Review

Strong FtsZ is with the force: mechanisms to constrict bacteria

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FtsZ, the best-known prokaryotic division protein, assembles at midcell with other proteins forming a ring during septation. Widely conserved in bacteria, FtsZ represents the ancestor of tubulin. In the presence of GTP it forms polymers able to associate into multi-stranded flexible structures. FtsZ research is aimed at determining the role of the Z-ring in division, describing the polymerization and potential force-generating mechanisms and evaluating the roles of nucleotide exchange and hydrolysis. Systems to reconstruct the FtsZ ring *in vitro* have been described and some of its mechanical properties have been reproduced using *in silico* modeling. We discuss current research in FtsZ, some of the controversies, and finally propose further research needed to complete a model of FtsZ action that reconciles its *in vitro* properties with its role in division.

Introducing the ring: the role of FtsZ in bacterial cell division

FtsZ is the main cell division protein in most prokaryotes and many eukaryotic organelles [1]. It is essential for assembly of the division machinery, and is very likely to be the protein responsible for driving cell constriction. In eubacteria, the *ftsZ* gene is usually found in the *dcw* gene cluster, a DNA region containing division and cell-wall synthesis genes (Box 1) [2]. The pattern of conservation of these genes across eubacterial taxa suggests that FtsZ-driven cell division dates back at least to the origin of the peptidoglycan cell wall [3,4]; protein sequence analyses have shown that FtsZ is an ancient protein that might have originated soon after cells evolved [1,5].

Prior to cell division, FtsZ localizes beneath the cytoplasmic face of the cell membrane at the cell center forming the Z-ring (Figure 1). This Z-ring is a stationary structure, continuously remodeled by polymerization and depolymerization [6]. It has a protein turnover time of a few seconds and quickly disappears if the cells are depleted of energy [7,8]. Fluorescence microscopy analysis of Z-ring assembly and dynamics suggests that it is a single continuous structure, an idea further supported by the observation of dynamic helical structures arising from the ring and extending towards the cell poles [9,10]. Nevertheless, cryoelectron tomography of dividing *Caulobacter* cells has rendered images of a discontinuous Z-ring apparently formed by short, around 100 nm long, independent filaments sparsely distributed within a narrow region at the site of septation [11], and single-molecule microscopy

Glossary

Atomic force microscopy (AFM): generates topographic maps of surfaces at nanometer resolution. Samples can be dry or immersed in solution, and a sharp silicon nitride tip a few nanometers in diameter, placed at the edge of a very soft cantilever, is used to scan over a surface by gently touching the sample to follow the profile of the scanned surface. Piezoelectric elements on electronic command facilitate accurate and precise movements to allow very precise scanning.
Cooperative polymerization: a polymerization process characterized by two phases, formation of a nucleus and polymer elongation from this nucleus. The nucleation phase is energetically unfavorable, whereas elongation from the nucleus is favorable.
Isodesmic polymerization: as opposed to cooperative polymerization, this is a process in which the addition of a monomer to a growing polymer occurs always with the same affinity, independently of polymer size.
Kinetic models: mathematical models of complex chemical or biochemical reactions based on the identification of all the steps of the global reaction and the calculation of the flux through the different steps using chemical equilibrium and kinetics parameters. They require measurements of the values of as many equilibrium constants and reaction rates as possible.
Lattice models: physical models used to define the behavior of a collection of particles in a discrete space according to a limited and specified set of rules defining particle interactions.
Light scattering: the scatter of light produced by particles in solution can be measured at 90° using a fluorimeter, setting the detector to the same wavelength of the source (that is, excitation and emission wavelengths are the same). Usually wavelengths in the range of 350–450 nm are used. Measurements are useful to follow polymerization or assembly reactions in which there are large size-variations between reagents and products. More sophisticated procedures, using more complex equipment, such as multiangle light scattering and dynamic light scattering can provide information on the size and shape of the particles.
Molecular dynamics (MD): a computational simulation technique in which atoms within molecules are allowed to interact for a period of time accordingly to the principles of physics. In the case of proteins, nucleic acids and other biological macromolecules, the parameters that are relevant include electrostatic attraction and repulsion forces, Van der Waals interactions, and the lengths, angles, and dihedral angles related to the properties of the covalent bond. Usual simulation times for protein complexes vary from 100 ps to 1 ns for short trajectories, and up to 10–20 ns for longer ones.

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Box 1. What is in a name?

The first cell-division mutants isolated in *E. coli* were called *fts* for filamentous temperature sensitive mutant and were assigned the letters A to G [71]. Soon, several other *fts* genes were described and these were named in alphabetic order corresponding to their publication date. Some of these names were later abandoned because they turned out to be already described genes largely unrelated to cell division. The *ftsB* locus, for example, was found to be an allele of *nrdB*, coding for a subunit of ribonucleoside diphosphate reductase [72,73], so the name disappeared from the literature, to be recycled several years later to name a new division gene [74]. In 1980, after the advent of molecular subcloning and complementation mapping [75], it was found that a region at minute 2.5 of the *E. coli* genetic map, named *ftsA*, contained not just one, but two different loci. The name *ftsA* was maintained for one of these, the locus in which a mutation had already been described and mapped by van de Putte *et al.* [76]. Jumping over the alphabet to avoid conflicts with the already shifting list of *fts* genes, the other locus was called *ftsZ*. Subsequent work led to the identification of other genes mapping without interruption in the same *E. coli* chromosomal region and that also had roles in cell division or murein synthesis, among them other *fts* and *mur* genes. After a similar region was described in the *Bacillus subtilis* chromosome it was finally named the *dcw* cluster to acknowledge its role in encoding division and cell-wall biosynthesis genes [77].

shows that the protein in helical patterns is most likely in monomeric or oligomeric form [6]. In *Escherichia coli*, FtsZ filaments appear anchored to the membrane, possibly by interacting with two other division proteins, FtsA and ZipA. For septation, these three proteins seem to assemble simultaneously forming the proto-ring. Assembly of the proto-ring at midcell triggers the localization of about ten other division proteins to the septal zone to form the full division ring or divisome [12] and, whereas removal of FtsZ leads to the simultaneous disappearance of the other proteins from the division site, the divisome complex, including FtsZ itself, is not totally stable until FtsN, allegedly the last element to assemble, is present [13]. This suggests that the FtsZ ring functions as a temporary initial scaffold for the other division proteins. In total a score of membrane and periplasmic proteins, several of them involved in the regulation of septation and in the synthesis and remodeling of the septal peptidoglycan, interact to yield a complex macromolecular structure that can be visualized in phase-contrast images of the dividing bacteria as a septum [12].

The role of FtsZ in prokaryotic cell division is still a matter of discussion. It is the first protein to localize to the division site, and is therefore thought to be the trigger of ring assembly. In addition, it is thought to work as a scaffold for the other division protein complexes, and might also take an active role in driving cell constriction. These roles are not mutually exclusive. Cell division is normally coupled to the synthesis of the septal cell wall, but in certain *E. coli* multiple murein hydrolase deletion mutants, such as the Δ(*sltY mltA mltB amiA amiB amiC mepA dacB pbpG*) mutant, these two processes are uncoupled, and cell division can occur in the absence of peptidoglycan synthesis [14]. This means that membrane constriction is likely to be driven by an intracellular mechanism independent from cell-wall synthesis and that cell-wall synthesis might not be absolutely essential for cytokinesis. Besides, FtsZ from *Mycoplasma pulmonis* and *Bacillus subtilis* with the C-terminus replaced by that of *E. coli* FtsZ can drive cell division of *E. coli* [15]. These proteins are highly divergent from *E. coli* FtsZ, so it is unlikely that they can interact with any other potential *E. coli* motor protein. Therefore, provided that FtsZ can

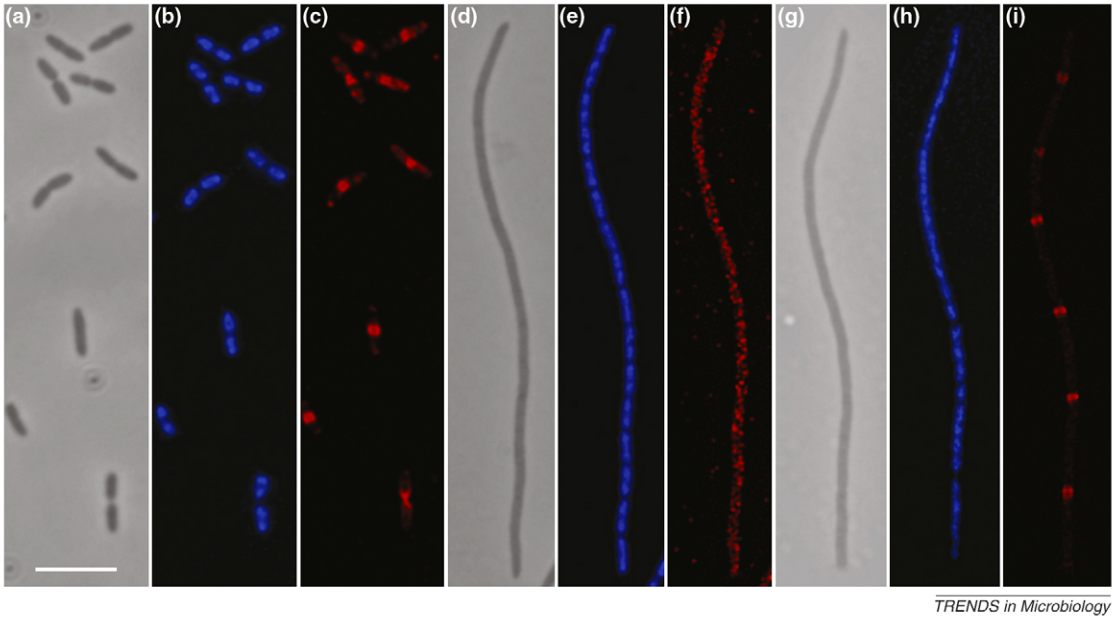

Figure 1. *In vivo* assembly of FtsZ at midcell. **(a–c)** When *ftsZ* expression proceeds at rates supporting the production of 140% FtsZ relative to the amounts present in the wild type, FtsZ locates at midcell in *E. coli* VIP205 cells, a strain in which expression of a single copy of *ftsZ* is regulated under the control of the *tac* promoter (P*tac*)–*lacI*^{q} and the inducer IPTG (isopropyl β-D-thiogalactopyranoside) [79]. **(d–f)** In the absence of IPTG, cells fail to divide and VIP205 cell filaments are formed because the amount of FtsZ drops to the levels supported by basal expression from P*tac* (20%). These low FtsZ levels do not support the assembly of the protein into localized rings, and the residual material remains as disorganized foci within the cells. **(g–i)** Upon restoration of its synthesis by IPTG induction, FtsZ assembles into rings (appearing as two opposing dots in the single deconvolution plane) that, prior to the resumption of septation, occupy regularly spaced positions along the filament. **(a, d, g)** Phase contrast and **(b, e, h)** DAPI (4′,6-diamidino-2-phenylindole) staining of the nucleoids; **(c, f, i)** immunostaining of FtsZ with MVC2 FtsZ antibody (purified by adsorption to purified FtsZ) revealed with antirabbit serum conjugated with Alexa-594. Scale bar, 5 μM.

Box 2. Is FtsZ a tubulin blueprint?

FtsZ is often dubbed 'the prokaryotic homolog' of eukaryotic tubulin. This statememt implies that FtsZ is somehow a primitive and simpler tubulin. Indeed, FtsZ is likely to have preceded tubulin, but their divergence entailed several qualitative changes that differentiated both proteins. As for their common properties, both have a similar fold, bind and hydrolyze GTP, and polymerize in a GTP-dependent manner. However, the basic subunit of the FtsZ polymer is a monomer, whereas tubulin evolved to become a dimer of α and β tubulins, possessing two distinct nucleotide-binding sites, one non-hydrolyzing, containing a GTP molecule that is not exchanged with the medium (non-exchangeable site), and another site between two dimers, which is the active site, that has catalytic activity and exchanges nucleotide. In addition, tubulin acquired several loops that are involved in microtubule assembly and in interactions with several microtubule-associated proteins and with molecular chaperones required for correct monomer folding and dimer assembly. *In vitro* the two proteins can be made to assemble into a variety of structures, but in cells FtsZ functions in close association with the inner membrane, in a size range of a few micrometers (even in the giant *Epulopiscium* cells the Z-rings are relatively small [78]), whereas microtubules work in the cytoplasm and usually are much larger (tenths of micrometers). It is likely that these differences have shaped the evolutionary divergence between the two proteins.

interact with FtsA and ZipA through the C-terminus, self-interaction plays a major role in division. These data, together with the central role of FtsZ throughout the prokaryotic world, suggest that FtsZ itself is the key element of the mechanism that generates the membrane constriction force.

During the past decade interest in FtsZ research has expanded beyond microbiology. Initially microbiologists studied FtsZ due to its prominent role in prokaryotic division, but its phylogenetic and structural properties attract scientists from other fields as well. FtsZ is a 'user-friendly' protein for biochemical and biophysical studies, the *ftsZ* genes from diverse bacteria are readily expressed in *E. coli*, and it is relatively easy to obtain milligrams of a highly pure and fairly stable protein. This provides a relatively simple experimental system that generates a large variety of dynamic structures and, being the ancestor of tubulin (Box 2) [16], FtsZ might be a useful model to study some aspects of microtubule function. Moreover, FtsZ fulfills several criteria for being a useful antibiotic target, and there are promising results along this line of study [17]. Despite this apparent simplicity, results obtained from measurements of the biochemical and biophysical properties of FtsZ have generated some controversial conclusions when analyzed in the biological context of cell division. We should be aware that, whereas FtsZ forms part of a complex ring structure during division, *in vitro* experiments are usually conducted in dilute solution after cell breakage. Several mathematical and physical models to explain polymerization dynamics and force generation have been proposed to try to explain the observations given the still scarce information available at the molecular level. New promising approaches, including single-molecule experiments and *in vitro* reconstituted systems, offer fresh insights into how the dynamics of FtsZ polymerization might relate to the assembly and function of the division ring within the cell.

Properties of FtsZ

FtsZ is a GTPase, and as such it binds to and hydrolyzes GTP, and polymerizes in a GTP-dependent manner [18]. The active site is not present in the protein monomer, instead it is formed by the interface between two monomers [19,20], and therefore the GTPase activity depends on

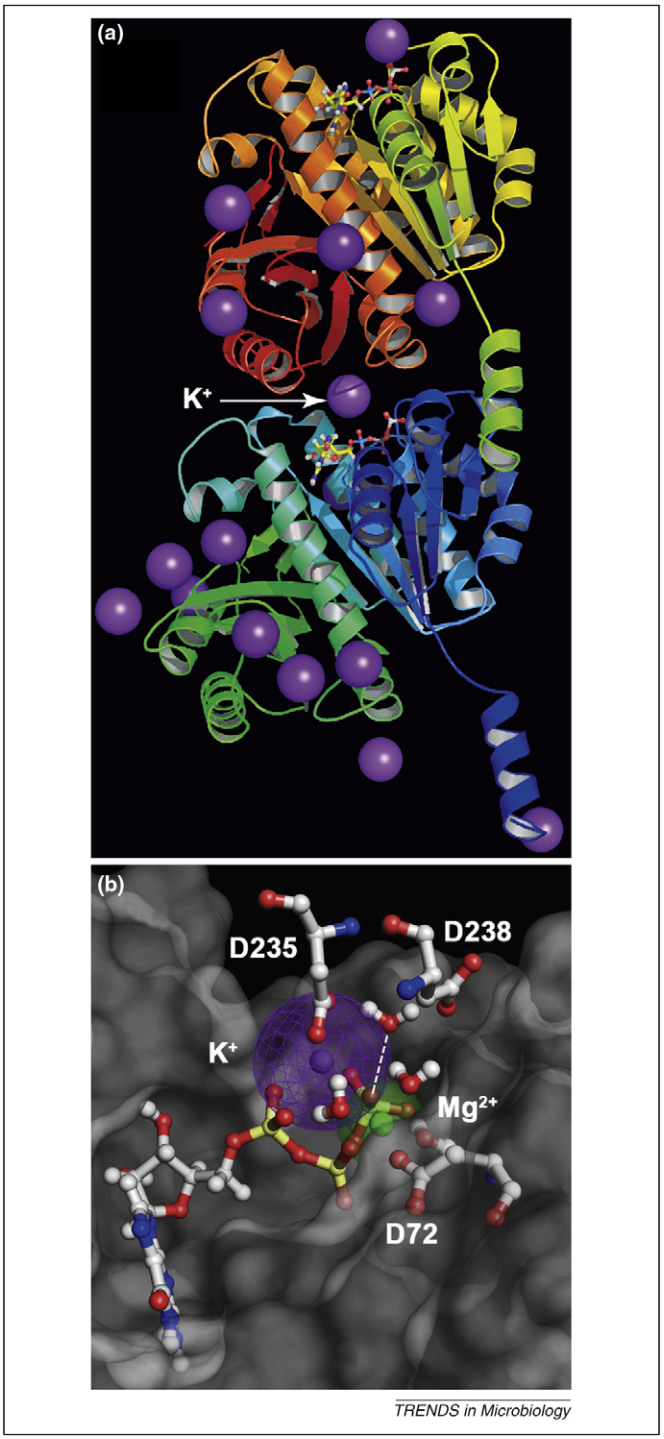

Figure 2. Molecular dynamics (MD) of FtsZ GTPase activity and polymerization. **(a)** Model for FtsZ dimer (ribbon) in the presence of GTP (ball-and-stick) and K^+ ions (purple spheres). A potassium atom localizes spontaneously in the interface between two monomers, close to the GTP molecule. **(b)** Scheme of the position of selected atoms surrounding GTP in the FtsZ dimer interface after 5 ns of MD in presence of K^+. A water molecule is located within the coordination sphere of K^+ in a position compatible with catalysis of the GTP molecule (discontinuous line). Modified, with permission, from Ref. [24].

subunit assembly. Both assembly and hydrolysis depend on the binding of nucleotide, Mg^{++}, and a monovalent cation [21–24]. The binding affinities are low, in the micromolar range for nucleotides and Mg^{++}, and in the millimolar range for K^+ or Na^+. The affinity is somewhat higher for GTP than for GDP, but the two nucleotides can compete with each other.

The FtsZ monomer is a globular protein formed by two closely packed domains. In addition, it has an N-terminal extension that contacts with the adjacent subunit [20] and a C-terminal extension that is important for binding to other cell-division proteins (ZipA and FtsA). The monomers interact head-to-tail, with the nucleotide bound at their interface. The interlocked monomers do not fit as tightly as tubulin monomers, and leave a wider junction that could allow for a rapid loss of the inorganic phosphate produced after hydrolysis, and even an easier exchange of the nucleotide within the polymer [25]. The negative charges of the phosphates are shielded by Mg^{++} (both in FtsZ–GTP and FtsZ–GDP) and K^+ (in FtsZ–GTP). Computer simulations using molecular dynamics [24] have suggested that, at neutral pH, one K^+ ion is located at a stable position between the γ-phosphate of GTP and the top monomer. This ion holds a water molecule in position for the catalytic attack on the phosphate bond (Figure 2). Under conditions simulating reduced pH, the protonated GTP promotes cation-independent polymerization, but is poorly hydrolyzed, if at all. These models, corroborated by *in vitro* experiments [24], indicated that polymerization and GTPase activities can be uncoupled by decreasing the pH. Molecular dynamics and light scattering results (Glossary) coincide to indicate that FtsZ polymers are particularly stable at low pH conditions [24]. Stable polymers are also obtained with *Methanococcus jannaschii* FtsZ in the absence of nucleotide, and even with GDP at high protein concentrations [26,27]. This suggests that the GTPase activity and the nucleotide exchange rate are related to the polymerization–depolymerization dynamics of the protein.

In the presence of GDP and Mg^{++}, FtsZ assembles into short oligomers. This is a non-cooperative equilibrium process where the size distribution of the oligomers depends on protein and Mg^{++} concentration and ionic strength [28]. Upon addition of saturating amounts of GTP, Mg^{++} and a monovalent cation, FtsZ polymerizes, and the polymers hydrolyze GTP with a Michaelis constant (K_m) of around 80 μM [22], at a rate in the order of 5 molecules of GTP per molecule of FtsZ per minute. Polymerization can be monitored by light scattering or by electron microscopy [29,30]. Polymer formation is very fast and maximal polymerization is obtained within a few seconds, a feature that hampers detailed analysis of the kinetics of protein assembly. Depletion of GTP and/or an increase of GDP result in depolymerization at a slower rate, taking place within a few minutes of the increase in the GDP:GTP ratio [31]. The inclusion of a GTP-regenerating system extends the lifetime of the polymerized state up to over an hour, whereas fast depolymerization can be induced by addition of an excess of GDP [25].

Polymerization and bundling of FtsZ are additionally regulated *in vivo* by several proteins including MinC, SulA, ZapA or ClpX [32] that contribute to permit ring assembly only at the right place and at the right time. Although the saturating concentrations of FtsZ, GTP, Mg^{++} and K^+ found in the growing bacterial cell would favor FtsZ to be in the polymeric state at all times during the cell cycle and throughout the whole cell length, there are at least two negative regulatory systems (Noc and Min) [32] that inhibit polymerization when and where it is not needed.

A closer look at the FtsZ polymers

The first FtsZ electron microscopy images showed disordered networks of very long, multistranded filaments of different widths [30]. Later findings showing single-stranded thin filaments led to further work aimed at discerning the structure of the FtsZ polymers [33]. These results indicated that FtsZ polymerization is very sensitive to the reaction conditions, and that the polymers are polymorphic. Surface adsorption promotes polymer growth, so that the electron microscope grid could yield images of the FtsZ polymer with a different structure than those present in solution. Nevertheless, it is widely accepted that the basic FtsZ polymer is a single-stranded filament. In solution these filaments are relatively short, and have a narrow size distribution, ranging from 50 to roughly 150 subunits [34,35].

FtsZ protofilaments have a strong tendency to associate further to form multi-stranded polymers. This trend can be regulated by the concentration of protein, by the pH or the presence of Ca^{++}, charged surfaces and macromolecular crowding agents. Depending on the reaction conditions a variety of structures can be observed, including filament

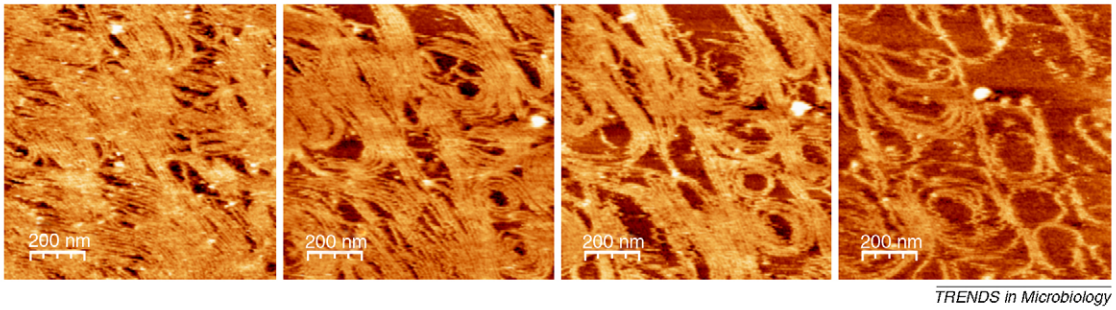

Figure 3. Atomic force topographic images of FtsZ filaments on mica. *E. coli* FtsZ was polymerized in solution in the presence of GTP and incubated on mica for 1 minute before rinsing excess protein. Images were taken with the surface immersed in buffer solution. The sequence (left to right) illustrates the evolution of the shape of the single filament aggregates formed on the surface as protein density decreases along time. The time difference between the first and last image is 80 minutes.

networks, bundles, ribbons, tubes, sheets, spirals or toroids [36–39]. Based on molecular modeling and on the roles of pH and Ca^{++}, it has been proposed that an electrostatic component is involved in Ca^{++}-induced bundling and the formation of closely packed, ordered structures [40]. In addition, electron microscopy and atomic force microscopy (AFM) show that, under different conditions, the distance between pairs of protofilaments fluctuates and that their interaction is more of a loose association, suggesting that the lateral interactions between protofilaments are weak, plastic and heterogeneous (Figure 3) [41,42]. The analysis of morphological variation of *M. tuberculosis* FtsZ polymers as a function of cation valence, ionic strength and pH indicates that lateral interactions might be explained by a polyelectrolyte condensation mechanism [43].

Occasionally, depending on some specific conditions, the protofilaments have well-defined curvatures. For example, in the presence of GDP at high dextran concentrations, small circles are formed [44], whereas in the presence of the GTP analog GDP-AlF_3 the circles are wider [41]. This suggests that a switch might function by FtsZ adopting two conformations, either straight or curved, each associated with the GTP- or GDP-bound state respectively [44]. Higher resolution techniques compatible with imaging in solution have shown that indeed FtsZ filaments show a dynamic morphology and that they can actively explore different conformations. For example, when the FtsZ protein polymerizes over a mica surface it forms long filaments that pack closely next to neighboring filaments, and these tend to cover the entire surface [41,42]. Far from being rigid, these filaments are quite flexible and curve gently, adapting their shape to the topology of the surrounding surfaces. Whether this curvature is restricted to one plane or whether it is the same in the GDP- and GTP-bound states are open questions.

The contents and the exchange dynamics of the nucleotide

The chemical kinetics view of protein polymerization assumes that the state of the protein-bound nucleotide uniquely controls the association and dissociation rates of monomers to or from polymers [45]. From this point of view, the nucleotide contents of the polymer, and the way the nucleotide turns over, are relevant parameters for understanding the role of FtsZ *in vivo* because they set limits on the possible mechanisms of action. If the phosphorylation state of the nucleotide were associated with different conformational states, then we might envisage two alternatives. A polymer containing a high ratio of GDP-bound subunits might store the energy of hydrolysis in the form of structural strain, and this energy could be used later to produce a mechanical force when the strain is released. Alternatively, a polymer containing a high ratio of GTP would not be appropriate for storing energy, and the role of the GTPase activity would be more likely to be associated with polymerization dynamics.

Measuring the nucleotide contents within dynamic polymers is difficult, and different approaches have yielded values ranging from near zero to 50% for the fraction of GDP–FtsZ versus GTP–FtsZ [25,46–48], respectively, raising questions regarding the actual *in vivo* values of GTP/GDP contents and whether the differences between GDP–FtsZ and GTP–FtsZ translate into structural diversity.

Another related question is whether GDP in the polymer can be replaced by GTP, or if it is replaced only in the free monomers. Because the first available electron microscopy images showed very long polymers, it was proposed that exchange should occur throughout the polymers [25]. This interpretation is challenged by the behavior of the much shorter filaments present in solution and by kinetic analyses using fluorescently labeled mutants or fluorescent derivatives of GTP. Together they suggest that nucleotide exchange can take place in free monomers in solution [27,48,49].

All these results still leave open questions concerning the activity of the FtsZ polymers, among which are the role of nucleotide hydrolysis and the localization of nucleotide hydrolysis within single filaments. Information about the interactions between neighboring nucleotide-binding sites, and the degree of coupling between the nucleotide phosphorylation states and the structural states of the subunits, will help to understand the relationship between the enzymatic activity of FtsZ and its role in constriction and to evaluate if the nucleotide state is the only element governing polymerization, as suggested by the chemical kinetics view, or whether the coupling is less strict than previously thought [45].

The polymerization process

In the presence of GDP, FtsZ oligomerization is an equilibrium process that follows an isodesmic polymerization scheme, resulting in a relatively broad distribution of short oligomers [28,46]. By contrast, GTP-induced polymerization has turned out to be more difficult to analyze because it proceeds very rapidly and yields a mixture of polymers of heterogeneous sizes that can themselves participate as reagents. A cooperative polymerization mode based on the simultaneous operation of longitudinal and lateral interactions upon addition of GTP was suggested by the presence of very long multistranded polymers observed by electron microscopy. This was further supported by centrifugation assays able to quickly separate long polymers from monomers and short oligomers. At the end of the process, cooperative systems should always yield a constant amount of unpolymerized protein equivalent to the critical concentration above which polymerization is triggered. The centrifugation assays determined a critical protein concentration, around 1 μM, for FtsZ polymerization, finding that no polymers were produced at lower values [38,46,50]. In a theoretical nucleation scheme to describe the production of multistranded polymers, cooperativity arises from the existence of two different kinds of interactions between the monomers, usually longitudinal and lateral. Because only one type of interaction can occur in the initial encounters between two monomers, their assembly is not energetically favorable until a short multistranded nucleus is formed, when the incorporation of monomers into the growing polymer is then favored. Because this theoretical scheme cannot explain the cooperative assembly of single-stranded filaments, alternative mechanisms have been invoked.

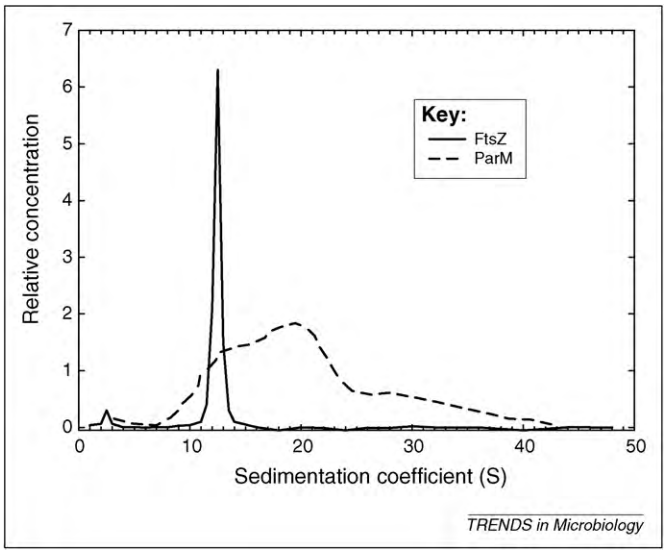

Figure 4. Sedimentation velocity analysis of FtsZ and ParM. Schematic representation of the sedimentation coefficient distributions *E. coli* FtsZ (solid line) and ParM (discontinuous line) showing the different behaviors of the two proteins. Both protein preparations were equilibrated in a neutral saline buffer in the presence of GTP (FtsZ) or ATP (ParM), $MgCl_2$ and an enzymatic nucleotide-regenerating system. ParM polymers are long linear filaments with a broad size distribution; by contrast, FtsZ shows a narrow distribution that suggests the presence of a well-defined, stable intermediate.

Sedimentation velocity and fluorescence resonance energy transfer (FRET) have been used to quantitatively characterize FtsZ polymers in solution. Sedimentation analysis of polymers at steady-state in the presence of a GTP-regenerating system revealed an unexpectedly narrow size distribution [34], in contrast with the polydisperse sedimentation behavior expected for cooperatively assembly systems, such as that of ParM, a bacterial segregation protein (Figure 4). These results suggest that FtsZ polymer formation in the presence of GTP might involve the formation of a stable and relatively short intermediate. Another relevant observation on FtsZ polymerization in solution derives from kinetic analysis of FRET using fluorescently labeled FtsZ mutant proteins. The resultant FRET signal was directly related to FtsZ assembly [48,49] and is compatible with a cooperative polymerization reaction obeying a mechanism that requires the presence of a weakly-associated dimer nucleus.

Several equilibrium models have been proposed to explain the cooperative behavior of FtsZ polymers in solution. A model that involves an isodesmic assembly with preferential cyclation of naturally curved polymers can account for the condensation-like behavior observed in the sedimentation experiments, and is consistent with the presence of cyclic structures observed by AFM and electron microscopy images at low surface protein density where lateral protofilament interactions are minimized [34,41]. The possibility of an allosteric change associated with polymerization was proposed as an alternative to explain the cooperativity of single-stranded filaments [51,52]. Further research into this previously unexplored idea showed that there is a full range of possible allosteric models to describe the polymerization of single-stranded filaments [35,53]. These involve a conformation switch either induced by subunit assembly or stabilized in the polymeric form. Nevertheless, analyses of several crystallographic structures have not detected conformation variations that might represent different FtsZ states [54]. Therefore the conformation change involved in a putative allosteric switch must be subtle. In addition, an independent mechanism needs to be included in the allosteric models to explain the narrow polymer size distribution deduced from the measurements of sedimentation velocity. It must be kept in mind that these models are equilibrium approximations that do not take into account nucleotide hydrolysis and exchange or subunit turnover. Reliable experimental measurements are therefore required to be fed into non-equilibrium models of the polymerization process before a complete picture can be obtained.

All the above discussion concerning the different models used to describe in detail the process of FtsZ polymerization applies to the protein in solution, a condition that is seldom, if ever, found within the structure of a cell. Because surface adsorption plays a role in stabilizing the filaments, a different picture is observed when protein polymerization occurs on a surface, where filaments grow to be very long and show a more varied behavior, including processes such as filament fragmentation and reannealing (Figure 3) [41,42,55].

Mathematical modeling of FtsZ inside virtual cells

A variety of computer-assisted models have been advanced to simulate or recreate *in silico* the polymerization of FtsZ and, moreover, to fit the assembly of FtsZ filaments into cell-sized ring structures. This has been facilitated by the initial simplicity, together with the behavioral richness, of the FtsZ system. As noted above, whereas the mechanisms and rates of FtsZ polymerization have been measured in the absence of spatial constraints (i.e. in homogeneous solution), the *in vivo* assembly of FtsZ into a complex division ring able to effect constriction of the cell envelope occurs within a structured cell. Two main kinds of models, kinetic and lattice, have been advanced. They aim at bridging the gap between the results of *in vitro* polymerization experiments and the function of FtsZ in cell division. Kinetic models, constructed to reproduce kinetic data, explicitly describe the different reactions of the polymerization process such as nucleation, elongation, monomer exchange or filament bending [56–58]. Reaction rates and equilibrium constants built into these models have been derived from published experimental values or, in the absence of more reliable data, were assumed to adopt plausible arbitrary values. These models are limited by the large number of parameters that need to be fed into the simulation making it difficult, or even unfeasible, to measure them all experimentally. Nevertheless, a description of the details of how the Z-ring functions is one of the goals of the research on FtsZ. Some kinetic models have been used to explore force-generating mechanisms [59,60], and these have found that the range of forces compatible with the experimental data (on the order of piconewtons) is within that required for membrane constriction.

An *in silico* alternative for modeling the collective behavior of subunits in filament polymerization and aggregate formation, and to quantitatively explore the forces

generated by polymerization, is the use of lattice models. These models use a more elementary approach in which the monomers are depicted as simple geometric particles that are allowed to move and interact on a discrete space (the lattice) while following a set of rules and bond energies. In contrast to the kinetic models, the biochemical details underlying the interactions are not relevant for lattice models and are therefore not considered. The number of parameters involved in the simulations is therefore limited, and these include the longitudinal binding energy between monomers and the lateral interaction between neighboring filaments. This restricted number of variables are fed into the model after assigning values of similar magnitudes to those observed experimentally, or are approximated by comparing the results of the simulations with experimental results. In addition, the spatial modulation of the longitudinal energy to simulate the effect of inhibition of polymerization by MinC has been used in one model [59], whereas a preferential angle of dimer curvature was considered to operate in another [61,62]. Lattice models have been used to reproduce the formation of division ring-like structures and, interestingly, all point to a central role for the lateral interactions between filaments in force generation. This role is also supported by experimental evidence obtained *in vivo* [63]. Although the intrinsic lack of biochemical detail prevents the simulations using lattice models from yielding clues on the molecular basis underlying the nature of lateral interactions, their conclusions about the involvement of lateral interactions on force generation are robust. Indeed, different approaches converge in identifying filament curvature and lateral interactions as the basic elements of the mechanisms generating a contractile force [63–66].

Reconstitution of the division ring outside the bacterial cell

The interactions between the different components of the cell division machinery were first inferred from genetic analysis of mutants defective in different components of the divisome, and complemented later by using specialized yeast and bacterial two-hybrid systems. Limited reconstitution of the division ring *in vitro* has been used to analyze the interactions between FtsZ and other cytoplasmic proteins such as ZapA or MinC [12,32]. The interaction of FtsZ with membranes and membrane proteins has been studied using partially reconstituted systems. One of the natural membrane-anchoring components of the proto-ring, the ZipA protein, was adhered by means of a histidine tail to a planar lipid bilayer extended over a mica surface. This structure served as an anchor to monitor FtsZ polymerization by AFM. In this case the exact composition of the lipids used to construct the bilayer was found to be of crucial importance. FtsZ bundles were formed over the lipid surface and evolved into more complex structures only when an *E. coli* lipid extract was used [67]. In a different approach, FtsZ was modified by fusion of a membrane-attachment helix, and tagged with a fluorescent protein to allow its detection by fluorescence microscopy. This protein could be spontaneously incorporated into a fraction of collapsed tubular, multilamellar vesicles where it formed rings able to force a limited GTP-dependent constriction. This same construction, when added to already-formed liposomes, induced their bending into different shapes, suggesting that filaments of the FtsZ protein adopt a preferential curvature, and this was argued to support a bending model of force generation [68–70].

Concluding remarks

FtsZ is the major prokaryotic cell division protein and is most likely an important element for generating the force required for constriction. It is a GTPase that polymerizes in a GTP-dependent manner to form single-stranded filaments that interact laterally to form a variety of higher-order structures. The finding of cooperativity in the generation of single-stranded filaments cannot be easily fitted into current models describing cooperativity in the assembly of protein polymers. This has led to the formulation of models based on the formation of stable structures or the occurrence of allosteric transitions to describe FtsZ polymerization. The difficulties in bridging the gap between the behavior of FtsZ – as measured in solution and as observed in the dividing cell – have led to testing alternative approaches including *in vitro* reconstitution and computer-assisted modeling. From these studies, filament curvature and lateral interactions between FtsZ filaments emerge as two potential elements to generate the force needed for constriction. Because computer models can be used to simulate the behavior of complex systems in which different parameters can be easily adjusted, it should be possible to test *in silico* the effects of a variety of modifications affecting the polymerization and lateral interactions exhibited by FtsZ. Those found to significantly

Box 3. Outstanding questions

- Is the Z-ring a continuous structure? Cryoelectron tomography suggests that the ring is not continuous, and could be formed by short filaments dispersed over a narrow area. In other reports helical FtsZ structures that move away from the ring have been detected [9,10]. Single molecule tracking suggests that the protein is polymeric in the ring, and monomeric or oligomeric in the helical pattern [6]. How can these divergent observations be explained?
- Is the Z-ring a force-generating structure, or just a scaffold to localize the division machinery? And what is the role of nucleotide hydrolysis by FtsZ in the constriction of the cell?
- Does structural plasticity play a role in FtsZ polymer dynamics? If so, what is the degree of coupling between the phosphorylation states of the nucleotide and the structural states of the monomer?
- What are the fine structural and functional details of the FtsZ filament? Where does hydrolysis occur within the filament? Are GDP-bound and GTP-bound subunits mixed within a filament, or do they form different phases? Does nucleotide exchange occur throughout the filament or only in free monomers? Are the two ends of the filaments equivalent in terms of polymerization dynamics, or are the filaments polar?
- What is the nature of the lateral interactions between filaments? Are these interactions different in closely packed tubes, ribbons, or loosely bound multistranded filaments? Is there a unique mechanism underlying the diversity of FtsZ assemblies [43]?
- Is the surface adsorption of FtsZ observed *in vitro* related to the behavior of the protein when associated to the cell membrane? If so, how does surface adsorption modify the kinetics and structural properties of FtsZ? And how do these FtsZ properties respond to intracellular crowding?

alter the properties of the protein can then be introduced by site-directed mutagenesis into the real protein and their effects on its behavior analyzed *in vitro* and *in vivo*. Finally our goal should be to integrate these observations into a detailed description of the mode of action of FtsZ as forming part of the divisome complex and exerting a function in the accurate division of the living cell (Box 3).

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References

1 Faguy, D.M. and Doolittle, W.F. (1998) Cytoskeletal proteins: the evolution of cell division. *Curr. Biol.* 8, R338–R341
2 Vicente, M. *et al.* (1998) Regulation of transcription of cell division genes in the *Escherichia coli dcw* cluster. *Cell Mol. Life Sci.* 54, 317–324
3 Tamames, J. *et al.* (2001) Bringing gene order into bacterial shape. *Trends Genet.* 17, 124–126
4 Mingorance, J. *et al.* (2004) Genomic channeling in bacterial cell division. *J. Mol. Recognit.* 17, 481–487
5 Davis, B.K. (2002) Molecular evolution before the origin of the species. *Prog. Biophys. Mol. Biol.* 79, 77–133
6 Niu, L. and Yu, J. (2008) Investigating intracellular dynamics of FtsZ cytoskeleton with photo-activation single molecule tracking. *Biophys. J.* 95, 2009–2016
7 Stricker, J. *et al.* (2002) Rapid assembly dynamics of the *Escherichia coli* FtsZ-ring demonstrated by fluorescence recovery after photobleaching. *Proc. Natl. Acad. Sci. U. S. A.* 99, 3171–3175
8 Rueda, S. *et al.* (2003) Concentration and assembly of the division ring proteins FtsZ, FtsA, and ZipA during the *Escherichia coli* cell cycle. *J. Bacteriol.* 185, 3344–3351
9 Thanedar, S. and Margolin, W. (2004) FtsZ exhibits rapid movement and oscillation waves in helix-like patterns in *Escherichia coli*. *Curr. Biol.* 14, 1167–1173
10 Michie, K.A. *et al.* (2006) Trapping of a spiral-like intermediate of the bacterial cytokinetic protein FtsZ. *J. Bacteriol.* 188, 1680–1690
11 Li, Z. *et al.* (2007) The structure of FtsZ filaments in vivo suggests a force-generating role in cell division. *EMBO J.* 26, 4694–4708
12 Vicente, M. *et al.* (2006) Septum enlightenment: assembly of bacterial division proteins. *J. Bacteriol.* 188, 19–27
13 Rico, A.I. *et al.* (2010) Role of the *Escherichia coli* FtsN protein in the assembly and stability of the division ring. *Mol. Microbiol.* 76, 760–771
14 Heidrich, C. *et al.* (2002) Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in *Escherichia coli*. *J. Bacteriol.* 184, 6093–6099
15 Osawa, M. and Erickson, H.P. (2006) FtsZ from divergent foreign bacteria can function for cell division in *Escherichia coli*. *J. Bacteriol.* 188, 7132–7140
16 Nogales, E. *et al.* (1998) Tubulin and FtsZ form a distinct family of GTPases. *Nat. Struct. Biol.* 5, 451–458
17 Haydon, D.J. *et al.* (2008) An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. *Science* 321, 1673–1675
18 Romberg, L. and Levin, P.A. (2003) Assembly dynamics of the bacterial cell division protein FTSZ: poised at the edge of stability. *Annu. Rev. Microbiol.* 57, 124–154
19 Scheffers, D.J. *et al.* (2002) GTP hydrolysis of cell division protein FtsZ: evidence that the active site is formed by the association of monomers. *Biochemistry* 41, 521–529
20 Oliva, M.A. *et al.* (2004) Structural insights into FtsZ protofilament formation. *Nat. Struct. Mol. Biol.* 11, 1243–1250
21 Lu, C. *et al.* (1998) FtsZ from *Escherichia coli*, *Azotobacter vinelandii*, and *Thermotoga maritime* – quantitation, GTP hydrolysis, and assembly. *Cell Motil. Cytoskeleton* 40, 71–86
22 Sossong, T.M., Jr *et al.* (1999) Self-activation of guanosine triphosphatase activity by oligomerization of the bacterial cell division protein FtsZ. *Biochemistry* 38, 14843–14850
23 Tadros, M. *et al.* (2006) Activation of the *Escherichia coli* cell division protein FtsZ by a low-affinity interaction with monovalent cations. *FEBS Lett.* 580, 4941–4946
24 Mendieta, J. *et al.* (2009) Structural and functional model for ionic (K^+/Na^+) and pH dependence of GTPase activity and polymerization of FtsZ, the prokaryotic ortholog of tubulin. *J. Mol. Biol.* 390, 17–25
25 Mingorance, J. *et al.* (2001) *Escherichia coli* FtsZ polymers contain mostly GTP and have a high nucleotide turnover. *Mol. Microbiol.* 41, 83–91
26 Huecas, S. and Andreu, J.M. (2004) Polymerization of nucleotide-free, GDP- and GTP-bound cell division protein FtsZ: GDP makes the difference. *FEBS Lett.* 569, 43–48
27 Huecas, S. *et al.* (2007) The interactions of cell division protein FtsZ with guanine nucleotides. *J. Biol. Chem.* 282, 37515–37528
28 Rivas, G. *et al.* (2000) Magnesium-induced linear self-association of the FtsZ bacterial cell division protein monomer. The primary steps for FtsZ assembly. *J. Biol. Chem.* 275, 11740–11749
29 Mukherjee, A. and Lutkenhaus, J. (1994) Guanine nucleotide-dependent assembly of FtsZ into filaments. *J. Bacteriol.* 176, 2754–2758
30 Mukherjee, A. and Lutkenhaus, J. (1999) Analysis of FtsZ assembly by light scattering and determination of the role of divalent metal cations. *J. Bacteriol.* 181, 823–832
31 Small, E. and Addinall, S.G. (2003) Dynamic FtsZ polymerization is sensitive to the GTP to GDP ratio and can be maintained at steady state using a GTP-regeneration system. *Microbiology* 149, 2235–2242
32 Adams, D.W. and Errington, J. (2009) Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat. Rev. Microbiol.* 7, 642–653
33 Romberg, L. *et al.* (2001) Polymerisation of FtsZ, a bacterial homolog of tubulin: is assembly cooperative? *J. Biol. Chem.* 276, 11743–11753
34 González, J.M. *et al.* (2005) Cooperative behavior of *Escherichia coli* cell-division protein FtsZ assembly involves the preferential cyclization of long single-stranded fibrils. *Proc. Natl. Acad. Sci. U. S. A.* 102, 1895–1900
35 Huecas, S. *et al.* (2008) Energetics and geometry of FtsZ polymers: nucleated self-assembly of single protofilaments. *Biophys. J.* 94, 1796–1806
36 Yu, X.C. and Margolin, W. (1997) Ca^{2+}-mediated GTP-dependent dynamic assembly of bacterial cell division protein FtsZ into asters and polymer networks *in vitro*. *EMBO J.* 16, 5455–5463
37 Löwe, J. and Amos, L. (2000) Helical tubes of FtsZ from *Methanococcus jannaschii*. *Biol. Chem.* 381, 993–999
38 González, J.M. *et al.* (2003) Essential cell division protein FtsZ assembles into one monomer-thick ribbons under conditions resembling the crowded intracellular environment. *J. Biol. Chem.* 278, 37664–37671
39 Popp, D. *et al.* (2009) FtsZ condensates: an *in vitro* electron microscopy study. *Biopolymers* 91, 340–350
40 Beuria, K. *et al.* (2006) Effects of pH and ionic strength on the assembly and bundling of FtsZ protofilaments: a possible role of electrostatic interactions in the bundling of protofilaments. *Int. J. Biol. Macromol.* 40, 30–39
41 Mingorance, J. *et al.* (2005) Visualization of single *Escherichia coli* FtsZ filament dynamics with atomic force microscopy. *J. Biol. Chem.* 280, 20909–20914
42 Sugimoto, S. *et al.* (2010) AAA+ chaperone ClpX regulates dynamics of prokaryotic cytoskeletal protein FtsZ. *J. Biol. Chem.* 285, 6648–6657
43 Popp *et al.* (2010) Suprastructures and dynamic properties of *Mycobacterium tuberculosis* FtsZ. *J. Biol. Chem.* 285, 11281–11289
44 Lu, C. *et al.* (2000) Straight and curved conformations of FtsZ are regulated by GTP hydrolysis. *J. Bacteriol.* 182, 164–170
45 Kueh, H.Y. and Mitchison, T.J. (2009) Structural plasticity in actin and tubulin polymer dynamics. *Science* 325, 960–963
46 Huecas, S. and Andreu, J.M. (2003) Energetics of the cooperative assembly of cell division protein FtsZ and the nucleotide hydrolysis switch. *J. Biol. Chem.* 278, 46146–46154

47 Romberg, L. and Mitchison, T.J. (2004) Rate-limiting guanosine 5′-triphosphate hydrolysis during nucleotide turnover by FtsZ, a prokaryotic tubulin homologue involved in bacterial cell division. *Biochemistry* 43, 282–288
48 Chen, Y. and Erickson, H.P. (2009) FtsZ filament dynamics at steady state: subunit exchange with and without nucleotide hydrolysis. *Biochemistry* 48, 6664–6673
49 Chen, Y. and Erickson, H.P. (2005) Rapid *in vitro* assembly dynamics and subunit turnover of FtsZ demonstrated by fluorescence resonance energy transfer. *J. Biol. Chem.* 280, 22549–22554
50 Mukherjee, A. and Lutkenhaus, J. (1998) Dynamic assembly of FtsZ regulated by GTP hydrolysis. *EMBO J.* 17, 462–469
51 Michie, K.A. and Löwe, J. (2006) Dynamic filaments of the bacterial cytoskeleton. *Annu. Rev. Biochem.* 75, 467–492
52 Dajkovic, A. and Lutkenhaus, J. (2006) Z ring as executor of bacterial cell division. *J. Mol. Microbiol. Biotechnol.* 11, 140–151
53 Miraldi, E.R. *et al.* (2008) Allosteric models for cooperative polymerization of linear polymers. *Biophys. J.* 95, 2470–2486
54 Oliva, M.A. *et al.* (2007) Structural insights into the conformational variability of FtsZ. *J. Mol. Biol.* 373, 1229–1242
55 Hamon *et al.* (2009) Mica surface promotes the assembly of cytoskeletal proteins. *Langmuir* 25, 3331–3335
56 Surovtsev, I.V. *et al.* (2008) Kinetic modeling of the assembly, dynamic steady state, and contraction of the FtsZ ring in prokaryotic cytokinesis. *PloS Comput. Biol.* 4, e1000102
57 Allard, J.F. and Cytrynbaum, E.N. (2009) Force generation by a dynamic Z-ring in *Escherichia coli* cell division. *Proc. Natl. Acad. Sci. U. S. A.* 106, 145–150
58 Lan, G. *et al.* (2008) Polymerization and bundling kinetics of FtsZ filaments. *Biophys. J.* 95, 4045–4056
59 Lan, G. *et al.* (2009) Condensation of FtsZ filaments can drive bacterial cell division. *Proc. Natl. Acad. Sci. U. S. A.* 106, 121–126
60 Drew *et al.* (2009) Analyses of mechanisms for force generation during cell septation in *Escherichia coli*. *Bull. Math. Biol.* 71, 980–1005
61 Páez, A. *et al.* (2009) Self-organization of curved living polymers: FtsZ protein filaments. *Soft Matter* 5, 2625–2637
62 Páez, A. *et al.* (2009) Simple modeling of FtsZ polymers on flat and curved surfaces: correlation with experimental *in vitro* observations. *PMC Biophys.* 2, 8
63 Monahan, L.G. *et al.* (2009) Lateral FtsZ association and the assembly of the cytokinetic Z ring in bacteria. *Mol. Microbiol.* 74, 1004–1017
64 Ghosh, B. and Sain, A. (2008) Origin of contractile force during cell division of bacteria. *Phys. Rev. Lett.* 101, 178101
65 Hörger, I. *et al.* (2008) Langevin computer simulations of bacterial protein filaments and the force-generating mechanism during cell division. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 77, 011902
66 Shlomovitz, R. and Gov, N.S. (2009) Membrane-mediated interactions drive the condensation and coalescence of FtsZ rings. *Phys. Biol.* 6, 46017
67 Navajas, P.L. *et al.* (2008) *In vitro* reconstitution of the initial stages of the bacterial cell division machinery. *J. Biol. Phys.* 34, 237–247
68 Osawa, M. *et al.* (2008) Reconstitution of contractile FtsZ rings in liposomes. *Science* 320, 792–794
69 Osawa, M. *et al.* (2009) Curved FtsZ protofilaments generate bending forces on liposome membranes. *EMBO J.* 28, 3476–3484
70 Erickson, H.P. (2009) Modeling the physics of FtsZ assembly and force generation. *Proc. Natl. Acad. Sci. U. S. A.* 106, 9238–9243
71 Ricard, M. and Hirota, Y. (1973) Process of cellular division in *Escherichia coli*: physiology study on thermosensitive mutants defective in cell division. *J. Bacteriol.* 116, 314–320
72 Kren, B. and Fuchs, J.A. (1987) Characterization of the *ftsB* gene as an allele of the *nrdB* gene in *Escherichia coli*. *J. Bacteriol.* 169, 14–18
73 Taschner, P.E. *et al.* (1987) Genetic and morphological characterization of *ftsB* and *nrdB* mutants of *Escherichia coli*. *J. Bacteriol.* 169, 19–25
74 Buddelmeijer, N. and Beckwith, J. (2004) A complex of the *Escherichia coli* cell division proteins FtsL, FtsB and FtsQ forms independently of its localization to the septal region. *Mol. Microbiol.* 52, 1315–1327
75 Lutkenhaus, J.F. *et al.* (1980) Organization of genes in the *ftsA–envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus: *ftsZ*. *J. Bacteriol.* 142, 615–620
76 van de Putte, P. *et al.* (1964) The selection of mutants of *Escherichia coli* with impaired cell division at elevated temperature. *Mutat. Res.* 106, 121–128
77 Ayala, J.A. *et al.* (1994) Molecular biology of bacterial septation. In *Bacterial Cell Wall* (Ghuysen, J.M. and Habenbeck, R., eds), Elsevier Science, pp.73–101
78 Angert, E.R. and Clements, K.D. (2004) Initiation of intracellular offspring in *Epulopiscium*. *Mol. Microbiol.* 51, 827–835
79 Garrido, T. *et al.* (1993) Transcription of *ftsZ* oscillates during the cell cycle of *Escherichia coli*. *EMBO J.* 12, 3957–3965