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Bacterial Cell Division: Nonmodels Poised to Take the Spotlight

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Abstract

The last three decades have witnessed an explosion of discoveries about the mechanistic details of binary fission in model bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus*. This was made possible not only by advances in microscopy that helped answer questions about cell biology but also by clever genetic manipulations that directly and easily tested specific hypotheses. More recently, research using understudied organisms, or nonmodel systems, has revealed several alternate mechanistic strategies that bacteria use to divide and propagate. In this review, we highlight new findings and compare these strategies to cell division mechanisms elucidated in model organisms.

Contents

INTRODUCTION

The field of bacterial cell division has relied heavily on model organisms, such as the gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis*, largely because of the abundance of available genetic tools for these organisms. Since *E. coli* and *B. subtilis* are both rod-shaped cells that divide symmetrically along their short axis, it has been relatively straightforward to compare and contrast the mechanisms involved in regulating cell division in these two species. Tremendous progress has been accomplished over the last three decades in understanding the fundamentals of bacterial cell division. To gain a deeper appreciation of how bacterial cells divide, several research groups have begun to examine differently shaped organisms that may undergo more complex cell cycles and occupy a variety of ecological niches; much of what has been learned from studying model organisms appears not to apply to these organisms. Certainly, our understanding of molecular details in these systems is still in its infancy compared to our knowledge of model systems, but a wide array of interesting cell division mechanisms is already being reported (**Figure 1**). Therefore, this review highlights new research in traditionally understudied systems and compares these systems to cell division mechanisms elucidated in well-studied model organisms.

FtsZ: almost universally conserved bacterial tubulin homolog that is the major component of the cell division machinery

Z-ring: ring-shaped supramolecular structure at the division septum composed of polymerized FtsZ and cell division machinery components

Divisome: bacterial cell division machinery, composed of FtsZ and associated proteins

Nucleoid occlusion (NO): negative cell division regulatory system that prevents cytokinesis over the chromosome

Min system: negative cell division regulatory system that prevents improper Z-ring assembly

FtsZ, the bacterial homolog of tubulin (the eukaryotic cytoskeletal protein that polymerizes to form microtubules), is almost universally conserved in different bacterial species. FtsZ assembles as a ring (termed the Z-ring) and marks the site for division by subsequently recruiting components of the divisome to initiate cytokinesis (58). A central question has been to understand how the correct placement of the Z-ring initially occurs. In *E. coli*, two negative regulatory systems influence Z-ring assembly and localization: (*a*) nucleoid occlusion (NO), mediated by the SlmA protein, which prevents cell division atop the nucleoid and (*b*) the Min system, composed of three proteins in *E. coli*, which prevents cell division near the polar regions of the cell (132) (**Figure 2*a***). *B. subtilis* also harbors a NO system, mediated by the Noc protein, which is not homologous to the *E. coli* SlmA protein and also functions in a different fashion (132). In *E. coli*, the Min system oscillates from one cell pole to another, thereby creating a low time-averaged concentration at midcell, permitting Z-ring assembly to take place only near that location (90) (**Figure 2*a***). In the absence of Min proteins, cell division occurs near the poles, resulting in the formation of small cells lacking nucleoids at that site, referred to as minicells. Although *B. subtilis* harbors components of the Min system, this system functions to mediate the fidelity of cell division via the cell division protein DivIVA instead of determining the spatial placement of the Z-ring (45, 56, 137) (**Figure 2*b***). Curiously, both well-studied systems are dispensable for correct Z-ring placement, suggesting the presence of other, heretofore undiscovered, division factors that are the major focus of current

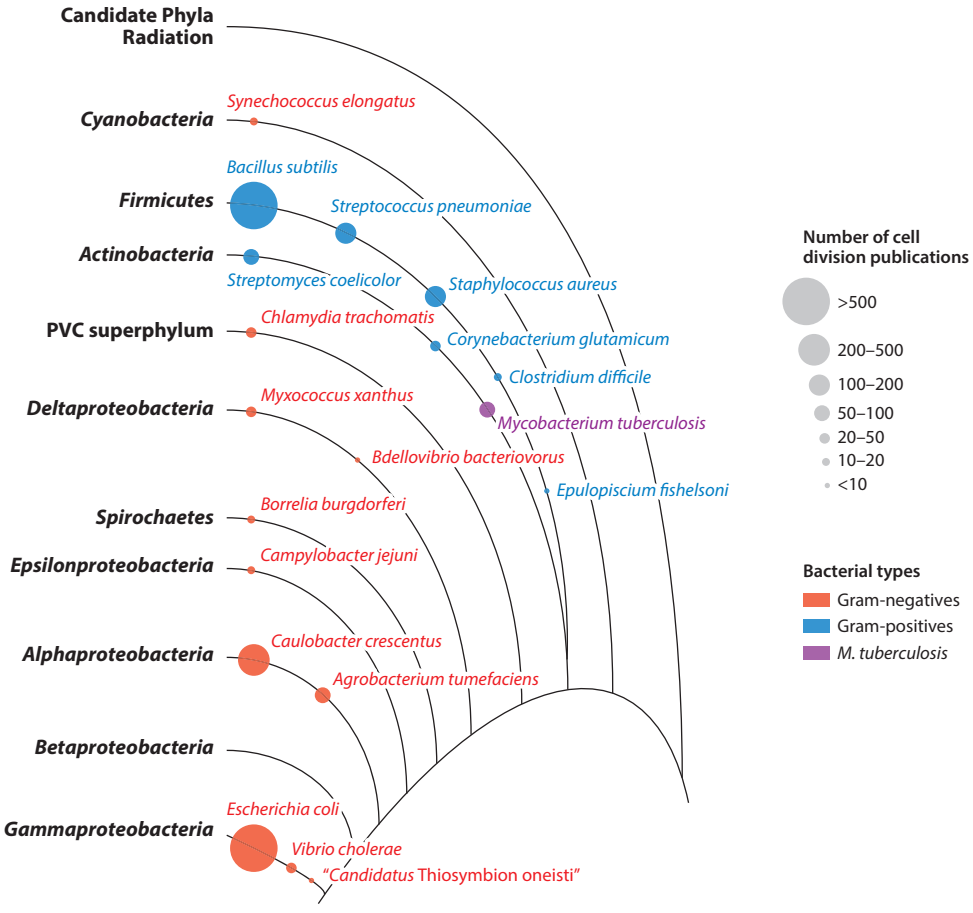

Figure 1

Representation of the relative number of reports describing cell division in various bacterial species. The diameters of the circles roughly indicate the number of cell division publications available. Note: The diameter of the circles for *Escherichia coli* and *Bacillus subtilis* are capped at an arbitrary number so that other circles are visible. Lines depict phyla and lineages, loosely based on the bacterial domain of the tree of life (adapted from 64). Several phyla and lineages were omitted for clarity. PVC superphylum comprises *Planctomycetes*, *Verrucomicrobia*, and *Chlamydiae*. Branch length, space between branches, and the order in which organisms are listed are not based on phylogeny.

research (7, 117). The notion that negative regulation can determine Z-ring positioning was also supported by observations in another model organism, *Caulobacter crescentus*, that lacks both Min and NO systems but instead employs a protein termed MipZ to prevent Z-ring assembly near the cell poles (discussed in detail below; **Figure 2*c***) (53, 130).

Study of model systems established a fundamental concept that placement of the division septum is largely the result of negative regulation, but recent results have indicated that Z-ring placement in multiple species may be positively influenced, thereby setting up an entirely new paradigm for bacterial cell division. We also highlight studies in several systems that are less well established but point to other novel mechanisms for cell division regulation. Finally, we review cell division behaviors of bacteria that break fundamental rules learned from model systems more

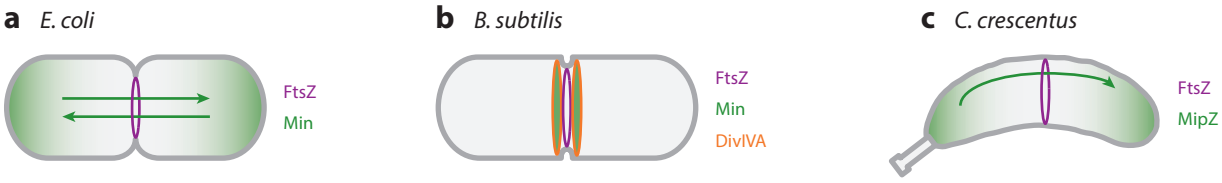

Figure 2

Regulation of cell division in model organisms is achieved predominantly by negative regulators. (*a*) In *Escherichia coli*, proteins that make up the Min system (*green*), which prevents FtsZ ring assembly (*purple*), oscillate between the poles and inhibit cell division close to cell poles. (*b*) In *Bacillus subtilis*, the Min system is recruited to sites adjacent to newly forming septa by DivIVA (*orange*); it does not mediate division site selection but maintains cell division fidelity by preventing aberrant septation from occurring at midcell adjacent to a newly formed septum. (*c*) In *Caulobacter crescentus*, MipZ interacts with chromosome-bound ParB, comigrates to the stalkless pole, and displaces polar-localized FtsZ (not shown), to permit FtsZ ring assembly at midcell. The negative regulators of FtsZ are shown in green. Green arrows indicate movement. The nucleoid occlusion system is not depicted, for clarity.

dramatically: dividing along alternate axes of the cell and not utilizing the nearly universally conserved FtsZ protein at all.

POLAR FLAGELLATES

The monotrichous (one polar flagellum per cell), dimorphic alphaproteobacterium *C. crescentus* lacks both MinCD and NO systems to regulate the placement of the FtsZ ring. Instead it employs the ParA-like ATPase MipZ (midcell positioning of FtsZ) to regulate the assembly site of the Z-ring (34, 112, 130). MipZ forms a gradient by interacting directly with origin-proximal DNA-bound ParB-*parS* complexes at the flagellated (stalked) pole prior to cell division and translocating with the newly replicated origin to the nonflagellated pole (**Figure 2*c***). At the new pole, the presence of the MipZ gradient displaces polar-localized FtsZ through direct interaction, thereby creating an FtsZ polymerization-permissive zone near midcell, where FtsZ is allowed to assemble into a Z-ring and form the division septum (73, 130). The formation of minicells has been observed in this bacterium dating back to 1978 (108), and not surprisingly, cells in which MipZ is depleted produce minicells, owing to the misregulated assembly of FtsZ at nonpermissive subcellular regions (130). Similarly, the multifunctional polar-localized protein PopZ (pole-organizing protein that affects FtsZ) transitions from unipolar to bipolar and captures the ParB-*parS* complex at the nonflagellated pole. Cells lacking *popZ* were unable to produce stalks, formed minicells, and appeared elongated because of erroneous cell division (14, 38). These phenotypes were due to a malfunction of chromosome segregation and subsequent incorrect MipZ localization, linking stalk formation with cell division. TipN (tip of new pole) is another protein involved in marking the new pole (the site of flagellar assembly) after cell division. Interestingly, overproduction of TipN resulted in the formation of both minicells and elongated cells (65, 80, 82). Absence of TipN and TipF, a protein essential for flagellar assembly, results in cell elongation and filamentation (65). In this manner, a mechanism that coordinates cell division with flagellar assembly in this freshwater organism may provide a dispersal mechanism for progeny cells.

Campylobacter species exploit the formation of amphitrichous flagella (one flagellum per pole) to regulate FtsZ placement. These organisms require the correct number of flagella on each pole to be present to achieve efficient motility as well as for successful host colonization (120, 127). *Campylobacter* species lack a MinCD system and instead utilize a MinD/ParA-like ATPase protein, FlhG (FleN), a known regulator of flagellar number (**Figure 3*a***). In *Campylobacter jejuni*, cells lacking *flhG* often produce more than one flagellum per pole, but intriguingly they also

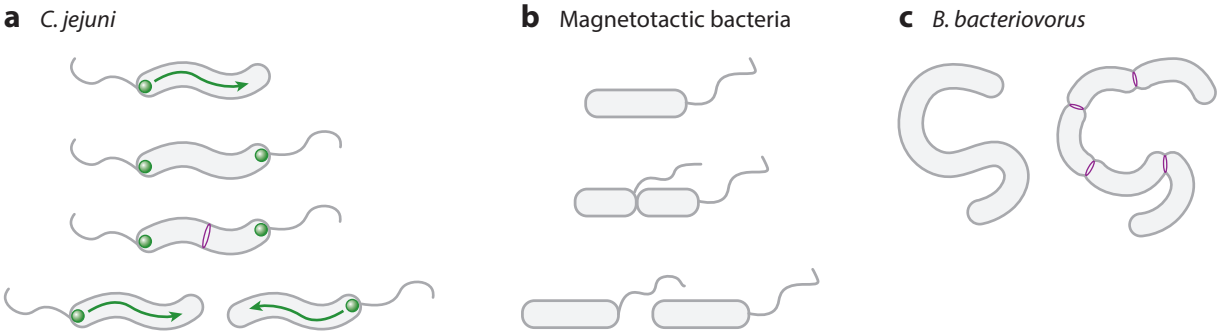

Figure 3

Cell division regulation in polar flagellates. (*a*) In *Campylobacter jejuni*, FlhG (*green spheres*) is a MinD/ParA-like ATPase that regulates flagellar copy number and participates in negatively regulating FtsZ (*purple*) assembly near the poles. (*b*) In a magnetotactic bacterium, flagella arise from the site of cell division (suggesting positive regulation of cell division), perhaps to ensure that the flagella of daughter cells are oriented in the same direction as the parental cells along Earth's magnetic dipole. (*c*) Cells of *Bdellovibrio bacteriovorus* grow as filaments inside the periplasm of gram-negative bacteria. Just prior to host cell lysis, the filamentous cell undergoes synchronous septation (reminiscent of sporulating *Streptomyces coelicolor* cells) to liberate motile daughter cells. Possible sites of FtsZ ring (*purple*) assembly are shown; flagella arising from the site of septation are not depicted.

form minicells, suggesting a role in cell division for FlhG (8). Consistent with a MinD-like role for FlhG in cell division regulation, cells of an FlhG mutant lacking ATPase activity exhibit cell length elongation—a phenotype that was suppressed by increasing the levels of FtsZ (8, 71). Amphitrichous arrangement of flagella in *Campylobacter* species ensures that cell division occurs away from the poles, improving the likelihood of the formation of flagella at cell poles in the daughter cells while preventing minicell formation. Interestingly, *flhG* of *Helicobacter pylori*, a lophotrichous organism containing several flagella at one pole, is able to complement the defect of a *flhG* null mutant of *C. jejuni*, indicating that the use of flagellar assembly to regulate cell division may not be unique to *Campylobacter* species (8).

In contrast, in a monotrichous magnetotactic bacterium of the *Gammaproteobacteria* class, the site of the cell division septum appears to dictate the site for the construction of the new flagellum: specifically, at the side of the septum facing the daughter cell that has no flagellum (84) (**Figure 3*b***). It is hypothesized that this mechanism permits offspring to align themselves along the same polarity of magnetic dipole as the parent cell and position their flagellated pole accordingly (84).

Together, these data indicate that regulation of flagella or stalk formation may be tightly intertwined with cell division. Further research in these organisms will shed light on the precise molecular mechanisms by which flagellar assembly regulates cell division.

The monotrichous gram-negative bacterium *Bdellovibrio bacteriovorus* belongs to the class of *Deltaproteobacteria*, which parasitize other gram-negative bacteria. In this organism, the nonflagellated pole is utilized to recognize and penetrate the outer membrane of the prey (122). Once inside the periplasm of prey such as *E. coli*, *B. bacteriovorus* undergoes unipolar growth in the form of filamentation (40) and feeds off of the nutrients provided by the prey. Just prior to inducing host lysis, *B. bacteriovorus* produces multiple motile daughter cells (18, 46) (**Figure 3*c***). The manner in which this bacterium spatially and temporally regulates this remarkable cell division event and the identity of cell division factors required remain to be elucidated. Although this organism encodes FtsZ, which is speculated to be involved in cell division (33, 115), the Min system is completely absent (115). Unlike most bacteria, *B. bacteriovorus* encodes two copies of MreB (112), a protein that regulates peptidoglycan synthesis, and at least one MreB homolog appears to play a role

in regulating cell division and nucleoid organization (20, 47). Concurrent septation that appears similar to the event that occurs during *Streptomyces* sporulation (see below) has been reported in this organism (**Figure 3*c***). It was also observed that flagellation occurs before daughter cell separation (18), similar to the mechanism of the magnetotactic bacterium described above. At this time, there is no knowledge of any positive regulatory mechanism in *B. bacteriovorus* similar to the one present in *Streptomyces* (discussed in the section titled "Examples of Positive Regulation"); nor do we know if flagellation has a role in regulating cell division in *B. bacteriovorus*. Because it is a small bacterium (1 μm long and 0.25 μm wide, about one-third of the size of *E. coli* or *B. subtilis*), light and fluorescence microscopy studies have been limited (46), but perhaps the increased use of super-resolution microscopy techniques will help renew interest in understanding the cell division process in *B. bacteriovorus*.

POLAR GROWTH

Unlike *E. coli* and *B. subtilis*, which grow by incorporating peptidoglycan near midcell, the rod-shaped, gram-negative alphaproteobacterium *Agrobacterium tumefaciens* grows by adding peptidoglycan specifically at the new poles (22, 63) (**Figure 4*a***). The nongrowing pole, usually the old pole, is reserved for the secretion of an adhesive molecule to facilitate attachment to various surfaces. This organism possesses three copies of *ftsZ*. One copy encodes a protein equivalent to that of *E. coli*, where the gene is present within an operon coding for FtsQ and FtsA. The other two *ftsZ* copies exhibit varying levels of truncation and are encoded elsewhere on the genome. A recent study has elegantly shown that after septation, which occurs near midcell in *A. tumefaciens*, FtsA and FtsZ stay at the newly formed growth pole and presumably facilitate polar growth. Subsequent to cell enlargement, FtsA and FtsZ relocate to midcell to form an FtsZ ring that, upon completion of

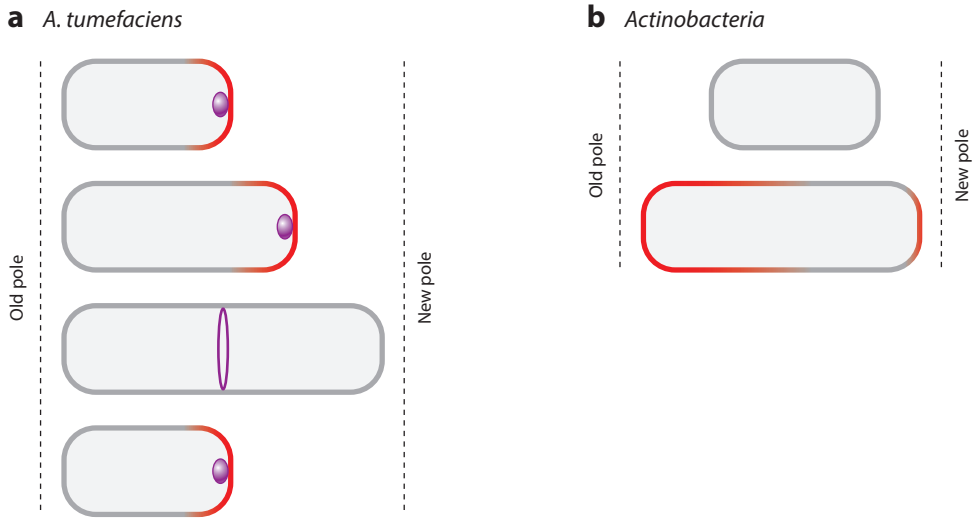

Figure 4

Cell division in bacteria that display polar growth. (*a*) FtsZ (*purple spheres*) in *Agrobacterium tumefaciens* remains localized at the pole after cell division and likely facilitates polar elongation specifically at the new pole (*red line*). Subsequently, FtsZ relocates to the midcell site (*purple ring*) to assemble the divisome and mediate cell division. (*b*) Cells of both *Corynebacterium glutamicum* and *Mycobacterium tuberculosis* (from the phylum *Actinobacteria*) undergo uneven growth at cell poles, with the old poles exhibiting faster growth than the new ones.

septum formation, marks the new pole for polar growth (15, 55, 147) (**Figure 4*a***). Proteins PopZ and PodJ were identified and reported to mark the growth pole and old pole, respectively (55). An interesting feature that is present in both *A. tumefaciens* and *C. crescentus*, also an alphaproteobacterium, is the pole-localized intermediate population of FtsZ (130, 147), although the significance of this in *C. crescentus* is unclear. How the division site is determined in *A. tumefaciens* and whether there is a MipZ-like factor regulating FtsZ placement are also unknown.

Another group of bacteria that display polar growth is predominantly in the *Actinobacteria* phylum. Some examples are *Mycobacterium tuberculosis*, *Streptomyces coelicolor* (see section below titled "Examples of Positive Regulation"), and *Corynebacterium glutamicum*. The latter is reported to undergo uneven polar growth in which the old poles appear to grow faster than the newly formed poles (121) (**Figure 4*b***). This rod-shaped bacterium lacks both Min and NO systems, and the midcell division site is not precisely chosen, unlike its well-studied rod-shaped counterparts *B. subtilis* and *E. coli*. As a result, cell division often produces daughter cells grossly unequal in size (31). *C. glutamicum* contains two ParA proteins, one encoded by the *parAB* operon and the other encoded elsewhere as a standalone gene (32). The orphan ParA-like protein, referred to as PldP, appears to play a role in regulating cell division. Deletion of *pldP* results in a minicell phenotype, whereas overexpression of *pldP* results in increased cell length, consistent with its role in regulating cell division. In further support of this idea, PldP localizes to the nascent division sites (31).

In *M. tuberculosis*, which also undergoes polar growth, cell division produces two daughter cells of unequal size, and it appears that the old poles elongate faster than the new pole (74) (**Figure 4*b***). The well-studied FtsZ anchoring proteins, such as FtsA, are curiously absent in this organism (25). However, *M. tuberculosis* harbors other FtsZ anchoring proteins, such as FhaB (also referred to as FipA), FtsW, and CrgA (26, 74, 107, 126). Mycobacteria lack both a Noc-like system and complete Min system (60). However, a protein homologous to MinD, termed septum site-determining protein, or Ssd (Rv3660c), plays a critical role in determining the site of FtsZ ring assembly and appears to link cell division with environmental adaptation (41). The AAA+ family chaperone ClpX, which is known to regulate and recycle FtsZ in *E. coli* (21, 50, 125) and *B. subtilis* (57, 140), also plays a role in negatively regulating cell division in *M. tuberculosis* (37). A cell wall hydrolase, ChiZ, helps regulate FtsZ localization in this organism (135). Additionally, it was noted that a Ser/Thr phosphatase (PstP) is important for cell division regulation (119), and that the GTPase activity of FtsZ and interaction with other divisome partners of FtsZ are negatively affected via phosphorylation by Ser/Thr kinase PknA (126, 129). Several studies reported that the transcription factor–like WhiB homologs may be involved in nontranscriptional regulation of cell division in the genus *Mycobacterium*, either by directly interacting with FtsZ or by acting as a chaperone, depending on the species (11, 54, 78). Furthermore, there is evidence that *ftsZ* levels can be upregulated or downregulated for adapting to various extracellular environments (75). Regulation of cell division in nonpathogenic *Mycobacterium smegmatis* may harbor a largely similar cell division regulation system as in *M. tuberculosis*, since *M. smegmatis ftsZ* can complement the deletion of *ftsZ* in *M. tuberculosis* (36). Bacteria that undergo polar growth therefore appear to use neither the Min system nor the NO system to divide; they may instead use several other components to couple cell division to cell growth.

EXAMPLES OF POSITIVE REGULATION

The Min and NO systems exemplify a mechanism that for many years has been understood to be a common theme in bacterial cell division: Placement of the cell division machinery might be primarily mediated by negative regulation, whereby the Z-ring would assemble at subcellular locations where negative regulators would be largely absent. However, several recent examples in

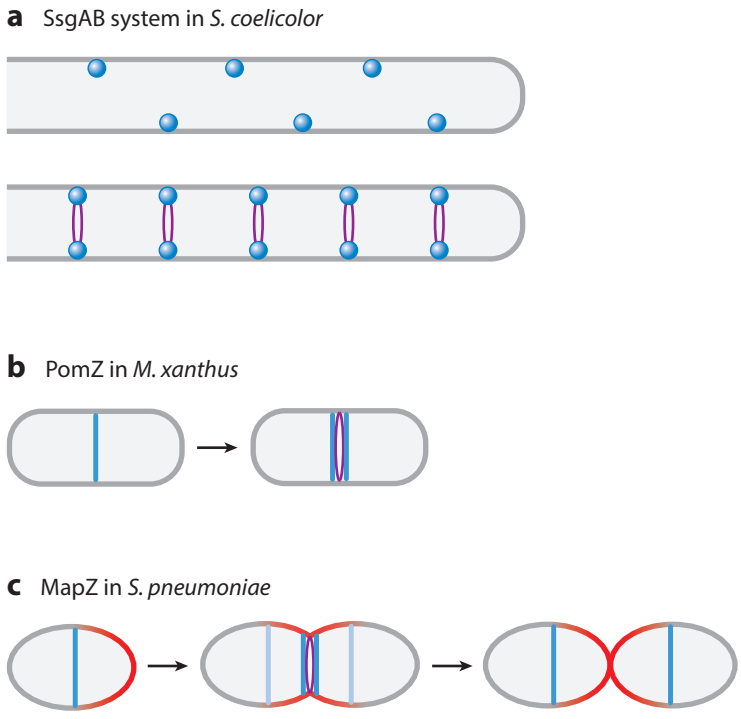

Figure 5

Examples of positive regulation of cell division. (*a*) During sporulation in *Streptomyces coelicolor*, SsgB interacts with SsgA (*blue spheres*) to mark potential division sites and recruit FtsZ (*purple*). (*b*) *Myxococcus xanthus* uses PomZ (*blue lines*) to indicate the site for FtsZ localization and assembly. (*c*) In *Streptococcus pneumoniae*, MapZ (LocZ; *blue lines*) localizes to the midcell division site to facilitate the process of FtsZ ring assembly. Just prior to septum formation, a subpopulation of MapZ (*light blue lines*) binds to the nascent peptidoglycan during lateral cell wall synthesis (*red line*) and hitchhikes to mark the future division sites.

S. coelicolor, *Myxococcus xanthus*, and *Streptococcus pneumoniae*, which all lack both Min- and NO-like systems (106, 118), suggest that positive regulatory elements in some species may prelocalize to the future site of cell division to position the cell division machinery (**Figure 5**).

In *S. coelicolor*, *ftsZ* is a nonessential gene that is likely only required for sporulation (94). Although it does contain a negative FtsZ regulator, CrgA, that plays a poorly defined function (27), it harbors another system mediated by SsgAB that positively affects FtsZ assembly by actively recruiting FtsZ to the division sites (141) (**Figure 5*a***). SsgB, the protein that brings FtsZ to the division sites during sporulation, mislocalizes in the absence SepG, a transmembrane protein that may be involved in nucleoid compaction (145), suggesting that chromosome organization and cell division may be coordinately regulated during sporulation in *S. coelicolor*. Consistent with this notion, the chromosome segregation proteins ParA and ParB in this organism are able to regulate cell tip elongation and FtsZ assembly (30, 77), and the broadly conserved multifunctional cell division protein DivIVA interacts with ParA at least indirectly via a cytoskeletal protein, Scy (29, 62). Finally, it is worth noting that although most of the cell division research in *S. coelicolor* has focused on sporulation in this organism due to the dispensability of *ftsZ* during normal growth, new observations suggest that this naturally filamentous bacterium does indeed routinely separate its cytosol and achieve compartmentalization by formation of FtsZ-independent cross membranes

that represent a form of cell division whose molecular details of formation and regulation await further study (23, 144).

Another example of positive regulation is observed in the deltaproteobacterium *M. xanthus*. In this organism, the ParA-like protein PomZ localizes to midcell division sites immediately before localization of FtsZ, and does so in an FtsZ-independent manner (131) (**Figure 5*b***). Cells lacking *pomZ* formed filamentous cells and nucleoid-less minicells, suggesting that PomZ plays a central (currently undefined) role in determining where FtsZ localizes and polymerizes within the cell to form Z-rings.

A third case of positive regulation is reported in the ovoid bacterium *S. pneumoniae*, where a recently identified protein, MapZ (LocZ), localizes to the site of cell division prior to FtsZ and the FtsZ-anchoring protein FtsA and recruits downstream divisome proteins, beginning with FtsZ (48, 61) (**Figure 5*c***). Deletion of *mapZ* resulted in suboptimal septum placement and production of nucleoid-less minicells. MapZ is conserved only within *Streptococcaceae* and *Enterococcaceae* families among *Firmicutes* (52). MapZ is capable of binding nascent peptidoglycan, and due to cell elongation, which slightly precedes septation during cell division, it has been proposed that a MapZ subpopulation splits from the midcell associated population to migrate and subsequently mark new cell division sites (52). MapZ is regulated after translation via phosphorylation by the Ser/Thr kinase StkP, which also localizes to the division site and acts as a critical switch coordinating peptidoglycan synthesis during elongation and septation (48, 49). Depending on the strain background, the Δ*mapZ* phenotype may be less dramatic, indicating that there could be other redundant factors that perform a role similar to that of MapZ (12). Another report shows that FtsA, an essential protein in *S. pneumoniae* (81), is required for proper localization of FtsZ and may play a role in regulating peptidoglycan synthesis during both septation and lateral cell elongation (99).

BACTERIA THAT CONTAIN MULTIPLE CHROMOSOMES

A gammaproteobacterial cousin of *E. coli*, *Vibrio cholerae*, contains two chromosomes. As there are many bacterial species with multiple chromosomes that are being discovered, there is an accumulating interest in understanding how the faithful segregation of chromosomes is coordinated with cell division, and *V. cholerae* has emerged as a model to address these questions (39, 67, 109). Chromosome 1 of *V. cholerae*, the larger chromosome, communicates with chromosome 2 and signals the proper time to initiate DNA replication while undergoing replication itself (6, 110, 136). A recent report has provided evidence that mutants lacking both Min and NO systems are viable (51). However, the Min system becomes critical in maintaining cell division fidelity when chromosome partitioning is perturbed. Similar to *C. crescentus*, this bacterium displays pole-localized FtsZ. The absence of SlmA, which mediates NO in this organism, results in the untimely accumulation of FtsZ at midcell, suggesting that chromosome segregation in *V. cholerae* is linked to the proper timing of cell division. Specifically, it has been proposed that the SlmA binding sites, especially on chromosome 2, may act as a cell division timing device that allows FtsZ assembly based on the status of nucleoid segregation (51).

COCCI

In the gram-positive spherical bacterium *Staphylococcus aureus*, new cell division planes arise orthogonal to the two previous division planes, resulting in the formation of its signature grape-like clusters (79, 134) (**Figure 6*a***). This organism is an increasingly studied model for interrogating cell division in spherical bacteria, where the lack of distinctly symmetrical planes imposes an

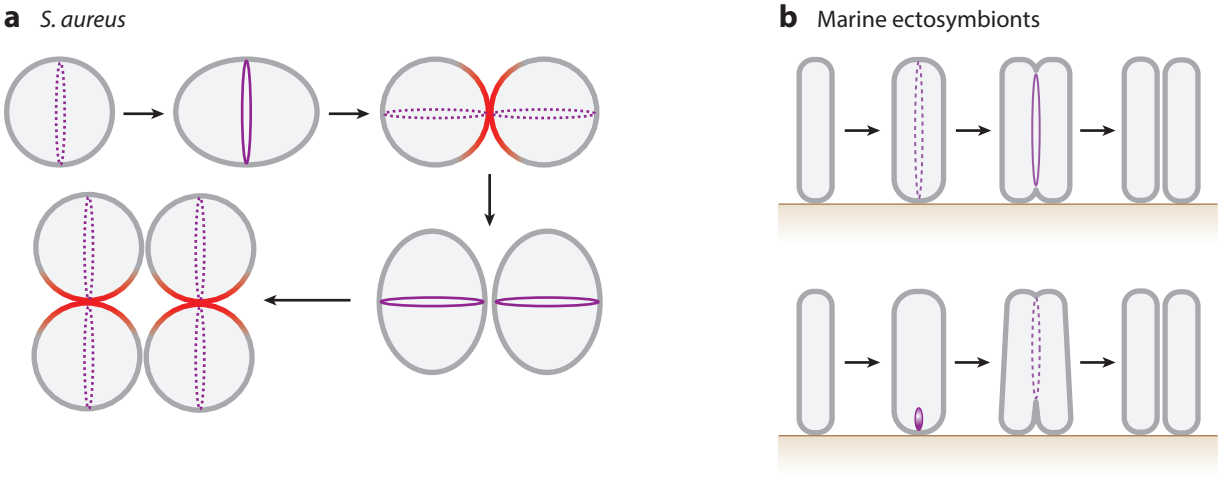

Figure 6

Other modes of bacterial cell division. (*a*) Subsequent generations of the spherical *Staphylococcus aureus* divide in a plane orthogonal to that of the two previous generations. Although cell wall synthesis was speculated to occur only during septum synthesis, new evidence suggests that circumferential cell wall synthesis also occurs in this organism. Subsequent to cell division, the septum-derived cell wall material (*red*) contributes to less than half of the newly formed daughter cell. The division plane of the next generation lies orthogonal to that of the preceding generation. (*b*) (*Top*) The marine ectosymbiont "*Candidatus* Thiosymbion oneisti" attaches at one pole to the surface (*brown*) of the nematode *Laxus oneistus*. This bacterium grows wider along its short axis prior to FtsZ (*purple*) assembly, which occurs at midcell parallel to the long axis of the cell, presumably to permit both daughter cells to remain adhered to the host. (*Bottom*) In a gammaproteobacterial relative of this organism (yet to be named) that lives on the surface of a different nematode, *Robbea hypermnestra*, FtsZ localizes closer to the host-proximal pole and triggers septum formation. Subsequently, several fragmented FtsZ foci continue to mediate septum synthesis toward the distal pole. The factors involved in the regulation of longitudinal division remain to be elucidated.

additional challenge in establishing the plane of cell division (106). *S. aureus* lacks a Min system but possesses a NO system that may link nucleoid segregation and cell division (138). The gene coding for DivIVA, which spatially regulates the Min system in *B. subtilis* (45), is present in *S. aureus*, but deletion of *divIVA* does not obviously affect cell division or chromosome segregation (105). The cell shape–determining protein MreB is widely conserved in rod-shaped bacteria but is absent in *S. aureus*. Interestingly, the shape-determining proteins MreC and MreD are present in this organism and localize to division sites. However, deletion studies indicated that these proteins are not major factors in regulating septal or peripheral peptidoglycan synthesis (128). The homolog of a negative regulator of FtsZ, EzrA, which is conserved among several gram-positive bacteria (87), directly interacts with FtsZ in *S. aureus* (123) and localizes to division sites and participates in regulating cell size in *S. aureus* by controlling peptidoglycan synthesis and cell division (69, 124). Recent reports have shown that, subsequent to cell division, daughter cell separation happens very quickly, within a span of 2 ms, suggesting that a nonenzymatic mechanism is responsible for cell separation (97, 146). A traditional view of *Staphylococcus* cell division was that the biosynthesis of peptidoglycan was restricted to division sites. This view also suggested a mechanism for the identification of sequential orthogonal midcell division plane selection that invoked the presence of peptidoglycan-based, scar-like marks that appear after each round of divisions (133). In fact, DivIB, which was shown to bind peptidoglycan, was speculated to be a pointer for previously used sites for division, since depletion of DivIB resulted in incorrect placement of septa (13). However, newer measurements, generated with advanced microscopy and newly developed cell wall–labeling techniques, indicated that septal cell wall material accounted for less than half of the cell wall

material in a newly separated cell (97, 146) (**Figure 6*a***), which contradicted the previously held model of restricted deposition of peptidoglycan at the septum (104, 133). The newer measurements also indicated that peptidoglycan synthesis occurs along the entire circumference of *S. aureus* cells, mediated by at least one of the four penicillin-binding proteins (97, 146). Thus, given new observations that revealed the nonrestricted insertion of peptidoglycan, a model based on a peptidoglycan-based division marker appears less likely, and the question of how cell division plane selection occurs in *S. aureus* remains open.

BACTERIA THAT LACK FtsZ

Since FtsZ is apparently the central bacterial cell division factor, the discovery of a growing number of organisms that do not encode *ftsZ* raised the question of how cell division occurs in these FtsZ-less bacteria (10, 43, 68, 116). One such FtsZ-less organism is the obligate intracellular pathogen *Chlamydia trachomatis*. Adding to this anomaly of the absence of FtsZ was the longstanding conundrum that although these organisms possess the genes encoding peptidoglycan biosynthesis machinery, peptidoglycan production could not be measured directly. Nonetheless, *Chlamydia* cells were sensitive to antibiotics that target peptidoglycan synthesis, indicating that cell wall material was likely present (98). With the aid of fluorescent D–amino acid labeling techniques that measure nascent peptidoglycan incorporation, it was recently shown that peptidoglycan is indeed present in *C. trachomatis* and other related organisms (89, 101, 103). However, an observation that likely explains the unsuccessful earlier attempts at detecting peptidoglycan in *C. trachomatis* is that peptidoglycan synthesis primarily occurred transiently during cell division and predominantly at the division site. This led to the proposed model of peptidoglycan synthesis driving cytokinesis in this organism (89). Indeed, in *C. trachomatis* and a related organism, MreB and RodZ (9), which are involved in spatially regulating peptidoglycan incorporation, localize to cell division sites (66, 72, 88). Interestingly, recent observations have suggested that even in *E. coli*, FtsZ may not provide the force for membrane invagination and constriction during cell division and that peptidoglycan assembly may instead be the rate-limiting step that deforms the membrane (143), such that cell wall–driven cytokinesis may be a widely conserved mechanism driving bacterial cell division. Recent work has shown that *C. trachomatis* can also exhibit asymmetric, budding-like cell division, which is documented in the FtsZ-less evolutionary relative *Gemmata obscuriglobus*, a member of the phylum *Planctomycetes* (1, 83, 88), and in L-forms of *B. subtilis* that do not produce FtsZ or peptidoglycan (44). Interestingly the *C. trachomatis* FtsQ ortholog, similar to the *E.coli* divisome protein FtsQ (100), localizes to the site where budding occurs and stays at the asymmetric division septum, suggesting that other conserved divisome components may help mediate alternate cell division strategies in this organism (1).

EXTRAORDINARY MODES OF CELL DIVISION

Beyond molecular anomalies, such as the absence of certain conserved factors (Min and NO systems, or FtsZ, for example) that is increasingly apparent in the course of studying cell division in diverse bacterial species, more fundamental physical differences in certain organisms have emerged that violate long-held central premises of bacterial cell division. Chief among these are the notions that a bacterium must produce exactly two daughter cells by binary fission and that cell division of a rod-shaped cell must occur along its short axis.

An endosymbiont that belongs to the genus *Epulopiscium* and is one of the largest known bacteria, at more than 600 μm × 80 μm (5), inhabits the intestines of surgeonfish, where it "gives birth" to multiple live offspring (3). In these organisms, it has been shown that FtsZ assembles

into polar rings, and a minimum of 2 (and sometimes up to 12) live offspring emerge from each pole and grow side by side and are then "birthed" subsequent to programmed mother cell lysis (4, 139), in a manner reminiscent of endospore formation in *Firmicutes*. Thus, in *Epulopiscium* spp., asymmetric division of the progenitor cell gives rise to multiple progeny and lysis of the progenitor, not as a stress response but as part of the normal cell cycle.

A longstanding challenge in bacterial cell biology has been to determine how FtsZ assembles at midcell, but septum placement has been assumed to occur along the short axis of a rod-shaped cell—not from pole to pole. A stunning mode of cell division was recently observed in some marine *Gammaproteobacteria* ectosymbionts that are in the process of receiving a proper binomial name (17, 102). A rod-shaped bacterium now referred to as "*Candidatus* Thiosymbion oneisti" grows as a monolayer, with each bacterium attached at one pole to the surface of the marine nematode *Laxus oneistus*. In this organism, fragmented FtsZ rings form parallel to the long axis of the cell, instead of perpendicular to the long axis, as in commonly studied model organisms (86) (**Figure 6*b***). This organism possesses an operon that encodes a Min system, but it is not yet clear if Z-ring placement is regulated by the Min system. In another ectosymbiont of the same family that also undergoes longitudinal division, it was recently shown that FtsZ accumulation occurs first at a pole proximal to the host (85) (**Figure 6**). Interestingly, instead of initiating cell division from both poles at the same time, this organism initiates the process at the host-proximal pole and drives daughter cell separation toward the other pole, making use of several FtsZ foci aligned at the division plane rather than an FtsZ ring. One model posits that division along the long axis permits continued association of daughter cells with the host surface on which the monolayer of bacteria is formed. Division along the long axis has also been reported for a helix-shaped *Drosophila melanogaster* endosymbiont, *Spiroplasma poulsonii*, in which longitudinal branching occurs and FtsZ localizes to the branching sites (111).

CONCLUDING THOUGHTS

In recent years, studies of nonmodel organisms have been increasing. For example, an operon containing novel midcell-associated genes that are probably involved in cell division has been reported for the obligate anaerobe *Clostridium difficile* (114). It was also reported that the *C. difficile* Min system, which includes an *E. coli*–like MinE protein in addition to the *Firmicutes*-like DivIVA, is capable of oscillation when produced in *B. subtilis*, suggesting that *C. difficile* may exhibit features that integrate both systems (92). These advances were aided by new cell biology tools that now permit the use of fluorescence microscopy probes in anaerobes such as *C. difficile* (16, 113). Further variations on existing themes are also emerging from studies of nonmodel organisms. In a twist on positive regulation of cell division site selection in certain spirochetes such as in the etiological agent of Lyme disease, *Borrelia burgdorferi*, peptidoglycan growth was observed in precise subregions within a growing cell, so that the daughter cell division site is marked one generation in advance (70). Even the well-studied Min system displays variety, in that Min oscillation can occur in the cyanobacterium *Synechococcus elongatus* even in the presence of physical barriers set up by thylakoid membranes (91). Similar to *C. difficile*, *S. elongatus* harbors both MinE-like and DivIVA-like proteins to regulate cell division (91).

Discoveries continue even in model organisms. MinD, for example, may have an additional role in DNA segregation in *E. coli* and *B. subtilis* (28, 76). In *B. subtilis*, the NO protein Noc does not directly interact with FtsZ in the way that the NO protein in *E. coli* (SlmA) does (24, 35, 142). Instead, it was recently proposed that Noc anchors the chromosome to the membrane to physically prevent FtsZ ring assembly (2). Another example of linking chromosome replication to cell division involves a nucleoid replication-terminus region organization factor in *E. coli* (MatP) that, together

with other divisome proteins, positively facilitates Z-ring assembly and constriction (7, 19, 93). There is also evidence suggesting the existence of hitherto undiscovered FtsZ regulators, since division site selection may still occur in the absence of the Min and NO systems in *E. coli* and *B. subtilis* (7, 117), and new evidence continues to emerge that links nutrition status to cell division (59, 96). Finally, FtsZ, the well-studied protein central to cell division in most bacteria, is still under active investigation, as new evidence has questioned whether it is the predominant force generator that drives membrane invagination during cytokinesis (42, 95, 143). Instead, an emerging model proposes that assembled FtsZ is a platform to recruit peptidoglycan synthetic enzymes to provide directionality for peptidoglycan synthesis, and that it is peptidoglycan synthesis per se that directly contributes the force for septum formation (143).

The well-established genetics afforded by model systems has provided detailed molecular insights into how these species grow and give rise to progeny. Moving forward, the increasing availability of genome sequences even for unculturable bacteria, combined with the advent of molecular tools that increase the ease of genetic manipulation, promises to open avenues of research into traditionally understudied systems to reveal the full repertoire of prokaryotic cell division strategies.

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