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Review Article

REVIEW ARTICLE

Bacterial cell division: modeling FtsZ assembly and force generation from single filament experimental data

Pablo Mateos-Gil[1], Pedro Tarazona[2] and Marisela Vélez[3,*]

[1]Institute of Molecular Biology and Biotechnology, FO.R.T.H, Vassilika Vouton, 70013 Heraklion, Greece, [2]Condensed Matter Physics Center (IFIMAC) and Instituto de Ciencia de Materiales Nicolás Cabrera, Universidad Autónoma de Madrid, E-28049 Madrid, Spain and [3]Instituto de Catálisis y Petroleoquímica CSIC, c/ Marie Curie 2, Cantoblanco, 28049 Madrid, Spain

*Corresponding author: Instituto de Catálisis y Petroleoquímica CSIC, c/ Marie Curie 2, Cantoblanco, 28049 Madrid, Spain. Tel: +34 91 585 4802; Fax: +34 91 585 476; E-mail: marisela.velez@icp.csic.es

One sentence summary: This review summarizes recent experiments and theoretical models that address how the bacterial cytoskeletal protein FtsZ polymerizes and exerts force on the bacterial membrane during the process of cell division.

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ABSTRACT

The bacterial cytoskeletal protein FtsZ binds and hydrolyzes GTP, self-aggregates into dynamic filaments and guides the assembly of the septal ring on the inner side of the membrane at midcell. This ring constricts the cell during division and is present in most bacteria. Despite exhaustive studies undertaken in the last 25 years after its discovery, we do not yet know the mechanism by which this GTP-dependent self-aggregating protein exerts force on the underlying membrane. This paper reviews recent experiments and theoretical models proposed to explain FtsZ filament dynamic assembly and force generation. It highlights how recent observations of single filaments on reconstituted model systems and computational modeling are contributing to develop new multiscale models that stress the importance of previously overlooked elements as monomer internal flexibility, filament twist and flexible anchoring to the cell membrane. These elements contribute to understand the rich behavior of these GTP consuming dynamic filaments on surfaces. The aim of this review is 2-fold: (1) to summarize recent multiscale models and their implications to understand the molecular mechanism of FtsZ assembly and force generation and (2) to update theoreticians with recent experimental results.

Keywords: FtsZ; bacterial cytoskeleton; theoretical models; cell division; single filaments

INTRODUCTION

Dividing a cell into two daughter cells requires mechanical work. Eukaryotic cells use an ATP fueled cytokinetic ring self-assembled from actin and the motor protein myosin to achieve this task (Pollard 2010). In contrast, bacteria have no motor proteins involved in division. The contractile force required for cell partition is, at least partly, provided by the dynamic self-assembly of FtsZ (Osawa, Anderson and Erickson 2008; Mingorance *et al.* 2010; Lutkenhaus, Pichoff and Du 2012; Meier and Goley 2014; Erickson and Osawa 2017). Understanding the different ways nature uses to transform chemical energy into mechanical work constitutes an enormous challenge to which theoretical models have made important contributions. Models of both the assembly process and force generation mechanism can be built from different experimental information. Reaction rates obtained from experiments can feed kinetic models to propose

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possible paths by which FtsZ assembles and disassembles. Another approach is to use detailed structural and dynamic information of the protein aggregates and to build computational models based on interactions between neighboring monomers to explore how they associate. The system can be described focusing at different length scales. The aggregates can be described from an atomistic representation of the individual proteins, or at a larger scale using coarse-grained force fields in which several atoms or protein monomers constitute the beads that interact to form higher order organized structures. These multiscale computational methods are essential tools in the statistical physics of soft-condensed matter systems and are becoming increasingly useful in biophysical studies to picture and analyze quantitatively the collective behavior of protein assemblies (Ayton, Noid and Voth 2007; Ghosh and Sain 2008; Surovtsev, Morgan and Lindahl 2008; Allard and Cytrynbaum 2009; Paez *et al.* 2009; Sun, Walcott and Wolgemuth 2010; Dow *et al.* 2013; Stachowiak *et al.* 2014; Braun *et al.* 2016; Ruiz-Martinez *et al.* 2016). In particular, they have been very useful to understand the structure, assembly and function of tubulin microtubules (Fygenson, Braun and Libchaber 1994; Flyvbjerg, Holy and Leibler 1996; Chowdhury 2013).

FtsZ is a tubulin homolog that orchestrates bacterial cell division. It polymerizes to form a ring-like structure that is both a scaffold for the assembly of the bacterial cytokinetic machinery and an active element in cell constriction (Bi and Lutkenhaus 1991; de Boer, Crossley and Rothfield 1992; RayChaudhuri and Park 1992; Löwe and Amos 1998; Mukherjee and Lutkenhaus 1998; Nogales *et al.* 1998). During the first decade after the discovery of FtsZ in 1992, at least 10 proteins were identified to modulate the assembly and membrane attachment of the so-called Z-ring in order to constitute the functional division complex (Mosyak *et al.* 2000; Rueda, Vicente and Mingorance 2003; Adams and Errington 2009; Alexeeva *et al.* 2010; Mingorance *et al.* 2010; Natale, Pazos and Vicente 2013; Szwedziak *et al.* 2014; Haeusser and Margolin 2016; den Blaauwen, Hamoen and Levin 2017). However, experiments have shown that the complexity of the full Z-ring is not needed for membrane constriction. Experiments on reconstituted systems revealed that FtsZ alone can exert force and deform membranes through GTP-dependent polymerization and depolymerization (Osawa, Anderson and Erickson 2008, 2009).

In spite of great efforts in the last decade, both experimental and theoretical, the question of how this ancestral bacterial cytoskeletal protein self-assembles and generates a constriction force remains unanswered. Experiments describe cooperative assembly, dynamic monomer exchange, treadmilling, lateral bundling and monomer flexibility, a complexity that is difficult to include in a clear picture of how this monomeric GTPase self-associates, attaches and deforms the underlying membrane.

In the last 10 years, atomic force microscopy (AFM), single molecule fluorescence microscopy, electron microscopy and cryo-electron microscopy have provided high resolution and dynamic data of filaments, both on reconstituted systems and inside bacteria (Mingorance *et al.* 2005; Mateos-Gil *et al.* 2012a,b; Loose and Mitchison 2013; Kretschmer and Schwille 2016; Bisson-Filho *et al.* 2017; Wagstaff *et al.* 2017; Yang *et al.* 2017; Yao *et al.* 2017; Ramirez-Diaz *et al.* 2018). Furthermore, new information on the structure and dynamics of monomers constituting the filaments has also become available from mutational and structural analysis combined with computational studies (Martín-Galiano *et al.* 2010; Chen and Erickson 2011; Ramírez-Aportela *et al.* 2014).

We want to contribute to the 25th anniversary of the discovery of the prokaryotic cytoskeleton (Erickson 2017b), celebrated in 2017, by contrasting prevailing models with recent experiments to reevaluate our current understanding of the FtsZ bacterial dynamic filaments. The aim of this review is also to facilitate information flow between theoretical physicists and experimental microbiologists and biophysicist hoping this bridge will advance the understanding of this ancestral protein molecular machine.

MODELING THE FORMATION OF FTSZ PROTOFILAMENTS *IN VITRO*

Bacteria are small entities with a well-defined shape that cleave in the place where the division furrow is formed, with subsequent formation of two daughter cells. The relative simplicity of the system, as compared to more complex dividing mechanism of eukaryotic cells, has attracted the attention of theoretical biophysicists. Well-established physical membrane models allow simulating the deformations of a closed cylindrical membrane as a response to a localized tension. This review will not address models that consider the whole cell and the deformations induced by a constriction force generated by a localized ring. We will focus instead on modeling how FtsZ monomers assemble into filaments and how filaments condense and exert force on the underlying attached membrane. We will point out that the geometry of both protein filaments and lipid membrane could be essential to understand how this protein–lipid composite material participates in cell constriction. More information on modeling whole cell membrane deformation can be found in Hörger *et al.* (2010), Tsukanov *et al.* (2011), Almendro-Vedia, Monroy and Cao (2013), He *et al.* (2015), Picallo *et al.* (2015) and the references therein.

Filaments in solution

FtsZ filaments, both *in vivo* and *in vitro*, have been seen to aggregate into complex and dynamic structures. *In vivo*, the filaments can condense not only into rings, but also into spirals and arcs (Addinall and Lutkenhaus 1996; Margolin 2002; Michie *et al.* 2006). The Z-ring has a dynamic behavior and exchanges FtsZ monomers with ones found in the cytosol within seconds, as originally determined by fluorescence recovery after photobleaching of fluorescently-tagged FtsZ (Stricker *et al.* 2002). Filaments of FtsZ polymerized in solution *in vitro* were also found to exchange monomers and to assemble into a large variety of shapes, both as individual filaments and as filament aggregates. Transmission electron microscopy (TEM) showed curved, straight, single or double FtsZ filaments, as well as FtsZ assembled as filament sheets, toroids, helices or bundles in response to ion composition of the buffer as well as concentration and presence of crowding agents (Erickson and Stoffler 1996; Erickson *et al.* 1996; Löwe and Amos 1999; Hale, Rhee and de Boer 2000; Lu, Reedy and Erickson 2000; González *et al.* 2003; Marrington *et al.* 2004; Huecas *et al.* 2008; Popp *et al.* 2009, 2010; Milam, Osawa and Erickson 2012; Housman *et al.* 2016).

Experiments with light scattering, fluorescence anisotropy and fluorescence energy transfer (FRET) that confirmed the dynamic behavior of the filaments also revealed that polymerization only took place above a critical monomer concentration. This raised questions about the structure of the minimal polymerization unit, given that the simplest explanation to this cooperativity was to consider an initial nucleation that led the

formation of double filaments. Polymerization assays based on light scattering or sedimentation assays cannot quantitatively detect initial small-sized polymers. Careful fluorescence assays together with theoretical kinetic models that fitted experimental results were necessary to conclude that a monomer activation step could account for the cooperativity observed (Mukherjee, Dai and Lutkenhaus 1993; Bramhill and Thompson 1994; Erickson and Stoffler 1996; Erickson *et al.* 1996; Mukherjee and Lutkenhaus 1998; Mingorance *et al.* 2001; Romberg, Simon and Erickson 2001; Scheffers *et al.* 2002; Caplan and Erickson 2003; Chen *et al.* 2005; Miraldi, Thomas and Romberg 2008).

This is a good example of how kinetic models, assuming reaction rates governing different steps involved in the formation of the fibers, capture the polymerization behavior and contribute to rationalize experimental results. Initially, cooperative filament assembly was attributed to the presence of double filaments, similarly to what happens in actin (Frieden 1985). Evidence that filaments were single stranded (Mingorance *et al.* 2005) demanded alternative explanations. Models including a monomer activation step, nucleation and elongation of filaments were able to reproduce the kinetics of the initial seconds of polymerization. This led to the conclusion that a conformation change in the monomer as it polymerizes could account for the cooperativity observed in filament assembly (Chen and Erickson 2005; Chen *et al.* 2005; Miraldi, Thomas and Romberg 2008). Recent structural studies are documenting this proposed conformational switch associated to polymerization (Elsen *et al.* 2012; Wagstaff *et al.* 2017). Another important implication of this conformational switch is that it provides a potential explanation for how treadmilling might occur within a single-stranded filament. Treadmilling has been recently observed both *in vivo* an *in vitro*. Experiments using high-resolution fluorescence techniques have shown that it is associated with peptidoglycan synthesis in cells (Bisson-Filho *et al.* 2017; Yang *et al.* 2017). Filaments on supported lipid membranes also show complex asymmetric growth, as discussed in the next section.

Another intriguing aspect of FtsZ dynamic polymerization is its dependence on GTP hydrolysis (Mukherjee, Dai and Lutkenhaus 1993; Bramhill and Thompson 1994; Mukherjee and Lutkenhaus 1998). How is the GTP hydrolysis rate associated with filament integrity? Experiments designed to measure nucleotide turnover rate and content within the filament showed that GTP hydrolysis is the major rate-limiting step in GTP turnover. Phosphate and nucleotide exchange are significantly faster, meaning that FtsZ filaments predominantly contain GTP (Mingorance *et al.* 2001; Romberg and Mitchison 2003). A series of FRET experiments demonstrated that rapid *in vitro* subunit turnover took place both with and without nucleotide hydrolysis (Chen and Erickson 2005; Chen *et al.* 2005; Chen and Erickson 2009).

Taken altogether, this dynamic and structural experimental evidence confirms that, despite the structural homology of the FtsZ monomer with tubulin and frequent attempts to compare their behaviors, the strategies they follow to assemble and exert mechanical force are significantly distinct. One difference resides in how they couple the rates of GTP hydrolysis and nucleotide exchange with the resident time of monomers in the polymer. In tubulin, GTP hydrolysis is orders of magnitude faster than the life time of the assembled microtubule (Vandecandelaere *et al.* 1999), but the exchange of the hydrolyzed nucleotide is much slower (Piedra *et al.* 2016). FtsZ, however, has a much slower GTP hydrolysis rate, only 2-fold faster than monomer turnover time, and a much faster hydrolyzed nucleotide exchange (Romberg and Mitchison 2003). The rapid release of hydrolysis products from individual FtsZ filaments implies that the energy from GTP hydrolysis is quickly dissipated, in contrast to microtubules, where this energy from hydrolysis is stored as strain in the polymer (Caplow, Ruhlen and Shanks 1994) and released during disassembly to perform mechanical work. The other major difference is that FtsZ does not assemble into a predominant form, double filaments as actin or cylindrical tubes as tubulin. It condenses into larger bundles of varying number of individual filaments that can bend to different extents to form sheets, helices or toroids even in the absence of other proteins. A good way to describe this polymorphism is to picture the assemblies as soft fluctuating structures that can adopt a large range of shapes easily malleable through environmental conditions.

The question that arises then is how can these 'soft' filaments, formed through the hydrolysis and exchange of GTP and monomers, couple their highly dynamic and ductile behavior with the formation of a stable ring that exerts force? Modeling the assembly of FtsZ monomers into supramolecular structures can contribute to find an answer. To date, kinetic models have been most frequently used. They use kinetic data from filament polymerization and depolymerization, GTP hydrolysis and monomer exchange, complemented with structural data, e.g. filament curvature and lateral aggregation or bundling, to address questions regarding Z-ring formation and force generation mechanisms. They can be adapted to include or exclude different elements depending on the complexity of the experiments. For example, in order to account for the dynamic polymerization rates observed in bulk experiments, models do not need to include filament cyclization (Chen *et al.* 2005; Lan *et al.* 2008; Miraldi, Thomas and Romberg 2008; Ghosh and Sain 2011; Ruiz-Martinez *et al.* 2016). However, filament curvature is required to explain membrane constriction and the formation of a ring on the inner side of the bacterial membrane (Ghosh and Sain 2008; Surovtsev, Morgan and Lindahl 2008; Allard and Cytrynbaum 2009; Erickson 2009).

In spite of their relevance, kinetic models have important limitations. Filament formation involves many steps, so models require a large number of parameters. This leads to uncertainties that make their refinement difficult. Fig. 1 shows the FRET fluorescence signal registered during FtsZ polymerization (Chen *et al.* 2005). Fig. 1B shows how three different polymerization schemes, i.e. monomer activation followed by (i) polymerization of single-stranded filaments, (ii) simple bundling or (iii) multiple filament bundling, were successful to fit the same experimental data, highlighting the difficulty in discriminating between model assumptions. Other limitations of these models are that they cannot handle long times after the onset of assembly or the high FtsZ concentrations found *in vivo* (Lan *et al.* 2008). In addition, they fail to account for GTP hydrolysis or transformation of filaments into bundles. More complex models that consider protein binding to the membrane, GTP hydrolysis, cyclization (Surovtsev, Morgan and Lindahl 2008) and lateral bundling (Lan *et al.* 2008) are computationally prohibitive and can only be applied under restrictive conditions of single-polymer or simple bundling. One approach to overcome the need to include excessive information is the use of multiscale kinetic models for polymer assembly, reducing the complexity of the calculations while still accounting for bundling effects at high protein concentrations (Ruiz-Martinez *et al.* 2016). Notwithstanding these limitations, force generation models have mainly used kinetic models of filament assembly to explain constriction. We will come back to this issue in the next section.

In recent years, available high-resolution structural and dynamic data about filament formation have opened the door

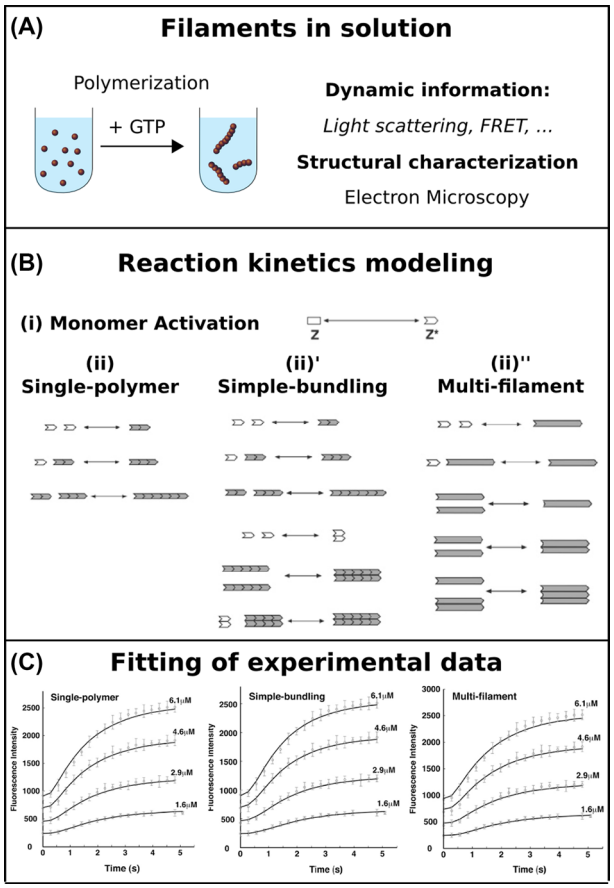

Figure 1. Modeling FtsZ polymerization in solution. Panel (**A**) shows a cartoon illustrating how experiments performed in bulk solution where FtsZ polymerization upon addition of GTP can be followed in real time using different techniques such as light scattering or FRET to extract dynamic information. In parallel, electron microscopy studies provide structural information on the filaments and FtsZ aggregates formed. Panels (B) and (C) depict three kinetic models proposed to explain the same experimental data obtained with FRET during the initial seconds of FtsZ polymerization triggered with GTP, adapted from Chen *et al.* (2005) and Lan *et al.* (2008). Panel (B) illustrates three different kinetic models considering a common initial stage of FtsZ monomer activation followed by three different scenarios considering (i) single polymerization, (ii) simple-bundling and (iii) multifilament aggregation. Panel (C) shows fitting of experimental data to the three alternative models depicted in (B) indicating the difficulty of kinetic models to discern between different mechanisms underlying the dynamic behavior of FtsZ.

to different modeling approaches successfully used in other scientific fields. In physics, chains of monomers in which bonds are broken and remade by thermal fluctuations are called 'living polymers'. These systems share some of the main characteristics of FtsZ filaments and the theoretical framework used to study them can be of great help to shed light into FtsZ dynamic behavior. These theoretical models can propose quantitative answers and suggest assembly mechanisms using the newly available structural and kinetic experimental information. The information about filament shape and size distributions can be used in multiscale molecular dynamics (MD) simulations that integrate information from different time and length scales. Models can include some details of the interactions to describe a specific behavior at the molecular scale, e.g. GTP hydrolysis at the FtsZ monomer–monomer interface, and a coarser model to accurately represent the higher order behavior observed, like the structures formed by the bundles of FtsZ filaments. The fact that the fine-grained details become irrelevant at a larger scale is useful to reduce the computational cost while still providing a conceptual clue of the 'universality class' to which the studied system belongs. Modern statistical physics establishes the existence of 'universality classes' in which the same collective behavior, e.g. a characteristic kind of phase transition, may arise from very different molecular interactions. The details of these interactions become 'irrelevant', except to provide values of some characteristic parameters, such as the temperature at which the transition appears or the value of the order parameter. In the same way, the atomistic description of FtsZ monomer–monomer interactions is irrelevant for some models in which the values of this monomer–monomer interaction are fine-tuned to reproduce the collective behavior observed in experiments (Paez *et al.* 2009; Gonzalez de Prado Salas *et al.* 2013, 2014; González de Prado Salas and Tarazona 2016). As high-resolution information comes preferentially from experiments on surfaces, it is better to discuss these models in that context.

Filaments on surfaces

Studies of FtsZ filaments adsorbed on surfaces revealed more dynamic and structural complexity than anticipated from bulk solution experiments. AFM and single molecule fluorescence microscopy provide real-time images of polymerization and depolymerization of filaments on surfaces (Mingorance *et al.* 2005; Lan *et al.* 2009; Mateos-Gil *et al.* 2012a,b; Encinar *et al.* 2013; Loose and Mitchison 2013; Márquez *et al.* 2017).

The first AFM images of single filaments adsorbed on mica in solution showed they were curved with a tendency to interact laterally (Mingorance *et al.* 2005; Hörger *et al.* 2008a,b; Hamon *et al.* 2009; Paez *et al.* 2009). More detailed analysis of the evolution of isolated individual filaments without lateral contacts on mica provided overwhelming evidence that filaments are curved in the presence of GTP and that all interfaces between adjacent monomers behave equally (Mateos-Gil *et al.* 2012b). Simulations showed that nucleotide exchange and monomer separation followed by fast reannealing of neighboring monomers reproduces experimental results. This GTP exchange rate, however, dependent on the kinetics of GTP hydrolysis and the size of the neighboring fragments, is associated to the probability at which irreversible breaking of the FtsZ bond appears.

A major surprise came when filaments were formed on supported lipid membrane with a well-defined orientation. Filaments adopted linear or curved shapes depending on the lipid composition (Mateos-Gil *et al.* 2012a; Encinar *et al.* 2013; Gonzalez de Prado Salas *et al.* 2013). The switch between straight and curved filaments, considered to be relevant for force generation (discussed below) previously associated only to the phosphorylated state of the GTP nucleotide, was found to be also sensitive to the orientation of FtsZ monomers on the surface (Osawa, Anderson and Erickson 2009; Gonzalez de Prado Salas *et al.* 2014).

Other experiments on lipid surfaces controlling monomer orientation also showed unexpected results. For example, filaments bound to the membrane through protein FtsA formed complex patterns such as fast-moving filament bundles and chiral rotating rings in the presence of GTP (Loose and Mitchison 2013). It was later shown that FtsZ anchored through ZipA, or even in its absence, also formed vortexes (Krupka *et al.* 2018; Ramirez-Diaz *et al.* 2018). This is a strong indication that this is an intrinsic property of FtsZ dependent on surface protein density, GTP hydrolysis and protein orientation, and not on the presence of the membrane anchoring protein. Simple models based on individual straight-curved filaments associated to GDP-GTP

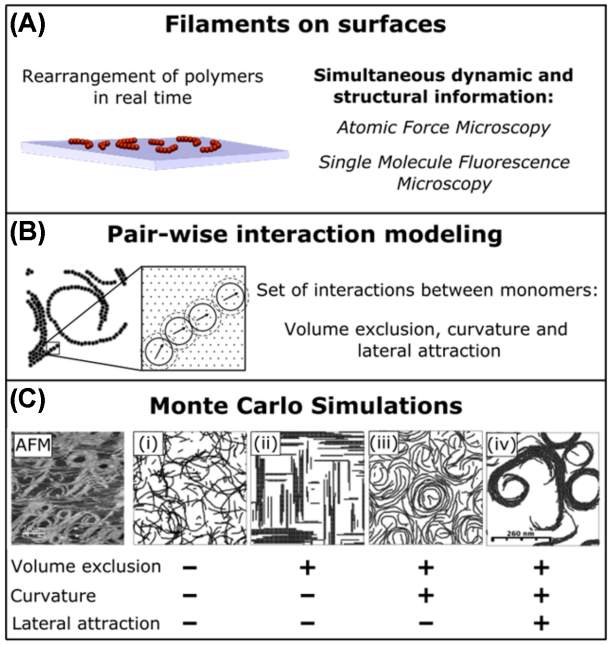

Figure 2. Modeling FtsZ filaments on surfaces. Panel (**A**) shows a cartoon illustrating experiments performed with atomic force microscopy or single molecule fluorescence microscopy allow to follow the reorganization of FtsZ polymers attached to surfaces in real time providing simultaneously dynamic and structural information. Panels (**B**) and (**C**) depict non-deterministic theoretical models used to extract quantitative information on the interactions between FtsZ monomers from experimental AFM images, adapted from Paez *et al.* (2009). Panel (B) shows FtsZ filaments formed by monomers positioned in a fine-grained lattice. Each monomer is defined as a coarse bead for volume exclusion, with a head-to-tail directionality and a surrounding area for lateral contacts, depicted with a solid circle, an arrow and a dashed circle, respectively. Instead of a fit, panel (C) shows Monte Carlo simulations where thousands of FtsZ monomers explore stochastically different configurations in absence (–) or presence (+) of different pairwise interactions proposed till the shapes and sizes of FtsZ filaments observed experimentally are reproduced.

content cannot explain this collective behavior, underlining the fact that the role of GTP is more complex than affecting only the bending angle between FtsZ monomer interfaces (Lu *et al.* 2000).

The new modeling approaches mentioned above now become essential to understand these complex results. Analyzing the shape of the filament aggregates observed on surfaces is done using soft matter models that feed a set of interaction energies between monomers into a Monte Carlo (MC) simulation that allows the system to evolve stochastically, instead of deterministically, to a minimal energy configuration at equilibrium. Fig. 2 illustrates how non-deterministic theoretical models can simulate experimental data providing quantitative information of monomer–monomer interactions (Paez *et al.* 2009). Fig. 2B shows that curvature and lateral interactions between monomers are enough to reproduce the experimental observations. The effective lateral interaction strength was estimated to be around 20 times less than the longitudinal bond energy, not a hundred times lower, as estimated indirectly (Lan *et al.* 2009) from fitting bulk polymerization experiments with a kinetic model (Lan *et al.* 2008). This quantitative estimate revealed the important role of lateral interactions in the formation of the Z-ring *in vivo*. When the parameters used to reproduce experimental results on planar surfaces were used in simulations of filaments on curved cylindrical surfaces resembling the bacterial membrane, lateral interaction between curved filaments were able to drive the formation of rings. Moreover, a small percentage decrease in the interaction between monomers outside the central region, which could be provided by a weak concentration gradient of other proteins perturbing the FtsZ monomer–monomer interaction, was enough to position the rings in the center (Paez *et al.* 2009). This illustrates how the balance of several comparable weak interactions, i.e. preferential curvature, longitudinal and lateral monomer–monomer interactions, can be tuned to generate a rich and adaptable behavior.

These models were taken a step further to understand the higher complexity of the results observed when the orientation of monomers relative to the surface affects the collective behavior of filament aggregates. Multiscale hierarchical modeling using coarse-grained models and computer simulations were used. First, the distribution of curvature and torsion angles between monomers were obtained from a MD simulation of a pentamer, run over 80 ns at an atomistic scale. This information was then used in much coarser MC simulations to explore the dynamic collective behavior of filaments and bundles, made by thousands of monomers, and to compare with AFM experiments on a scale of minutes or hours (Gonzalez de Prado Salas *et al.* 2014; González de Prado Salas and Tarazona 2016). MD at the atomistic scale showed the presence of a twist in the filament. The coarse-grained model showed that including this twist provided enough flexibility to the filament to adopt some of the conformations observed experimentally. The model also suggested that regulating the stiffness of the monomer attachment to the surface, and not only its orientation, could modulate the mechanical properties of the filament (Gonzalez de Prado Salas *et al.* 2014). Fig. 3 illustrates the elements used in this multiscale model. Fig. 3A shows the information obtained from model MD simulations at an atomistic scale, and Fig. 3B depicts MC simulations integrating the bending and torsion angles obtained from MD simulations.

These studies revealed, for the first time, that the interaction of the filaments with the neighboring surface plays an important role in defining polymer structure and dynamics. Two new elements essential to explain the observations stood out as relevant: the presence of a twist in the free-standing filaments and the importance of stiffness in the attachment of the oriented monomers to the underlying lipid surface. The details of the monomer attachment become relevant to modulate the competition between the curvature and twist of the filaments near the surface (Gonzalez de Prado Salas *et al.* 2014; González de Prado Salas and Tarazona 2016), which also strongly affect the tension exerted on the underlying membrane, as will be discussed in the following section.

A robust and comprehensive model should be able to explain the observed structural diversity of filaments on surfaces and also their dynamic behavior. It is therefore interesting to contrast the model with the complex dynamic rearrangements observed *in vitro* on supported lipid membranes (Loose and Mitchison 2013; Ramirez-Diaz *et al.* 2018). Two theoretical approaches have been used to explain these experimental observations (Denk *et al.* 2016; González de Prado Salas and Tarazona 2016). One describes the FtsZ filaments as an 'active matter' system (Marchetti *et al.* 2013), i.e. as elastic polymers presenting purely steric repulsions with fixed intrinsic curvature that follow Brownian movements with a drive to maintain constant tangential velocity (Denk *et al.* 2016). The model cannot provide mechanistic insight into how the known properties of FtsZ polymerization contribute to the rotation assumed to be present in the system. Nevertheless, disregarding the underlying mechanisms, this model provides evidence that this active rotation

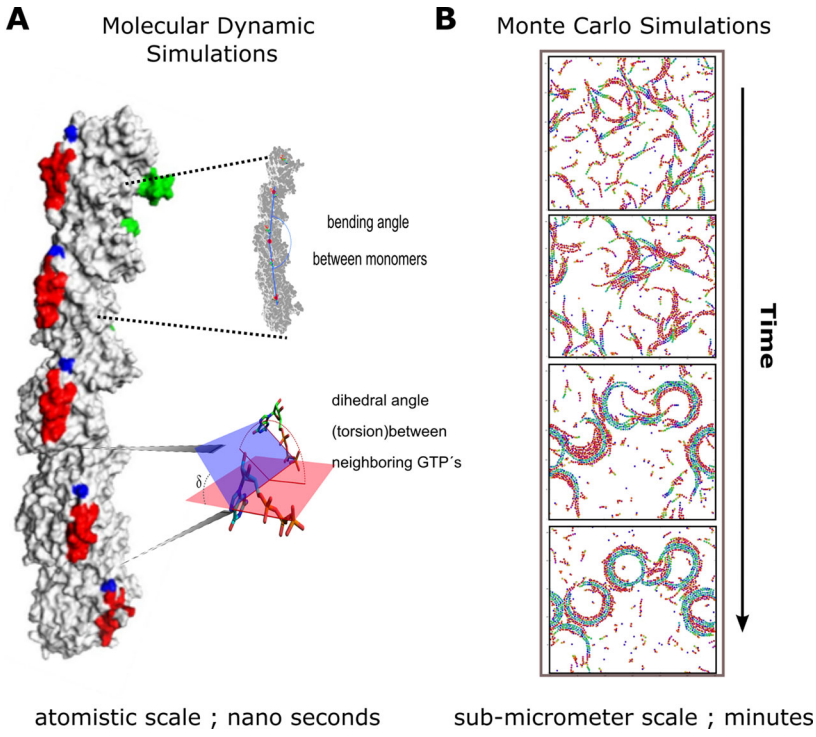

Figure 3. Multiscale modeling of FtsZ filament. Panel (**A**) shows the information obtained from molecular dynamics (MD) simulations at atomistic scale where the bending curvature and torsion angle between FtsZ monomers within a pentamer were obtained during several nanoseconds, adapted from Gonzalez de Prado Salas *et al.* (2014). Panel (**B**) depicts monte carlo (MC) simulations integrating the bending and torsion angles obtained from MD simulations to evaluate the evolutions of filaments on a surface for a certain surface concentration. Dynamic bundles reorganize and condense into circular aggregates. Each monomer color represents a different anchoring angle of the monomers to the surface, adapted from González de Prado Salas and Tarazona (2016). Simulation box in (**B**) 0.3 μm $\times$ 0.26 μm.

produces rings and C-shaped filament aggregates similar to those observed for FtsZ on mica and reproduced in MC simulations including lateral attraction between filaments (Paez *et al.*, 2009). The rotation of the filaments produces the circular motion of isolated rings at low protein density. In contrast, at higher densities, rings are arrested by the formation of jammed bundles, also similar to those formed in models of 'non-active' but interacting filaments.

The second approach is based on the previously described multiscale model that includes the torsional structure of bonds between monomers, the formation of live filaments that break and anneal under thermal equilibrium fluctuations, and the condensation into polymorphic bundles due to lateral attraction between filaments (González de Prado Salas and Tarazona 2016). The qualitative addition to the previous models describing the structures formed by FtsZ adsorbed on mica is that the anchoring to the substrate is considered to act as a soft angular spring on a random fraction of the protein monomers, rather than as a strong restriction on the orientation for all the monomers along the filament (see Fig. 3B). This generates a subtle interplay between the anchoring with the optimal curvature and torsion. Altogether, this approach shows that flexible monomers that interact longitudinally with a twist and a preferential curvature with transient interactions simulating GTP hydrolysis can originate dynamic bundles that condense into circular aggregates when assembled on a two-dimensional surface.

Interestingly, the experimentally observed rotating vortexes do not appear if the simulations are restricted to a two-dimensional surface (González de Prado Salas and Tarazona 2016). However, ongoing refinement of this theoretical model indicates that when the filaments are only partially linked to the membrane, with one free end that can move away from it, an asymmetry that could originate rotation becomes apparent. The geometric constraints on the curved and twisted filaments attached through a flexible linker to the membrane could favor successive monomer addition on one end and depolymerization on the other. Fig. 4 shows a cartoon that illustrates this concept, which still requires experimental and theoretical verification. Fig. 4A depicts the geometry of *in vitro* experiments on a flat surface (Loose and Mitchison 2013; Ramirez-Diaz *et al.* 2018). The spontaneous torsion of the filament renders a chiral orientation that could induce asymmetric growth, strongly dependent on surface protein and GTP concentration, as well as on the flexibility of the anchor, as has been observed experimentally. In those experiments, the observed treadmilling, i.e. preferential growth on one end of the filament bundles, appeared only at an intermediate range of protein concentration on the surface and high GTP concentration, suggesting that polarized growth is only present when protein monomers coming from the surface and from solution incorporate at a different rate to the growing filament end. Furthermore, the observed changes in the direction of the rotation could be due to the large sensitivity of the system to membrane attachment. If FtsZ is bound through the amino terminal region, presumably more sterically hindered, the rotation follows the anticlockwise direction of the filament twist. If it is bound through its carboxy-terminal domain that contains the flexible region to space the protein from the membrane, this looser attachment could balance the effect of the anticlockwise twist (Gonzalez de Prado Salas *et al.* 2014).

The additional geometric constraint described above could add directionality to simulations that already reproduce observed breaking, annealing and condensation of flexible curved

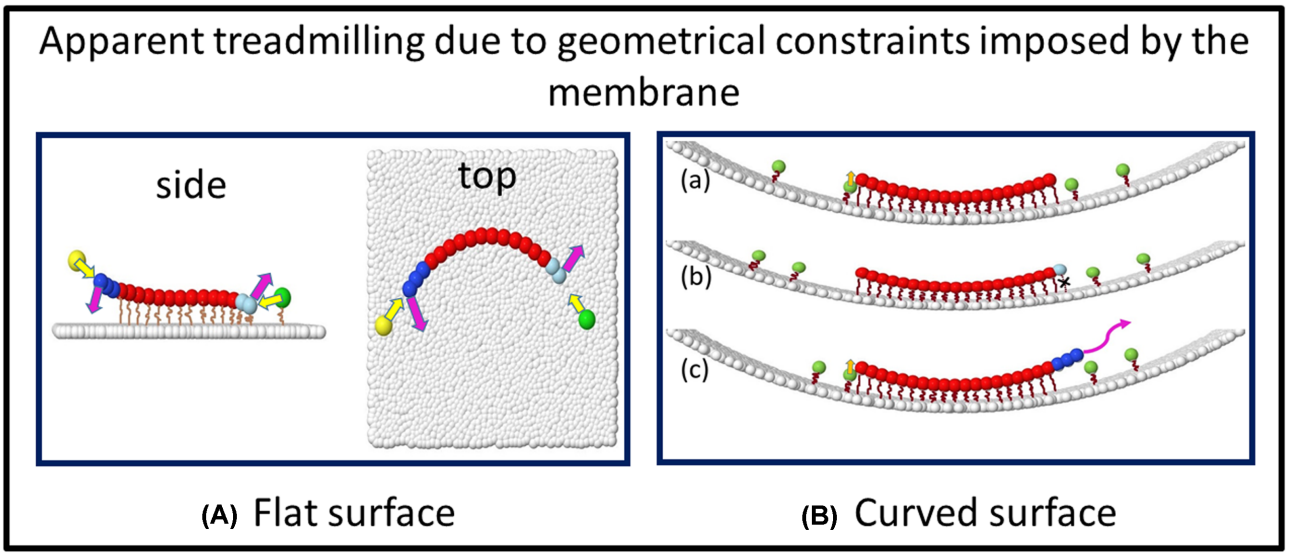

Figure 4. Apparent treadmilling due to geometric constrains imposed by the membrane. Panel (**A**) illustrates how the partial attachment of the filament to the membrane could originate an asymmetry in the polymerization rate at the ends of the filament on a flat surface. The yellow arrows indicate polymerization and magenta depolymerization. The red monomers are attached to the membrane, and the dark blue monomers to the least restricted end that could in principle grow faster. Panel (**B**) shows the corresponding effect on a curved surface, in which the addition of a monomer at one end (**a**) generates a tension at the other end (**b**) facilitating attachment of a new monomer to the same end until a depolymerization event brings back the symmetric (fully linked) filament (**c**).

dynamic bundles that close to form rings (González de Prado Salas and Tarazona 2016). It could also provide an explanation to the GTP and protein concentration dependence of the chiral rotating vortexes, as well as the dynamic and reversible directionality of the fast-moving filaments bundles that coexist with them (Loose and Mitchison 2013). Rotating vortexes are observed only over a transient time, and depending on the protein accumulated at the surface. At very high protein concentration filaments formed a three-dimensional network, supporting the idea that partially attached filaments can grow away from the surface under certain conditions (Ramirez-Diaz *et al.* 2018).

The same line of thought adapted to a different geometry can be applied to explain *in vivo* observations, when filaments are attached to the interior of the cell membrane (Bisson-Filho *et al.* 2017; Yang *et al.* 2017). Fig. 4B illustrates a plausible explanation for reversible treadmilling of filaments semi-attached to a curved membrane. In this case, the membrane curvature introduces a mismatch between the relaxed linker of the free monomer and the tense linker at the end of the filament. When a monomer attaches to one end, it tenses the filament asymmetrically, facilitating further growth on that end and breakage on the opposite one. This asymmetry would be fully reversible, after a depolymerization event of the loose tail, strongly dependent on the details of the attachment and filament length, and would also coexist with filament bundles breaking and annealing, as observed *in vivo* (Bisson-Filho *et al.* 2017; Yang *et al.* 2017). The consideration that a geometric mismatch between the filament and membrane curvatures contributes to generate an apparent treadmilling does not contradict the hypothesis of an intrinsic polarity of filaments growth recently suggested (Wagstaff *et al.* 2017), and both elements could be relevant *in vivo*.

A pertinent question to ask is to what extent the multiscale model described above, developed from experiments performed in model systems, supports experimental evidence from *in vivo* or other *in vitro* studies? This will help assess its potential contribution to understand FtsZ function inside the cell. The strength and orientation of filament attachment highlighted as important in the model have been also identified as relevant in *vivo*. There is conclusive evidence that a flexible tether between FtsZ and the membrane is essential to sustain bacterial division (Buske and Levin 2013; Gardner, Moore and Erickson 2013; Buske *et al.* 2015; Sundararajan *et al.* 2015). This anchoring flexibility could come partly from an intrinsically disordered region present in FtsZ between the protofilament-forming globular domain and the C-terminal (Ct) peptide that binds FtsA and ZipA and tethers FtsZ to the membrane (Gardner, Moore and Erickson 2013) and partly from flexible regions in the membrane anchoring proteins (Ohashi *et al.* 2002).

The presence of a twist in the FtsZ filaments, i.e. the other new element introduced in the model to account for the single filament experimental results, has also been suggested previously from *in vitro* studies. Both MD simulations (Hsin, Gopinathan and Huang 2012) and experiments (Arumugam *et al.* 2012; Wagstaff *et al.* 2017) described a filament twist, but its impact on the structure and mechanical properties of filament aggregates was not explored. It is tempting to suggest that this filament twist, due to a 20º rotation angle around the longitudinal axes of two neighboring monomers, could be associated to the large flexibility between the two monomer domains recently described. MD simulations (Hsin, Gopinathan and Huang 2012; Natarajan and Senapati 2013; Ramírez-Aportela *et al.* 2014) and mutational studies (Díaz-Espinoza *et al.* 2007; Martín-Galiano *et al.* 2010; Chen and Erickson 2011; Matsui *et al.* 2014; Montecinos-Franjola *et al.* 2014; Wagstaff *et al.* 2017) have addressed the important question of how monomer conformation, in its GDP or GTP forms, determines the cooperative assembly and the curved and straight protofilament configuration. Recent bioinformatics analysis and experiments also suggest that the interdomain interaction is stronger than the interaction of the protofilament longitudinal interfaces involved in the GTPase activity of FtsZ (Díaz-Espinoza *et al.* 2007; Martín-Galiano *et al.* 2010; Matsui *et al.* 2014). The N-terminal GTP-binding domain and the C-terminal hydrolysis activation domain can rotate and shift apart, giving rise to a closed conformation in the

isolated monomer and an open conformation associated to the monomer in the protofilament. This conformational switch, in FtsZ from *Staphylococcus aureus,* can imply rotations of the N-terminal GTP-binding domain and the C-terminal GTPase activation domain of up to ∼27° (Matsui *et al.* 2012; Wagstaff *et al.* 2017). A conformational switch has also been observed in FtsZ from *Mycobacterium tuberculosis* (Leung *et al.* 2004).

High-resolution data of the FtsZ monomer conformation within the protofilament architecture are starting to become available (Wagstaff *et al.* 2017). In this study, TEM micrographs show that mutations that modify monomer flexibility affect filament morphology, but it is still not clear how different monomer conformations relate to the functionally relevant collective mechanical, structural and dynamic properties of protofilament aggregates. It has been suggested that this conformational switch could favor treadmilling (Wagstaff *et al.* 2017), and most likely, other properties such as the bending rigidity or the overall filament shape would also respond to monomer flexibility. It will be interesting to use advances in structural techniques, such as solid-state NMR (Habenstein and Loquet 2016), that can provide atomistic resolution of large macromolecular assemblies associated to membranes in liquid to fill this gap. This information could relate high-resolution structural and dynamic information of the monomers to that of the filament aggregates and provide clues about how monomer flexibility underlies the extremely rich structural and dynamic behavior of FtsZ filaments.

MODELING FORCE GENERATION

FtsZ filaments have been considered, until recently, the main force generation elements in bacterial cell division (Erickson and Osawa 2017). However, experiments *in vivo* using quantitative super-resolution microscopy propose that cell wall synthesis and chromosome segregation are the limiting processes of cytokinesis, suggesting that the main role of FtsZ is to recruit all divisome proteins (Coltharp *et al.* 2016; Xiao and Goley 2016; Coltharp and Xiao 2017). Nevertheless, whether it provides the full force for cell division or not, *in vitro* experiments on artificial membrane systems have shown that only FtsZ is capable of deforming the membrane (Osawa, Anderson and Erickson 2008, 2009; Osawa and Erickson 2018). Moreover, it is widely accepted that nucleotide-dependent dynamic assembly of eukaryotic cytoskeletal proteins such as actin and tubulin can exert mechanical forces in different ways (Dogterom *et al.* 2005; Murrell *et al.* 2015). Although other processes could play a relevant role in separating the two newly formed bacterial cells, there is convincing evidence that FtsZ filament aggregates assembled in the Z-ring modify membrane shape. An interesting option is that force exerted by FtsZ on the inner side of the cell membrane could serve not only to divide the cell, as previously thought, but also as a mechano-transducer to activate and/or regulate the cell wall synthesis machinery located in the periplasmic space, i.e. across the cytoplasmic membrane (Gray *et al.* 2015; Coltharp and Xiao 2017).

The fact that FtsZ requires no other protein to form a contractile ring (Osawa, Anderson and Erickson 2008) has inspired much work to explain and model how the dynamic self-assembly of protofilaments can exert force on an underlying membrane (Lan, Wolgemuth and Sun 2007; Ghosh and Sain 2008; Hörger *et al.* 2008a; Surovtsev, Morgan and Lindahl 2008; Allard and Cytrynbaum 2009; Erickson 2009; Lan *et al.* 2009; Paez *et al.* 2009; Ghosh and Sain 2011; Gonzalez de Prado Salas *et al.* 2014).

Role of filament curvature and lateral interactions

Deterministic kinetic models that propose force generation mechanisms have put major emphasis on the longitudinal interaction between the monomers, where the phosphorylated nucleotide is located. Hydrolysis takes place in the pocket formed by neighboring monomers (Romberg and Mitchison 2003), so force generation has been attributed to conformational changes at monomers interface associated with nucleotide hydrolysis. Early electron microscopy studies showed that the curvature of individual filaments was associated with GTPase activity (Lu *et al.* 2000), indicating that GTP favored a straight filament conformation and GDP a curved one. This information suggested a 'hydrolyze and bend' simple model, where the curving of the filament was responsible for the filament 'power stroke' needed to drive constriction (Allard and Cytrynbaum 2009; Erickson 2009). However, experimental evidence indicating that the FtsZ-GDP form is curved and the FtsZ-GTP bound is straight is not conclusive, and the relation between monomer structure and nucleotide phosphorylated state is revealing to be more complex. X-ray crystallography analyzing FtsZ from different organisms found no evidence of a monomer conformational change due to nucleotide phosphorylation state (Oliva, Trambaiolo and Löwe 2007). More recently, structural, mutational and computational studies highlight the importance of monomer flexibility and reveal that conformational changes associated to nucleotide state reside mostly in the motion between the N-terminal and C-terminal domains of the protein and are transmitted to neighboring monomers through the T7 synergy loop (Matsui *et al.* 2012, 2014; Wagstaff *et al.* 2017). This implies that the conformational switch is due to polymerization, not to nucleotide hydrolysis, and is based on the monomer internal flexibility and not on the interfacial region, as has been also suggested (Hsin, Gopinathan and Huang 2012; Li *et al.* 2013; Wagstaff *et al.* 2017).

However, if this GTP-dependent structural change is the main source of force generation, it has to be associated with monomer exchange occurring on the timescale of seconds (Stricker *et al.* 2002). Mathematical, computational and deterministic models including kinetic parameters to account for the GTP hydrolysis rate, mechanisms of filament attachment to the surface, rigidity of the filaments, protein distribution between the membrane and the cytosol, and preferential curvature of the FtsZ filaments, have been proposed to explain how monomer assembly and disassembly could account for generating force (Ghosh and Sain 2008; Surovtsev, Morgan and Lindahl 2008; Allard and Cytrynbaum 2009; Erickson 2009). In most of these models, the force generating capacity was mainly attributed to bending of the filaments, not always assumed to be coupled to GTP hydrolysis (Ghosh and Sain 2011). Other models can explain force generation by only consider bundling and cross-linking between cytoskeletal filaments (Drew *et al.* 2009; Sun, Walcott and Wolgemuth 2010).

Another set of models emphasizes the role played by lateral interactions between filaments (Lan *et al.* 2009; Monahan, Robinson and Harry 2009; Paez *et al.* 2009) in condensing the filaments into higher order structures and in stabilizing filament curvature. Additionally, lateral interactions could also contribute to contraction of the FtsZ ring by promoting sliding to increase the number of lateral bonds. As mentioned in the previous section, *in vitro* single filament experiments confirm that both preferential curvature and lateral interactions, only one order of magnitude weaker than the longitudinal interactions, are necessary to reproduce the experimental results, suggesting that both types of interactions are likely to play an important role *in vivo*.

In addition to proposing mechanisms behind force generation, models also allow a quantitative estimate of the forces generated. They support the assumption that a dynamic filament subject to continuous monomer exchange, accompanied by changes in the curvature, could account for the generation of a 20–90 pN bending force on the surface. Although this is enough to deform a membrane in reconstituted systems, it might not provide the ≥ 400 pN to invaginate the cell envelope including the peptidoglycan layer and compensate for turgor pressure (Lan, Wolgemuth and Sun 2007; Jiang *et al.* 2011; Banerjee, Scherer and Dinner 2016). However, it has been recently suggested that this turgor pressure might not play an important role in cell division and that constricting the inner membrane and building new peptidoglycan to form a septum might take place within a high osmolarity environment, in which case 20–90 pN generated by the FtsZ ring would be sufficient (Erickson 2017a). Existing models do not yet provide an unambiguous explanation of how the balance between curvature, lateral interactions, dynamic polymerization and filament bundling lead to generate force. Different alternatives provide plausible scenarios, so more quantitative experimental results associating membrane deformations with high spatial and time resolution of the FtsZ polymers bound to a membrane or the contribution of additional elements would be needed to discard alternatives and advance the field.

Role of the membrane: an underappreciated element

It was shown in the section 'Filaments on surfaces' how constraining filaments to a two-dimensional surface offers new possibilities to guide and modulate FtsZ filament plasticity. Considering that inside the bacteria, filaments are attached to the cell membrane surface to perform their biological role, it is not far-fetched to assume that this configuration also participates in modulating the constriction force. Until now, most of the force generation models extrapolated data obtained from solution experiments (curved to straight nucleotide-dependent switches, lateral associations) and the surface was mostly considered as a passive support.

The emerging picture generated from experiments and theoretical models discussed in the previous section indicates that the structural and dynamic plasticity rooted in the intrinsic flexibility of the monomers, their nucleotide dependent dynamic, longitudinal and lateral interactions, and their flexible anchoring to the surface can be strongly modulated by the geometric restrictions imposed by the surface attachment. As a consequence, the proximity of the surface can regulate the curvature, rigidity and dynamic behavior of the membrane-anchored filaments. Moreover, the presence of this soft and malleable protein material at the membrane interface should also modulate physical membrane parameters such as preferential curvature or bending energies, both important for membrane constriction (Hörger *et al.* 2010).

FtsZ monomers are linked to the surface always with the same orientation through the conserved C-terminal peptide that binds to ZipA or FtsA, restricting the rotational mobility of individual monomers with the possibility of canceling out their twist. The energy required to untwist the filament is estimated to be lower than the bending energy (Gonzalez de Prado Salas *et al.* 2014). This implies that if the filaments are attached to the membrane through the C-terminal region, leaving the preferential curvature lying perpendicular to the membrane surface, the filaments would stiffen, exerting a force caused by their curvature oriented perpendicular to the membrane. This scenario is compatible qualitatively with experimental results with FtsZ oriented and attached to reconstituted membrane systems (Osawa, Anderson and Erickson 2009). The switch from straight to curved conformation could be now triggered by the strength of the attachment to the membrane, instead of solely because of nucleotide hydrolysis. Fig. 5 summarizes this new concept of including filament torsion, curvature and flexible attachment to the surface in the model to explain how FtsZ could exert force on the underlying membrane.

Filaments manifest a wealth of collective energy-dependent emergent properties characteristic of other active soft matter systems: they condense to form aggregates, adopt different configurations, respond to protein and GTP concentration, surface lipid composition, orientation of surface attachment, interaction with protein partners, or the geometry and curvature of the surface (Mateos-Gil *et al.* 2012a; Loose and Mitchison 2013; Marchetti *et al.* 2013; Arumugam, Petrášek and Schwille 2014; Zieske 2014; de Prado Salas *et al.* 2015; Kretschmer and Schwille 2016; Ramirez *et al.* 2018). The condensation of FtsZ filaments near the surface could modulate their curvature, mechanical properties and growth, as well as membrane properties, to facilitate local deformations. The details of how monomers are exchanged from individual filaments or whether or not they form a continuous ring might lose relevance in favor of the importance of the overall collective properties of the soft and dynamic protein lattice coupled to the membrane. It is interesting to note that the underlying lipid membrane is also a soft deformable material. It is likely that to fully understand the way that force is generated and transmitted along and across the lipid membrane we will have to include in the picture the crosstalk between the organization of membrane lipids and the FtsZ filaments forming on their surface (de Prado Salas *et al.* 2015). The membrane lying underneath the Z-ring seems to be enriched in cardiolipin (Matsumoto *et al.* 2006), although the consequences of this enrichment on local membrane properties are not well understood.

Recent high-resolution analysis of the septal ring structure and function *in vivo* have highlighted its complexity, and the new elements included in this new model could provide clues to understand some of the observations. One of the questions that has not yet found a definite answer is whether the ring is continuous or not and whether it extends or not into the center of the cell. Forces generated by this new proposed mechanism would be independent of the formation of a continuous ring lying close to the membrane surface. Current observations of ring structure are conflicting. Some high-resolution structural *in vivo* studies, using cryo-EM (Li *et al.* 2007; Szwedziak *et al.* 2014), indicate the presence of long continuous filaments, but other experiments using super-resolution fluorescence microscopy (Fu *et al.* 2010; Holden *et al.* 2014; Rowlett and Margolin 2014; Coltharp *et al.* 2016; Holden 2018) and also cryo-EM (Li *et al.* 2007) indicate that the ring is not continuous. The Z-ring also appears to extend into the center of the cell with a density and continuity that fluctuate depending on FtsZ and GTP concentration, looking more like a ring divided in patches, (Strauss *et al.* 2012; Piro *et al.* 2013; Lyu *et al.* 2016; Söderström and Daley 2017; Vedyaykin *et al.* 2016). There is already considerable evidence that force generation does not require full ring formation. For example, non-ring FtsZ structures can promote asymmetric constriction in a number of cases (Addinall and Lutkenhaus 1996; Yao *et al.* 2017) and non-ring-forming FtsZ has also been described in symbiotic bacteria (Leisch *et al.* 2016).

The highly dynamic character of the ring inside the cell as well as a large plasticity in its orientation and surface

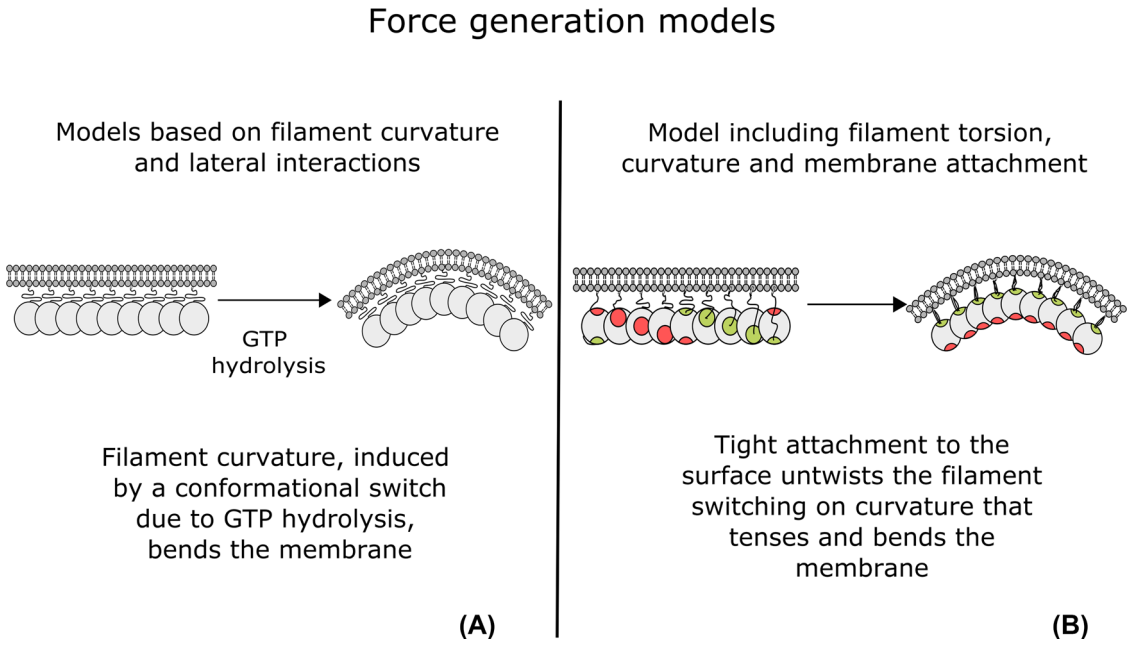

Figure 5. Force generation models. The cartoon depicts a simplified version of the two main concepts referred to in the text to account for the force exerted by FtsZ polymers on the membrane. Panel (**A**) refers to models that consider filament curvature switched on by GTP hydrolysis and filament bundling due to lateral interactions cause membrane bending (Allard and Cytrynbaum 2009; Erickson and Osawa 2017). Panel (**B**) illustrates the concept of how, including filament torsion, curvature and flexible attachment to the membrane could provide a way to switch filaments into a force generating configuration (Gonzalez de Prado Salas *et al.* 2014). The figure is not drawn to scale, and the membrane curvature is exaggerated to highlight the main concept.

attachment predicted from the model are also supported by experiments *in vivo*. The treadmilling of FtsZ associated with synthesis of peptidoglycan at the division site (Bisson-Filho *et al.* 2017; Yang *et al.* 2017) could be explained by the model, although work in progress will refine the details. Polarized fluorescence microscopy data suggest a disordered organization indicating that some filaments, or at least some monomers within the filaments, are aligned in the cell-axis direction and the orientation appears to depend on the bacterial species (Si *et al.* 2013).

The scenario created by the model suggests an additional way to tune or trigger the force exerted by the filaments on the surface: controlling the attachment strength of the filaments to the membrane. As mentioned above, there is evidence for the importance of the non-structured region *in vivo*. Moreover, it is also known that the Z-ring forms in the cell before constriction begins (Den Blaauwen *et al.* 1999; Tsukanov *et al.* 2011). Therefore, the ring assembles and remains in latent form until constriction is triggered, probably by interaction with proteins that bundle and restrict the flexibility of the non-structured anchoring domains (Den Blaauwen *et al.* 1999; Aarsman *et al.* 2005; Meier and Goley 2014; Eun *et al.* 2015; Haeusser and Margolin 2016; Holden 2018).

The multiscale model also enriches our picture of how the multiple interactions between monomers and filaments participate in the generation of complex 'soft structures' and might help to understand why experiments that explore the effect of mutants in cell division are so difficult to predict. The picture of filaments constituted by simple stacking of subunits on top of each other and interacting laterally is not consistent with various ambiguous results. For example, the effect of mutants in the longitudinal interface shows that protofilaments, unexpectedly, require a functional interface at both the top and bottom of the monomer in order to allow for cell division (Redick *et al.* 2005). This might reflect the relevance of recent results underlining the importance of monomer flexibility. Bottom mutants could affect the internal flexibility of the monomer by indirectly altering interactions at sites located away from the mutation site.

Defining the nature and role of lateral interactions has also remained elusive. A straightforward association between their effect on *in vitro* bundling and their role *in vivo* has been difficult to establish. Lateral interfaces between FtsZ protofilaments have been extensively probed and several residues have been genetically implicated in lateral interactions (Lu *et al.* 2001; Stricker and Erickson 2003; Koppelman *et al.* 2004; Jaiswal *et al.* 2010; Shin *et al.* 2013; Haeusser, Rowlett and Margolin 2015; Márquez *et al.* 2017; Moore *et al.* 2017). However, some results are ambiguous and it is difficult to relate alterations in the tendency to form protofilament bundles *in vitro* with their function *in vivo*. Recent structural analysis detected two different weak interprotofilament lateral interfaces (Guan *et al.* 2018) that were demonstrated to be relevant *in vivo*.

The authors propose that transient lateral interactions, rather than mediating the formation of stable and static higher order architectures, induce changes in treadmilling velocities of single protofilaments. This hypothesis is more in agreement with the ideas proposed by the multiscale model that suggests that monomer flexibility could be associated with filament twist, implying that lateral interactions are probably more complex and elusive than previously thought. Depending on the monomer conformation, different lateral interactions might be favored over others (Gonzalez de Prado Salas *et al.* 2013). Some could affect the degree of untwisting and vice versa, giving rise to different degrees or types of bundling, probably affecting GTP hydrolisis/exchange rates and filament dynamics. This might indicate that not all the bundles are equivalent, explaining why

so many different proteins have been found to bundle FtsZ filaments (Huang, Durand-Heredia and Janakiraman 2013). It might be interesting to consider that lateral mutants could also affect flexible regions needed for monomer conformational switches.

PERSPECTIVES AND CONCLUSIONS

Bringing physics tools and concepts to study FtsZ filaments has generated a multiscale model that constitutes a rational framework to understand the peculiarities of the bacterial cytoskeletal protein FtsZ. Its more extensively studied eukaryotic homolog, tubulin, self-assembles into microtubules of well-defined shapes allowing the use of relatively simple models to analyze their polymerization and depolymerization. In contrast, FtsZ assembles into flexible and polymorphic structures. The formation of protofilament aggregates and bundles *in vitro*, and the full septal ring *in vivo*, is produced by the entangled effects of several soft driving forces leading to different forms and dynamic effects depending on the conditions: formation and breaking of 'living-polymer' protofilaments tied to the GTP-GDP hydrolysis cycle, condensation of those filaments into bundles and larger structures through lateral interactions, balance between the spontaneous curvature and torsion of the filaments, anchoring to the membrane through membrane proteins and the potential remodeling of the membrane itself. Cumulatively, these effects likely result in a complex landscape of possible soft structures, connected by multiple dynamic paths, which do not exclude each other. The efficiency of each pathway can be affected by different control mechanisms in various ways, and the overall robustness and plasticity of the system comes precisely from both the softness of the structures and the entanglement of the drives that control their pathways.

Statistical physics models for FtsZ over the last decade have shown that such soft polymorphism and entangled pathways observed experimentally may be reproduced in MC simulations, with relatively simple models, as long as the interaction parameters remain within a restricted range of values. Outside that range we find either very disordered structures or 'too ordered', i.e. rigid, aggregate structures, like the microtubules formed by tubulin with very well defined paths of assembly and disassembly. We may speculate that it is precisely the bacterial 'choice for robustness through softness' what has driven the biological evolution of FtsZ and its associated proteins. The picture emerging from the rapidly growing experimental evidence, both *in vivo* and *in vitro*, may benefit of this more flexible perspective, rather than trying to fit the results into more rigid and deterministic concepts that were useful to understand eukaryotic structural proteins but do not seem to apply to this bacterial cytoskeletal protein.

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