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Diversification and specialization of the bacterial cytoskeleton

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The past decade has witnessed the identification and characterization of bacterial homologs of the three major eukaryotic cytoskeletal families: actin, tubulin and intermediate filaments. These proteins play essential roles in organizing bacterial subcellular environments. Recently, the ParA/MinD superfamily has emerged as a new bacterial cytoskeletal class, and imaging studies hint at the existence of even more, as yet unidentified, cytoskeletal systems. Much as the cytoskeleton is used for different purposes in different eukaryotic cells, the specific identities, functions and regulatory mechanisms of cytoskeletal proteins can vary between different bacterial species. In addition, extensive cross-talk between bacterial cytoskeletal systems may represent an important mode of cytoskeletal regulation. These themes of diversity, species-specificity and crosstalk are emerging as central properties of cytoskeletal biology.

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Introduction

Through advances in cellular imaging, it has become clear that the subcellular environments of bacterial cells are highly organized and precisely regulated in both space and time [1]. Cytoskeletal proteins appear to be central to this subcellular organization. The bacterial homologs of eukaryotic cytoskeletal proteins have recently been reviewed in extensive detail [2–6]. This review will briefly summarize our current understanding of these proteins, and then focus on new directions in the bacterial cytoskeletal field, including the identification of new cytoskeletal families, the species-specific nature of cytoskeletal biology, and the potential for crosstalk between cytoskeletal systems.

All three eukaryotic cytoskeletal families have homologs in bacteria

FtsZ and BtubA/B are tubulin homologs

The tubulin homolog, FtsZ, is the most widely conserved bacterial cytoskeletal protein, and is found in nearly all

bacteria and archaea, as well as several eukaryotic organelles [5]. FtsZ is required for cytokinesis, which it organizes by forming a ring at the plane of division [7]. The localization of this Z-ring to the right place at the right time in the cell cycle is tightly controlled by species-specific regulators of FtsZ assembly, which are illustrated in Figure 1 and detailed below. In order for a proper Z-ring to form, the cytoplasmic FtsZ protein must be linked to the membrane, which in *E. coli* is achieved through two anchors, FtsA and ZipA [8,9]. Once the Z-ring is assembled at the division site, it is thought to function as a scaffold for the recruitment and assembly of a cascade of at least eight additional cell division proteins [5,10]. The specific mechanisms by which this multi-protein ring mediates the membrane and cell wall rearrangements of cytokinesis remain unknown.

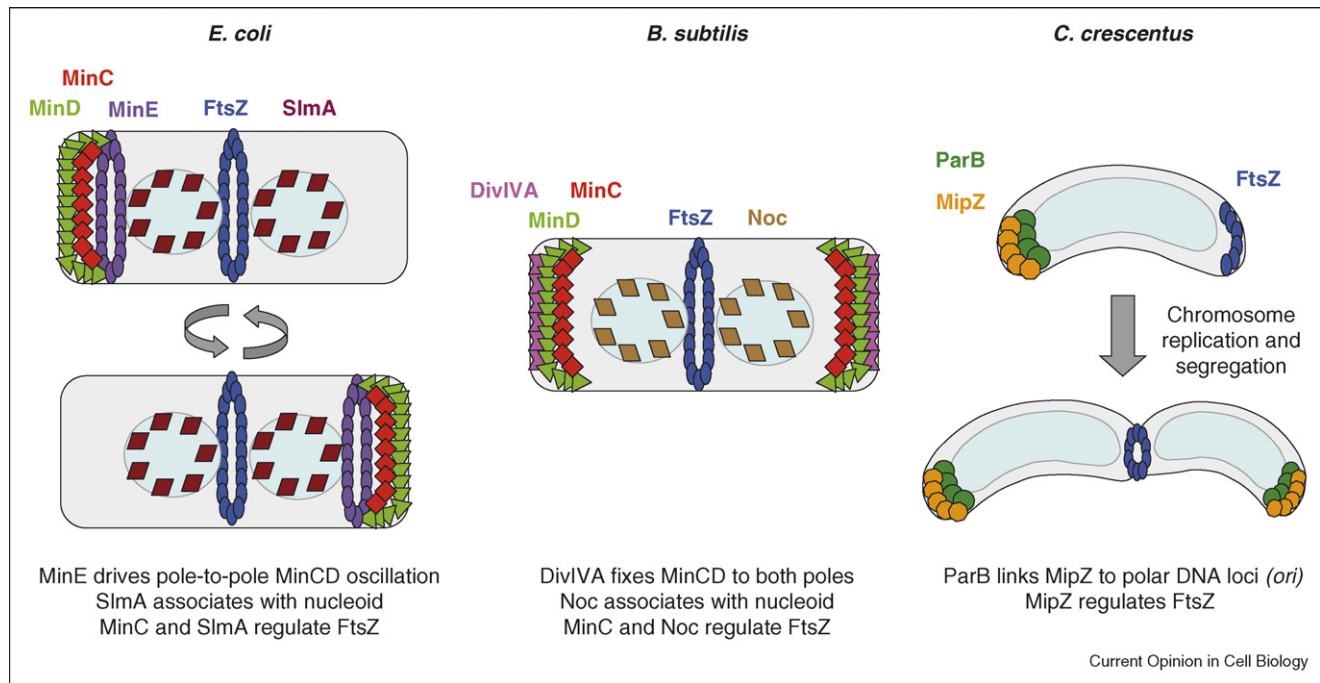
The structure of FtsZ resembles that of both α - and β -tubulin [11], and both FtsZ and tubulin polymerize into linear protofilaments in a GTP-dependent fashion [12,13]. *In vivo*, the monomers within an FtsZ filament have been shown to turn over dynamically [14]. Unlike microtubules, however, there is no convincing evidence that FtsZ protofilaments assemble into higher-order tubules, or that FtsZ filaments have an FtsZ-GTP cap. Despite these differences, the ease of expressing and purifying FtsZ point mutants has been successfully exploited to confirm models for the polymerization of tubulin, which is notoriously challenging to produce recombinantly [15,16]. FtsZ is also the sole bacterial cytoskeletal protein for which direct regulators of filament assembly have been identified and characterized both *in vivo* and *in vitro*. These include positive regulators that promote FtsZ stability and assembly (ZapA in *Bacillus subtilis*) or bundling and anchoring (ZipA in *E. coli*), as well as negative regulators that inhibit FtsZ assembly (MinC, SulA and SlmA in *E. coli*; MinC, YneA and Noc in *B. subtilis*; and MipZ in *Caulobacter crescentus*) [6,17,18**].

The BtubA and BtubB proteins represent a second class of bacterial tubulin homologs that to date have only been identified in the genus *Prostheco bacter* from the division *Verrucomicrobia* [19]. BtubA/B have far greater similarity in sequence, structure, and assembly to tubulin than does FtsZ [20**,21], fueling speculation that these genes have emerged by horizontal transfer from a eukaryote. The cellular functions of *btubA/B* in *Prostheco bacter* are unknown.

MreB and ParM are actin homologs

Bacterial actin homologs can be divided into two main classes, MreB and ParM, that differ in their sequences

Figure 1



E. coli, *B. subtilis*, and *C. crescentus* all divide by assembling a ring of the FtsZ tubulin homolog at the division plane. However, each of these species uses different proteins and mechanisms to direct FtsZ to its proper location.

and polymerization dynamics. The MreB class is always encoded in the bacterial chromosome and regulates a wide array of cellular functions, whereas the ParM class is always carried on extrachromosomal plasmids and appears to be dedicated to the proper segregation of those plasmids.

The MreB homologs of *E. coli*, *B. subtilis* and *C. crescentus* all form a helical structure that extends from pole to pole. In *C. crescentus*, this structure is dynamic; during each cell cycle it contracts into a division-plane-associated ring that expands back into an extended helix before cell division is complete [22[•],23[•]]. Both *E. coli* and *C. crescentus* have a single MreB homolog. When MreB is perturbed in these species, either genetically or with the MreB-disassembly compound, A22, cells lose their characteristic shapes and round up [23[•],24], polar proteins become mislocalized [22[•],25,26], and chromosome segregation is impaired [27^{••},28^{••}]. MreB thus appears to function as a key integrator of spatial and temporal information in bacterial cells. *B. subtilis* has three MreB homologs (MreB, Mbl and MreBH) [29]. Mutants in each of these genes have different phenotypes [30], but all three proteins colocalize in a single structure [31^{••}]. In the absence of double and triple mutant analysis, it thus remains unclear whether the differences among the *B. subtilis* MreB homologs are a consequence of specialized functions or differential expression.

To date, all of the analysis of MreB assembly *in vitro* has been performed on MreB from the thermophilic bacterium *Thermotoga maritima*. *T. maritima* MreB self-assembles into linear filaments in the presence of ATP or GTP, and its structure closely resembles that of actin, despite their low level of sequence similarity [32]. Like actin, ATP hydrolysis is thought to occur after MreB monomer polymerization, and MreB filaments have been observed to bundle into a rigid structure [33,34[•]]. *In vivo*, MreB monomers have been shown to be highly dynamic in both *B. subtilis* and *C. crescentus* [35,36[•]]. By tracking single molecules in living cells, *C. crescentus* MreB was shown to directionally treadmill through short filaments. Such treading suggests that, like actin, MreB filaments are assembled in a polar manner, with preferential polymerization and depolymerization from opposite poles [36[•]]. Though individual MreB filaments appear to be polarized, this polarity might not be coupled to the overall cellular polarity, as studies in both *B. subtilis* and *C. crescentus* detected no global polarity to the MreB helix [35,36[•]].

No proteins have been shown to directly regulate MreB assembly, although several proteins have been genetically or biochemically implicated as functioning in concert with MreB. These candidate interactors include the cell shape regulators MreC, MreD, Pbp2 and LytE [31^{••},37[•]–39[•]], and the potential regulators of

chromosome segregation RNA polymerase and SetB [28^{••},40]. With the development of new assays for MreB function and organization, the steadily increasing number of MreB-interacting proteins should ultimately shed light on the specific mechanisms by which MreB carries out its many functions.

The second, plasmid-specific class of bacterial actin homologs is typified by the ParM protein of the R1 *E. coli* plasmid. Though the structure of ParM is similar to that of actin [41,42], the kinetics of its polymerization are more similar to tubulin, as it exhibits dynamic instability (distinct phases of steady assembly and rapid collapse) [43^{••}]. Together with the ParR protein and the *parS* locus on the plasmid DNA, the R1 ParM system represents the simplest and best-characterized system for the segregation of bacterial DNA. ParR proteins bind the *parS* sites on two R1 plasmids and stabilize the assembly of a rigid ParM filament in between the two plasmids. This ParM structure acts as a tension rod to push the two plasmids apart — one to each of the two extreme cell poles [44]. The evolutionary relationships between MreB, ParM and actin are unclear.

Crescentin is an intermediate-filament-like protein

Intermediate filaments (IFs) are a large class of coiled-coil-rich proteins that were thought to have evolved in animals, as they are generally absent from plant, fungal and bacterial genomes [45]. A screen for cell shape mutants in *C. crescentus* identified a new protein, Crescentin, with striking domain similarity to animal IFs [46]. Cells lacking Crescentin are perfectly viable, but take on a straight rod-like morphology, in contrast to the curved banana-like shape of wild-type *C. crescentus*. Both Crescentin and animal IFs affect cell shape, can self-assemble *in vitro* in the absence of divalent cations, nucleotides or other accessory factors, and have four coiled-coil domains, the fourth of which is interrupted by a discontinuous ‘stutter’ [2]. Though no other proteins with the same organization have been identified in bacteria, there are proteins with slightly divergent domain structures that could carry out analogous functions in other species [46]. Neither the evolutionary origin of Crescentin nor the mechanism by which it influences *C. crescentus* shape are understood.

Bacteria have additional families of cytoskeletal proteins

ParA/MinD homologs represent a new family of bacterial cytoskeletal proteins

With the discovery of bacterial homologs for each of the eukaryotic cytoskeletal families, the list of bacterial cytoskeletal types was thought to be complete. It is now clear, however, that bacteria have additional cytoskeletal families without clear eukaryotic counterparts. The first of these families to be characterized was the ParA/MinD superfamily of ATPases. This superfamily includes

proteins that have a deviant Walker A motif within their nucleotide-binding P-loop [47].

One subgroup of the ParA/MinD family consists of the MinD proteins that contain a deviant Walker A ATPase, a highly conserved MinD-box, and a C-terminal membrane targeting sequence [48]. MinD proteins regulate cell division by regulating FtsZ assembly [49]. MinD binds the plasma membrane in its ATP-bound form [50–52], a state in which it has been shown to polymerize into filaments both *in vivo* [53] and *in vitro* [54]. MinD-ATP filaments recruit a second protein, MinC, which directly inhibits FtsZ polymerization [55,56]. The membrane-associated MinD pool is driven to the cell poles by two different mechanisms in *E. coli* and *B. subtilis* (Figure 1). In *E. coli*, a third Min protein, MinE, stimulates the hydrolysis of MinD-ATP, forcing it off the membrane [51,57]. Successive waves of MinD polymerization, MinC and MinE recruitment, and MinD depolymerization set up a stable oscillation of MinC and MinD from pole to pole [58–60]. The result of these oscillations is that the time-averaged lowest MinC concentration is at the cell center, which is therefore the favored site of FtsZ assembly. In *B. subtilis*, the DivIVA protein is constitutively associated with both poles and recruits MinD and subsequently MinC, again driving FtsZ assembly towards the cell center [61,62]. In both species, the cell must elongate to generate a central region where the concentration of MinC becomes low enough to accommodate FtsZ polymerization. Consequently, the Min system regulates both the localization and timing of FtsZ-mediated cell division.

The ParA subgroup of the ParA/MinD superfamily consists of proteins encoded by both plasmids and chromosomes. All known plasmid partitioning systems include either a ParM-like actin homolog (type II partitioning system) or a plasmidic ParA homolog (type I partitioning system) [63]. ParA functions with a *cis*-acting DNA element (*parC*) and a ParB protein that binds both the *parC* DNA element and ParA [63]. *In vitro*, the SopA and ParF plasmidic ParA homologs have been shown to polymerize into filaments in an ATP-dependent manner that can be regulated by ParB homologs [64^{••},65[•]]. *In vivo*, plasmidic ParA homologs form dynamic filaments that oscillate in between the two copies of the separating plasmids [64^{••},66[•]]. ATP is likely to be an important regulator of these dynamics, since mutants that perturb ATP binding or hydrolysis also perturb both filament dynamics and plasmid partitioning [64^{••},67].

The chromosomally encoded ParA homologs have been most extensively characterized in *B. subtilis* and *C. crescentus*, as *E. coli* is one of the rare bacterial species that lacks a chromosomal *par* system. Like the plasmidic ParA homologs, the chromosomal ParA homologs generally function with a *cis*-acting DNA element and a ParB-like

linker [68]. In *B. subtilis*, ParA (Soj) has been shown to bind near the origin of replication through a ParB-like (Spo0J) intermediate [69–71]. Intracellular oscillations may be a general feature of the ParA/MinD superfamily since Soj localization oscillates in a manner similar to both MinD and plasmidic ParA homologs [70,71]. *soj* mutants do not have a striking chromosome segregation defect, but do affect the transcription of genes involved in sporulation [72–74]. In *C. crescentus*, the ParA protein also binds near the origin through ParB, and both *parA* and *parB* mutants affect chromosome dynamics, potentially as a secondary consequence of effects on cytokinesis and cell cycle progression [75,76].

A second, more distantly related ParA homolog, MipZ, was recently characterized in *C. crescentus* [18**]. Like ParA, MipZ localizes near the origin through association with ParB. However, MipZ has a very different function from ParA: it directly interacts with FtsZ to inhibit its polymerization. Since the *C. crescentus* origins of replication are localized to the extreme poles, the association of MipZ with the origins inhibits FtsZ polymerization near the poles, driving the Z-ring towards the cell center (Figure 1) [18**]. This mechanism may represent an interesting cell-cycle checkpoint, as it ensures that the cytokinetic FtsZ ring is not assembled until the chromosomes have begun to segregate. Since *C. crescentus* has no obvious Min protein homologs, MipZ may simultaneously play the roles of both MinC and MinD. It remains to be seen whether ParA homologs regulate FtsZ dynamics in other bacterial species.

High-resolution imaging suggests the presence of additional cytoskeletal elements

Recent advances in electron cryotomography have enabled the imaging of bacterial cells at extremely high resolutions, roughly two orders of magnitude greater than is theoretically possible by light microscopy [77]. These tomograms use tilted series of thin sections to reconstruct three-dimensional images of samples that are not fixed, but rather rapidly frozen under high pressure to preserve their internal organization. Since some bacteria are small enough to fit in a single such section, bacterial imaging is at the forefront of this exciting new technique [78]. When electron cryotomography was applied to *Spiroplasma melleiferum*, two distinct filamentous structures were observed [79**]. Protein labeling methods have yet to be implemented in the context of electron cryotomography, such that the proteins that make up these *Spiroplasma* structures have not been identified.

Recently, *C. crescentus* was also imaged by electron cryotomography, and four distinct filament types were observed [80**]. Since *C. crescentus* cells have FtsZ, MreB, Crescentin and MipZ filaments, it seemed plausible that these four known cytoskeletal structures could account for the four observed filament types. However, perturbations

of Crescentin and MreB suggested that neither of these proteins make up any of the four filament types [80**]. These tantalizing results point to the possible existence of several additional bacterial cytoskeletal systems that remain to be discovered.

Candidates for some of these novel cytoskeletal structures may emerge from a growing number of bacterial proteins that appear to localize to filamentous structures by low-resolution fluorescence microscopy. These proteins include SecA, SecY, SetB and Tar from *E. coli* [40,81]; SecG and SecE from *B. subtilis* [82]; MreC and Pbp2 from *C. crescentus* [23*,38*,39*]; and CfpA from *Treponema phagodenis* [83]. It remains to be seen whether these proteins are actually cytoskeletal elements that can directly polymerize, or whether their apparent filamentous organization relies on their association with other cytoskeletal scaffolds.

Cytoskeletal components, functions and regulations can vary between bacterial species

Cytoskeletal proteins are essential for the cell biology of every bacteria examined. However, there are species-specific differences in which cytoskeletal elements are present in which bacteria, how they function and how they are regulated. For example, while FtsZ is present in nearly all bacteria, Crescentin has only been identified in a single species [46], and a rough census performed in 2003 found that ~65% of bacterial species have an MreB homolog [30]. Meanwhile, the ParA/MinD superfamily is highly diverse, such that while *E. coli* cells have MinD but no ParA, *C. crescentus* cells have ParA and MipZ but no MinD [18**].

Different bacterial species have also diversified the functions of cytoskeletal proteins. *C. crescentus* uses a second ParA homolog, MipZ, to carry out the function preformed by MinD and MinC in *E. coli* and *B. subtilis* [18**]. In an analogous scenario, the magnetotactic bacterium *Magnetospirillum magneticum* has both a conventional MreB homolog and a second, chromosomally encoded MreB homolog, MamK, that lies in a gene cluster associated with the specialized iron-filled membranous invaginations that mediate magnetotaxis [84*]. MamK forms a filament down the length of the cell that associates with a second filamentous structure, MamJ [84*,85]. These filaments are not required for the formation of the iron-filled structures, but are required for their organization into a linear array, without which they fail to function as a coordinated cellular magnet. Since many bacterial species possess more than one MreB homolog, it should prove interesting to see if there are other cases where MreB is co-opted to fill a species-specific function.

Bacteria have diversified both the identities and functions of their cytoskeletons, so it should come as no surprise that they have also diversified the mechanisms by which

cytoskeletal proteins are regulated. As discussed above, three different species direct FtsZ assembly to the mid-cell by three different mechanisms: *E. coli* uses oscillating polar MinC and MinD, *B. subtilis* uses fixed polar MinC and MinD, and *C. crescentus* uses MipZ, which is anchored to the polar origins of chromosomal replication (Figure 1) [10,18^{••}]. *E. coli* and *B. subtilis* also both have mechanisms for nucleoid occlusion: a protein (SlmA or Noc, respectively) associates with the nucleoid and blocks FtsZ assembly, thereby preventing inappropriate division through a chromosome [86[•],87[•]]. However, the SlmA and Noc proteins that serve this nucleoid occlusion function in these two species are unrelated and act by different biochemical mechanisms [86[•],87[•]]. Not to be left out, MreB also appears to be differentially regulated in different species: *C. crescentus* MreB dynamically condenses from a spiral to a ring during the cell cycle [22[•],23[•]], whereas MreB homologs exist only in spiral form in *E. coli* and *B. subtilis* [29,53,88], and MreB has only been observed as a ring in *Rhodobacter sphaeroides* [89].

How much crosstalk is there between the different bacterial cytoskeletal elements?

An emerging theme in eukaryotic cytoskeletal biology is the existence of extensive interactions between the microtubule, actin and intermediate filament cytoskeletons [90,91]. There are intriguing suggestions that extensive crosstalk may also exist between bacterial cytoskeletal elements. The MinD and MipZ proteins of the ParA/MinD cytoskeletal superfamily function to regulate the assembly of a different cytoskeletal protein, FtsZ [10,18^{••}]. In *C. crescentus*, FtsZ may in turn regulate MreB, as FtsZ is required for the condensation of MreB spirals into rings [23[•]]. Since FtsZ, MreB and Crescentin all affect aspects of cell shape determination through largely mysterious mechanisms, it should prove interesting to discover just how much interaction really exists between these cytoskeletal systems.

Conclusions

The discovery that bacterial cells have cytoskeletons was groundbreaking for the field of bacterial cell biology. As we learn more about the intricacies of the bacterial cytoskeleton, it is becoming clear that this is not a singular system. Bacteria have diversified and specialized the identities, functions and regulation of their cytoskeletal proteins. It is tempting to speculate that these themes are universal features of cytoskeletal biology. If so, are there additional eukaryotic cytoskeletal systems that have yet to be discovered? Perhaps specialized cytoskeletons, such as the unique Major Sperm Protein of nematodes [92], are not such outliers after all. Similarly, could other cytoskeletal regulators share the enormous diversity seen in the myosin superfamily [93]? With time, this field thus promises to hold significant advances for our understanding of both bacterial cell biology and the general features of cytoskeletal systems.

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12 Cell structure and dynamics

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