Bacterial cell division: assembly, maintenance and disassembly of the Z ring

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Abstract | Bacterial cell division is orchestrated by a tubulin homologue, FtsZ, which polymerizes to form a ring-like structure that is both a scaffold for the assembly of the bacterial cytokinetic machinery and, at least in part, a source of the energy for constriction. FtsZ assembly is tightly regulated, and a diverse repertoire of accessory proteins contributes to the formation of a functional division machine that is responsive to cell cycle status and environmental stress. In this Review, we describe the interaction of these proteins with FtsZ and discuss recent advances in our understanding of Z ring assembly.

Cytokinesis in bacteria is achieved by a macromolecular machine called the divisome[1–3]. Divisome assembly is initiated by polymerization of the tubulin homologue FtsZ into a ring-like structure that lies close to the cytoplasmic membrane at the prospective division site[4]. In rod-shaped cells such as *Escherichia coli* and *Bacillus subtilis*, the division site usually lies at the midpoint of the rod, perpendicular to the long axis of the cell (FIG. 1a). Constriction of the ring gives rise to two equal daughter cells, each of which is half the length of the parent cell. This 'Z ring' serves as a scaffold for the recruitment of the downstream components of the divisome and it persists throughout division, guiding the synthesis, location and shape of the division septum[5]. FtsZ is almost universally conserved throughout the Bacteria (BOX 1); it is also present in the Euryarchaeota and has an active role in the division of the plastids and mitochondria of several groups of the Eukarya[6]. FtsZ was recently shown to be an important new target for antibiotics, as specific inhibitors of FtsZ were found to protect against a lethal dose of *Staphylococcus aureus* in a mouse model of infection[7].

Although the lifestyle and developmental behaviour of some bacteria (for example, *Caulobacter crescentus*) dictates tight regulation of both FtsZ synthesis and stability[8,9], in the two best studied bacteria, *E. coli* and *B. subtilis*, no significant changes seem to occur in the concentration of FtsZ, either throughout the cell cycle or under different growth conditions[10,11]. In these bacteria, cell division must therefore be regulated at the level of FtsZ assembly[12] (BOX 2; FIG. 1b). Indeed, the number and diversity of proteins that are known to interact directly with FtsZ has grown steadily over the past decade (TABLE 1). Many of these proteins have roles that are similar to those of the microtubule-associated proteins (MAPs) of the Eukarya and affect the assembly, organization and stability of the Z ring, although none seems to be a true MAP homologue. Some of these proteins are essential for survival but many are not, and there is considerable functional overlap in the group. How then do these proteins ensure the correct assembly of FtsZ into a Z ring that is both competent for cell division and responsive to cell cycle status and environmental stresses? This Review discusses the proteins that interact with FtsZ during the assembly of the Z ring and highlights the recent progress in our understanding of how these proteins intervene in the mechanism of cell division and its regulation once the Z ring has been established.

FtsZ polymerization and the nature of the Z ring

FtsZ is a cytoplasmic protein that is composed of two globular domains separated by a central core helix (FIG. 2a). Despite the low level of sequence similarity, the tertiary structure of FtsZ is remarkably similar to that of tubulin, supporting the theory that these proteins have common ancestry[13–15]. Furthermore, FtsZ polymerizes into tubulin-like protofilaments by the head-to-tail association of individual subunits[16–19] (FIG. 2b). FtsZ, like tubulin, is a self-activating GTPase[20–22]: the insertion of the synergy loop from the base of one subunit into the nucleotide-binding site of the subunit below places the catalytic residues close to the γ-phosphate, thereby allowing nucleotide hydrolysis to proceed[19,23]. The GTPase activity of FtsZ therefore depends on FtsZ polymerization[24].

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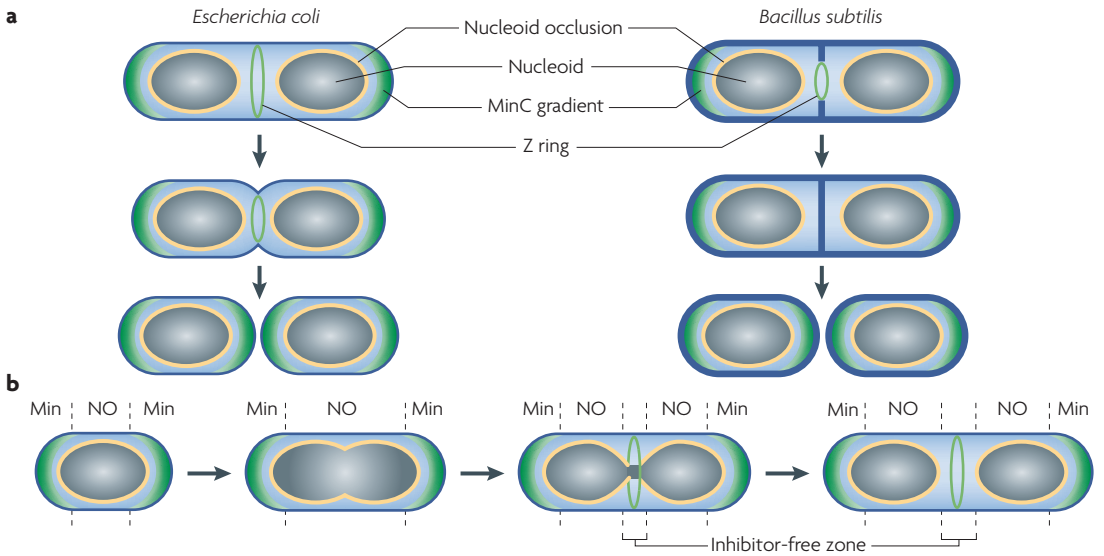


Figure 1 | **Cell division in rod-shaped bacteria. a** | Two different modes of division. After chromosome replication and segregation into nucleoids the Z ring assembles at mid-cell. The ring then constricts to bring about division. Cell wall synthesis follows the ring inwards. In *Escherichia coli*, synthesis of the division septum is accompanied by constriction of the outer membrane. In *Bacillus subtilis*, a cross wall of peptidoglycan initially divides the cell before it is degraded and remodelled to form the new, hemi-spherical cell poles. **b** | The spatial regulation of Z ring assembly. Nucleoid occlusion (NO), which is mediated by Noc (in *B. subtilis*) or SlmA (in *E. coli*), inhibits Z ring assembly close to the nucleoid. The Min system acts to prevent Z ring assembly at the cell poles (for simplicity, the Min dynamics in *E. coli* have been omitted). From left to right: in 'newborn' cells, both systems initially inhibit Z ring assembly throughout the cell; following cell elongation and chromosome replication, NO maintains inhibition in the cylindrical part of the cell; and finally, the progression of chromosome segregation reveals an inhibitor-free region at mid-cell, allowing the Z ring to assemble.

Cooperative assembly
Assembly that is characterized by the increased affinity of individual subunits for the growing polymer rather than for each other. Polymerization displays a sigmoidal relationship with concentration and has a defined critical concentration, below which no significant assembly occurs.

Cryo-electron microscopy tomography
A technique that allows the visualization of biological molecules in a near-native state. Unfixed samples are flash frozen, held at cryogenic temperature and visualized by transmission electron microscopy. Tomography uses a series of tilted images from which a three-dimensional image of the sample can be deduced.

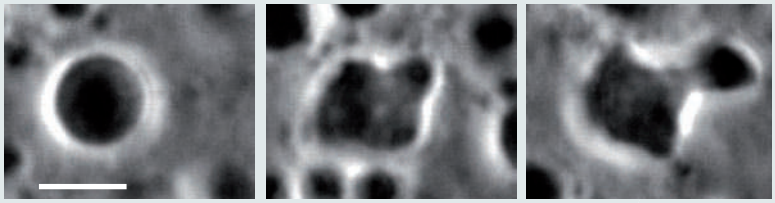
Fluorescence recovery after photobleaching
A live-cell imaging technique used to monitor the intracellular dynamics of fluorescently tagged molecules. A target region is irreversibly photobleached by a laser and then monitored for recovery of fluorescence. Dynamics are expressed in terms of the half-time for recovery (the time taken to reach half of the original fluorescence intensity).

In vitro, FtsZ displays cooperative assembly[25–30] into a range of polymeric forms, from single-stranded protofilaments to rings, bundles, tubules and sheets, depending on the experimental conditions used[16,17,31,32]. However, the architecture of the Z ring that is formed *in vivo* is not well understood, and recent cryo-electron microscopy tomography of *C. crescentus* cells suggests that it might consist of a large number of short, overlapping protofilaments, rather than forming a continuous ring[33]. *In vitro*, FtsZ exhibits at least two important transitions during its assembly[34]. The initial assembly of single-stranded protofilaments occurs at a critical concentration of around 0.5–1 μM[28,30,34,35], but there is a further transition at around 3 μM, which might represent the assembly of higher-order structures with extensive lateral interactions between the protofilaments[34]. Puzzlingly, the FtsZ protofilament is only one subunit thick[25,36], so the origin of this cooperativity remains unclear. Recently, modelling has suggested that a monomer isomerization step could be the source of this behaviour[35–37]. Importantly, the intracellular concentration of FtsZ greatly exceeds that of the critical concentration for assembly, at 3–10 μM[10,38].

FtsZ polymerization depends on GTP binding but not hydrolysis[16]. The combination of a partially solvent, accessible nucleotide-binding site and the abundance of GTP in the cytoplasm means that GTP hydrolysis is probably a rate-limiting step in the polymerization cycle and, in contrast to tubulin, FtsZ polymers might contain mainly GTP[19,29,39]. Fluorescence recovery after photobleaching (FRAP), as well as other studies, has revealed that the Z ring is not a static structure: it undergoes constant remodelling throughout its existence, with rapid turnover dynamics (in the order of 8 seconds) that depend on the intrinsic GTPase activity of the FtsZ polymer[10,40,41]. Indeed, GTP hydrolysis promotes the disassembly of the protofilament and drives the highly dynamic nature of the FtsZ polymers that are observed both in the cell and *in vitro*, as well as limiting the length of individual protofilaments[30,34,40,41]. Accordingly, FtsZ mutations that impair GTP hydrolysis but not GTP binding stabilize polymers by reducing subunit turnover[41,42].

In addition to a regulatory function, nucleotide hydrolysis has been proposed to have a force-generating role during septation, whereby a nucleotide-dependent transition from a straight, GTP-bound polymer to a curved, GDP-bound polymer can transmit mechanical work to the membrane[33,43]. However, the physiological role of this force remains controversial and is complicated by the fact that mutants that are severely impaired in nucleotide hydrolysis can still divide[44,45]. Moreover, recent work suggests that the relationship between the morphology of polymers assembled *in vitro* and GTP hydrolysis is not as straightforward as was initially thought[46]. Nevertheless, the recent demonstration that Z rings can impart a force on a tubular membrane *in vitro*[47], combined with the observation of both straight and curved filaments *in vivo*[33], reinforces the possibility of force generation by the Z ring. In addition, mathematical modelling of Z ring dynamics has shown that force generation could be achieved independently of FtsZ GTPase activity, with hydrolysis instead being required for subunit turnover and polymer remodelling[35,48].

Box 1 | **FtsZ-independent division**



The wide conservation of FtsZ throughout the Bacteria and the euryarchaeotal branch of the Archaea, which is supported by the ever-increasing wealth of genome sequence information, has cemented its position as an essential cell division protein. There are, however, several intriguing examples of species in which FtsZ is absent, has been replaced or is simply no longer required for cell division.

Missing FtsZ

Several distinct groups of bacteria, including Chlamydiae and Planctomycetes, as well as *Ureaplasma urealyticum* and *Mycoplasma mobile*, are unusual among bacteria in that they lack a recognizable homologue of FtsZ[52,124,125]. Although it has been suggested that *Chlamydia* spp., which are obligate intracellular parasites, might draw on a host cell division machinery[126], there is compelling evidence to suggest that they divide independently of their hosts and might instead use the subset of division genes that they contain to synthesize a peptidoglycan division septum[127]. However, it is not clear how this site is chosen or how any putative synthetic machinery is assembled in the absence of FtsZ. Planctomycetes and *U. urealyticum* are free-living organisms and so must be capable of independent cell division, but to date little progress has been made in understanding how this might be accomplished.

Replacing FtsZ

Substantial progress has been made in understanding the division of the Crenarchaeota, which completely lack FtsZ, owing to the discovery of a new cell division machine[128,129]. The products of three cell cycle-regulated genes, *cdvA*, *cdvB* and *cdvC*, constitute a protein complex that forms a contractile-ring structure between segregated nucleoids in dividing cells that is essential for cell division. Although CdvA is unique to the Crenarchaeota, CdvB and CdvC are homologous to proteins of the endosomal sorting complex required for transport (ESCRT) III complex of the Eukarya, which is involved in endosomal protein sorting, viral budding and membrane abscission during cytokinesis. These proteins are found in all but one order of the Crenarchaeota and are absent from the FtsZ-dependent Euryarchaeota.

Life before FtsZ

Recently, the L-form (wall-less derivatives of common bacteria) of *Bacillus subtilis* was found to propagate independently of FtsZ by a new mode of division that is defined by a remarkable extrusion–resolution process (see the figure, which shows phase-contrast images of division in L-form cells of *B. subtilis*) that discharges viable progeny[130]. This might be related to the surprising finding that *ftsZ* can be deleted in *Streptomyces* spp.[131]. It is possible that these sick mutants use a similar extrusion–resolution process to propagate. Although the mechanisms underlying extrusion–resolution remain unclear, the process could provide a glimpse into the distant past, to a time before the FtsZ-based division machinery had evolved.

Scale bar represents 3μm. Images courtesy of P. Domínguez-Cuevas, Newcastle University, UK.

Although the carboxy-terminal domain of FtsZ is similar in its fold and acidic nature to that of tubulin, it lacks the extended surface loops that are thought to aid the assembly of the microtubule and its interaction with motor proteins[49]. However, FtsZ contains a unique, highly conserved peptide (~9 amino acids) at its extreme C terminus; this site is thought to be unstructured and is dispensable for polymerization but essential for cell division, as it is the binding site for several division proteins[50–52] (discussed below).

MAPs can alter the stability, bundling and disassembly of the tubulin polymer in response to cellular cues but, as mentioned above, to date no true homologues have been found in the Bacteria. Nonetheless, the similarity of the polymerization dynamics of FtsZ and tubulin has afforded valuable insights into how the unique regulatory proteins that interact with FtsZ might modulate its polymerization. First, proteins can prevent the initial assembly of FtsZ by preventing nucleotide binding, sequestering individual monomers or capping growing polymers. Second, proteins that promote polymerization or stabilize existing polymers could do so by concentrating FtsZ at the membrane, cross-linking separate protofilaments or facilitating the formation of stabilizing lateral interactions, as well as by preventing the disassembly of the polymer by inhibiting nucleotide hydrolysis. Finally, proteins that disassemble or destabilize the polymer might do so by directly counteracting the mechanisms used by positive-acting factors — that is, by stimulating GTPase activity, facilitating disassembly by sequestering monomers or inhibiting the formation of lateral interactions.

Assembling the Z ring

A crucial element of Z ring assembly is the association between FtsZ and the cell membrane. FtsZ does not seem to have any direct affinity for the membrane, but all models for Z ring formation require its attachment to the membrane, both to maintain its structural integrity during septation and probably to transmit any constrictive force to the membrane[1,33,51,53]. In *E. coli* there is good evidence that two proteins, FtsA and ZipA, collaborate in anchoring FtsZ to the membrane[54]. Both of these proteins are essential for cell division[54,55]: depletion of either single protein results in the formation of elongated filaments with regularly assembled Z rings that are positioned correctly between segregated nucleoids but are non-functional for division. When both proteins are removed, Z rings do not form[54,56].

ZipA. ZipA is a bitopic protein that is composed of three domains: a transmembrane anchor, a long, basic arm that is rich in proline and glutamine residues and a large, globular C-terminal domain[55,57,58]. ZipA has a particularly rare membrane topology, as its sole transmembrane anchor is at the amino terminus and the C-terminal domain is in the cytoplasm[55,59]. This unusual topology is shared by the division protein extra Z rings A (EzrA) in *B. subtilis*[1,60]. However, these two proteins seem to have considerably different roles in cell division, so the potential importance of their shared membrane topology is unclear.

In the cell, ZipA is dispersed throughout the inner membrane until the initiation of cell division, when it is recruited to the assembling Z ring by a direct interaction with FtsZ[55,56,61]. The division block that occurs in cells that are depleted of ZipA seems to be due to the failure of the Z ring to recruit FtsK, a protein that is essential for the recruitment of downstream division proteins[54,62]. ZipA interacts directly with the conserved C-terminal tail of FtsZ[57–59,61,63]. This interaction is mediated by the C-terminal domain of ZipA, which is both necessary and sufficient for its recruitment to the Z ring[59]. Interestingly, the transmembrane domain does not seem to be simply an inert

Bitopic protein
A protein that contains a single transmembrane segment between the cytoplasmic and extracytoplasmic domains.

Box 2 | **Spatial regulation of cell division**

In both *Bacillus subtilis* and *Escherichia coli*, assembly of the Z ring — and thus the initiation of cell division — is regulated by two distinct but partially overlapping systems (FIG. 1b). These systems have been reviewed in detail elsewhere but are briefly described here[132,133]. First, nucleoid occlusion prevents the assembly of the Z ring over the nucleoid by the action of two unrelated DNA binding proteins, Noc in *B. subtilis* and SlmA in *E. coli*[134,135]. Although SlmA interacts directly with FtsZ *in vitro*, the exact mechanism by which either protein inhibits the formation of the Z ring remains unclear[135]. Second, the Min system contributes to spatial regulation by inhibiting Z ring assembly at the cell poles, preventing the generation of anucleate mini-cells. This is achieved by creating a symmetrical concentration gradient of MinC and MinD, negative regulators of Z ring assembly, around the mid-cell position so that the time-averaged concentration is always highest at the cell poles. MinC interacts directly with FtsZ and is thought to prevent Z ring assembly at several levels by inhibiting lateral interactions and weakening longitudinal bonds, thereby compromising the integrity of the assembled polymer[92,93,110,136,137]. In addition, in *E. coli* MinC has been shown to act early during the assembly of the Z ring, competing with FtsA for binding at the extreme carboxy-terminal tail of FtsZ[138]. The removal of FtsA further destabilizes the assembling Z ring and concentrates the inhibitor in direct contact with its target[138].

In *E. coli* topological specificity is imparted by the periodic oscillation of MinC and MinD from pole to pole, a process that is driven by MinE[139–141]. In *B. subtilis*, two unrelated proteins, DivIVA and MinJ, are thought to recruit MinC and MinD to division sites and retain it at the new cell poles[142–147]. Recruitment to the nascent division site allows MinC to prevent the re-initiation of cell division and to ensure that only one division event occurs per cell cycle.

anchor, suggesting that it has a specific function in division, perhaps by interacting with one or more of the many membrane-bound components of the divisome[59] (BOX 3).

Structural studies have revealed that the C-terminal domain of ZipA consists of a six-stranded anti-parallel β-sheet laid against three α-helices, with a core domain containing a split β–α–β fold that is similar to the folds found in many RNA-binding proteins[57,58]. The solvent-exposed face of this motif forms a shallow hydrophobic cleft that extends across the domain[57,58]. The final 20 amino acids of the C-terminal domain of FtsZ are sufficient for the interaction with ZipA, and a co-crystal structure of the complex has been obtained[57], which shows that the hydrophobic cleft of ZipA is occupied by the C-terminal fragment of FtsZ[57]. This binding is driven by the burial of exposed hydrophobic residues, and most of the binding affinity is provided by only a few key residues, which are highly conserved[57,58].

In vitro, the C terminus of ZipA promotes the assembly of FtsZ into thick bundles and sheets of protofilaments that are aligned laterally[59,64]. Consistent with this positive role in assembly and in the formation of stabilizing lateral interactions, twofold overproduction of ZipA suppresses the division defect of cells bearing the thermosensitive *ftsZ84* mutation at the non-permissive temperature[64].

Although the mechanism by which ZipA organizes FtsZ protofilaments is not known, it is clear that the individual domains of ZipA have separable functions *in vivo*, and several models describing the activity and physiological role of ZipA have been proposed[51,55,57,59,64]. The globular C-terminal domain might facilitate bundle formation either by a chaperone-like activity, through which it stabilizes the unstructured C-terminal tail, or by simply shielding the solvent-exposed hydrophobic residues[57]. In addition, the essential nature of the transmembrane domain and the ability of the Z ring to assemble in the absence of FtsA show that ZipA can function as a membrane tether for FtsZ[51,54–56]. The flexibility of the unstructured linker domain between the transmembrane anchor and the C-terminal domain is likely to be pivotal in this tethering role, allowing the C-terminal domain to reach out from the membrane and bind the C-terminus of FtsZ[51,65].

ZipA is not widely conserved outside the Gammaproteobacteria, which suggests that its role has either been replaced or become redundant[55]. In support of the second suggestion, a single amino acid substitution in FtsA renders ZipA non-essential in *E. coli*, allowing efficient growth and division in its absence[66] (discussed below).

FtsA. FtsA is widely conserved throughout the Bacteria and is often found upstream of *ftsZ*, in an operon within the *dcw* cluster[67]. The crystal structure of FtsA from *Thermotoga maritima* showed that it is structurally related to actin and consists of two domains, each of which can be further divided into two subdomains[68]. In one structure, the nucleotide-binding site, which is formed by an inter-domain cleft, was found to contain both ATP and a Mg^{2+} ion[68], supporting reports that FtsA can bind ATP[69–71]. Interestingly, FtsA purified from *Streptococcus pneumoniae* can bind various nucleotide phosphates in a manner that is directly proportional to the length of the phosphate moiety; ATP and GTP are favoured over ADP and GDP, which are favoured over AMP and GMP[71]. In *B. subtilis*, FtsA has been shown to exist as a dimer in solution[70], and various two-hybrid assays have shown that FtsA can interact with itself[72–76]. Furthermore, FtsA from *S. pneumoniae* has been reported to reversibly polymerize into corkscrew-like helices that are composed of pairs of paired protofilaments[71]. These polymers exhibited nucleotide-dependent stability, forming extremely stable polymers in the presence of ATP and less stable polymers in the presence of ADP[71]. This report suggests a role for nucleotide hydrolysis in the regulation of FtsA polymerization; however, ATPase activity was not found in *S. pneumoniae* FtsA and has so far only been reported in FtsA from *B. subtilis*[70]. Although the role of nucleotide binding is not yet known, it is intriguing that mutation of residues that have been shown to contact the nucleotide in the crystal structure abolishes the ability of FtsA to interact both with itself and with FtsZ[72]. Structural modelling suggests that FtsA dimerizes in a head-to-tail fashion, with the individual subunits rotated 180° to one another; however, the importance of this model remains unclear[77].

In *E. coli*, FtsA interacts with the conserved extreme C terminus of FtsZ[51,54,56]. In both *E. coli* and *B. subtilis* the ratio of FtsZ to FtsA is approximately 5 to 1 (REFS 10,70), and maintenance of this balance is crucial for cell division[78]. This is exemplified by the fact that the toxic overproduction of either protein can be counteracted by overproduction of the other[78]. The ability of FtsA to support the initial assembly of the Z ring without ZipA, as well as the ability of an *ftsA* gain-of-function mutant to bypass the need for ZipA, suggested that FtsA can also tether the Z ring to the inner membrane[56,66]. Indeed, the re-examination of a highly conserved motif at the extreme C terminus of FtsA revealed a membrane-targeting amphipathic helix that is separated from the core protein by a flexible linker

ftsZ84
A mutation that results in the substitution of glycine to serine at position 105 in *Escherichia coli* FtsZ. This substitution reduces both the GTP binding and the GTPase activity of FtsZ *in vitro* and renders the protein temperature sensitive *in vivo*.

Table 1 | **FtsZ and the proteins that modulate its polymerization**

Protein	Role	Size* (kDa)	Structural characterization	*In vitro* data available?	Essential?	Conservation
Assembly of the Z ring						
FtsZ	A structural subunit of the Z ring that is required for initiation of cell division and serves as a scaffold for divisome assembly	40.2	A self-assembling GTPase that is structurally related to tubulin	Yes	Yes‡	Widely conserved
FtsA	A principal membrane tether that is required for Z ring assembly, organization of FtsZ polymers and recruitment of downstream proteins	48	An ATP-binding protein that is structurally related to actin but that has the uniquely positioned subdomain 1C	No§	Yes‖	Widely conserved; often found in an operon with *ftsZ*
ZipA	Promotes Z ring assembly and is a secondary membrane anchor	36.3	A large globular domain that is linked to a single amino-terminal TM domain by an extended linker	Yes	Yes	Gammaproteobacteria
Regulators of Z ring dynamics						
ZapA	A positive modulator of Z ring assembly and stability	9.7	Forms a dimer through its extensive coiled-coil region	Yes	No	Widely conserved
ZapB	Has a redundant role in ensuring proper Z ring assembly	9.5	A dimer consisting of a single coiled-coil region; self-assembles into large polymer bundles	No§	No	A subset of Gammaproteobacteria
SepF	Has an overlapping role with FtsA in Z ring assembly and is required for proper septal morphology	17	NA	No	No¶	Gram-positive bacteria and Cyanobacteria
EzrA	A negative regulator of Z ring assembly throughout the cell membrane that contributes to mid-cell Z ring dynamics and has a role in coordinating cell elongation with division	64.8	Predicted to contain four coiled-coil regions in its carboxyl terminus and a single N-terminal TM anchor	Yes	No	Gram-positive bacteria with a low GC content
Cell cycle-responsive regulators						
UgtP	A growth rate-dependent inhibitor of cell division	43.4	A membrane-associated protein that self-assembles into tightly wound spirals *in vitro*	Yes	No	A widely conserved metabolic pathway
SulA	A cell division inhibitor of the SOS response that prevents the assembly of new Z rings and facilitates the disassembly of existing Z rings	18.7	Forms a dimer, either face of which can interact with the T7 loop of FtsZ	Yes	No	Gammaproteobacteria
MciZ	Contributes to the inhibition of Z ring assembly following the initiation of sporulation	4.8	A small peptide that is predicted to bind close to the nucleotide-binding pocket of FtsZ	Yes	No	*Bacillus* spp.
General regulators						
ClpX	Helps to maintain the cytoplasmic pool of free FtsZ subunits	46.2	A hexameric substrate-recognition complex of ClpXP protease	Yes	No	Widely conserved

*The stated protein sizes are for *Bacillus subtilis* or, where appropriate, *Escherichia coli* and are calculated values, published at the SubtiList or Colibri World-Wide Web Servers. ‡This protein is not essential for viability in *Streptomyces coelicolor*. §*In vitro* data are only available on the proteins themselves, not on their potential to modulate FtsZ polymerization. ‖This is essential in *E. coli* but not for vegetative cell division in *B. subtilis*. ¶This protein is essential in *Synechococcus elongatus*. EzrA, extra Z rings A; MciZ, mother cell inhibitor of FtsZ; NA, no data available; TM, transmembrane.

region[67]. This amphipathic helix is essential for the function of FtsA under normal laboratory conditions: deletion of this region renders FtsA non-functional and causes it to assemble into deleterious cytoplasmic rods[67]. Importantly, and in contrast to ZipA[59], the membrane targeting region of FtsA can be replaced by various unrelated membrane-targeting determinants[67,79].

FtsA differs from actin in that it is missing the 1B domain and it has an unrelated subdomain called 1C[68]. Deletion of 1C renders FtsA unable to interact with itself, although it retains the ability to localize to the Z ring[73]. The inability of FtsA lacking the 1C domain to allow the progression of cell division has been explained by the finding that the 1C domain is involved in recruiting downstream components of the divisome to the Z ring[73,80].

Although the role of the extreme C terminus of FtsZ in the interaction with FtsA has been extensively characterized, little was known about the nature of the binding interface in FtsA until recently[50]. A screen for *ftsA* mutations that cause the resultant protein to interact with the membrane but not with FtsZ identified important FtsA residues, including three charged residues that are strictly conserved and are clustered mainly on the surface of the 2B subdomain[72]. Substitutions of these residues do not affect the ability of FtsA to self-interact, but they do abolish its ability to interact with FtsZ[72]. The 2B subdomain is likely to have additional functions in cell division, as deletion of a small anti-parallel β-sheet at the apex of this domain seems to affect the correct placement of the Z ring[73]. Furthermore, the *ftsA** mutation is found in the same region[66] (discussed below).

Aside from its role in Z ring assembly, FtsA is now known to affect the integrity of the Z ring at various stages of divisome assembly[67,72,74,81]. The *ftsA** gain-of-function

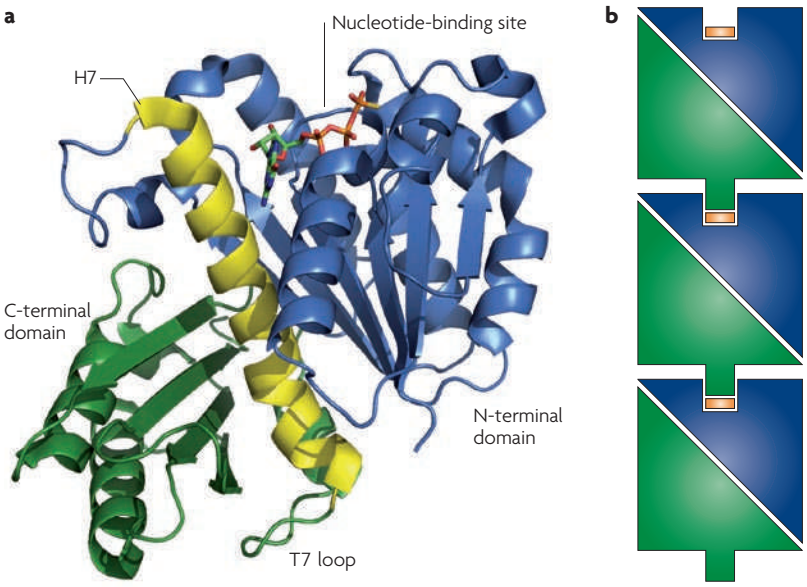


Figure 2 | **FtsZ polymerization. a** | The crystal structure of a *Bacillus subtilis* FtsZ monomer bound to GTP-γS (Protein Data Bank entry 2RHO). FtsZ is composed of two independently folding domains and a central core helix, H7. The amino-terminal domain contains the tubulin signature motif, GGGTGTG, and forms the nucleotide-binding site. At the base of the carboxy-terminal domain, following H7, is the catalytic T7 or synergy loop. The extreme C-terminal tail, which forms the binding site for several division proteins, is not visible in the crystal structure. **b** | FtsZ polymerizes by the head-to-tail association of individual subunits, shown in blue and green, to form a single-stranded protofilament with a longitudinal subunit repeat that is similar to that of tubulin. Insertion of the T7 loop into the nucleotide-binding site (orange) of the subunit below places two highly conserved aspartate residues in the vicinity of the FtsZ γ-phosphate; these aspartate residues are thought to activate the GTPase activity of FtsZ by polarizing an attacking water molecule.

mutation bypasses the need for ZipA and partially suppresses the requirement for FtsK[66]. Cells bearing only *ftsA** assemble Z rings earlier in the cell cycle and can tolerate higher levels of MinC than wild-type cells, indicating that both the initial assembly and the stability of the Z ring are enhanced by FtsA* (REF. 82). FtsA* seems to confer enhanced stability on the Z ring at the level of initial assembly, as it affects neither the intrinsic dynamics of FtsZ subunits nor the proportion of FtsZ present in the ring[82]. In support of this model, the interaction of FtsZ with FtsA* is much stronger than the interaction with wild-type FtsA[82]. In addition, a further gain-of-function mutation in FtsA that seems to act by stabilizing the Z ring has recently been reported[81].

The assertion that FtsA probably constitutes the principal membrane anchor for the Z ring is supported by several lines of evidence[67,72] and most notably by the absence of recognizable homologues of ZipA in most bacterial lineages[55]. The situation in *B. subtilis*, however, is somewhat different. FtsA is not essential for vegetative cell division, although the cells are highly filamentous and the frequency of functional Z ring formation is severely reduced[83–85]. This is consistent with the proposed role of FtsA in promoting Z ring integrity[84]. However, the ability of the Z ring to form, even at low frequency, is puzzling and suggests that another protein might also have a similar role to FtsA, albeit at a reduced level of efficiency (discussed below).

Synthetic lethal
Lethality due to a combination of two non-lethal mutations.

Z ring assembly and polymer dynamics

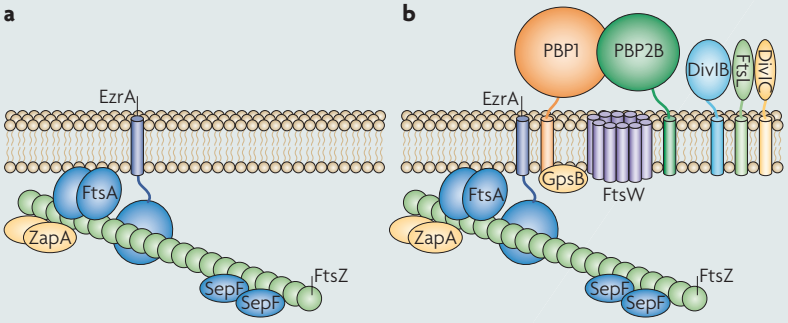
Many mainly non-essential proteins are thought to directly interact with FtsZ to regulate the dynamics of the Z ring that is assembled at mid-cell. Although individually these proteins are not required for Z ring assembly, their various roles in modulating the polymerization of FtsZ mean that the combined loss of certain regulatory proteins often produces a synthetic lethal division phenotype.

ZapA. ZapA is a small protein of 85 amino acids that was identified in a genome-wide screen for proteins that, when overexpressed, can overcome the lethality that is induced by MinD overexpression in *B. subtilis*[86]. In both *B. subtilis* and *E. coli*, ZapA is recruited to the divisome early, probably by a direct interaction with FtsZ, where it enhances both the assembly and the stability of the Z ring[86]. Cells lacking ZapA exhibit no phenotype in either organism[86,87] but, similar to other non-essential regulatory proteins, this loss is conditionally lethal in various sensitized backgrounds[60,85,88], including in cells with artificially lowered FtsZ levels[86]. Surprisingly, given its apparently positive role in Z ring assembly, null mutations in *zapA* are synthetically lethal when combined with null mutations in *ezrA*[86]. This synergism probably results from the assembly of a less dynamic Z ring in the absence of EzrA (discussed below), which reduces the cytoplasmic pool of subunits that are available for exchange. This, combined with the increased concentration of FtsZ that is required for assembly in the absence of ZapA, leads to the synthetic lethal phenotype[86].

The crystal structure of ZapA revealed an anti-parallel tetramer that is composed of a pair of dimers which associate through extensive coiled-coil interactions along the C termini of the individual subunits[89]. Dimerization of ZapA is mediated mainly by contacts between the small, globular N-terminal domains[89]. In solution, oligomerization is constrained by concentration and the protein exists in a dimer–tetramer equilibrium. However, taking into account the predicted abundance of ZapA *in vivo*, the dimer is more likely to be the physiologically relevant species[86,89,90].

In vitro, ZapA specifically interacts with FtsZ and, consistent with its positive role in polymerization *in vivo*, enhances both the initial polymerization of FtsZ and the stability of the resulting polymers[86,89,90]. ZapA does not form higher-order structures under any conditions examined to date[86,89,90]. FtsZ polymers that have assembled in the presence of equimolar amounts of ZapA form higher-order structures, such as branched networks that are composed of thick protofilament bundles[86,89,90], which are similar to those observed in the presence of artificial bundling agents such as diethylaminoethyl dextran and Ca^{2+} (REF. 91). The enhancement of polymerization and, in particular, polymer stability is directly correlated with the inhibition of FtsZ GTPase activity[86,90]. Intriguingly, one report identified a potential change in the conformational state of the bound nucleotide, using the new technique of linear dichroism to monitor changes in the FtsZ polymer[90,91]. The authors suggested that this conformational change might be the result of a general activity of bundling agents and proposed a model in which

Box 3 | **Assembly of the divisome**



Following the relief of spatial regulation as the cell grows and its chromosomes segregate, the Z ring forms at mid-cell, tethered to the inner face of the cytoplasmic membrane. It is not clear whether there are early intermediates in ring formation, but dynamic helical polymers of FtsZ have been described, which suggests that the Z ring forms by the collapse of a spiral intermediate[148–151]. Once assembled, the Z ring begins the process of recruiting the downstream components of the divisome.

In *Bacillus subtilis*, assembly of the divisome occurs by a two-step mechanism, in which the early-assembling proteins (see the figure, part **a**) are directly recruited to the Z ring by FtsZ and, after a substantial delay, are followed by the concerted and interdependent assembly of the later-assembling proteins (see the figure, part **b**)[1,152]. In *Escherichia coli*, classical localization studies had suggested an essentially linear pathway of divisome assembly[153]. However, recent work has shown that this process probably occurs by the sequential assembly of at least three subcomplexes[2,154]. Similarly to *B. subtilis*, a time delay between the assembly of the Z ring and the recruitment of the later-assembling proteins has been detected[155].

Following the completion of divisome assembly and, possibly, an as-yet-undiscovered signal, the Z ring constricts ahead of the leading edge of the newly synthesized division septum. Finally, the daughter cells separate, and this involves the action of specialized peptidoglycan hydrolases. The overlap between invagination and cell separation varies between organisms: in *B. subtilis* a complete cross wall is usually formed before cell separation begins, whereas in *E. coli* the two processes occur almost simultaneously, leading to a characteristic furrow at the active division site (FIG. 1a). These distinctions probably relate to the differing cell envelope structures of Gram-positive and Gram-negative bacteria but might also reflect flexibility in the relative timing of septal wall hydrolysis, which is tightly coupled to septum synthesis in *E. coli* but is much more separable in *B. subtilis*.

EzrA, extra Z rings A; GpsB, guiding PBP1-shuttling protein B; PBP1, penicillin-binding protein 1; PBP2B, penicillin-binding protein 2B.

ZapA binding at a site proximal to the active site favours an alternate conformation of GTP, rendering it less amenable to hydrolysis and thereby stabilizing the polymer[90]. A second, not necessarily mutually exclusive model suggests that the multimeric nature of ZapA might allow it to cross-link individual protofilaments and, through this bridging activity, help to promote the assembly of FtsZ bundles by strengthening lateral interactions[86,89]. Orthologues of ZapA are found throughout the Bacteria, and its ability to promote the formation of stable bundles of FtsZ has been shown in three different organisms[86,89,90]. The possibility that ZapA acts positively in Z ring formation is supported by its ability to antagonize the inhibitory activity of MinC *in vitro*[92,93].

ZapB. ZapB is a small protein of 81 amino acids that was first identified in *E. coli* and has an anti-parallel coiled-coil dimeric structure[94]. ZapB is recruited early to the assembling Z ring, and this process requires FtsZ but neither FtsA nor ZipA. Cells bearing a null mutation in *zapB* are viable, although slightly elongated, and tend to form filamentous cells that are blocked in cell division. In the absence of ZapB, Z ring formation is both reduced in frequency and altered in morphology, and short, tight-pitched helices are frequently formed. *In vitro*, ZapB dimers can interact through their termini to form large bundles. As ZapB is abundant (approximately 13,000 molecules per cell), ZapB polymers might bridge shorter FtsZ protofilaments in the assembling Z ring to promote their organization into a functional ring. However, the contribution of ZapB to Z ring assembly seems to be subtle, as mutation of *zapB* generates a striking division phenotype only in bacteria carrying the *ftsZ84* mutation, not in bacteria with a range of other cell division mutations, including of *zapA*. The interaction of ZapB with FtsZ or its potential to modulate FtsZ polymerization *in vitro* has not yet been reported.

EzrA. EzrA was identified in *B. subtilis* as a negative regulator of Z ring assembly in a screen for extragenic suppressors of a temperature-sensitive *ftsZ* mutant tagged with green fluorescent protein (*ftsZ*-GFP)[60], but it probably has multiple functions in division. EzrA is an integral membrane protein and is predicted to have an N-terminal transmembrane anchor and a cytoplasmic C-terminal domain that consists of four coiled-coil regions[60,95]. This domain structure is conserved throughout the low-GC-content Gram-positive bacteria in which it is present[95]. EzrA is constitutively expressed and is abundant, with an estimated 10,000–20,000 molecules per cell[95,96]. It is distributed throughout the plasma membrane of elongating cells and then recruited to the Z ring by a recently identified patch of seven amino acids, known as the QNR patch, that follows the last coiled-coil region in the C-terminal region[60,97]. *ezrA* mutants have an increased frequency of Z ring formation and additional Z rings are often observed at one or both cell poles, which suggests a Min-like function in preventing Z ring assembly at cell poles[42,60,95,97]. In support of its role as a negative regulator, a lack of EzrA lowers both the concentration of FtsZ that is required for Z ring assembly and the ability of MinC and MinD overproduction to inhibit this process[42,60,97]. In addition, twofold overproduction of EzrA in a sensitized genetic background is sufficient to efficiently block Z ring assembly[95]. Taken together, these reports suggest that FtsZ assembly is stabilized in the absence of EzrA.

Despite the apparent contradiction posed by the presence of a negative regulator of FtsZ at the divisome, it has been proposed that EzrA participates in maintaining the dynamic nature of the medial Z ring[40]; indeed, several studies point to an active role for EzrA at mid-cell[60,97,98]. Cells lacking EzrA are notably longer than wild-type cells, which suggests that their cell division is delayed[60,97,98]. One group has suggested that this delay results from the recruitment of other division proteins to additional polar Z rings, resulting in a dilution of the available pool of limiting division proteins. In support of this model, they found that the overproduction of FtsL could suppress the increased cell length phenotype[98]. However, another group found that *ezrA*-null cells were longer than wild-type cells even under growth conditions that do not support the formation of

polar Z rings, which led them to propose that the delay in cell division results from the increased stability of the medial Z ring[60,97]. In support of this model, it was shown that the cell length phenotype, as well as the ability to overcome the inhibition caused by MinC and MinD, correlated with the extent to which EzrA could localize to the Z ring; further support came from the finding that in the absence of EzrA, the turnover of FtsZ in the Z ring is reduced[41,97].

Several groups have now established that there is a direct interaction between EzrA and FtsZ *in vitro* using a wide range of techniques and, in support of bacterial two-hybrid analyses, have found that EzrA can self-interact[95,99,100]. Using both sedimentation analysis and 90°-angle light scattering, EzrA has been found to inhibit FtsZ polymerization in a concentration-dependent manner, with a concurrent fourfold increase in the critical FtsZ concentration that is required for Z ring assembly[95,99,100]. The use of a fluorescently conjugated nucleotide analogue revealed that EzrA inhibits FtsZ polymerization at two distinct levels[99]: first, at the monomeric level, by reducing the affinity of FtsZ for GTP; and second, at the polymeric level, by increasing the rate of GTP hydrolysis, potentially by stabilizing the transition state of the hydrolysis reaction, and thereby promoting the disassembly of FtsZ polymers. Although only modest, the increased rate of hydrolysis is supported by the kinetics of the reaction, which show statistically significant increases in both K_{cat} and K_m. In all cases, the *in vitro* data showed maximal activity of EzrA at a 1/1 to 2/1 molar ratio of EzrA to FtsZ[95,99,100]. In addition, it has recently been shown that EzrA modulates FtsZ assembly primarily by interacting with the conserved extreme C terminus of FtsZ[100], and that the conserved patch responsible for the mid-cell localization of EzrA is not necessary for its inhibitory activity[97]. This implies that EzrA can either interact with FtsZ at more than one site or, more probably, that the QNR patch mediates an interaction with another core member of the divisome. One notable discrepancy in the *in vitro* data is that only one study showed that EzrA can disassemble preformed FtsZ polymers[100].

Recently, EzrA has been shown to have a third role: the coordination of the elongation–division cycle along with a newly identified component of the divisome, guiding PBP1-shuttling protein B (GpsB)[101,102]. In this pathway, EzrA was shown to be primarily involved in the recruitment of the bifunctional penicillin-binding protein 1 (PBP1) to the divisome and, in agreement with cell biology data, was found to interact with the transmembrane domain of PBP1 (REF. 101).

SepF. Recently, two groups independently identified SepF as a new component of the divisome in *B. subtilis* that is required for correct septal morphology[85,88]. *In vivo*, SepF interacts both with itself and with FtsZ, and its recruitment to the Z ring is dependent on FtsZ but not on the later-assembling proteins[85,88]. In addition, SepF can be isolated from cells in complex with FtsZ, FtsA, EzrA and ZapA[85]. *sepF*-null mutants are viable and have only a mild defect in cell division[85,88]. However, the septa of these cells are abnormally thick and, in some cases, cell separation is initiated before septal completion[88].

The defective septa pointed to a role for SepF in the period following Z ring completion, during the constriction period of cell division[88]. In support of this hypothesis, Z rings can assemble in the absence of SepF and EzrA and can recruit the late-assembling division protein PBP2B but cannot initiate septum synthesis[88]. This model also explains the observed increase in cell length, as it predicts a delay in cell division in cells that lack SepF, owing to the synthesis of thick septa[88]. However, *sepF* is also synthetically lethal with *ftsA*, which suggests that it might act early during the assembly of the Z ring[85]. Indeed, Z ring assembly is abolished in cells lacking both proteins; instead, FtsZ forms loose spiral structures in the internucleoid spaces of these cells[85]. In strong support for a role in Z ring assembly, the overproduction of SepF in an *ftsA*-null background can restore both normal Z ring formation and cell growth rate in a concentration-dependent manner[85].

SepF is conserved throughout the Gram-positive bacteria. In *S. pneumoniae* it forms part of the *ftsAZ* operon[85,88,103], and its absence causes gross morphological changes, including septal defects[103]. Perhaps more importantly, SepF is essential in the cyanobacterium *Synechococcus elongatus*, which does not have an identifiable homologue of FtsA[104]. The two disparate models discussed above could be reconciled if we consider that the constriction of an incorrectly assembled Z ring leads to a morphological abnormality of the septum (M. Gündoğdu, personal communication). SepF overproduction allows essentially wild-type growth and division in the absence of FtsA, a fact that contrasts with the overlapping roles of ZipA and FtsA in *E. coli* (see previous section) and that has important implications for the role of FtsA in this organism[85]. FtsA is not essential in *B. subtilis*[83,84], which suggests that it does not recruit any essential downstream proteins and that a correctly formed Z ring is sufficient for their recruitment and for efficient division. Finally, as FtsA probably has a structural role in tethering the Z ring to the inner membrane, these findings also predict that another division protein, such as EzrA or SepF itself, can efficiently support this function.

ClpX. Recently, ClpX, the substrate recognition complex of the ClpXP protease, was identified as a negative regulator of Z ring assembly in *B. subtilis*, during a screen for suppressors of the temperature-sensitive *ftsZ*–GFP mutant[105]. Consistent with ClpX functioning as a negative regulator, a *clpX*-null mutation also suppresses the severe division defect that is associated with MinC and MinD overproduction[105,106]. Moreover, despite producing only a modest increase in cell length in a wild-type strain, twofold overproduction of ClpX is sufficient to completely block Z ring assembly in a temperature-sensitive *ftsZ* background, even at the permissive temperature[105]. *In vitro*, ClpX specifically interacts with FtsZ and efficiently inhibits FtsZ polymerization in a concentration-dependent manner, independently of both its ATP-dependent chaperone activity and its proteolytically active partner, ClpP[105,106]. This inhibition is not accompanied by any change in the GTPase activity that is associated with FtsZ polymerization, which suggests that ClpX is active against FtsZ polymers[105].

90°-angle light scattering
A real-time assay that measures the amount of light that is scattered by FtsZ polymers. The increase in signal is proportional to the extent of Z ring assembly, and the method can also be used to follow disassembly.

Although the mechanism of inhibition remains unclear, the wide conservation of ClpX throughout the Bacteria supports the proposal that ClpX could be a general regulator of Z ring assembly[105].

Responding to cell cycle status

UgtP. Consistent with previous data showing that cell division in *B. subtilis* is subject to growth rate-dependent regulation[11], UgtP was recently identified by Weart *et al.* as a growth rate-dependent inhibitor of Z ring assembly[107]. UgtP is a terminal sugar transferase and uses UDP–glucose in the synthesis of the diglucosyl diacylglycerol anchor of lipoteichoic acid. It acts as a growth rate-dependent inhibitor of cell division during growth in nutrient-rich media, but is downregulated under nutrient-limiting conditions. UgtP associates reversibly with the divisome, and the transition between the divisome and sequestration into apparently random foci is controlled by the availability of UDP–glucose, which is supplied by the upstream members of the pathway, UTP–glucose-1-phosphate uridylyltransferase B (GtaB) and phosphoglucomutase A (PgcA). UgtP is present at approximately 2,400 molecules per cell and at a physiologically relevant molar ratio was shown to be a potent inhibitor of FtsZ assembly by disrupting the formation of the stabilizing lateral interactions between protofilaments[107]. The inhibitory activity of UgtP is partially dependent on the availability of UDP–glucose, although the main role of substrate availability seems likely to be in stimulating the re-localization of UgtP to the divisome.

The authors proposed a model in which UgtP responds to nutrient availability, as sensed by the supply of UDP–glucose, to coordinate cell division with the growth rate. They suggest that UgtP localizes to the Z ring to delay cell division until the cells reach a sufficient length to allow completion of the multi-fork DNA replication that is typical of rapidly growing cells. A similar cell length phenotype in *E. coli* cells lacking the PgcA homologue, Pgm, suggests that this might be a conserved mechanism for coordinating cell size and division throughout the Bacteria[107].

SulA. SulA is a cell division inhibitor that is produced in response to DNA damage, as part of the SOS response, in *E. coli*[108,109] and rapidly stalls cell division by both preventing the assembly of nascent Z rings and facilitating the disassembly of existing Z rings, including those that are actively constricting[110–112].

SulA is a potent inhibitor of FtsZ polymerization *in vitro*[35,110,112–115], as it increases the critical concentration that is necessary for assembly almost fivefold without affecting the cooperative nature of assembly[35]. Recent structural studies revealed that SulA interacts with the base of the C-terminal domain of FtsZ, completely masking the catalytic synergy loop[113]. SulA is therefore thought to act by binding to FtsZ monomers, sequestering them and thereby increasing the apparent critical concentration[112,113]. In support of this mode of action, when FtsZ is in molar excess it efficiently assembles even in the presence of SulA[35], arguing against a filament-capping mechanism for SulA[113,115]. A sequestration model also explains both the observed inhibition of FtsZ GTPase activity[112,113,115] by SulA and the finding that FtsZ mutants that are impaired in GTPase activity exhibit resistance to SulA, with the degree of resistance correlating with the extent of their remaining GTPase activities[45,115–117]. The key to understanding this finding came with the discovery that the dynamic nature of FtsZ polymers that is observed both in the cell and *in vitro*[34,40,41] depends on GTP hydrolysis[30]. Therefore, when SulA binds to FtsZ monomers and removes them from the pool that is available for exchange, ongoing nucleotide hydrolysis in the polymer promotes its rapid disassembly. This is consistent with reports that SulA can disassemble FtsZ polymers both in the cell and *in vitro*. Similarly, mutants of FtsZ that are impaired in GTP hydrolysis but not GTP binding form stable polymers, and so the rapid turnover of subunits does not occur, rendering them resistant to sequestration by SulA[42,45]. Interestingly, SulA was found to exist as a dimer both in the crystal structure and in solution, which suggests that it could cross-link FtsZ polymers and allow substoichiometric levels of the protein to rapidly inhibit Z ring formation[113]. However, a recent report found that in *E. coli* at least, and consistent with the proposed sequestration model, SulA is effective only once it reaches a critical concentration[35].

SulA is not well conserved, but recently two proteins responsible for the SOS-induced division arrest in *B. subtilis* and *Corynebacterium glutamicum* (YneA and DivS, respectively) have been identified[118,119]. These proteins are not homologous to SulA or to each other. The assembly of the Z ring is inhibited on the production of DivS[119], but YneA seems to act at a later stage in cell division, possibly by preventing the recruitment of FtsL or DivIC[98,118]. The presence of a lysin motif (LysM) peptidoglycan-binding domain in YneA is intriguing, but its importance is not yet understood[118].

MciZ. Recently, a 40-amino acid peptide, mother cell inhibitor of FtsZ (MciZ), was identified as an inhibitor of Z ring assembly that is produced during *B. subtilis* sporulation[120]. Cells lacking MciZ exhibit a markedly increased frequency of Z ring formation during the later stages of sporulation and, consistent with a role as a negative regulator of FtsZ, artificial expression of *mciZ* during vegetative growth prevents Z ring assembly[120]. *In vitro*, MciZ binds specifically to FtsZ and is a potent inhibitor of FtsZ polymerization[120]. A large reduction in the GTPase activity of FtsZ accompanies this inhibitory activity, suggesting that MciZ acts at the level of the monomer to inhibit the initial assembly of the FtsZ polymer. There is evidence to support a model proposing that MciZ binds at a site proximal to the nucleotide-binding pocket of FtsZ, so as to occlude GTP binding and FtsZ polymerization. First, an MciZ-resistant mutant of FtsZ that bears a substitution close to the nucleotide-binding site has been isolated, identifying the probable peptide-binding site. Second, the kinetics of GTPase activity in the presence of MciZ displayed a significant increase in K_m, which is characteristic of competitive inhibition[120]. Interestingly, the mode of action of MciZ is similar to that recently proposed for a chemical inhibitor of FtsZ assembly[121], reinforcing the

Lipoteichoic acid
A class of teichoic acids that is anchored in the cell membrane. Teichoic acids are anionic polymers that are ubiquitous in the cell walls of Gram-positive bacteria.

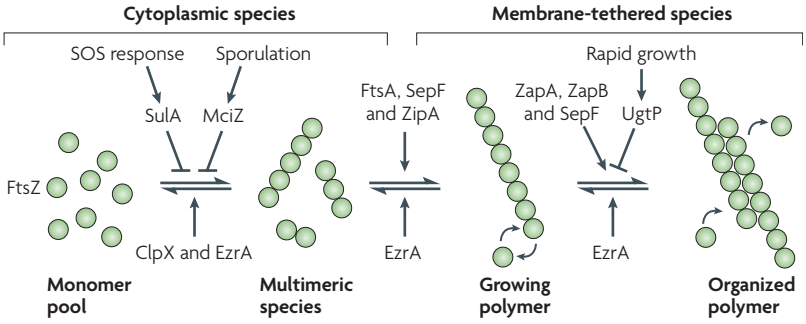


Figure 3 | **Regulation of FtsZ assembly by accessory proteins.** A large number of accessory proteins act in concert to regulate cell division at various levels of FtsZ assembly. Before division, FtsZ is thought to exist as a cytoplasmic population of monomers and multimeric species of various lengths. Inhibitors of FtsZ assembly, such as SulA in *Escherichia coli* (^{Ec}), which sequesters monomers, or mother cell inhibitor of FtsZ (MciZ) in *Bacillus subtilis* (^{Bs}), which prevents GTP binding, act on the monomer pool to prevent Z ring assembly and also facilitate Z ring disassembly owing to the rapid exchange of FtsZ subunits in the Z ring with those in the cytoplasm. The monomer pool that is available for exchange is maintained by both ClpX, which seems to prevent further assembly of FtsZ, and extra Z rings A (EzrA^{Bs}), which reduces the initial assembly of FtsZ and promotes its disassembly from the medial Z ring. Following the relief of spatial regulation, the Z ring assembles at mid-cell and is tethered to the inner face of the cytoplasmic membrane by FtsA or ZipA^{Ec}, which, along with ZapA, also contribute to the stability of the Z ring. Furthermore, early-assembling accessory proteins such as ZapB^{Ec} and SepF^{Bs} also have potential roles in organizing the ultrastructure of the Z ring; SepF seems to make a substantial contribution to this process in *Bacillus subtilis* and might itself act as a membrane tether. During rapid growth, further 'fine tuning' of the Z ring is carried out by UgtP^{Bs}, which delays Z ring completion until cells reach an appropriate size.

notion that an understanding of the cellular mechanisms that regulate FtsZ assembly is likely to inform future work in developing clinically relevant inhibitors of bacterial cytokinesis.

Future perspectives

The past few years have seen the discovery of many additional factors that are involved in Z ring formation and modulating polymer dynamics. Moreover, much progress has been made in understanding how Z ring assembly is both coordinated with the cell cycle and rendered responsive to cellular and environmental stresses. As we have seen, there is considerable overlap and redundancy present in the regulation of the Z ring (FIG. 3) and, as a result, it is likely that further, non-essential regulators remain to be discovered. Although, as we have described, the regulation of the initial assembly and the stability of the Z ring is now reasonably well understood, there is as yet no consensus on how the Z ring senses the completion of the divisome, initiates constriction and eventually disassembles. Similarly, the emerging model of Z ring formation following the collapse of a spiral intermediate might also involve additional regulators or might simply be the result of passive remodelling, driven by the relief of spatial regulation. Although to date no true bacterial homologues of MAPs have been found, as we advance our understanding of division proteins, tentative functional parallels can be drawn and further characterization of these proteins might reveal additional relationships with the MAPs[122].

The recent *in vitro* reconstitution of contractile Z rings in tubular liposomes as well as the assembly of a MinD–MinE gradient on the surface of a membrane are promising steps forward[47,123] that offer the potential to study the regulation of Z rings using *in vitro* systems, rather than simply studying the effects of individual proteins on the bulk polymer. Although this will undoubtedly be technically challenging, we must remain cautious when drawing conclusions on the basis of the effects of single accessory proteins on FtsZ polymers. In the cell, we also need to take into account the effects of membrane binding, the presence of numerous other regulatory proteins at the Z ring and the crowded nature of the cytoplasmic environment. The advances that we anticipate making in understanding Z ring assembly and its regulation promise to make this an enlightening and exciting field in the coming years.

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DATABASES

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FURTHER INFORMATION

Jeff Errington's homepage: http://www.ncl.ac.uk/camb/cbcb
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