HHS Public Access

Author manuscript

Nat Rev Mol Cell Biol. Author manuscript; available in PMC 2016 February 18.

Published in final edited form as:

Nat Rev Mol Cell Biol. 2005 November ; 6(11): 862–871. doi:10.1038/nrm1745.

FTSZ AND THE DIVISION OF PROKARYOTIC CELLS AND ORGANELLES

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Abstract

Binary fission of many prokaryotes as well as some eukaryotic organelles depends on the FtsZ protein, which self-assembles into a membrane-associated ring structure early in the division process. FtsZ is homologous to tubulin, the building block of the microtubule cytoskeleton in eukaryotes. Recent advances in genomics and cell-imaging techniques have paved the way for the remarkable progress in our understanding of fission in bacteria and organelles.

Duplication of cells occurs by the division of a mother cell into two daughter cells. This process, known as cytokinesis, provides the force to split cells and is spatially regulated to faithfully partition the genetic material. The cytoskeleton has a crucial role in cytokinesis. In animal and fungal cells, a medial ring of actin and myosin, helped by other proteins, contracts to divide the cell. Plant cells use a cell plate, and are guided by actin filaments and microtubules. Prokaryotes, which include bacteria and archaea, possess homologues of eukaryotic cytoskeletal proteins. Most prokaryotes use a tubulin homologue, a protein known as FtsZ, to divide. Eukaryotic organelles such as chloroplasts and mitochondria evolved from bacteria, and all chloroplasts and some mitochondria use FtsZ to divide.

FtsZ is thought to be the first protein to localize to the site of future division in bacteria[1], and it assembles into what is known as the Z ring (FIG. 1). In *Escherichia coli*, the Z ring recruits at least ten other proteins, all of which are required for the progression and completion of cytokinesis[2], as will be discussed below. Whereas the cytokinetic apparatus in eukaryotic cells and even organelles can be observed directly in stained thin sections by transmission electron microscopy, the Z ring and its associated factors are not detectable by these methods. This is probably because the protein machinery is largely membrane bound and resides in a densely populated cytoplasmic environment. However, the development of green fluorescent protein (GFP) fusion and improved immunofluorescence techniques over the past 10 years have been crucial in allowing the first direct visualization of many cellular components and their dynamics. For example, using GFP fusions, the dynamics of the Z ring and other components of the cell-division protein machinery can now be visualized in living bacterial cells. The combination of these new cytological tools with genomics and the

Competing interests statement

The author declares no competing financial interests.

already powerful genetics of several model systems have spurred rapid progress in our understanding of the molecular cell biology of bacteria and eukaryotic organelles. This review will discuss how the recent revolutions in genomics and cell biology have provided new evolutionary and mechanistic insights into how bacterial cells and organelles divide.

The FtsZ family of proteins

Conservation of FtsZ

FtsZ is a highly conserved protein that is found in most of the major groups of bacteria and in the EURYARCHAEAL branch of the Archaea. However, it is absent in the CRENARCHAEA, and is also missing in a few large bacterial groups[3]. One of these groups, the Planctomycetes, is unusual in that some species contain a membrane-bound NUCLEOID. One representative of this group, *Pirellula* spp., has been completely sequenced and lacks the *ftsZ* gene. Several species in another diverse bacterial group that includes Chlamydiae and Verrucomicrobia also lack *ftsZ*. Intriguingly, *Prosthecobacter dejongii*, a member of the verrucomicrobial group that lacks *ftsZ*, contains genes that are significantly more similar to the tubulin gene than *ftsZ*. Although the sequences of these bacterial tubulin genes are also divergent from eukaryotic tubulins, it is likely that they were acquired from a eukaryote by horizontal gene transfer. In support of this idea, species related to *P. dejongii* do not have these tubulin genes. Nothing is known about the functional significance of these bacterial tubulins, although recently they have been shown to assemble into protofilaments and hydrolyse GTP[4]. Finally, many of the wall-less mollicutes (MYCOPLASMAS) contain *ftsZ*, except for *Ureaplasma urealyticum*, a free-living species[5]. The mycoplasmas have also lost many other cell division genes, but it is not clear why *U. urealyticum* lost *ftsZ*, or how it can divide without it. In fact, the mechanism by which cell division occurs in any of the species that lack *ftsZ* is unknown.

FtsZ is also found in eukaryotic cells. Nuclear-encoded homologues of FtsZ are imported into chloroplasts and mitochondria of primitive eukaryotes such as protists[6]. As a result, primitive algae have separate FtsZ homologues for their chloroplasts and mitochondria (see below). Higher plants contain two distinct families of FtsZ homologues that seem to have diverged early in plant evolution, perhaps because they have distinct, conserved functions[7,8]. As would be expected from their ENDOSYMBIOTIC origins, mitochondria contain FtsZ proteins that are most closely related to those of α-proteobacteria, which are the progenitors of mitochondria, whereas chloroplast FtsZs are most closely related to those of cyanobacteria, the predecessors of chloroplasts.

FtsZ domain structure

FtsZ contains four main protein domains, as determined by the crystal structure of FtsZ from the thermophilic bacterium *Thermotoga maritima*[9] and by phylogenetic analysis[3]. These domains comprise a variable N-terminal segment, a highly conserved core region, a variable spacer, and a C-terminal conserved peptide (FIG. 2). The functions of the N-terminal segment and spacer have not been determined. The core region contains the tubulin signature motif and is responsible for GTP binding and hydrolysis, which is required for self-assembly of the protein (see below). Recently, this core region has been shown to

consist of two independently folding N-terminal and C-terminal segments[10] — the Nt core and Ct core, respectively. The Nt core contains the GTP-binding site and binds the bottom portion of the adjacent monomer in the protofilament (see below), whereas the Ct core binds the top portion of the adjacent monomer in the protofilament.

The C-terminal peptide is not required for assembly, but is essential for interactions with other membrane-associated cell division proteins, FtsA and ZipA. Deletion of the C-terminal peptide blocks FtsZ function, probably by preventing its interaction with both ZipA and FtsA[11]. The presence of either FtsA or ZipA is required for the stability of Z rings[12], which implies that their functions overlap (see also below). FtsZs from different species are generally unable to substitute for *E. coli* FtsZ[13], probably because of the specialized function of the C-terminal peptide.

Structure and assembly of FtsZ

FtsZ is a structural homologue of tubulin[9]. Like tubulin, purified FtsZ binds and hydrolyses GTP[14–16]. GTP binding induces FtsZ self-assembly into protofilaments that consist of a head-to-tail linear polymer of FtsZ[10,17–19]. These protofilaments and other structures, such as minirings, resemble structures formed by tubulin[20]. However, whereas tubulin assembles into microtubules, comprising 13 protofilaments arranged around a hollow core, FtsZ protofilaments do not assemble into microtubule-like structures. Instead, FtsZ protofilaments associate laterally to form bundles or sheets. This bundling can be induced by several factors, such as Ca^{2+} (REF. 21), macromolecular crowding[22] and the binding of partner proteins such as ZipA[23] (see FIG. 3). It is likely that FtsZ protofilament bundling is important *in vivo*, although without direct visualization only indirect conclusions can be drawn. Also, most biochemical studies have been done with *E. coli* FtsZ, but significant variations in assembly dynamics have been observed with FtsZ from *Mycobacterium tuberculosis*[24], which emphasizes the importance of other model systems in correlating the *in vivo* and *in vitro* properties of FtsZ.

The GTP-dependent assembly of FtsZ protofilaments *in vitro* is probably driven by the affinity between GTP-bound FtsZ monomers. GTP is subsequently hydrolysed by an active site formed between the two associated monomers, as in microtubule assembly[25,26]. However, GTP hydrolysis and subsequent nucleotide exchange seems to differ from that of microtubules. Recent data indicate that the nucleotide-binding site in FtsZ is accessible to the cytoplasm, which is rich in GTP and should therefore rapidly restore GTP binding[10,27]. If this conclusion is correct, then GTP hydrolysis should be the limiting step for the FtsZ assembly cycle and FtsZ protofilaments probably consist of mostly FtsZ–GTP, which is resistant to depolymerization[10,28]. Indeed, it has been shown *in vitro* that once the pool of GTP is exhausted, FtsZ protofilaments disassemble[29]. By contrast, microtubule protofilaments consist mostly of GDP–tubulin with a GTP cap and are susceptible to rapid depolymerization once the cap is hydrolysed[30]. Interestingly, GDP can allow FtsZ assembly as well, resulting in curved polymers[31,32]. The potential significance of such polymers *in vivo* is unclear, especially if nucleotide exchange from the large available pool of GTP is sufficiently rapid to saturate most FtsZ with GTP.

The actual mechanism of FtsZ assembly into protofilaments and protofilament bundles is also controversial. Most methods for monitoring FtsZ assembly indicate that the assembly of protofilaments is COOPERATIVE, with a defined critical concentration for assembly[33,34]. However, the presence of only one binding face for a new monomer to attach to an existing protofilament indicates an ISODESMIC ASSEMBLY mechanism[35,36], as does the appearance of single protofilaments in electron micrographs of assembled FtsZ in the absence of bundling factors. How can the apparent cooperativity of assembly be rationalized with the microscopic evidence of single-stranded protofilaments? One possible answer suggested recently[37] is that FtsZ initially assembles isodesmically as a curved protofilament, but after reaching a certain length the protofilament ends are able to contact each other. This process of cyclization would cause the formation of additional lateral bonds, resulting in cooperativity. In accord with this model, protofilament rings, some hundreds of nanometers in diameter, have been observed by atomic force microscopy and electron microscopy[37]. If cyclization could be partially suppressed *in vivo*, then it is conceivable that the Z ring could be a single cyclized protofilament; following longitudinal pulling, it could extend into a short spiral of cyclizing protofilaments. Spiral FtsZ structures are often observed in cells (see below). However, the 125–200 subunit circles would require 30–40 s to assemble at the diffusion-limited assembly rate of 4.6 per second[34] and are therefore not compatible with the kinetics of initial assembly, nor with the 8 s half-time turnover at steady state *in vitro*[38] and *in vivo*[39] (H. Erickson, personal communication). As it stands, there is currently no explanation for the cooperative assembly of a single-stranded protofilament.

Regulation of Z-ring assembly

Spatial regulation

The concentration of FtsZ in the cell is approximately 10 μM, which is significantly higher than the critical concentration of 1–2 μM that is needed for protofilament assembly *in vitro*. This indicates that assembly inhibitors keep FtsZ from assembling except at the right time and place[27]. One important inhibitory system, the MIN SYSTEM, is crucial for the precise positioning of the Z ring. In *E. coli*, this system consists of the FtsZ assembly inhibitor MinC, and the MinD and MinE proteins, which oscillate from one cell pole to the other[40,41]. MinD is an ATPase that binds to the membrane in its ATP form and is released from the membrane on ATP hydrolysis. MinE drives MinD off the membrane by binding to MinD and stimulating its ATP hydrolysis. Subsequent diffusion of MinD–ADP and nucleotide exchange causes MinD–ATP to rebind to the membrane; this new binding occurs far away from the original site because of the transient presence of MinE at the original site and the kinetics of nucleotide exchange[42–44]. Because MinC associates with the oscillating MinD protein, FtsZ assembly is inhibited most at the cell poles and least at midcell (FIG. 4). This causes an FtsZ disassembly wave to oscillate from pole to pole[45].

Whereas many bacterial species contain the MinCDE system, many others such as *Caulobacter crescentus* lack the Min proteins. And others, such as *Bacillus subtilis*, contain only MinCD. Consistent with the role of MinE in stimulating the oscillation of MinD, the MinC and MinD proteins of *B. subtilis* do not move but are tethered to the cell poles by another protein, DivIVA, which itself binds strongly to the cell poles[46]. Therefore, although

it has a completely different structure and function, DivIVA is analogous to MinE in that it restricts the action of MinC to the cell poles and away from the future division site. *Bacillus subtilis* MinCD is important for blocking polar divisions but not for the precise placement of the Z ring at midcell[47]. Some spherical-shaped cells such as *Neisseria gonorrhoeae* and *Synechocystis* spp. contain all three Min proteins, which are required for normal cell division in alternating perpendicular planes[48,49]. It is therefore likely that the more complex problem of identifying the plane of division in such cells is determined in part by Min oscillation, which tends to follow the cell's long axis[50,51].

Another important spatial regulatory system is nucleoid occlusion[52]. Like the Min system, nucleoid occlusion negatively regulates Z-ring assembly. Acting independently of the Min system[53], nucleoid occlusion prevents the assembly of the Z ring on top of unreplicated chromosomal DNA. As a result, Z rings do not form at the cell midpoint until after most of the chromosome has been duplicated and partitioned towards the cell poles (FIG. 4). In the absence of the Min system, multiple Z rings can assemble in DNA-free areas of the cell. This indicates that Z-ring assembly does not require a specific receptor, but instead can occur anywhere on the cytoplasmic membrane that is not blocked by nucleoid occlusion or Min[54,55]. Moreover, if nucleoid occlusion is inactivated, septa will form over unsegregated nucleoids, resulting in chromosome 'guillotining'. The inactivation of nucleoid occlusion can be achieved by decondensing the chromosome[56].

The recent discovery of proteins in *E. coli* and *B. subtilis* that mediate nucleoid occlusion (SlmA and Noc, respectively) has provided an important molecular framework for understanding this process[57,58]. Cells that lack these proteins seem normal, but when the Min system is also inactivated, FtsZ often assembles on top of nucleoids as well as between them. These multiple FtsZ assemblages sequester FtsZ subunits, thereby preventing the assembly of productive Z rings and blocking cell division. Nucleoid occlusion normally prevents Z rings from forming over unreplicated chromosomes, but artificially blocking replication in *slmA*$^{-}$ or *noc*$^{-}$ cells results in chromosome guillotining. Therefore, it seems that Noc and SlmA topologically restrict the assembly of FtsZ, enhancing the cooperativity of Z-ring assembly, and serve as a checkpoint to prevent guillotining of nucleoids. Noc and SlmA do not share sequence similarity, but both bind to multiple sites on chromosomal DNA. The normal appearance of *slmA*$^{-}$ or *noc*$^{-}$ cells indicates either that their Min systems are sufficient to restrict Z rings to the midcell, or that other, partially redundant, nucleoid occlusion systems exist. The latter is more likely, because even in the absence of Noc or SlmA and MinCD, the majority of FtsZ still localizes to spaces between segregated nucleoids, indicating that the process of nucleoid occlusion remains partially active. Curiously, purified SlmA protein from *E. coli* enhances the bundling of FtsZ protofilaments[57], which is at odds with its role in blocking Z-ring assembly but points to the existence of other nucleoid-occlusion-mediating factors in the cell.

Although there is no evidence for the existence of proteins that localize to the midcell before FtsZ, positive factors might have a role in its localization. For example, some *E. coli* plasmids such as F and P1 localize at the cell quarter positions — the future cells' midpoints — before cytokinesis. A factor that localizes to such positions might leave a mark that later helps to position the Z ring. There is some evidence that future cell poles (and therefore

division sites) are recognized by proteins in filamentous cells, which is consistent with the marking of division sites[59]. Membrane staining has indicated that certain lipid domains might be enriched at division sites, either as a cause or an effect of Z-ring localization[60]. Alternative factors in ring positioning will probably be important in the many species that lack the Min system, or in species that do not exhibit obvious nucleoid occlusion[61,62].

Cell-cycle timing

In *E. coli*, the Z ring assembles at approximately the same time as chromosome replication terminates[63]. Because chromosome segregation occurs continuously during replication, once replication origins are positioned near the cell poles, Z rings assemble between largely segregated bulk nucleoids. However, the basis for this temporal control is not known. No local signal from the nucleoid is required for Z-ring assembly in *E. coli*, as Z rings can assemble at a distance from non-replicating chromosomes in filamentous cells[53,64]. Moreover, although the initiation of chromosome replication is not necessary for Z-ring assembly, the proper positioning of the Z ring at the midcell is dependent on replication initiation[55,56,65]. In *B. subtilis*, the precision of Z-ring positioning at the midcell does not depend on REPLISOME positioning, because the replisome itself is highly mobile[66].

Once the Z ring assembles, its contraction also becomes subject to regulation. In *E. coli*, Z-ring assembly is followed by a period in which the ring remains relatively stable and does not contract significantly. The reason for the delay is not clear, although during this time the process of cell-wall synthesis switches from the elongation mode to the septal mode. This switch is dependent on FtsZ but not on other cell-division proteins that are recruited by FtsZ[67], which are described below. The number of Z rings that can contract in each cell cycle is also regulated. For example, cells with extra nucleoids limit the number of division events to one per cell cycle[68]. The factors that are involved in this cell-cycle timing of septation are not known, although DNA replication seems to be important for the proper expression of several genes, including *ftsZ*[69]. In *E. coli* and *B. subtilis*, FtsZ levels do not change significantly during the cell cycle[70], which indicates that the activity of FtsZ is cell-cycle controlled. By contrast, FtsZ levels in *C. crescentus* vary dramatically because of proteolysis and resynthesis[71]; nevertheless, the artificial increase of FtsZ levels at the wrong time in the cell cycle does not trigger Z-ring assembly or change the timing of Z-ring constriction[72]. Finding the source of this cell-cycle control of FtsZ activity remains an important challenge for the future.

Other regulators of Z-ring assembly

The fact that Z rings can form independently of nucleoids in cells that lack Min proteins indicates that there must be regulators of Z-ring assembly other than Min and nucleoid occlusion. Indeed, several positive and negative regulators of Z-ring assembly are known (FIG. 3). ZipA, a positive regulator, bundles FtsZ protofilaments *in vitro* and is essential for cell division[23,73]. It is only present in *E. coli* and a few other closely related species. The topology of ZipA is unusual, with its N terminus in the cytoplasmic membrane and its C terminus, which is sufficient for the bundling activity, in the cytoplasm. A protein that has a similar topology, known as EzrA, is present in *B. subtilis* and other Gram-positive bacteria; EzrA, however, is a negative regulator of Z-ring assembly and inhibits FtsZ polymerization

in vitro[74,75]. As ZipA and EzrA bind directly to FtsZ, they must therefore regulate FtsZ assembly at the membrane. Recently, the conserved chaperone ClpX was also found to inhibit Z-ring formation in *B. subtilis*[76]. Purified ClpX can inhibit FtsZ polymerization, although GTP hydrolysis is not affected, indicating that ClpX functions after the initial assembly of protofilaments.

ZapA, which was first found in *B. subtilis* but is present in a wide variety of species including *E. coli*, binds directly to FtsZ, has a strong tendency to dimerize through coiled coils and promotes the bundling of protofilaments[77,78]. However, as with Noc or SlmA, the loss of ZapA has no detectable consequences *in vivo*, except when combined with other defects[78,79], which indicates that the positive regulatory function of ZapA is redundant or required only under certain circumstances. Other cell-division genes found in cyanobacteria and conserved in other species are good candidates for other FtsZ-assembly regulators[61].

Finally, an inhibitor of FtsZ assembly, SulA, becomes active under conditions of DNA damage, to delay cell division until chromosomes can be properly duplicated and partitioned. Instead of being bound to DNA like Noc, the SulA protein is synthesized only following induction of the SOS RESPONSE[80] and prevents Z-ring formation[81], probably by directly binding FtsZ and preventing its assembly into protofilaments[82]. However, SulA is cleaved by the Lon protease, allowing Z rings to assemble after a delay. In *B. subtilis* and other Gram-positive bacteria, the YneA protein has a similar general role in disrupting Z rings after DNA damage. However, YneA has no structural similarity to SulA and its biochemical mechanism of inhibiting cell division is not yet known[83].

FtsZ function in bacteria

Assembly of the cytokinesis machine

Once assembled, the Z ring recruits several membrane-associated proteins that are essential for cell division[2] (FIG. 5). These proteins seem to be recruited in a linear order, with FtsA and ZipA required for all the others to arrive and the recruitment of FtsN, one of several BITOPIC membrane proteins that are essential for cell division, being dependent on all the others. FtsA and ZipA are required to anchor the Z ring to the membrane[73,84]. Because a point mutation in FtsA, R286W, can completely bypass the requirement for ZipA[85], FtsA probably has the main role in stabilizing the Z ring at the membrane in *E. coli*. The recent discovery of a membrane-targeting sequence at the C terminus of FtsA, which consists of an amphipathic helix[84], strongly supports this crucial role for FtsA in anchoring the Z ring to the membrane. FtsA and ZipA can bind to the same region of FtsZ because FtsZ is present at ~10,000 molecules per cell, and ZipA and FtsA are at least fivefold less abundant. In support of this idea, the presence of excess FtsA or ZipA has an inhibitory effect on cell division, which can be reversed by increasing the level of FtsZ. In *M. tuberculosis*, which lacks ZipA and FtsA, FtsZ might be tethered to the membrane through a unique FtsZ-binding domain that is present in another conserved cell-division protein, FtsW, which normally depends on FtsZ for its localization to the Z ring[86] (FIG. 5a).

The mechanism by which the other proteins are recruited, and how they are dependent not only on FtsZ, but also on FtsA and ZipA, is slowly being unravelled. For example, ZipA is

required for the recruitment of all downstream proteins, but because it can be bypassed, it cannot be recruiting these proteins by direct, unique protein–protein contacts. Instead, ZipA probably indirectly enhances the recruitment activity of the Z ring by stabilizing the ring components. In other cases, direct protein–protein interactions are important for subassemblies of later septal proteins. This is particularly clear for FtsQ, which is needed midway in the recruitment pathway. FtsQ can co-purify with its downstream partners FtsL and FtsB[87]. Moreover, when targeted prematurely to the Z ring by fusion to ZapA, FtsQ can recruit FtsL, FtsB and the later protein FtsI (but not FtsN) to the Z ring in the absence of the upstream proteins FtsW or FtsA[88]. The failure of FtsN to be recruited in this system indicates that the proper assembly of all components at the septum, particularly FtsA, might be essential for the localization of FtsN. In support of this, FtsA or a small subdomain of FtsA, known as 1c, is able to recruit FtsI and FtsN to cell poles when fused to DivIVA, a protein that localizes to the cell poles[89]. Other bacterial two-hybrid assays also show interactions among many cell-division proteins, including FtsA–FtsI and FtsA–FtsN, which is consistent with a patchwork of protein–protein interactions[90,91].

Assembly of the division machine and the timing of its function in other bacteria are likely to be variations on a similar theme. For example, there is more co-dependency of recruitment in *B. subtilis*[92]. In contrast to *B. subtilis* and *E. coli*, *C. crescentus* cell-division proteins are specifically synthesized and degraded during the cell cycle in parallel with their requirement: FtsA and FtsQ, which function after FtsZ, are synthesized and stable in predivisional cells, but are degraded post-division when they are no longer needed[93]. The septation process in round-shaped bacteria such as streptococci and staphylococci involves many of the same proteins, but their growth is normally dependent on the septation machinery[94]. As a result, there are significant differences in the timing of septal localization and function in round bacteria[95,96].

Contraction and recycling of the Z ring

During the period after assembly but before visible contraction, the Z ring seems to be stable. FRAP experiments with GFP fusion proteins, however, show that individual FtsZ monomers within the ring undergo rapid exchange with FtsZ in the cytoplasm, with an average recovery time of ~8–9 s[39]. This indicates that subunits within the Z ring are in a constant state of flux, not unlike microtubules or actin filaments in eukaryotic cells. The GTPase-defective FtsZ84 protein, on the other hand, displays much slower turnover in the cell, providing a clear correlation between GTPase activity and ring dynamics. Nevertheless, cells with FtsZ84 divide normally at 30°C, which indicates that the high level of turnover is not essential for ring function.

How does the visible contraction of the Z ring at the leading edge of the inner membrane translate into the pinching of cell membranes and cytokinesis? Perhaps the Z ring contracts passively ahead of invaginating septal wall growth, which, in turn, is promoted by proteins such as FtsI, the septum-specific cell wall transpeptidase, that are recruited by the Z ring. Another possibility is that the rapid net loss of FtsZ monomers, which are tethered to the membrane by the other proteins of the machine, exerts a pinching force on the membrane

that is analogous to the mechanical force exerted by DYNAMINS on their membrane substrates[97].

Experimental evidence indicates that Z-ring contraction might be accompanied by the loss of FtsZ subunits from the Z ring. In cells that express both FtsZ and FtsZ–GFP, fluorescent polymer-like structures seem to emanate from the closing ring[45,98]. Moreover, this fluorescence remains in a helical pattern throughout the cell even when no Z rings are present, suggesting that non-ring FtsZ is not randomly diffuse but tethered to the membrane in a structure, possibly through ZipA or FtsA. This structure is highly mobile, as evidenced from the rapid movement of fluorescence within the helical patterns. It is not clear whether FtsZ assembly causes these helical patterns or whether FtsZ localizes to a helical track made by some other cell-envelope component.

Other cellular functions of FtsZ

The functions of FtsZ are not limited to cytokinesis. Recent evidence indicates that the machineries responsible for cell shape and cell division interact. In *C. crescentus*, the extended coil of the actin homologue MreB, which contributes to the rod shape of this species, contracts towards the midcell at the time of division initiation[99]. This contraction is dependent on FtsZ, and might be a way to spatially and temporally regulate bulk septal wall synthesis at the time of cytokinesis. In *E. coli*, the simultaneous inactivation of FtsZ and certain penicillin-binding proteins such as the PBP5 carboxypeptidase, neither of which induces a significant shape alteration on its own, causes cells to branch and sometimes grow helically at their poles[100]. It is possible that non-ring FtsZ spirals are involved in interactions with the cell wall that cause the cells to change shape. Finally, FtsZ might have a role in the duplication of certain plasmids. In several *Bacillus* species, in addition to the conserved chromosomal copy of *ftsZ*, a weak homologue of *ftsZ* is encoded by a plasmid[3]. This raises the possibility that these plasmids, one of which (pX01) is required for virulence of *Bacillus anthracis*, use an FtsZ variant for replication or segregation.

FtsZ and organelle division

Plastid division

Chloroplast division is probably as complex as bacterial cell division, as multiple membranes need to be coordinately invaginated and sealed. Normal chloroplast fission and distribution require FtsZ, as shown by the dramatically enlarged organelles after the inhibition of FtsZ[101]. The protein machine of chloroplasts, unlike that of bacteria, can be observed in electron micrographs of thin sections. One possible reason for its visibility is that this machine consists of at least three layers: an innermost ring comprising FtsZ, followed by the plastid dividing ring that is visible by negative staining but is of unknown composition[102], and an outermost ring on the outside of the organelle that contains a dynamin-like protein (DRP)[103,104]. Dynamins are eukaryotic GTPases that assemble on membranes and mediate membrane fission[97]; dynamin also has a role in cytokinesis of animal and plant cells[105,106]. DRPs have no structural homology with FtsZ, and obvious homologues are absent from prokaryotes. Therefore, the plastid division apparatus consists of a chimaera of prokaryotic and eukaryotic fission systems (FIG. 6).

As mentioned above, two major homologues of FtsZ are found in plants, FtsZ1 and FtsZ2. They are found in the STROMAL COMPARTMENT and co-localize to a ring structure at the midpoint of the chloroplast[107]. An additional homologue of FtsZ that is distinct from FtsZ1 and FtsZ2, but is still related to cyanobacterial FtsZs, is also present in plastids of primitive algae. Homologues of cyanobacterial MinD and MinE proteins are found in plants, and are required for the proper localization of plastid Z rings in *Arabidopsis thaliana*[108,109], which indicates that the function of MinD and MinE in the spatial regulation of plastid Z rings has been conserved. Nevertheless, no homologues of MinC have been found in plants, so the factor that negatively regulates FtsZ assembly through MinD in plastids is not yet known.

Other regulators of FtsZ assembly in plastids have been identified. One protein, known as ARC6, is related to a cyanobacterial cell-division protein, Ftn2 (REF. 110). Ftn2 interacts directly with FtsZ and is important for cell division in several species of cyanobacteria[49]. ARC6 localizes to the membrane at the site of the plastid FtsZ ring and, like *E. coli* ZipA, seems to promote FtsZ assembly. Recently, a homologue of the SulA protein has been identified in *A. thaliana*[111]. However, unlike *E. coli* SulA, this version, which is also found in cyanobacteria, seems to have a positive role in cell and plastid division[111,112]. The reasons for the need for SulA in division of cyanobacteria and plastids are unknown, although it might function in the recycling of FtsZ. Other proteins might be involved in later stages of fission and in the coordination of the three membrane systems that need to be invaginated. One candidate for this function is ARTEMIS, a YidC homologue that helps to promote insertion of transmembrane proteins[113]. Recently, several additional cell-division genes have been discovered in cyanobacteria. Some of these, including the *ylm* genes, are conserved throughout most bacteria[114] and are often present in plants and algae[61], indicating that they might be involved in plastid division.

As in bacteria, plastid FtsZ has the potential to localize in a cytoskeletal-like array. An extensive network of filamentous structures is formed by FtsZ–GFP and a similar network is observed in electron micrographs[115]. This array, however, is not detected by immunostaining and might be an artefact of abnormally high expression levels[107]. Interestingly, in moss, one isoform of FtsZ can localize as rings in both chloroplasts and in the host-cell cytoplasm as detected by GFP fusions or by immunofluorescence[116], which indicates that it might have a role in division of the host cell.

Mitochondrial fission

Like chloroplasts, mitochondria need to distribute themselves to daughter cells after each division of the eukaryotic host cell. For many types of mitochondria, this requires a dynamic balance between organelle fusion and fission. Mitochondria of animals, fungi and higher plants lack FtsZ, and it is well-established that DRPs have important functions in fission of all mitochondria[6,117,118]. Nevertheless, mitochondria of some primitive eukaryotes contain nuclear-encoded FtsZ[119]. First discovered in primitive algae[120], FtsZ is also present in the mitochondria of *Dictyostelium discoideum*[121]. As with plastids, two distinct homologues of FtsZ (FszA and FszB) are found on the inside of *D. discoideum* mitochondria. Both are evolutionarily related to FtsZs from α-proteobacteria, which are the progenitors of

mitochondria. Genetic knockouts of either FtsZ homologue in *D. discoideum* result in mitochondrial elongation, which is consistent with a role of each FtsZ in mitochondrial fission. However, whereas FszA localizes to bands and foci at presumptive mitochondrial fission sites, FszB localizes to foci at the ends of the organelle[121]. This dual localization pattern is distinct from the common medial localization seen with plastid FtsZ homologues, and indicates that the two FtsZs have different roles.

The fission apparatus of primitive mitochondria appears to be similar to that of chloroplasts, with an inner FtsZ ring, an outer ring of DRP, and a mitochondrion-dividing ring sandwiched between them[117] (FIG. 6). This arrangement is consistent with the function of FtsZ in marking the initial site of division and recruiting the other two components. Once constriction by the mitochondrion-dividing ring initiates, the DRP ring functions in the late stages of fission. Apparently, this putative initial scaffolding function of FtsZ became dispensable at some point in evolution, leaving DRPs and other proteins to carry out fission. As no homologues of the Min proteins or other FtsZ regulators have been found for primitive mitochondria, it remains to be seen how the sites of fission are selected, and whether mitochondrial nucleoids have a role. It is even less clear how fission sites are selected in mitochondria that lack FtsZ.

Conclusions and future challenges

The revolutions in genomics and cell-imaging techniques have paved the way for the extraordinary progress in our understanding of fission in bacteria and organelles. Most of the players in these fundamental processes have been identified and localized to their sites of action. However, the essential nature of cytokinesis in prokaryotic cells, as well as the membrane association of most of the proteins, pose significant challenges. For example, does FtsZ function in cytokinesis by generating the constriction force itself, or is it merely a scaffold for other proteins that generate the force? How are FtsZ and other fission-associated factors such as DRPs in eukaryotes targeted to the site of fission, and what determines the timing of their action? What coordinates the invagination and fusion of multiple membranes? The present lack of biochemical assays for cytokinesis calls for novel genetic and cytological approaches that take advantage of the superb molecular genetics available for some of the bacterial and organelle model systems. In addition, the redundancy of many of the proteins, such as in *E. coli* cell division, means that obtaining a more minimal set of functional components is of high priority. This is where work done with diverse systems can help, as common themes will emerge that should simplify the study of the core proteins that are indispensable for cytokinesis.

Acknowledgments

Work in the Margolin laboratory is supported by grants from the National Institutes of Health and the National Science Foundation. I thank H. Erickson for helpful advice. I apologize to colleagues whose work was not cited here because of space limitations.

Glossary

EURYARCHAEAL	Pertaining to the group of archaea that includes the methanogens and extreme halophiles
CRENARCHAEA	The group of archaea that includes the extreme thermophiles
NUCLEOID	The organized form of a bacterial chromosome
MYCOPLASMAS	Wall-less bacteria
ENDOSYMBIOTIC	Describing the engulfment of one cell by another larger cell, with the engulfed cell evolving into an organelle
COOPERATIVE ASSEMBLY	The affinity of subunits for a polymer increases as more subunits are assembled, displaying a critical concentration below which little assembly occurs
ISODESMIC ASSEMBLY	The opposite of cooperative assembly, in that the affinity of each new subunit for a polymer is independent of the subunit concentration
MIN SYSTEM	A group of two or three bacterial proteins that inhibit unwanted formation of the Z ring at the cell poles
REPLISOME	The DNA-replication protein machinery
SOS RESPONSE	Inducible DNA repair system in bacteria invoked in response to a sudden increase in DNA damage
BITOPIC	Describes an integral membrane protein that has one cytoplasmic, transmembrane and periplasmic domain
FRAP	(Fluorescence recovery after photobleaching). A microscopic technique used to measure the movement (for example, diffusion rates) of fluorescently tagged molecules over time *in vivo*. Specific regions in a cell are irreversibly photobleached using a laser; fluorescence is restored by diffusion of fluorescently tagged unbleached molecules into the bleached area.
DYNAMINS	A family of GTPases that are important for membrane scission
STROMAL COMPARTMENT	The inner compartment of the chloroplast

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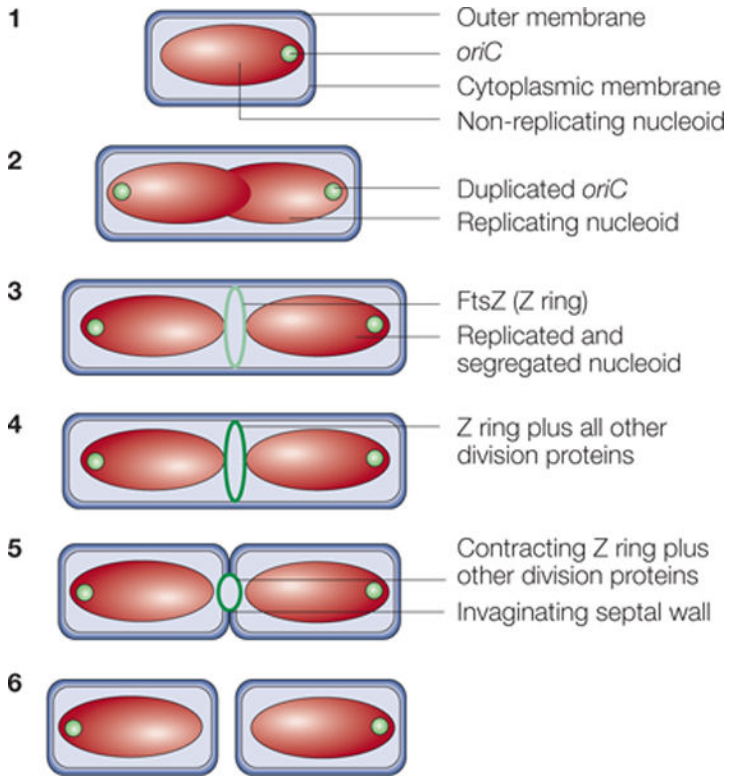

Figure 1. A typical cell-division cycle in *Escherichia coli*

In step 1, newborn cells grown at low growth rates contain a single, non-replicating chromosome in a structure known as a nucleoid. Soon after chromosome replication initiates, the replication origins (*oriC*) move towards the cell poles until both daughter chromosomes are segregated (step 2). Near the end of this process, the FtsZ protein assembles into the Z ring on the inner face of the cytoplasmic membrane (light green ring) at the cell centre, marking the future division site (step 3). In step 4, the Z ring recruits at least ten membrane-associated proteins (for details, see FIG. 6) to assemble the cell-division protein machinery (dark green ring). This machinery synthesizes the division septum, which consists of cell-wall material, with the Z ring at the leading edge of membrane invagination. Contraction of the Z ring and constriction of the outer membrane follow (step 5). The result is the production of two separate newborn daughter cells (step 6).

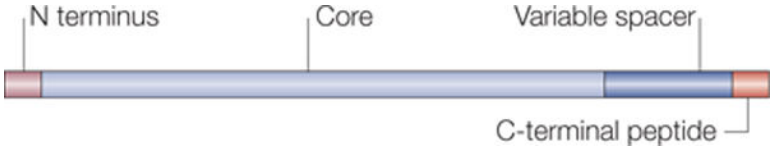

Figure 2. The domain structure of FtsZ

The domain structure applies to most FtsZ proteins. The N terminus and variable spacer domains are highly variable in length, and their precise functions are unknown. The core region displays most similarity to tubulin and is required for GTP binding and hydrolysis as well as assembly into protofilaments. The C-terminal peptide interacts with other cell-division proteins recruited by FtsZ such as ZipA, FtsA and FtsW, and might function mainly to anchor the Z ring to the membrane using these proteins.

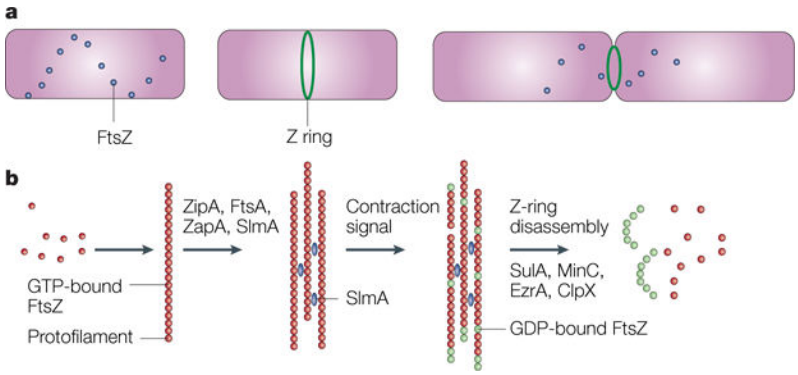

Figure 3. Assembly and disassembly of FtsZ and the Z ring

a | A time course, starting with a newborn *Escherichia coli* cell without a Z ring but with FtsZ in spiral patterns, followed by a cell with a Z ring, and finally, a dividing cell with a contracting Z ring. **b** | The putative time course of FtsZ assembly. Red dots indicate GTP-bound FtsZ dimers that assemble into protofilaments. GTP hydrolysis during assembly is probably balanced by rapid exchange of the GDP for the abundant GTP within the cell. Several protein factors, including ZipA, ZapA and probably also FtsA and the nucleoid occlusion protein SlmA (blue), bundle these protofilaments and anchor them to the cytoplasmic membrane. An unknown signal triggers ring contraction and probable disassembly; this might involve stimulation of GTP hydrolysis, which would increase the number of GDP-bound subunits at protofilament ends (shown in green), causing curved protofilaments to be formed. The free FtsZ then forms a spiral pattern throughout the cell, possibly by reassembling, and this FtsZ would then be available to form a new ring in the daughter cells. The presence of high levels of inhibitor proteins such as SulA, MinC, ClpX or EzrA (in *Bacillus subtilis*) might help to antagonize the ring-stabilizing proteins and tip the balance towards disassembly, although none of the inhibitor proteins is required for Z-ring contraction or disassembly.

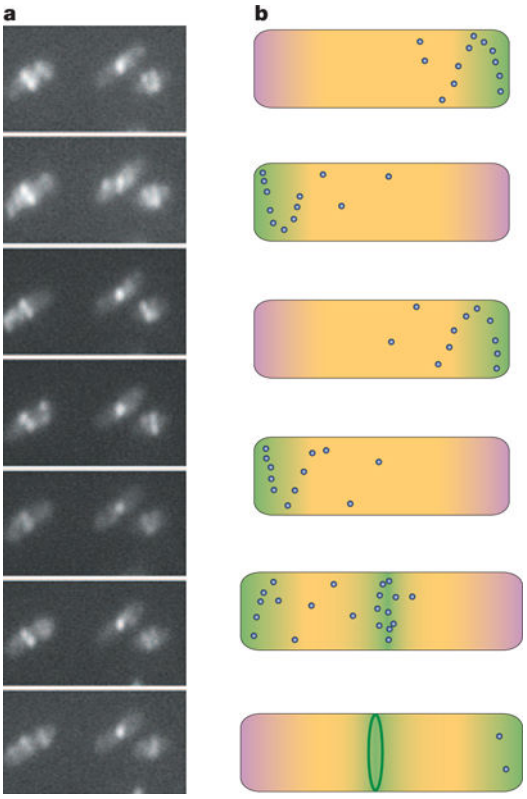

Figure 4. How the Z ring finds the centre of an *Escherichia coli* cell

a | A time course of a group of *E. coli* cells expressing FtsZ–GFP, showing the Z ring at the midcell but also displaying the oscillation of FtsZ from one side of the ring to the other. **b** | A schematic time course of the spatial regulation of FtsZ. The top four cells span approximately 2 minutes of growth of a cell early in the cell cycle, showing how the oscillation of Min proteins (pink) and FtsZ (green), along with nucleoid occlusion (yellow), prevent assembly of the Z ring (dark green) at all locations in the cell. The fifth cell is at a later point in the cell cycle, after chromosomal replication and segregation is well underway, relieving nucleoid occlusion at the cell centre. This, along with potential positive regulators, lowers the barrier for Z-ring assembly in a zone near the middle of the cell (green), allowing Z-ring assembly (final cell). Blue dots represent higher concentrations of non-ring FtsZ that oscillate in spiral patterns. Similar mechanisms might help to centre the Z ring in chloroplasts and in other bacteria that contain the Min system, such as cyanobacteria. Nucleoid occlusion might be the main spatial regulator in species that lack Min proteins.

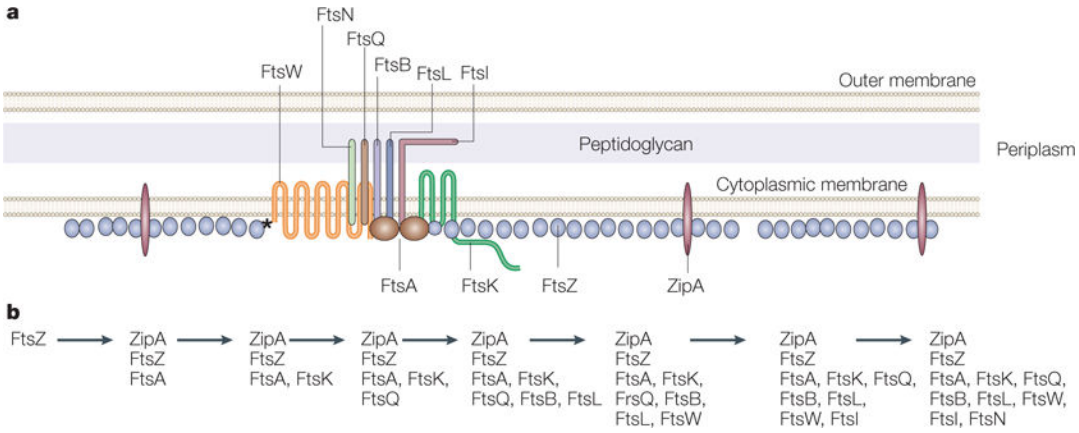

Figure 5. Fellowship of the ring

a | A model of the *Escherichia coli* Z ring and its essential protein partners is shown in cross section. FtsZ is shown as a series of single protofilaments at the membrane, although the actual structure of FtsZ in the Z ring is unknown. Both ZipA and FtsA contact FtsZ as well as the membrane in *E. coli*. However, FtsZ contacts FtsW directly in *Mycobacterium tuberculosis* (asterisk), which lacks ZipA and FtsA. A single transmembrane subassembly associated with an FtsA dimer is shown, based on the low relative amounts of most of the integral membrane proteins that are essential for cell division. These membrane proteins include FtsQ, FtsB, FtsL, FtsI and FtsN, which are bitopic proteins that each have a single transmembrane and periplasmic domain, and FtsW and FtsK, which are polytopic proteins with multiple transmembrane and periplasmic domains. The network of protein–protein associations is implied by the proximity of the proteins in the diagram. Proteins implicated in stabilization of the ring structure are labelled below the cytoplasmic membrane lines, whereas proteins implicated in later functions in septum formation, such as septum synthesis, are labelled above the lines. **b** | The dependency order of recruitment of essential cell-division proteins to the Z ring, as deduced from the requirement of a given protein for another’s localization to the Z ring.

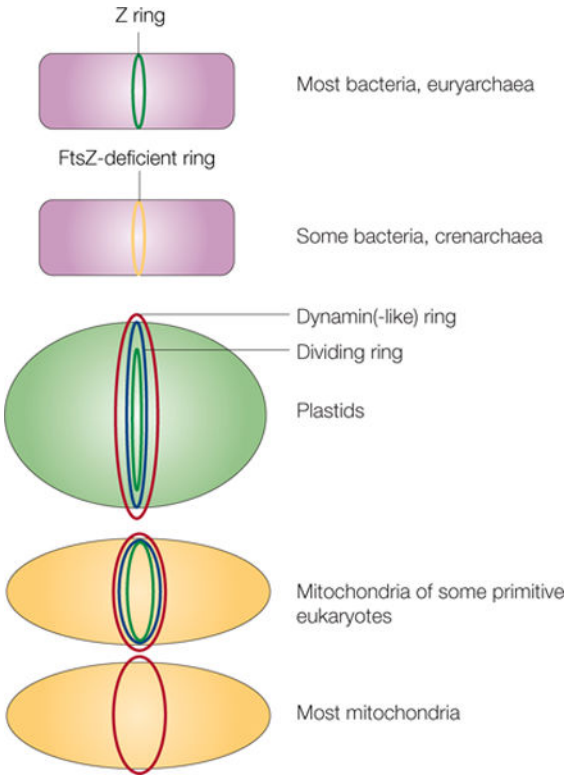

Figure 6. FtsZ and the evolution of cell and organelle fission

Different types of cells or organelles and their use of FtsZ or alternative proteins for fission are shown. In euryarchaea and many bacteria, FtsZ (green) localizes to the inner side of the inner membrane as the Z ring. Crenarchaea and some bacteria lack FtsZ, so some other protein must localize as a ring at the site of division (yellow). In plastids and mitochondria, dynamin or dynamin-like protein rings (red) localize to the cytoplasmic face. These organelles also contain a dividing ring (blue) and, in the case of chloroplasts and some primitive mitochondria, an innermost ring of FtsZ (green). Most mitochondria, including those of fungi and animals, lack FtsZ and a detectable dividing ring, but still rely on dynamin on the cytosolic face for fission (red).