pole-to-pole oscillations of the Min helical polar zones. The FtsZ oscillations were not seen in *minCDE* deletion mutants, suggesting a direct or indirect connection between the FtsZ and MinCDE cytoskeletal systems [60]. In contrast, the FtsZ oscillatory behavior is independent of the MreB cytoskeleton.

In addition, time-lapse studies have also shown that the helical FtsZ structures in both *B. subtilis* [57] and *E. coli* [60] undergo rapid short-range movements within the coiled structures on a time scale of <1 s. These short-range movements within the FtsZ spiral structures are independent of the presence of the FtsZ ring or the helical MreB cytoskeleton [60].

The Z-ring is a very dynamic structure at the molecular level. FRAP studies have shown rapid recovery of fluorescence within the Z-ring after photobleaching, with half-times of recovery of 8–9 s in *E. coli* and *B. subtilis* [61, 62]. This indicates that fluorescent FtsZ molecules (presumably from the cytoplasm) rapidly exchange for FtsZ molecules within the bleached region. This could represent exchange of subunits at the ends of

protofilaments within the ring or by exchange of subunits within FtsZ polymers [61, 62]. Alternatively, the rapid recovery could reflect complete disassembly of some filaments coupled with de novo synthesis of others. Expression of bacterial FtsZ-GFP in the fission yeast *S. pombe* also resulted in formation of filamentous cables and ring structures that showed rapid exchange in FRAP experiments, suggesting that the exchange process is intrinsic to FtsZ polymers and is not dependent on bacterial specific auxiliary proteins [63].

The rapid turnover of FtsZ molecules in the Z-ring led to the suggestion that the Z-ring is made of many short protofilaments, based on the assumption that subunit exchange only occurs at polymer ends and does not occur at internal sites within the filament. The idea that the Z-ring is composed of short protofilaments is also consistent with electron microscopic tomography studies of *C. crescentus* [64]. Atomic force microscopy studies of FtsZ filaments in vitro have shown that FtsZ polymers can undergo fragmentation and reannealing to the same or neighboring polymers [65]. If this also occurs in vivo, it would suggest a new level of complexity to the dynamic behavior of the filamentous polymers within the Z-ring.

Single molecule imaging studies of FtsZ in live *E. coli* cells did not reveal significant movement of molecules within the Z-ring [66]. This confirmed that the recovery of fluorescence after photobleaching can be attributed to movement of molecules in and out of FtsZ polymers, and cannot be attributed to movement of filaments in and out of the bleached area. It was also shown that FtsZ molecules not located near midcell undergo random movement with an apparent diffusion rate similar to the diffusion rate of intrinsic membrane proteins. Based on these findings, the authors suggested that FtsZ molecules outside the Z-ring are not filamentous structures, even though they appear to form helical patterns.

Despite the newly gained knowledge about FtsZ dynamics, the mechanism of Z-ring constriction remains a mystery. Because the single molecule experiments were performed on nondividing cells, studies on actively constricting cells during septal invagination will be needed before the significance of these results can be fully appraised.

Conclusions

Until the past decade, it was generally believed that bacteria did not contain internal cytoskeletons. However, it is now well established that despite their small size (usually about the size of the mitochondria of eukaryotic cells) bacterial cells contain a number of independent internal cytoskeletal elements that undergo striking dynamic changes in structural organization and cellular distribution during the life of the cell. It is interesting that the three most dynamic cytoskeletal elements—the MreB, MinCDE, and FtsZ cytoskeletal structures—exhibit very similar intracellular organizational patterns. In all three cases, the cytoskeletal elements can exist as filamentous structures that coil around the length of the cell or as annular structures that are assembled close to the midcell site of future division events (the FtsZ ring, MreB-associated cytoskeletal ring, and MinE ring). The helical and ring structures are very likely composed of filamentous polymers of the central cytoskeletal proteins (MreB, FtsZ, and MinD) since in all cases the purified proteins form long polymeric filaments when incubated in the presence of ATP or GTP under appropriate conditions. However, despite their superficial similarity in organization, the helical and ring structures in the different systems exhibit quite different dynamic behaviors, ranging from participating in septal invagination (FtsZ ring), playing a key role in pole-to-pole oscillatory reorganizational events (MinE ring and MinCDE helical elements), or undergoing divergent movement away from midcell (the MreB cytoskeletal doublet rings).

It is not known why these different cytoskeletal systems are organized into similar-appearing helical structures that extend around the long axis of the rod-shaped cells. It is possible that an, as yet unidentified, underlying helical track exists that is used by all the systems [16]. Alternatively, the filamentous helical organization may simply reflect the fact that any straight filamentous structure on the inner surface of a cylinder will automatically assume a helical configuration unless the filament is oriented parallel to the long or short axis of the cylinder. Since the cytoskeletal structures are each associated with the inner surface of the cytoplasmic membrane, this simple possibility cannot be ignored.

Since the study of bacterial cytoskeletal elements and their dynamic behavior is still in its infancy, a large number of questions remain to be answered. For example, what is the molecular organization of the filamentous helical and ring structures; if the structures are composed of many protofilaments, what holds the protofilaments together; what triggers the helix-to-ring transitions in these systems; what is the route taken by cytoskeletal molecules from helices to the ring assembly sites? The answers to these and many more questions must await future work. Considering the large amount of information that has been amassed in a relatively short period of time, we can anticipate major new advances and unanticipated discoveries during the next few years.

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References

1. Shih Y-L, Rothfield LI (2006) The bacterial cytoskeleton. Microbiol Mol Biol Rev 70:729–754
2. Cabeen MT, Jacobs-Wagner C (2007) Skin and bones: the bacterial cytoskeleton, cell wall, and cell morphogenesis. J Cell Biol 179:381–387
3. Pichoff S, Lutkenhaus J (2007) Overview of cell shape: cytoskeletons shape bacterial cells. Curr Opin Microbiol 10:601–605
4. Norris V, den Blaauwen T, Cabin-Flaman A, Doi RH, Harshey R, Janniere L, Jimenez-Sanchez A, Jin DJ, Levin PA, Mileykovskaya E, Minsky A, Saier M Jr, Skarstad K (2007) Functional taxonomy of bacterial hyperstructures. Microbiol Mol Biol Rev 71:230–253
5. Osborn MJ, Rothfield L (2007) Cell shape determination in *Escherichia coli*. Curr Opin Microbiol 10:606–610
6. Axelrod D, Koppel DE, Schlessinger J, Elson E, Webb WW (1976) Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. Biophys J 16:1055–1069
7. Thompson RE, Larson DR, Webb WW (2002) Precise nanometer localization analysis for individual fluorescent probes. Biophys J 82:2775–2783
8. Young K (2003) Bacterial shape. Mol Microbiol 49:571–580
9. Kim SY, Gitai Z, Kinkhabwala A, Shapiro L, Moerner WE (2006) Single molecules of the bacterial actin MreB undergo directed treadmilling motion in *Caulobacter crescentus*. Proc Natl Acad Sci USA 103:10929–10934
10. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF (2006) Imaging intracellular fluorescent proteins at nanometer resolution. Science 313:1642–1645
11. Lukyanov KA, Chudakov DM, Lukyanov S, Verkhusha VV (2005) Photoactivatable fluorescent proteins. Nat Rev Mol Cell Biol 6:885–890
12. Deich J, Judd EM, McAdams HH, Moerner WE (2004) Visualization of the movement of single histidine kinase molecules in live *Caulobacter* cells. Proc Natl Acad Sci USA 101:15921–15926
13. Elowitz MB, Surette MG, Wolf PE, Stock JB, Leibler S (1999) Protein mobility in the cytoplasm of *Escherichia coli*. J Bacteriol 181:197–203
14. Rothfield L, Taghbalout A, Shih Y-L (2005) Spatial control of bacterial division-site placement. Nat Rev Microbiol 31:959–968
15. Shih Y-L, Le T, Rothfield L (2003) Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. Proc Natl Acad Sci USA 100:7865–7870
16. Barak I, Muchova K, Wilkinson AJ, O'Toole PJ, Pavlendova N (2008) Lipid spirals in *Bacillus subtilis* and their role in cell division. Mol Microbiol 68:1315–1327
17. Shih YL, Kawagishi I, Rothfield L (2005) The MreB and Min cytoskeletal-like systems play independent roles in prokaryotic polar differentiation. Mol Microbiol 58:917–928
18. Hu Z, Gogol E, Lutkenhaus J (2002) Dynamic assembly of MinD on phospholipid vesicles regulated by ATP and MinE. Proc Natl Acad Sci USA 99:6761–6766
19. Suefuji K, Valluzzi R, RayChaudhuri D (2002) Dynamic assembly of MinD into filament bundles modulated by ATP, phospholipids, and MinE. Proc Natl Acad Sci USA 99:16776–16781
20. Szeto T, Rowland S, Rothfield L, King GF (2002) Membrane localization of MinD is mediated by a C-terminal motif that is conserved across eubacteria, archaea, and chloroplasts. Proc Natl Acad Sci USA 99:15693–15698
21. Szeto TH, Rowland S, Habrukowich C, King GF (2003) The MinD membrane targeting sequence is a transplantable lipid-binding helix. J Biol Chem 279:40050–40056
22. Hu Z, Lutkenhaus J (2003) A conserved sequence at the C-terminus of MinD is required for binding to the membrane and targetting MinC to the septum. Mol Microbiol 47:345–355
23. Ma L, Rothfield L (2004) Positioning of the MinE binding site on the MinD surface suggests a plausible mechanism for activation of the *Escherichia coli* MinD ATPase during division site selection. Mol Microbiol 54:99–108
24. Raskin D, de Boer P (1999) Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. Proc Natl Acad Sci USA 96:4971–4976
25. Rowland SL, Fu X, Sayed MA, Zhang Y, Cook WR, Rothfield LI (2000) Membrane redistribution of the *Escherichia coli* MinD protein induced by MinE. J Bacteriol 182:613–619
26. Drew D, Osborn M, Rothfield L (2005) A polymerization–depolymerization model that accurately generates the self-sustained oscillatory system involved in bacterial division site placement. Proc Natl Acad Sci USA 102:6114–6118
27. Meinhardt H, de Boer P (2001) Pattern formation in *Escherichia coli*: a model for the pole-to-pole oscillations of min proteins and the localization of the division site. Proc Natl Acad Sci USA 98:14202–14207
28. Huang KC, Meir Y, Wingreen NS (2003) Dynamic structures in *Escherichia coli*: spontaneous formation of MinE rings and MinD polar zones. Proc Natl Acad Sci USA 100:12724–12728
29. Kruse K (2002) A dynamic model for determining the middle of *Escherichia coli*. Biophys J 82:618–627
30. Pavin N, Paljetak HC, Krstic V (2006) Min-protein oscillations in *Escherichia coli* with spontaneous formation of two-stranded filaments in a three-dimensional stochastic reaction-diffusion model. Phys Rev E Stat Nonlin Soft Matter Phys 73:021904
31. Howard M, Rutenberg AD (2003) Pattern formation inside bacteria: fluctuations due to the low copy number of proteins. Phys Rev Lett 90:128102
32. Loose M, Fischer-Friedrich E, Ries J, Kruse K, Schwille P (2008) Spatial regulators for bacterial cell division self-organize into surface waves in vitro. Science 320:789–792
33. Wachi M, Doi M, Okada Y, Matsuhashi M (1989) New *mre* genes *mreC* and *mreD*, responsible for formation of the rod shape of *Escherichia coli* cell. J Bacteriol 171:6511–6516
34. Doi M, Wachi M, Ishno F, Tomioka S, Ho M, Matsuhashi M (1988) Determination of the gene products of the *mre* region that functions in formation of the rod shape of *Escherichia coli* cells and of the sequence of the *mreB* gene. J Bacteriol 170:4619–4624
35. Michie KA, Lowe J (2006) Dynamic filaments of the bacterial cytoskeleton. Annu Rev Biochem 75:467–492
36. Carballido-Lopez R (2006) The bacterial actin-like cytoskeleton. Microbiol Mol Biol Rev 70:888–909
37. Madabhushi R, Marians KJ (2009) Actin homolog MreB affects chromosome segregation by regulating topoisomerase IV in *Escherichia coli*. Mol Cell 33:171–180
38. Karczmarek A, MartÌnez-Arteaga R, Alexeeva S, Hansen F, Vicente M, Nanninga N, den Blaauwen T (2007) DNA and origin region segregation are not affected by the transition from rod to sphere after inhibition of *Escherichia coli* MreB by A22. Mol Microbiol 65:51–63
39. Kruse T, Gerdes K (2005) Bacterial DNA segregation by the actin-like MreB protein. Trends Cell Biol 15:343–345
40. Kruse T, Blagoev B, Lobner-Olesen A, Wachi M, Sasaki K, Iwai N, Mann M, Gerdes K (2006) Actin homolog MreB and RNA polymerase interact and are both required for chromosome segregation in *Escherichia coli*. Genes Dev 20:113–124
41. Defeu-Soufo HJ, Graumann PL (2005) *Bacillus subtilis* actin-like protein MreB influences the positioning of the replication machinery and requires membrane proteins MreC/D and other actin-like proteins for proper localization. BMC Cell Biol 6:10

42. Esue O, Cordero M, Wirtz D, Tseng Y (2005) The assembly of MreB, a prokaryotic homolog of actin. J Biol Chem 280:2628–2635
43. Esue E, Wirtz D, Tseng Y (2006) GTPase activity, structure and mechanical properties of filaments assembled from bacterial cytoskeleton protein MreB. J Bacteriol 188:968–976
44. Vats P, Rothfield L (2007) Duplication and segregation of the actin (MreB) cytoskeleton during the prokaryotic cell cycle. Proc Natl Acad Sci USA 104:17795–17800
45. Jones L, Carballido-Lopez R, Errington J (2001) Control of cell shape in bacteria: helical actin-like filaments in *Bacillus subtilis*. Cell 104:913–922
46. Carballido-Lopez R, Errington J (2003) The bacterial cytoskeleton: in vivo dynamics of the actin-like protein Mbl of *Bacillus subtilis*. Dev Cell 4:19–28
47. Figge RM, Divakaruni AV, Gober JW (2004) MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. Mol Microbiol 51:1321–1332
48. Gitai Z, Dye N, Shapiro L (2004) An actin-like gene can determine cell polarity in bacteria. Proc Natl Acad Sci USA 101:8643–8648
49. Slovak PM, Wadhams GH, Armitage JP (2005) Localization of MreB in *Rhodobacter sphaeroides* under conditions causing changes in cell shape and membrane structure. J Bacteriol 187:54–64
50. Vats P, Shih Y, Rothfield L (2009) Assembly of the MreB-associated cytoskeletal ring of *Escherichia coli*. Mol Microbiol 72:170–182
51. Carballido-Lopez R, Formstone A, Ying L, Ehrlich S, Noirot P, Errington J (2006) Actin homolog MreBH governs cell morphogenesis by localization of the cell wall hydrolase LytE. Dev Cell 11:399–409
52. Defeu-Soufo HJ, Graumann PL (2004) Dynamic movement of actin-like proteins within bacterial cells. EMBO Rep 5:789–794
53. Defeu-Soufo HJ, Graumann PL (2006) Dynamic localization and interaction with other *Bacillus subtilis* actin-like proteins are important for the function of MreB. Mol Microbiol 62:1340–1356
54. Srinivasan R, Mishra M, Murata-Hori M, Balasubramanian MK (2007) Filament formation of the *Escherichia coli* actin-related protein, MreB, in fission yeast. Curr Biol 17:266–272
55. Erickson H, Taylor D, Taylor K, Bramhill D (1996) Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers. Proc Natl Acad Sci USA 93:519–523
56. Bi E, Lutkenhaus J (1991) FtsZ ring structure associated with division in *Escherichia coli*. Nature 354:161–164
57. Peters PC, Migocki MD, Thoni C, Harry EJ (2007) A new assembly pathway for the cytokinetic Z ring from a dynamic helical structure in vegetatively growing cells of *Bacillus subtilis*. Mol Microbiol 64:487–499
58. Michie KA, Monahan LG, Beech PL, Harry EJ (2006) Trapping of a spiral-like intermediate of the bacterial cytokinetic protein FtsZ. J Bacteriol 188:1680–1690
59. Ben-Yehuda S, Losick R (2002) Asymmetric cell division in *B. subtilis* involves a spiral-like intermediate of the cytokinetic protein FtsZ. Cell 109:257–266
60. Thanedar S, Margolin W (2004) FtsZ exhibits rapid movement and oscillation waves in helix-like patterns in *Escherichia coli*. Curr Biol 14:1167–1173
61. Anderson DE, Gueiros-Filho FJ, Erickson HP (2004) Assembly dynamics of FtsZ rings in *Bacillus subtilis* and *Escherichia coli* and effects of FtsZ-regulating proteins. J Bacteriol 186:5775–5781
62. Stricker J, Maddox P, Salmon ED, Erickson HP (2002) Rapid assembly dynamics of the *Escherichia coli* FtsZ-ring demonstrated by fluorescence recovery after photobleaching. Proc Natl Acad Sci USA 99:3171–3175
63. Srinivasan R, Mishra M, Wu L, Yin Z, Balasubramanian MK (2008) The bacterial cell division protein FtsZ assembles into cytoplasmic rings in fission yeast. Genes Dev 22:1741–1746
64. Li Z, Trimble M, Brun Y, Jensen G (2007) The structure of FtsZ filaments in vivo suggests a force-generating role in cell division. EMBO J 26:4694–4708
65. Mingorance J, Tadros M, Vicente M, Gonzalez JM, Rivas G, Velez M (2005) Visualization of single *Escherichia coli* FtsZ filament dynamics with atomic force microscopy. J Biol Chem 280:20909–20914
66. Niu L, Yu J (2008) Investigating intracellular dynamics of FtsZ cytoskeleton with photoactivation single-molecule tracking. Biophys J 95:2009–2016