

Review

# The Role of Cytoskeletal Elements in Shaping Bacterial Cells

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Beginning from the recognition of FtsZ as a bacterial tubulin homolog in the early 1990s, many bacterial cytoskeletal elements have been identified, including homologs to the major eukaryotic cytoskeletal elements (tubulin, actin, and intermediate filament) and the elements unique in prokaryotes (ParA/MinD family and bactofilins). The discovery and functional characterization of the bacterial cytoskeleton have revolutionized our understanding of bacterial cells, revealing their elaborate and dynamic subcellular organization. As in eukaryotic systems, the bacterial cytoskeleton participates in cell division, cell morphogenesis, DNA segregation, and other important cellular processes. However, in accordance with the vast difference between bacterial and eukaryotic cells, many bacterial cytoskeletal proteins play distinct roles from their eukaryotic counterparts; for example, control of cell wall synthesis for cell division and morphogenesis. This review is aimed at providing an overview of the bacterial cytoskeleton, and discussing the roles and assembly dynamics of bacterial cytoskeletal proteins in more detail in relation to their most widely conserved functions, DNA segregation and coordination of cell wall synthesis.

**Keywords:** Bacterial cytoskeleton, DNA segregation, FtsZ, MreB, ParA, peptidoglycan

## Introduction

The presence of a cytoskeleton was once thought to be a unique feature of eukaryotic cells as (i) no bacterial protein with obvious sequence similarity to eukaryotic cytoskeletal elements had been identified and (ii) it was erroneously assumed that bacteria were too simple to require a cytoskeletal system, as they are much smaller than eukaryotic cells and lack obvious intracellular organelles [15, 59]. However, after the initial recognition of the bacterial division protein FtsZ as a tubulin homolog in the early 1990s, various families of bacterial cytoskeletal proteins have subsequently been identified, thanks to advances in fluorescence and electron microscopies, as well as the availability of ample genomic and structural data [13]. The discovery and functional characterization of the bacterial cytoskeleton have led to an appreciation for the highly organized and dynamic nature of bacterial cells. As in eukaryotic systems, cytoskeletal elements play important roles in intracellular organization and participate in essential cellular processes such as DNA partitioning,

cytokinesis, and cell morphogenesis. However, the roles of the bacterial cytoskeleton in these processes are quite different from those in eukaryotes. For example, many bacterial cells utilize their cytoskeleton during cell morphogenesis for organizing and guiding the multiprotein complexes responsible for cell wall peptidoglycan (PG) biosynthesis, rather than providing mechanical support to maintain cell morphology as in eukaryotic cells [65]. In accordance with the functions different from eukaryotic cytoskeletal proteins, bacterial systems also show assembly dynamics distinct from those of eukaryotic counterparts [13, 77]. For example, an actin homolog, ParM, forms polymers that display dynamic instability, instead of treadmilling as observed for eukaryotic actin filaments [33, 34]. Likewise, the assembly and localization of the bacterial cytoskeleton are regulated by mechanisms distinct from those in eukaryotic cells [13].

In this review, I will first provide an overview of bacterial cytoskeletons with a brief history of their identification. Then, I will discuss the roles of bacterial cytoskeletal proteins along with the regulation of their

assembly and localization for the two most well-studied functions of the bacterial cytoskeleton, the coordination of PG synthesis and DNA segregation.

## Discovery of Bacterial Cytoskeletons

### Tubulin Homologs

The view that cytoskeletal elements are present only in eukaryotic cells started to change when immuno-electron microscopy imaging of FtsZ suggested that FtsZ forms a ring-like structure at the division site before the onset of septation, and the FtsZ ring constricts during the division process [7]. This observation, along with the identification of a GTP-binding motif similar to tubulin in FtsZ, raised a possibility that FtsZ might act as a GTP-binding cytoskeletal element. This hypothesis was supported by the demonstration of GTP-dependent polymerization and GTPase activity of FtsZ, the two properties essential for making dynamic polymers and functioning as a cytoskeletal element [22, 70, 84]. When the structures of alpha-/beta-tubulins and FtsZ were determined by electron crystallography and X-ray crystallography, respectively, striking structural similarity was observed, confirming the idea that FtsZ is a tubulin homolog despite the low sequence homology between them [58, 72, 73].

Several groups of bacterial tubulin homologs have since been identified. Among them, BtubA/B identified in *Prostheco bacter* show a higher sequence similarity to alpha- and beta-tubulins than to FtsZ, suggesting they might have been acquired by horizontal gene transfer [41]. Interestingly, an electron cryotomographic (ECT) study on *Prostheco bacter* sp. suggested that BtubA/B also form a microtubule-like structure that has not been observed with other bacterial tubulin homologs [82]. On the other hand, TubZ homologs responsible for the segregation of the plasmids and temperate phages of *Clostridium* and *Bacillus* species do not show significant sequence homology to either tubulin or FtsZ, and thus constitute a distinct subfamily of tubulin homologs [51, 74]. TubZ homologs show overall folding similar to both tubulin and FtsZ, except for the rotation between N-terminal and C-terminal domains [3, 71]. Recently, another distinct family of bacterial tubulin homologs, named PhuZ, was identified in the genome of large lytic phages and shown to position phage DNA in the center of the cell for efficient virion production [49].

### Actin Homologs

Bacterial actin homologs were initially predicted by bioinformatics work. Bork *et al.* [8] performed a motif

search based on the unexpected structural similarity between the three functionally distinct ATP-binding proteins that share low sequence homology; namely actin, sugar kinases, and hsp70. Their search predicted three bacterial proteins (FtsA, MreB, and ParM (SbtA)) as additional members of the actin/sugar kinase/Hsp70 superfamily. FtsA is a division protein identified from the *fts* (filamentation temperature sensitive) screen for division-defective mutants of *E. coli* [64], whereas MreB was identified as a rod-shaped determinant of *E. coli* [103]. ParM is encoded in the *par* locus of *E. coli* plasmid R1 and is responsible for segregation of the plasmid [10]. This prediction initially did not draw much attention because the bacterial proteins as well as sugar kinases and Hsp70 did not seem to have the properties similar to actin. However, several years later, it was demonstrated that FtsA, MreB, and ParM all share the actin fold in the crystal structures and can form ATP-dependent filaments *in vitro*, confirming them as actin homologs [95-97]. In addition, the initial localization studies with epifluorescence microscopy suggested that MreB and ParM formed filamentous structures *in vivo*, suggesting their role as cytoskeletal elements in bacteria [42, 67].

Many additional bacterial actin homologs have been identified since then. Visualization of *Magnetospirillum magnetotacticum* with ECT revealed filamentous structures important for the proper positioning of magnetosomes as well as the detailed structure of these membraneous organelles [47]. Deletion of *mamK*, a distant homolog of actin and *mreB* present in the *mamAB* gene cluster, led to the disappearance of the filamentous structure and improper positioning of the magnetosomes, identifying MamK as a bacterial cytoskeletal protein important for organizing membraneous organelles [47]. In addition, several dozen distinct families of bacterial actin homologs, AlfA (actin like filament A) and other ALPs (actin like proteins), have been identified in various bacterial plasmids and shown to be responsible for the segregation of the plasmids [5, 25]. Many of these bacterial actin homologs form filaments with distinct structural features and show unique biochemical properties [77].

### Intermediate Filament (IF) Protein Homologs

Besides tubulin and actin homologs, bacterial proteins showing similar properties to eukaryotic IF proteins have also been identified. A visual screen of a library of random transposon insertion mutants of *Caulobacter crescentus* led to the identification of Crescentin as a protein critical for maintaining the crescent shape of this bacterium [2]. Crescentin was suggested to be an IF-like protein, based on

the similarity in primary amino acid sequence and in predicted domain organization, as well as its ability to form a filament without cofactors [2]. No additional bacterial protein with the typical domain architecture of IF proteins has been identified. However, proteins rich in coiled coils, termed CCRPs, have been identified in many bacterial genomes, and some CCRPs were shown to self-assemble into filaments without cofactors [4]. In addition, structural roles of CCRPs for proper cell shape maintenance have been demonstrated in several bacteria [4, 29, 104].

### ParA/MinD Family ATPases

Besides the homologs to eukaryotic cytoskeletal elements, bacteria employ a group of noncanonical ATPases for subcellular organization. Most NTPases have a Walker A motif, or P loop for phosphate binding, and a Walker B motif, a hydrophobic beta strand terminating with aspartate [105]. A subfamily of these P-loop NTPase superfamily is known as the deviant Walker A motif ATPases, as they have a deviant Walker A motif with an additional lysine, XKGGXXK[T/S], instead of the classic Walker A motif, GXXGXGK[T/S] [48]. The deviant Walker A motif ATPases dimerize upon ATP binding, which increases the affinity of the ATPases to the partner proteins or biological surfaces such as DNA or membrane [63]. As ATP hydrolysis is also coupled to dimerization, the deviant Walker A motif ATPases show ATP-dependent dynamic interaction with their binding partners.

ParA homologs, a subgroup of the deviant Walker A motif ATPases, are responsible for the segregation of many bacterial plasmids and chromosomal origins. In addition, some ParA homologs were shown to be involved in the proper localization of protein complexes, such as chemotaxis protein complexes in *Rhodobacter sphaeroides* and carboxysomes in cyanobacteria *Synechococcus* sp. [86, 91]. MinD, another ParA-like Walker ATPase, is responsible for positioning FtsZ assembly in the mid-cell. Based on their roles in subcellular organization and their ability to form filaments on biological surfaces, ParA on DNA and MinD on membrane, ParA/MinD family ATPases were suggested as a new class of bacterial cytoskeletal elements and named as the Walker A Cytoskeletal ATPases (WACAs) [59, 66].

ParA/MinD family ATPases show ATP-dependent interaction with biological surfaces. ATP binding induces the dimerization of the ATPases, which localizes the ATPases on DNA or membranes by increasing their affinity to these surfaces. On the contrary, the ATPases are released from the surfaces upon ATP hydrolysis owing to dimer dissociation. As the dimerization also increases the affinity of

ParA/MinD family proteins to their regulatory proteins that stimulates the ATPase activity, the association of the ATPases to the surfaces is transient in the presence of the regulatory proteins. Thus, ParA/MinD proteins dynamically associate with the biological surfaces with a repeated cycle of ATP binding and hydrolysis. This dynamic association leads to the formation of a concentration gradient in a spatially confined structure. As will be discussed in more detail later, the concentration gradient formation of ParA/MinD family ATPases, rather than the filament formation, is now recognized as the property more relevant for their cellular activities [45, 61].

### Bactofilins and Other Cytoskeletal Elements

Recently, another group of filament-forming proteins, named as bactofilins, have been identified among all major phyla of bacteria [50]. Bactofilins from *Caulobacter crescentus* and *Myxococcus xanthus* self-assemble into filaments *in vitro* without any cofactor requirement [46, 50]. Bactofilins have been shown to be required for proper cell shape maintenance and implicated to have a role in PG synthesis [37, 46, 50, 89].

Although the term “cytoskeleton” was originally coined to describe the filamentous structures in eukaryotic cells, some bacterial cytoskeletal elements, including the actin homologs MreB and FtsA, do not form visibly long structures in bacterial cells. Thus, the definition of bacterial cytoskeleton is not necessarily restricted to filament-forming proteins [81]. In this regard, proteins that form structures other than filaments, such as lattice-forming polar landmark proteins, DivIVA of gram-positive bacteria, and PopZ of alpha-proteobacteria, are also considered bacterial cytoskeletal elements [54].

### Bacterial Cytoskeletons and Peptidoglycan Synthesis

Most bacterial cells maintain remarkably uniform shapes and sizes in a given condition, rarely exhibiting irregular shapes. Bacterial cytoskeletons play critical roles in establishing and maintaining cell morphology. However, unlike eukaryotic cytoskeletons that provide mechanical support for maintaining cell morphology, bacterial cytoskeletons participate in shape maintenance by guiding the synthesis of PG, the wall structure that determines the bacterial shape and resists the osmotic pressure from the cytoplasm [17, 65]. Although bacteria display a wide diversity of shapes, the rod shape is the most prevalent [12, 109]. Moreover, other shapes, such as vibrioid or spiral

shapes, are maintained by the same PG synthetic machineries that are required for rod-shape maintenance, with a few additional factors [2, 88]. Thus, understanding the mechanisms of rod-shape maintenance is expected to provide insight into cell-shape determination for a variety of bacteria with differing cell morphology.

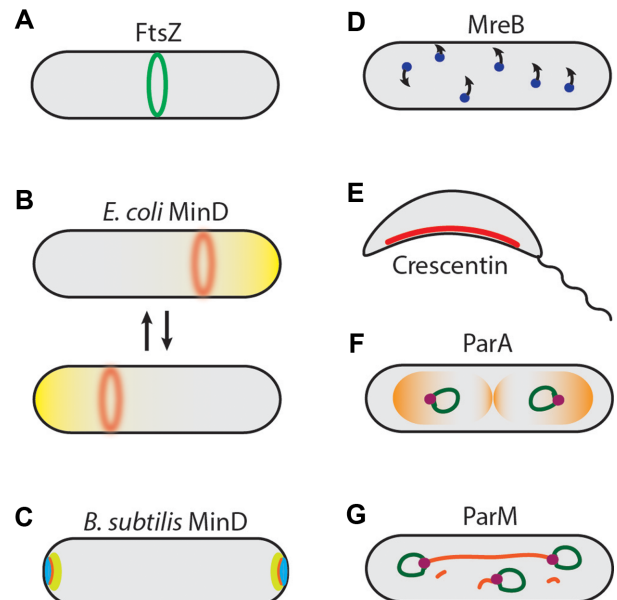
The rod shape is maintained by two distinct modes of PG synthesis; namely, sidewall synthesis for cell elongation and septal PG synthesis for division [93]. The sidewall is synthesized by a protein complex organized by a bacterial actin homolog, MreB, in most rod-shaped bacteria, except for several groups of tip-growing bacteria [17]. On the other hand, septal PG synthesis is coordinated by the tubulin homolog FtsZ and the actin homolog FtsA in almost all bacteria.

### FtsZ, FtsA, and Septal PG Synthesis

Septal PG is synthesized by a multiprotein complex called the divisome that assembles at the prospective division site. The cytoskeletal proteins FtsZ and FtsA are critical for initiating divisome assembly. FtsZ polymers assemble into a ring-like structure, termed the Z-ring, along with several FtsZ-interacting proteins, including FtsA, underneath the cytoplasmic membrane at the prospective division site (Fig. 1A) [23, 62]. After a time delay of at least 20% of the cell cycle, more than a dozen other components of the divisome are recruited to the Z-ring to form a mature cell division complex that carries out septal PG synthesis as well as the constriction of inner and outer membranes, and chromosome translocation at the division site [1, 31, 62].

In addition to its role in initiation of divisome assembly, it was suggested FtsZ provides the mechanical force for constriction, based on the observation that FtsZ fused to a fluorescent protein and a membrane anchor can produce a visible constriction in tubular liposomes in the presence of GTP [76]. The mechanical force was suggested to be generated from the conformational change between a straight protofilament and a highly curved filament, depending on the bound nucleotide [28, 39, 52, 76]. However, this model should be considered with some reservation, as some FtsZ mutants with markedly decreased GTPase activity can still support cell division [20]. In principle, septal PG synthesis is another process that can provide the mechanical force for constriction [36, 87]. Consistent with this idea, septal PG synthesis still occurs in some bacteria that are mostly devoid of PG in the cell envelope, such as L-form-like *E. coli* and *Chlamydia* [43, 53], suggesting that septal PG synthesis plays an essential role in the division process other than osmoprotection.

FtsA plays a role in recruiting downstream division proteins, in addition to serving as a membrane anchor to facilitate FtsZ assembly underneath the cytoplasmic membrane [79]. Recent study of FtsA mutants impaired for self-interaction suggested that the self-interaction of FtsA serves as a switch that regulates the interaction with the FtsA-interacting division proteins and thus the recruitment of the downstream division proteins for divisome maturation and initiation of constriction [80]. A recent reconstitution experiment with FtsZ and FtsA on the membrane surface showed that FtsZ forms dynamic ring-like structures that showed a directional circular movement based on FtsZ polymerization dynamics similar to treadmilling [57]. Interestingly, this treadmilling behavior was dependent on FtsA, suggesting that FtsA might also regulate the dynamic assembly of FtsZ.



**Fig. 1.** Localization of bacterial cytoskeletal elements.

(A) Tubulin homolog FtsZ ring (green circle) assembled at mid-cell. (B) *E. coli* MinD (yellow gradient) oscillating from pole to pole owing to the interaction with the topological determinant MinE ring (red), resulting in time-averaged gradient being highest at the poles. (C) *B. subtilis* MinD (yellow-green) localizing to the cell poles via interaction with MinJ (orange), which is recruited to the cell poles by the pole landmark protein DivIVA (light blue). (D) Actin homolog MreB patches (blue dots) rotating around the cell, perpendicular to the long axis. (E) IF-like protein Crescentin (red line) localizing along the concave side of *Caulobacter crescentus*. (F) SopA (ParA) gradient (orange) mediating the unidirectional movement of F (P1) plasmid (green circle) over the nucleoids. Purple dots represent the SopB (ParB)/sopS (*parS*) complexes. (G) Actin homolog ParM filaments (orange lines) segregating *E. coli* plasmid R1 (green circle). Purple dots represent the ParR/*parS* complexes.

### Division Site Placement by Regulation of FtsZ Assembly

In accordance with its critical role in divisome assembly, the assembly of FtsZ is spatiotemporally regulated by several negative regulators, such as SulA, the Min system, nucleoid occlusion factors, and MipZ. SulA is an FtsZ antagonist whose expression is induced as part of the SOS response upon DNA damage [69]. SulA inhibits FtsZ assembly by sequestering FtsZ monomers [21]. Whereas SulA simply delays Z-ring assembly during SOS response, other FtsZ antagonists function to promote Z-ring assembly at mid-cell.

In *E. coli*, the Min system consists of three proteins, MinC, MinD, and MinE [24]. MinC is the antagonist of FtsZ assembly that has a high affinity to MinD. MinD is a ParA/MinD family ATPase that dimerizes and localizes on the membrane upon ATP binding [60]. MinE is a topological determinant that enhances the ATPase activity of MinD and releases MinD from the membrane. The consequence of the interaction between MinD and MinE is the pole-to-pole oscillation of MinD that results in a time-averaged concentration of MinC/MinD complexes being highest at the poles and lowest at mid-cell, promoting Z-ring assembly at mid-cell (Fig. 1B) [55, 56, 83]. MinC/MinD localization is achieved by a different mechanism in *B. subtilis*. Instead of MinD oscillation, MinC/MinD is recruited to the poles by the pole landmark protein DivIVA via an adaptor protein, MinJ (Fig. 1C) [9, 78].

In many bacteria, the Min system is not essential owing to another partially redundant division site placement system, called nucleoid occlusion (NO), that inhibits Z-ring assembly over the nucleoids [107]. The specific factors for NO, Noc in *B. subtilis* and SlmA in *E. coli*, were identified based on the synthetic lethality with a defective Min system [6, 106]. Although NO factors perform a similar function, they belong to distinct families of DNA-binding proteins; Noc is a ParB family protein, and SlmA is a TetR family protein. SlmA has been shown to antagonize the formation of FtsZ protofilaments, whereas the target of Noc has not been identified yet [18, 19, 27]. In addition, the FtsZ-antagonistic activity of SlmA is enhanced dramatically upon DNA binding, providing a mechanism for precise spatiotemporal regulation of NO-factor activity in the cell [19]. Interestingly, the binding sites for both Noc and SlmA are enriched at the origin-proximal region of the chromosomes and nearly absent in one third of the chromosome close to the terminus [19, 92, 108]. As the replication termini are localized at mid-cell in the late phase of chromosome replication/segregation, this distribution of binding sites suggests a role of NO factors in coordinating

chromosome replication/segregation and cell division.

A new class of negative regulator of FtsZ assembly, MipZ, was shown to be responsible for the spatiotemporal regulation of Z-ring assembly in *Caulobacter crescentus*. MipZ is a ParA/MinD family ATPase that also shows an antagonistic activity on FtsZ assembly [90]. MipZ forms a gradient whose concentration is highest near ParB, because ParB has a high affinity to MipZ monomers and induces MipZ binding to DNA near ParB by enhancing MipZ dimerization [44]. As ParB localizes at the poles after chromosome segregation, the MipZ concentration becomes highest at the poles and lowest at mid-cell, promoting Z-ring assembly at mid-cell.

### Sidewall Synthesis and MreB

Except for coccoid bacteria, bacterial growth requires expansion along the cylindrical sidewall. Synthesis of the sidewall in most rod-shaped bacteria requires the activity of the actin homolog MreB, except for some groups of bacteria that grow from the poles, such as *Actinobacteria* and *Rhizobium*. Initial localization studies of MreB homologs suggested that they form dynamic helical cables along the sidewall of rod-shaped bacteria [14, 42]. As MreB homologs are required for rod-shape maintenance, MreB cables were suggested to serve as a track that guides the uniform synthesis of peptidoglycan along the sidewall. However, recent localization studies with high-resolution microscopic techniques suggested that MreB forms discrete patches that rotate along the cell periphery perpendicular to the long axis of the cell, rather than forming a helical track for PG synthesis that spans the entire cell (Fig. 1D) [26, 32, 98]. Moreover, the directional movement of MreB was inhibited by treatment of the drugs that target peptidoglycan synthesis, showing that MreB rotation is dependent on PG synthesis, rather than MreB polymerization driving the movement of PG synthetic machineries. As an alternative to the helical track model, it was recently suggested that MreB helps maintain the rod shape by directing PG synthesis to the regions of negative cell wall curvature, based on the enrichment of MreB at the regions of negative cell wall curvature and strong correlation of MreB localization and PG synthesis [94].

### PG Synthesis and Nucleotide-Independent Cytoskeletal Elements

Other than the actin and tubulin homologs, several nucleotide-independent cytoskeletal elements have been shown to affect cell morphology by regulating PG synthesis [54]. IF-homolog Crescentin polymers were shown to result

in the crescent shape by limiting cell wall synthesis on the side it polymerizes and thus causing uneven expansion of the sidewall (Fig. 1E) [11]. BacA and BacB, the bactofilins identified in *Caulobacter crescentus*, were shown to interact with the PG synthase PbpC to generate a stalk with optimal length [50]. CcmA, the bactofilin homolog in *Helicobacter pylori*, was shown to participate in the helical-shape generation by affecting the PG endopeptidase activity [88]. The recurring theme of these studies of the nucleotide-independent cytoskeletal elements is that bacterial cytoskeletal proteins determine cell shape by directing the synthesis of PG, rather than providing the mechanical support for the observed cell shape.

### Bacterial Cytoskeletons Responsible for DNA Segregation

Just as the microtubule-based mitotic spindles segregate sister chromatids during cell division in eukaryotic cells, bacterial cytoskeletons are involved in partitioning DNA molecules to achieve faithful inheritance of genetic material. Bacterial DNA segregation systems can be classified into three groups based on the types of the cytomotive element encoded in the segregation system; namely, ParA (deviant Walker A ATPase), actin, and tubulin homologs [35]. All three types of systems have three essential components: the centromere-like *cis*-acting DNA sequence on the cargo DNA, the cytoskeletal element, and an adaptor protein that binds DNA and forms a partition complex. In the past few years, there has been significant progress in understanding the mechanisms of these bacterial DNA segregation systems, revealing distinct assembly dynamics of the cytoskeletal elements employed by each system.

#### ParA Homologs (Deviant Walker A ATPases)

ParA homologs-based partitioning systems were identified more than 30 years ago and are widely distributed in many plasmids and most bacterial chromosomes. However, the mechanism underlying the ParA homolog-mediated partitioning process has been ambiguous for a long time. ParB/*parS* complexes in the plasmid DNA and chromosomal origins contact the ParA cloud and move towards the poles following the ParA cloud receding to the poles (Fig. 1F) [30, 85]. Based on this observation, it was suggested that the plasmids and chromosomal origins are pulled to the cell poles by ParA filaments in a way analogous to the segregation of eukaryotic chromosomes by microtubules. However, this view has been questioned because *in vivo* ParA filaments have not been observed and a ParA

filament-mediated pulling model cannot explain the essentiality of ParA's DNA-binding activity in DNA segregation [16, 38, 99].

Vecchiarelli *et al.* [99] suggested a diffusion-ratchet model as an alternative to the pulling model, based on the similarity between the deviant Walker A ATPases, MinD and ParA. In the diffusion-ratchet model, the concentration gradient of ParA dimers on the nucleoids serves as the driving force for the movement of ParB/*parS* complexes; ParB/*parS* complexes move towards the higher concentration of ParA *via* ParB's affinity to ParA dimers [101]. Upon binding to ParA, ParB stimulates the ATPase activity of ParA, releasing ParA from the nucleoids. Thus, ParB moves unidirectionally as ParA dimers are depleted in the wake of ParB. Recently, the Mizuuchi group provided strong evidence for the diffusion-ratchet model by reconstituting the P1 and F plasmid segregation systems in the microfluidic flow cell coated with nonspecific DNA [40, 100, 102]. Consistent with the diffusion-ratchet model, *sopS* (*parS*)-coated beads showed SopB (ParB)-dependent unidirectional movement, releasing SopA (ParA) from the DNA carpet in their wake [102].

#### ParM and Actin Homologs

The mechanism of plasmid segregation is best understood for an actin homolog, ParM, which is responsible for the segregation of *E. coli* plasmid R1. Visualization of the segregating R1 plasmids at the ends of ParM filaments in *E. coli* cells by dual-labeling immunofluorescence microscopy suggested ParM functions as a cytoskeletal element in the partitioning of R1 plasmid (Fig. 1G) [67]. ParM forms double helical filaments as eukaryotic actin, but the assembled polymer shows opposite handedness to actin (left-handed for ParM and right-handed for actin) because ParM uses a different assembly interface from actin [75]. Interestingly, ParM also shows distinct assembly dynamics. ParM filaments display symmetrical, bidirectional polymerization instead of the treadmilling behavior of actin [33]. In addition, ParM filaments nucleate ~300 times faster than actin filaments and exhibit dynamic instability. As a result, ParM is present as short dynamic filaments that constantly assemble and disassemble in the absence of ParR/*parS* complexes [33]. In an *in vitro* reconstitution experiment using ParM, ParR, and beads coated with *parS* DNA, long ParM filaments with ParR/*parS* beads at both ends were observed, suggesting the inhibition of the dynamic instability of ParM filament by binding of ParR/*parS* beads [34]. In addition, ParM filaments always aligned with the long axis of the micro-fabricated channels, demonstrating that ParM, ParR, and

*parS* DNA are sufficient for partitioning R1 plasmids along the long axis of the bacterial cell. As long ParM filaments form only when both ends of the ParM filament is associated with ParR/*parS* complexes, this bipolar stabilization also provides a mechanism to segregate the equal number of R1 plasmids into two daughter cells [34].

### TubZ and Tubulin Homologs

Plasmid segregation systems based on tubulin homologs, TubZ, have only recently been identified [51, 74]. TubZ homologs employ assembly dynamics distinct from eukaryotic tubulins for their function. TubZ of the pBtoxis plasmid forms dynamic, linear polymers that move by treadmilling, rather than showing dynamic instability [51]. Treadmilling was shown to be important in TubZ-mediated plasmid segregation, although it is not yet clear how treadmilling of TubZ contributes to the stability of the plasmid [51]. Crystallography of TubZ polymerized with nonhydrolyzable GTP $\gamma$ S revealed right-handed double helical filaments [3]. Recently, cryoelectron microscopy on the full-length TubZ polymerized with GTP showed that TubZ forms four-stranded filaments, suggesting that the two-stranded filaments might be an intermediate to the four-stranded filaments that form upon GTP hydrolysis [68]. However, it is still unclear how these different polymer forms are related to the plasmid segregation by TubZ polymers.

### Concluding Remark

Research on bacterial cytoskeletal proteins is at the heart of an emerging field of bacterial cell biology that studies the cellular processes and subcellular organization of bacterial cells with multidisciplinary approaches, encompassing advanced microscopy, structural biology, genomics, genetics, physiology, and biochemistry. The recent advances in bacterial cell biology have revealed numerous exciting aspects that have revolutionized our view on the complexity of bacterial cells. In addition to contributing to a deeper understanding of biological systems, bacterial cell biology provides critical scientific insights required for the discovery and development of new antibiotics. The achievements of bacterial cell biology also uncovered a vast amount of intriguing cellular phenomena that warrant further investigation. Thus, bacterial cell biology will continue to be an exciting field of research for many years to come.

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### References

1. Aarsman ME, Piette A, Fraipont C, Vinkenvleugel TM, Nguyen-Disteche M, den Blaauwen T. 2005. Maturation of the *Escherichia coli* divisome occurs in two steps. *Mol. Microbiol.* **55**: 1631-1645.
2. Ausmees N, Kuhn JR, Jacobs-Wagner C. 2003. The bacterial cytoskeleton: an intermediate filament-like function in cell shape. *Cell* **115**: 705-713.
3. Aylett CH, Wang Q, Michie KA, Amos LA, Lowe J. 2010. Filament structure of bacterial tubulin homologue TubZ. *Proc. Natl. Acad. Sci. USA* **107**: 19766-19771.
4. Bagchi S, Tomenius H, Belova LM, Ausmees N. 2008. Intermediate filament-like proteins in bacteria and a cytoskeletal function in *Streptomyces*. *Mol. Microbiol.* **70**: 1037-1050.
5. Becker E, Herrera NC, Gunderson FQ, Derman AI, Dance AL, Sims J, et al. 2006. DNA segregation by the bacterial actin AlfA during *Bacillus subtilis* growth and development. *EMBO J.* **25**: 5919-5931.
6. Bernhardt TG, de Boer PA. 2005. SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over chromosomes in *E. coli*. *Mol. Cell* **18**: 555-564.
7. Bi EF, Lutkenhaus J. 1991. FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354**: 161-164.
8. Bork P, Sander C, Valencia A. 1992. An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. *Proc. Natl. Acad. Sci. USA* **89**: 7290-7294.
9. Bramkamp M, Emmins R, Weston L, Donovan C, Daniel RA, Errington J. 2008. A novel component of the division-site selection system of *Bacillus subtilis* and a new mode of action for the division inhibitor MinCD. *Mol. Microbiol.* **70**: 1556-1569.
10. Breuner A, Jensen RB, Dam M, Pedersen S, Gerdes K. 1996. The centromere-like *parC* locus of plasmid R1. *Mol. Microbiol.* **20**: 581-592.
11. Cabeen MT, Charbon G, Vollmer W, Born P, Ausmees N, Weibel DB, Jacobs-Wagner C. 2009. Bacterial cell curvature through mechanical control of cell growth. *EMBO J.* **28**: 1208-1219.
12. Cabeen MT, Jacobs-Wagner C. 2005. Bacterial cell shape. *Nat. Rev. Microbiol.* **3**: 601-610.
13. Cabeen MT, Jacobs-Wagner C. 2010. The bacterial cytoskeleton. *Annu. Rev. Genet.* **44**: 365-392.
14. 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.
15. Carballido-Lopez R, Errington J. 2003. A dynamic bacterial cytoskeleton. *Trends Cell Biol.* **13**: 577-583.
16. Castaing JP, Bouet JY, Lane D. 2008. F plasmid partition

- depends on interaction of SopA with non-specific DNA. *Mol. Microbiol.* **70**: 1000-1011.
17. Cava F, Kuru E, Brun YV, de Pedro MA. 2013. Modes of cell wall growth differentiation in rod-shaped bacteria. *Curr. Opin. Microbiol.* **16**: 731-737.
  18. Cho H, Bernhardt TG. 2013. Identification of the SlmA active site responsible for blocking bacterial cytokinetic ring assembly over the chromosome. *PLoS Genet.* **9**: e1003304.
  19. Cho H, McManus HR, Dove SL, Bernhardt TG. 2011. Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist. *Proc. Natl. Acad. Sci. USA* **108**: 3773-3778.
  20. Dai K, Mukherjee A, Xu Y, Lutkenhaus J. 1994. Mutations in *ftsZ* that confer resistance to SulA affect the interaction of FtsZ with GTP. *J. Bacteriol.* **176**: 130-136.
  21. Dajkovic A, Mukherjee A, Lutkenhaus J. 2008. Investigation of regulation of FtsZ assembly by SulA and development of a model for FtsZ polymerization. *J. Bacteriol.* **190**: 2513-2526.
  22. de Boer P, Crossley R, Rothfield L. 1992. The essential bacterial cell-division protein FtsZ is a GTPase. *Nature* **359**: 254-256.
  23. de Boer PA. 2010. Advances in understanding *E. coli* cell fission. *Curr. Opin. Microbiol.* **13**: 730-737.
  24. de Boer PA, Crossley RE, Rothfield LI. 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*. *Cell* **56**: 641-649.
  25. Derman AI, Becker EC, Truong BD, Fujioka A, Tucey TM, Erb ML, et al. 2009. Phylogenetic analysis identifies many uncharacterized actin-like proteins (Alps) in bacteria: regulated polymerization, dynamic instability and treadmilling in Alp7A. *Mol. Microbiol.* **73**: 534-552.
  26. Dominguez-Escobar J, Chastanet A, Crevenna AH, Fromion V, Wedlich-Soldner R, Carballido-Lopez R. 2011. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science* **333**: 225-228.
  27. Du S, Lutkenhaus J. 2014. SlmA antagonism of FtsZ assembly employs a two-pronged mechanism like MinCD. *PLoS Genet.* **10**: e1004460.
  28. Erickson HP. 2009. Modeling the physics of FtsZ assembly and force generation. *Proc. Natl. Acad. Sci. USA* **106**: 9238-9243.
  29. Fiuza M, Letek M, Leiba J, Villadangos AF, Vaquera J, Zanella-Cleon I, et al. 2010. Phosphorylation of a novel cytoskeletal protein (RsmP) regulates rod-shaped morphology in *Corynebacterium glutamicum*. *J. Biol. Chem.* **285**: 29387-29397.
  30. Fogel MA, Waldor MK. 2006. A dynamic, mitotic-like mechanism for bacterial chromosome segregation. *Genes Dev.* **20**: 3269-3282.
  31. Gamba P, Veening JW, Saunders NJ, Hamoen LW, Daniel RA. 2009. Two-step assembly dynamics of the *Bacillus subtilis* divisome. *J. Bacteriol.* **191**: 4186-4194.
  32. Garner EC, Bernard R, Wang W, Zhuang X, Rudner DZ, Mitchison T. 2011. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science* **333**: 222-225.
  33. Garner EC, Campbell CS, Mullins RD. 2004. Dynamic instability in a DNA-segregating prokaryotic actin homolog. *Science* **306**: 1021-1025.
  34. Garner EC, Campbell CS, Weibel DB, Mullins RD. 2007. Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. *Science* **315**: 1270-1274.
  35. Gerdes K, Howard M, Szardenings F. 2010. Pushing and pulling in prokaryotic DNA segregation. *Cell* **141**: 927-942.
  36. Gerding MA, Ogata Y, Pecora ND, Niki H, de Boer PA. 2007. The trans-envelope Tol-Pal complex is part of the cell division machinery and required for proper outer-membrane invagination during cell constriction in *E. coli*. *Mol. Microbiol.* **63**: 1008-1025.
  37. Hay NA, Tipper DJ, Gygi D, Hughes C. 1999. A novel membrane protein influencing cell shape and multicellular swarming of *Proteus mirabilis*. *J. Bacteriol.* **181**: 2008-2016.
  38. Hester CM, Lutkenhaus J. 2007. Soj (ParA) DNA binding is mediated by conserved arginines and is essential for plasmid segregation. *Proc. Natl. Acad. Sci. USA* **104**: 20326-20331.
  39. Hsin J, Gopinathan A, Huang KC. 2012. Nucleotide-dependent conformations of FtsZ dimers and force generation observed through molecular dynamics simulations. *Proc. Natl. Acad. Sci. USA* **109**: 9432-9437.
  40. Hwang LC, Vecchiarelli AG, Han YW, Mizuuchi M, Harada Y, Funnell BE, Mizuuchi K. 2013. ParA-mediated plasmid partition driven by protein pattern self-organization. *EMBO J.* **32**: 1238-1249.
  41. Jenkins C, Samudrala R, Anderson I, Hedlund BP, Petroni G, Michailova N, et al. 2002. Genes for the cytoskeletal protein tubulin in the bacterial genus *Prostheco bacter*. *Proc. Natl. Acad. Sci. USA* **99**: 17049-17054.
  42. Jones LJ, Carballido-Lopez R, Errington J. 2001. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* **104**: 913-922.
  43. Joseleau-Petit D, Liebart JC, Ayala JA, and D'Ari R. 2007. Unstable *Escherichia coli* L forms revisited: growth requires peptidoglycan synthesis. *J. Bacteriol.* **189**: 6512-6520.
  44. Kiekebusch D, Michie KA, Essen LO, Lowe J, Thanbichler M. 2012. Localized dimerization and nucleoid binding drive gradient formation by the bacterial cell division inhibitor MipZ. *Mol. Cell* **46**: 245-259.
  45. Kiekebusch D, Thanbichler M. 2014. Spatiotemporal organization of microbial cells by protein concentration gradients. *Trends Microbiol.* **22**: 65-73.
  46. Koch MK, McHugh CA, Hoiczky E. 2011. BacM, an N-terminally processed bactofilin of *Myxococcus xanthus*, is crucial for proper cell shape. *Mol. Microbiol.* **80**: 1031-1051.
  47. Komeili A, Li Z, Newman DK, Jensen GJ. 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**: 242-245.
  48. Koonin EV. 1993. A superfamily of ATPases with diverse



- functions containing either classical or deviant ATP-binding motif. *J. Mol. Biol.* **229**: 1165-1174.
49. Kraemer JA, Erb ML, Waddling CA, Montabana EA, Zehr EA, Wang H, *et al.* 2012. A phage tubulin assembles dynamic filaments by an atypical mechanism to center viral DNA within the host cell. *Cell* **149**: 1488-1499.
  50. Kuhn J, Briegel A, Morschel E, Kahnt J, Leser K, Wick S, *et al.* 2010. Bactofilins, a ubiquitous class of cytoskeletal proteins mediating polar localization of a cell wall synthase in *Caulobacter crescentus*. *EMBO J.* **29**: 327-339.
  51. Larsen RA, Cusumano C, Fujioka A, Lim-Fong G, Patterson P, Pogliano J. 2007. Treadmilling of a prokaryotic tubulin-like protein, TubZ, required for plasmid stability in *Bacillus thuringiensis*. *Genes Dev.* **21**: 1340-1352.
  52. Li Y, Hsin J, Zhao L, Cheng Y, Shang W, Huang KC, *et al.* 2013. FtsZ protofilaments use a hinge-opening mechanism for constrictive force generation. *Science* **341**: 392-395.
  53. Liechti GW, Kuru E, Hall E, Kalinda A, Brun YV, VanNieuwenhze M, Maurelli AT. 2014. A new metabolic cell-wall labelling method reveals peptidoglycan in *Chlamydia trachomatis*. *Nature* **506**: 507-510.
  54. Lin L, Thanbichler M. 2013. Nucleotide-independent cytoskeletal scaffolds in bacteria. *Cytoskeleton (Hoboken)* **70**: 409-423.
  55. 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.
  56. Loose M, Kruse K, Schwille P. 2011. Protein self-organization: lessons from the min system. *Annu. Rev. Biophys.* **40**: 315-336.
  57. Loose M, Mitchison TJ. 2014. The bacterial cell division proteins FtsA and FtsZ self-organize into dynamic cytoskeletal patterns. *Nat. Cell Biol.* **16**: 38-46.
  58. Lowe J, Amos LA. 1998. Crystal structure of the bacterial cell-division protein FtsZ. *Nature* **391**: 203-206.
  59. Lowe J, Amos LA. 2009. Evolution of cytomotive filaments: the cytoskeleton from prokaryotes to eukaryotes. *Int. J. Biochem. Cell Biol.* **41**: 323-329.
  60. Lutkenhaus J. 2007. Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu. Rev. Biochem.* **76**: 539-562.
  61. Lutkenhaus J. 2012. The ParA/MinD family puts things in their place. *Trends Microbiol.* **20**: 411-418.
  62. Lutkenhaus J, Pichoff S, Du S. 2012. Bacterial cytokinesis: from Z ring to divisome. *Cytoskeleton (Hoboken)* **69**: 778-790.
  63. Lutkenhaus J, Sundaramoorthy M. 2003. MinD and role of the deviant Walker A motif, dimerization and membrane binding in oscillation. *Mol. Microbiol.* **48**: 295-303.
  64. Lutkenhaus JF, Wolf-Watz H, Donachie WD. 1980. Organization of genes in the *ftsA-envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus (*ftsZ*). *J. Bacteriol.* **142**: 615-620.
  65. Margolin, W. 2009. Sculpting the bacterial cell. *Curr. Biol.* **19**: R812-822.
  66. Michie KA, Lowe J. 2006. Dynamic filaments of the bacterial cytoskeleton. *Annu. Rev. Biochem.* **75**: 467-492.
  67. Moller-Jensen J, Borch J, Dam M, Jensen RB, Roepstorff P, Gerdes K. 2003. Bacterial mitosis: ParM of plasmid R1 moves plasmid DNA by an actin-like insertional polymerization mechanism. *Mol. Cell* **12**: 1477-1487.
  68. Montabana EA, Agard DA. 2014. Bacterial tubulin TubZ-Bt transitions between a two-stranded intermediate and a four-stranded filament upon GTP hydrolysis. *Proc. Natl. Acad. Sci. USA* **111**: 3407-3412.
  69. Mukherjee A, Cao C, Lutkenhaus J. 1998. Inhibition of FtsZ polymerization by SulA, an inhibitor of septation in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **95**: 2885-2890.
  70. Mukherjee A, Dai K, Lutkenhaus J. 1993. *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. *Proc. Natl. Acad. Sci. USA* **90**: 1053-1057.
  71. Ni L, Xu W, Kumaraswami M, Schumacher MA. 2010. Plasmid protein TubR uses a distinct mode of HTH-DNA binding and recruits the prokaryotic tubulin homolog TubZ to effect DNA partition. *Proc. Natl. Acad. Sci. USA* **107**: 11763-11768.
  72. Nogales E, Downing KH, Amos LA, Lowe J. 1998. Tubulin and FtsZ form a distinct family of GTPases. *Nat. Struct. Biol.* **5**: 451-458.
  73. Nogales E, Wolf SG, Downing KH. 1998. Structure of the alpha beta tubulin dimer by electron crystallography. *Nature* **391**: 199-203.
  74. Oliva MA, Martin-Galiano AJ, Sakaguchi Y, Andreu JM. 2012. Tubulin homolog TubZ in a phage-encoded partition system. *Proc. Natl. Acad. Sci. USA* **109**: 7711-7716.
  75. Orlova A, Garner EC, Galkin VE, Heuser J, Mullins RD, Egelman EH. 2007. The structure of bacterial ParM filaments. *Nat. Struct. Mol. Biol.* **14**: 921-926.
  76. Osawa M, Anderson DE, Erickson HP. 2008. Reconstitution of contractile FtsZ rings in liposomes. *Science* **320**: 792-794.
  77. Ozyamak E, Kollman JM, Komeili A. 2013. Bacterial actins and their diversity. *Biochemistry* **52**: 6928-6939.
  78. Patrick JE, Kearns DB. 2008. MinJ (YvjD) is a topological determinant of cell division in *Bacillus subtilis*. *Mol. Microbiol.* **70**: 1166-1179.
  79. Pichoff S, Lutkenhaus J. 2002. Unique and overlapping roles for ZipA and FtsA in septal ring assembly in *Escherichia coli*. *EMBO J.* **21**: 685-693.
  80. Pichoff S, Shen B, Sullivan B, Lutkenhaus J. 2012. FtsA mutants impaired for self-interaction bypass ZipA suggesting a model in which FtsA's self-interaction competes with its ability to recruit downstream division proteins. *Mol. Microbiol.* **83**: 151-167.
  81. Pilhofer M, Jensen GJ. 2013. The bacterial cytoskeleton: more than twisted filaments. *Curr. Opin. Cell Biol.* **25**: 125-133.
  82. Pilhofer M, Ladinsky MS, McDowall AW, Petroni G, Jensen GJ. 2011. Microtubules in bacteria: ancient tubulins build a

- five-protofilament homolog of the eukaryotic cytoskeleton. *PLoS Biol.* **9**: e1001213.
83. Raskin DM, de Boer PA. 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.
  84. RayChaudhuri D, Park JT. 1992. *Escherichia coli* cell-division gene *ftsZ* encodes a novel GTP-binding protein. *Nature* **359**: 251-254.
  85. Ringgaard S, van Zon J, Howard M, Gerdes K. 2009. Movement and equipositioning of plasmids by ParA filament disassembly. *Proc. Natl. Acad. Sci. USA* **106**: 19369-19374.
  86. Savage DF, Afonso B, Chen AH, Silver PA. 2010. Spatially ordered dynamics of the bacterial carbon fixation machinery. *Science* **327**: 1258-1261.
  87. Schwille P. 2014. Bacterial cell division: a swirling ring to rule them all? *Curr. Biol.* **24**: R157-R159.
  88. Syculo LK, Pincus Z, Gutierrez KD, Biboy J, Stern CA, Vollmer W, Salama NR. 2010. Peptidoglycan crosslinking relaxation promotes *Helicobacter pylori*'s helical shape and stomach colonization. *Cell* **141**: 822-833.
  89. Syculo LK, Wyckoff TJ, Biboy J, Born P, Pincus Z, Vollmer W, Salama NR. 2012. Multiple peptidoglycan modification networks modulate *Helicobacter pylori*'s cell shape, motility, and colonization potential. *PLoS Pathog.* **8**: e1002603.
  90. Thanbichler M, Shapiro L. 2006. MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. *Cell* **126**: 147-162.
  91. Thompson SR, Wadhams GH, Armitage JP. 2006. The positioning of cytoplasmic protein clusters in bacteria. *Proc. Natl. Acad. Sci. USA* **103**: 8209-8214.
  92. Tonthat NK, Arold ST, Pickering BF, Van Dyke MW, Liang S, Lu Y, et al. 2011. Molecular mechanism by which the nucleoid occlusion factor, SlmA, keeps cytokinesis in check. *EMBO J.* **30**: 154-164.
  93. Typas A, Banzhaf M, Gross CA, Vollmer W. 2012. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat. Rev. Microbiol.* **10**: 123-136.
  94. Ursell TS, Nguyen J, Monds RD, Colavin A, Billings G, Ouzounov N, et al. 2014. Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization. *Proc. Natl. Acad. Sci. USA* **111**: E1025-E1034.
  95. van den Ent F, Amos LA, Lowe J. 2001. Prokaryotic origin of the actin cytoskeleton. *Nature* **413**: 39-44.
  96. van den Ent F, Lowe J. 2000. Crystal structure of the cell division protein FtsA from *Thermotoga maritima*. *EMBO J.* **19**: 5300-5307.
  97. van den Ent F, Moller-Jensen J, Amos LA, Gerdes K, Lowe J. 2002. F-actin-like filaments formed by plasmid segregation protein ParM. *EMBO J.* **21**: 6935-6943.
  98. van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, Gitai Z. 2011. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proc. Natl. Acad. Sci. USA* **108**: 15822-15827.
  99. Vecchiarelli AG, Han YW, Tan X, Mizuuchi M, Ghirlando R, Biertumpfel C, et al. 2010. ATP control of dynamic P1 ParA-DNA interactions: a key role for the nucleoid in plasmid partition. *Mol. Microbiol.* **78**: 78-91.
  100. Vecchiarelli AG, Hwang LC, Mizuuchi K. 2013. Cell-free study of F plasmid partition provides evidence for cargo transport by a diffusion-ratchet mechanism. *Proc. Natl. Acad. Sci. USA* **110**: E1390-E1397.
  101. Vecchiarelli AG, Mizuuchi K, Funnell BE. 2012. Surfing biological surfaces: exploiting the nucleoid for partition and transport in bacteria. *Mol. Microbiol.* **86**: 513-523.
  102. Vecchiarelli AG, Neuman KC, Mizuuchi K. 2014. A propagating ATPase gradient drives transport of surface-confined cellular cargo. *Proc. Natl. Acad. Sci. USA* **111**: 4880-4885.
  103. Wachi M, Doi M, Tamaki S, Park W, Nakajima-Iijima S, Matsushashi M. 1987. Mutant isolation and molecular cloning of *mre* genes, which determine cell shape, sensitivity to mecillinam, and amount of penicillin-binding proteins in *Escherichia coli*. *J. Bacteriol.* **169**: 4935-4940.
  104. Waidner B, Specht M, Dempwolff F, Haeberer K, Schaetzle S, Speth V, et al. 2009. A novel system of cytoskeletal elements in the human pathogen *Helicobacter pylori*. *PLoS Pathog.* **5**: e1000669.
  105. Walker JE, Saraste M, Runswick MJ, Gay NJ. 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**: 945-951.
  106. Wu LJ, Errington J. 2004. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. *Cell* **117**: 915-925.
  107. Wu LJ, Errington J. 2012. Nucleoid occlusion and bacterial cell division. *Nat. Rev. Microbiol.* **10**: 8-12.
  108. Wu LJ, Ishikawa S, Kawai Y, Oshima T, Ogasawara N, Errington J. 2009. Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation. *EMBO J.* **28**: 1940-1952.
  109. Young KD. 2010. Bacterial shape: two-dimensional questions and possibilities. *Annu. Rev. Microbiol.* **64**: 223-240.