Modeling the physics of FtsZ assembly and force generation

Harold P. Erickson[1]

Department of Cell Biology, Box 3709, Duke University Medical Center, Durham, NC 27710

Edited by J. Richard McIntosh, University of Colorado, Boulder, CO, and approved April 24, 2009 (received for review February 28, 2009)

The tubulin homolog FtsZ is the major cytoskeletal protein in bacterial cytokinesis. It can generate a constriction force on the bacterial membrane or inside tubular liposomes. Several models have recently been proposed for how this force might be generated. These fall into 2 categories. The first is based on a conformational change from a straight to a curved protofilament. The simplest ''hydrolyze and bend'' model proposes a 22° bend at every interface containing a GDP. New evidence suggests another curved conformation with a 2.5° bend at every interface and that the relation of curvature to GTP hydrolysis is more complicated than previously thought. However, FtsZ protofilaments do appear to be mechanically rigid enough to bend membranes. A second category of models is based on lateral bonding between protofilaments, postulating that a contraction could be generated when protofilaments slide to increase the number of lateral bonds. Unfortunately these lateral bond models have ignored the contribution of subunit entropy when adding bond energies; if included, the mechanism is seen to be invalid. Finally, I address recent models that try to explain how protofilaments 1-subunit-thick show a cooperative assembly.

cooperativity | mathematical model | tubulin

FtsZ is the major cytoskeletal protein in bacterial cytokinesis. It assembles into a ring, called the Z ring, which attaches to the membrane at the center of the bacterium. The Z ring constricts to divide the cell. In addition to FtsZ, *Escherichia coli* has a dozen other proteins that associate with the Z ring and are essential for division. These proteins are mostly involved in remodeling the peptidoglycan wall. Our present picture is that the FtsZ ring generates a constriction force on the inner membrane, and also provides the scaffold to localize wall remodeling enzymes. In addition to invaginating the inner membrane, the constriction force may define the direction in which the peptidoglycan is remodeled.

A major question is what generates the constriction force. In eukaryotic cells, cytokinesis involves an antiparallel band of actin filaments that are pulled together by bipolar myosins. No analogous motor proteins have been identified in bacteria. Some bacteria and archaea, ones that do not have a peptidoglycan wall, have FtsZ but no recognizable homolog of any other *E. coli* division protein. This led to the "Z-centric hypothesis" that FtsZ may provide not just the cytoskeletal framework for division, but may also generate the constriction force by itself (1). An inspiration for this hypothesis was the observation that FtsZ assembles protofilaments with 2 conformations: A straight conformation, with no twist bend, and a highly curved conformation, where a 22° bend between subunits leads to a mini-ring of 16 subunits and an outside diameter of 24 nm (2). Lu et al. (3) advanced this model with observations suggesting that GTP favored the straight conformation and GDP the curved.

A completely different mechanism for force generation has been suggested in several recent papers (4–6). These propose that FtsZ forms a filament that can span the circumference of the cell, after which the 2 ends of the filament can interact with each other via lateral bonds. The lateral bonds provide a negative (favorable) free energy. A constriction force is generated as the filament ends slide to increase the number of lateral bonds.

Both models are consistent with the Z-centric hypothesis, that FtsZ can generate the constriction force without any other protein. This hypothesis has recently been confirmed by the reconstitution of Z rings in liposomes (7). Z rings assembled and generated a constriction force without the need of any other protein. This experiment did not, however, identify the mechanism by which the constriction force is generated.

Structure of the Z Ring. The Z ring is easily visualized by light microscopy using immunofluorescence or a GFP fusion. One would like to have higher resolution to visualize its fine structure, but conventional electron microscopy (EM) has failed to resolve any details of the Z ring. We have proposed the structure shown in Fig. 1, based on images of FtsZ protofilaments assembled in vitro and quantitative measurements of FtsZ in vivo. FtsZ assembles in vitro into protofilaments that are mostly straight, and average 125 nm, or 30 subunits, long (8, 9). We propose that these serve as the basic architectural unit to assemble the Z ring. The average protofilament is much shorter than the circumference of the cell, so they would have to be put together in a staggered overlap arrangement (Fig. 1).

An important point for this model is the number of protofilaments in the Z ring. We determined that there are 15,000 molecules of FtsZ in *E. coli* strain BL21, a B strain (10). A B/r K strain was determined to have 3,700 FtsZ per cell [I have corrected the authors' stated value for the 15% color difference between FtsZ and BSA (10)]. *Bacillus subtilis* has approximately 5,000 FtsZ per cell (11). Two studies using GFP-tagged FtsZ found that only 30% of the total FtsZ was in the Z ring in both *E. coli* and *B. subtilis* (12, 13). This would mean that the Z ring is 6–9 or 2–3 protofilaments thick for 15,000 or 5,000 FtsZ per cell, respectively. Our diagram of the Z-ring (Fig. 1) uses the lower value, for graphic simplicity and to emphasize that this minimal cytoskeleton can suffice for cell division.

The reason conventional EM fails to resolve the fine structure of the Z ring is now obvious—the small number of protofilaments is lost in the granular cytoplasm. Recently, however, Li et al. (14) used cryo-EM tomography to reconstruct an image of the Z ring of *Caulobacter crescentus*. They found an arrangement of short protofilaments remarkably similar to our model, but sparser. In particular, the protofilaments appeared to be scattered around the Z ring with no apparent lateral bonding to other protofilaments.

This structural model raises 2 questions. First, how are the protofilaments tethered to the membrane? This was solved by Pichoff and Lutkenhaus (15), who showed that FtsZ binds FtsA, and FtsA has a C-terminal amphipathic helix that binds it to the membrane. Thus, FtsZ is tethered to the membrane by FtsA [ZipA can also provide a tether, but it is dispensable if FtsA has an enabling mutation (16)]. The second question is, how are protofilaments connected to each other to make the staggered overlap? This would seem to require lateral bonds, but whether these exist and how they might contribute to Z-ring structure are still quite obscure (discussed below).

Author contributions: H.P.E. analyzed data and wrote the paper.

The author declares no conflicts of interest.

This article is a PNAS Direct Submission.

[1]E-mail: h.erickson@cellbio.duke.edu.

 www.pnas.org/cgi/doi/10.1073/pnas.0902258106

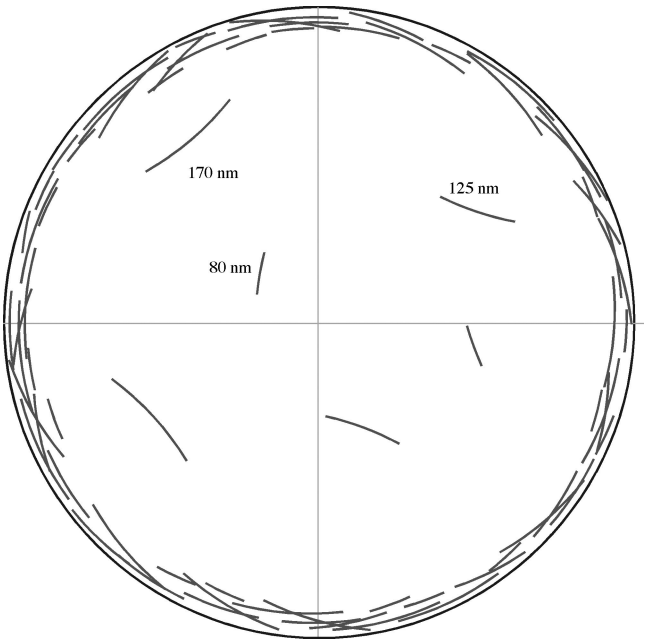

Fig. 1. A structural model of the FtsZ ring constructed from short protofilaments averaging 125 nm long. The outer circle represents the inner bacterial membrane. The ring is drawn here as 2- to 3-protofilaments thick. Z rings are 6- to 9-protofilaments thick in some strains, but in other strains 2–3 protofilaments can suffice for cell division. [Adapted with permission from ref. 7 (Copyright 2008, The American Association for the Advancement of Science).]

Considerations for Forces Generated by Protofilament Bending. The simplest model for force generation by bending postulates that the FtsZ protofilament has 2 preferred conformations, depending on whether the nucleotide is GTP or GDP. GTP favors the straight conformation, with zero bend between subunits, while GDP favors the curved conformation, with a 22° bend. If all subunits are in the curved conformation, they will form a mini-ring of 24 nm outside diameter (2) or a helix with a 24-nm diameter and a pitch of 16–22 nm (3). If the protofilament is tethered to a membrane of an undivided cell, of diameter 1,000 nm, and all its subunits have hydrolyzed the GTP to GDP, it might generate a constriction force as it tries to bend to its 24-nm diameter. Although the evidence for the highly curved, mini-ring conformation of protofilaments is clear, X-ray crystallography has not found a conformational switch within FtsZ monomers; regardless of species and nucleotide, the conformation of the monomers is unchanged (17). This suggests that the conformational switch is primarily at the interface between subunits.

The force that can be generated by a bending protofilament depends on its flexural rigidity. Gittes and Howard (18) measured the structural rigidity of microtubules and actin. Assuming that the microtubule wall is isotropic and knowing its dimensions, they calculated a Young's modulus of 1.2 GPa. This is the rigidity of hard plastic, like Plexiglas. The authors made the important point that globular protein subunits and the polymers they form are quite rigid. Mickey and Howard (19) calculated the flexural rigidity of a single protofilament, assuming it was a rectangular beam of 2.7 × 5.15 nm, and with their refined Young's modulus of 1.4 GPa. Their $EI = 1.2 \times 10^{-26}$ N m^2 corresponds to a persistence length Lp = 2.8 μm. This value of structural rigidity was used in the bend and hydrolyze model of Allard and Cytrynbaum (20).

Lan et al. (6) suggested that the bending rigidity of an FtsZ protofilament was much too low for it to generate a significant bending force. Their argument was based on a persistence length Lp = 0.18 μm, reported by Dajkovic et al. (21). An implicit assumption in this determination was that the relaxed state of the protofilaments is straight and that any observed curvature is the result of thermally induced bending. This is certainly the conventional wisdom, consistent with the simplest model stated above. However, several recent studies suggest that relaxed FtsZ protofilaments can have a gentle curvature, intermediate between the straight and mini-ring conformations. These naturally curved protofilaments could be as rigid as calculated by Mickey and Howard, but if are interpreted to result from thermal bending of a straight protofilament, the structural rigidity will be seriously underestimated.

A Moderately Curved Protofilament Conformation. Previous work has identified protofilaments in the 2 extreme conformations, straight, or maximally curved to make mini-rings or tubes. What would one expect for protofilaments containing a mixture of GTP and GDP? The simplest proposal would be that each subunit interface has either a 0° or 22° bend, depending on whether its nucleotide is GTP or GDP. Romberg and Mitchison (22) determined that the nucleotide content of FtsZ polymers was 20:80 GDP:GTP. This would give an average curvature of 4.4°, which would make a ring of 112 nm diameter. However, we have found that the GDP:GTP ratio is actually 50:50 if the external GTP is increased to 100 μM or above (Chen and Erickson, in preparation; the previous measurement was done at 20 μM GTP). The 50:50 mixture would predict an average bend between subunits of 11°, corresponding to a diameter of 45 nm. Experimentally we almost never see protofilaments with a curvature of 50–100 nm diameter. This poses another enigma for the hydrolyze and bend model that has not been previously addressed: Why are the protofilaments much less curved than expected from their GDP content?

Evidence has accumulated recently that there is a moderately curved conformation of FtsZ protofilaments, which is independent of GTP hydrolysis. The moderate curvature corresponds to a ring of approximately 200 nm diameter or an average 2.5° bend between subunits. Mingorance et al. (23) obtained AFM images of FtsZ protofilaments adsorbed to mica, showing a mixture of straight and curved protofilaments. The curved protofilaments were obtained with GTP, with the slowly hydrolyzable analog GMPCPP, or with a completely nonhydrolyzable analog GDP-AlF. The curvature thus seems not directly related to nucleotide state and certainly does not require hydrolysis. In a subsequent study of protofilaments on mica, Hörger et al. (4) measured by AFM the curvature for a selection of short protofilaments assembled in GDP-AlF and not distorted by contacts with other protofilaments. The curvature ranged from a diameter of 80 to 500 nm, with an average of 200 nm. Importantly, these authors measured the deviation from this average curvature and determined a persistence length Lp approximately 4 μm, essentially the same as that deduced by Mickey and Howard (19).

Similar curvature of protofilaments has been seen by conventional negative stain EM. Mingorance et al. (23) showed a striking image of 150 nm diameter circles assembled from FtsZ in AlF. Gonzalez et al. (24) showed circles of 160–250 nm diameter assembled from FtsZ with GTP. Chen et al. (25) found that assembly of the mutant L68W in EDTA (which blocks GTP hydrolysis) gave a mixture of straight and gently curved protofilaments, with a tendency to form closed circles of 200–300 nm diameter. Huecas et al. (26) showed a cryo EM image of several protofilaments with a uniform curvature about 200 nm diameter, mixed with some straight protofilaments. Looking back at one of our own figures, which we had selected to show the "straight conformation," it is clear that more than half of the protofilaments are actually gently curved [figure 2 *B* and *C* of Romberg et al. (9)].

The gently curved conformation sometimes leads to loose, multistranded bundles. Popp et al. (27) showed a bewildering array of curved and helical protofilaments associated into toroids and spiral bundles. The diameter was variable, but 200–300 nm was typical. Similar toroids were assembled from *B. subtilis* FtsZ in the

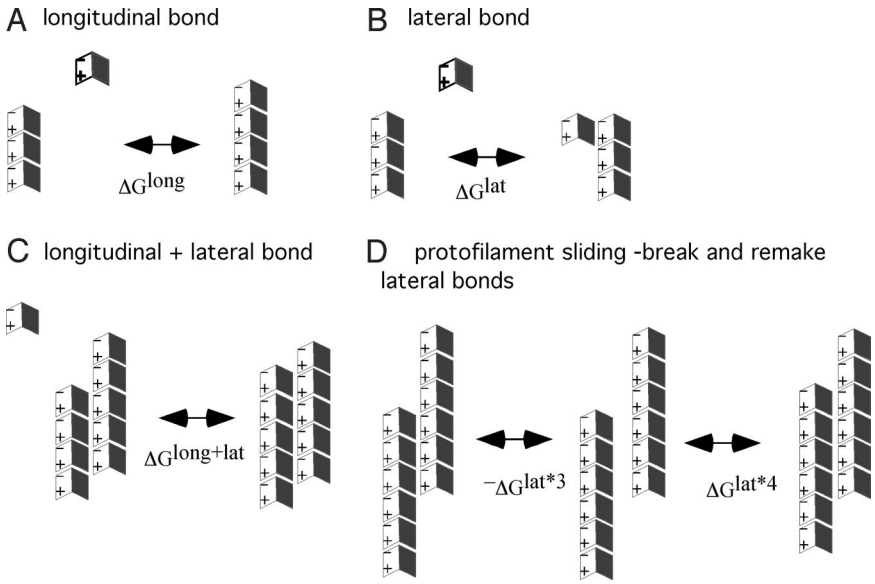

Fig. 2. Adding longitudinal and lateral bond energies in 2-stranded polymers. (*A–C*) Illustration of how a subunit can form a longitudinal bond, a lateral bond, or both at once. (*D*) How a protofilament pair could go from an association with 3 lateral bonds to one with 4.

presence of calcium (28). Srinivasan et al. (29) showed that FtsZ expressed in the cytoplasm of yeast formed donut structures of 500 nm diameter. Although of larger diameter than Popp's, these are almost certainly the same basic toroid structure, a bundle of curved protofilaments loosely attached by weak lateral attractions. It is remarkable that in both cases the toroids, which are free in solution and not tethered to a membrane, appear to be a relaxed structure and do not constrict to smaller diameter. If protofilaments in this moderately curved conformation were attached to the membrane, they could generate a bending force that constricts to 200 nm diameter. Whether this really happens, and how one could constrict further, are unanswered questions.

An important additional consideration for any model based on bending is the mechanical link that tethers FtsZ to the membrane. The simplest physical model proposes that the nonconserved C-terminal tail, amino acids 317–366 in *E. coli* FtsZ, is an unstructured polypeptide that provides a flexible tether to the FtsA membrane attachment. However, a simple flexible tether would permit a bending protofilament to simply roll over and bend in the plane of the membrane. Some mechanism is needed to keep the protofilament rigidly oriented so that its radius of curvature is perpendicular to the plane of the membrane.

We can now summarize the confusing situation with regard to curvature of FtsZ protofilaments. Under conditions of blocked GTP hydrolysis, protofilaments are sometimes straight, but many have a gentle but rigid curvature. Under conditions permitting hydrolysis, where 20–50% of the nucleotide is GDP, a similar mix of straight and gently curved protofilaments is found. The highly curved conformation with a 22° bend seems to be found only special circumstances, assembled in GDP on a cationic lipid monolayer or in DEAE dextran. Both the moderately and highly curved protofilament conformations are attractive candidates for force generation. The relationship to nucleotide state is less clear than originally thought.

Considerations for Forces Generated by Lateral Bonding and Sliding. Fig. 2 illustrates the second class of models, where subunits can assemble into protofilament pairs or sheets by forming 2 types of bonds. Longitudinal bonds join subunits to make a protofilament, and lateral bonds join subunits across 2 protofilaments. This 2-dimensional arrangement of longitudinal and lateral bonds is similar to that of the microtubule wall, for which the thermodynamics has been treated previously (30). One can postulate a bond energy, and a derived association constant, for the separate longitudinal and lateral bonds (Fig. 2 *A* and *B*). The central question is to determine the bond energy for a "cozy corner" association, where a subunit makes both a longitudinal and a lateral bond (Fig. 2*C*). A common mistake is to simply add the free energies for the lateral and longitudinal bonds. This would be equivalent to multiplying the association constants, which would give the wrong units (31). To do the calculation correctly, one needs to introduce the concept of subunit entropy (30–32).

It has long been recognized that the free energy for association of protein subunits is best considered as a balance of 2 components (32–34). First is the intrinsic bond energy, which contains all of the chemical bonds across the interface; this intrinsic bond energy is what favors association. Second is the intrinsic subunit entropy; this is the free energy needed to compensate for the loss of 3 translational and 3 rotational degrees of freedom when a subunit is immobilized in a polymer. This is discussed in detail in (32), where I argue that a value of 7 kcal/mol = 12 kT is an approximately universal number that applies to any protein subunit immobilized by any protein–protein bond. (The difference in entropy loss for a subunit entering a dimer versus a larger polymer (35) is small and will be ignored here.) Later, Horton and Lewis (36) compared the measured and calculated free energies for 15 protein pairs and deduced a value 6.2 kcal/mol for the comparable entropic parameter. For the present analysis, we will use +11 kT as the (unfavorable) free energy involved in immobilizing a subunit. [Note that solvent entropy, which is the driving force for hydrophobic bonding, is a part of the intrinsic bond energy; see (32) for details.] We can now write generally:

$$\Delta G = e^{bond} + e^{s} = e^{bond} + 11 \qquad [1]$$

$$K_A = \exp\frac{-1}{kT}(\Delta G) = \exp\frac{-1}{kT}(e^{bond}+e^{s}) = \exp\frac{-1}{kT}(e^{bond}+11) \qquad [2]$$

The net free energy for bond formation is ΔG, a negative free energy whose magnitude describes the strength of the association. e^{bond} is the (negative) intrinsic bond energy, including all of the chemical interactions across the interface. $e^{s} = +11$ kT is the

free energy needed to compensate for the 3 degrees of translational and rotational entropy. The term e^s may be considered an entropy tax that has to be paid to immobilize a subunit. The strength of the association is determined by how much $-e^{bond}$ exceeds the +11 kT entropy tax.

We can now write specific equations for the 3 reactions, Fig. 2 *A–C*. The key to this analysis is the assumption that it costs the same 11 kT when a subunit is immobilized by a longitudinal bond, a lateral bond, or both at once (32):

$$\Delta G^{long} = e^{long} + e^s = e^{long} + 11 \quad [3]$$

$$\Delta G^{lat} = e^{lat} + e^s = e^{lat} + 11 \quad [4]$$

$$\Delta G^{long+lat} = e^{long} + e^{lat} + e^s = e^{long} + e^{lat} + 11 \quad [5]$$

The key third equation calculates the bond energy for cozy corner association. The intrinsic bond energy is $e^{long} + e^{lat}$, and the 11 kT entropic tax is paid only once. If one approached this by simply adding ΔG, i.e., setting $\Delta G^{long+lat} = \Delta G^{long} + \Delta G^{lat}$, the entropy tax is counted twice. This would significantly underestimate the bond strength for the cooperative assembly, where 2 bonds are made at once.

The FtsZ modeling papers based on lateral bonds have ignored this subunit entropy term (4–6, 37, 38). It is instructive to revisit the model to see the magnitude of the effect. Lan et al. (6) proposed values for $\Delta G^{long} = -17$ kT, and $\Delta G^{lat} = -0.2$ kT, based on a previous analysis (37). The intrinsic bond energies are then $e^{long} = -28$ kT and $e^{lat} = -11.2$ kT. The $\Delta G^{long+lat}$ is now $-28 - 11.2 + 11 = -28.2$ kT. This is 11 kT stronger than the value calculated from the simple addition of ΔGs. This has major effects, in particular on the ability of protofilaments to slide.

If lateral bonding is to produce a constriction force, the 2 protofilaments need to be able to slide. This involves 2 steps (Fig. 2*D*). First, the existing lateral bonds need to be broken simultaneously, and second they need to be reformed after sliding to increase their number. A crucial question is, how rapidly can the 3 (or more) lateral bonds be broken (20)?

We can estimate the k_{-1} for this off reaction. We will assume that the on rate for lateral association, regardless of the number of bonds made, is $k_2 = 5 \times 10^6\ M^{-1}s^{-1}$. This is the generic, diffusion-limited on rate for protein–protein association (39), and the rate found experimentally for longitudinal bond formation by FtsZ (25). Since $K_A = k_2/k_{-1}$, $1/k_{-1}$ (s) $= K_A/(5 \times 10^6)$.

In Fig. 1*D*, the first step is to break 3 lateral bonds. The protofilaments are then free to slide and could reattach forming 4 lateral bonds. ΔG for making 3 lateral bonds is $(-3 \times 11.2 + 11)$ kT $= -22.6$ kT, corresponding to a $K_A = 6 \times 10^9\ M^{-1}$. This is a very strong total bond and would take an average of $1/k_{-1} = 1{,}200$ s to break. An attachment comprising 2 lateral bonds would be much weaker, with $K_A = 9 \times 10^4\ M^{-1}$, and $1/k_{-1} = 0.02$. The overall conclusion is that an attachment involving 1 or 2 lateral bonds would be too weak to achieve any significant effect, while an attachment involving 3 or more lateral bonds would be so strong that it would never release in a time relevant to the cell cycle.

Considerations of Specific Models for Z-Ring Assembly and Constriction. The goal of models is to devise a mechanism by which the Z ring can generate a constriction force. An initial consideration for any model is to determine how much force is needed to constrict the membrane and pull in the septum. Lan et al. (40) addressed this question by estimating effects of wall elasticity and turgor pressure and concluded that a force of 8 pN would be sufficient to initiate constriction in *E. coli*. Using a different approach of balancing forces, Drew et al. (41) concluded that a force of 1 pN would suffice. These numbers are of similar magnitude, and importantly, they are close to the approximately 5 pN that is generated by motor molecules myosin and kinesin.

The model of Lan et al. (6, 37) appears to be a classic sliding lateral bond, especially in Fig. 1 where the operational line is labeled "end sliding, mechanical work." However, the detailed description in their supporting information suggests that the dynamics involve primarily association and dissociation of individual subunits, and long protofilaments may not detach as a unit. The Ising model is an interesting and novel approach, and might appear to implicitly include the entropic effect. However, limiting subunits to 5 nm^3 lattice points, and only 6 rotational positions, would greatly underestimate the translational and rotational entropy. Also, the model seems to suggest that a lateral bond is formed by any lateral contact, regardless of rotation around the protofilament axis. This is very different from the known specificity of protein–protein bonds, and is similar to the Lennard-Jones potential discussed next. A driving force for the model seems to be Move 4 "attempt to delete an empty row and the ring decreases circumference by one lattice unit." But shouldn't a Brownian ratchet be unbiased, equally favoring adding or deleting a row? Another problem with the model is the need for a switch where the Z ring initially assembles with $\Delta G^{lat} = -0.2$ kT, and then ΔG^{lat} increases to -1 kT or more to initiate constriction. It was suggested that some "undetermined signal" might switch the strength of the lateral bonds. However, there are really no good candidates for such a switch. Moreover, a switch cannot be invoked for Z rings reconstituted in liposomes, which assemble and constrict using only purified FtsZ-YFP-mts (7). There is apparently a switch in vivo when the Z ring, which persists unconstricted over most of the cell cycle, finally constricts over a few minutes. My interpretation is that the Z ring is constantly exerting a constriction force on the membrane and that constriction finally occurs when the peptidoglycan remodeling permits the wall to follow this force.

Hörger et al. (4) described a variation of sliding lateral bond model. They assumed that FtsZ can form a protofilament longer than the circumference of the cell, allowing the 2 ends of the protofilament to overlap. They proposed that these ends interact with each other by a Lennard-Jones potential. This interaction is a kind of lateral bond but with a strangely different character. Instead of a typical protein–protein bond, where the subunits contact each other in a tightly fitting interface (34), the Lennard-Jones has a balanced attractive and repulsive potential, which in the model was set to bring the protofilaments to an optimal 13 nm apart, center-to-center. Since the protofilaments are only approximately 4 nm in diameter, their surfaces never contact and would have to interact by mysterious forces across the 9 nm of solution. They would be free to slide, and by moving in the direction that increased their overlap, they would increase the Lennard-Jones potential and thereby favor constriction. This model is very imaginative and seems to avoid the entropy problem, but it does so by postulating a type of protein–protein interaction with little precedent. I know of no attractive and repulsive forces whereby proteins can interact substantially (with enough free energy to generate a constriction force) with their surfaces separated by 9 nm of water.

The structure of FtsZ toroids (27, 29) might seem to be an example of relevant lateral attractions. Popp described the lateral association as weak and "liquid-like" with a diffuse diffraction peak corresponding to an average protofilament separation of 6.8 nm. An important observation was that the toroids, which were not tethered to a membrane, maintained their fixed diameter and did not constrict over periods of hours, confirming that whatever lateral attractions held the protofilaments in the structure did not generate a constriction force.

Surovtsev et al. (42) presented a model that has no lateral bonds. It is based on protofilament assembly through dimer nucleation and elongation, using kinetic values from in vitro studies. This model then introduced 3 new features that allow it to generate assembly of complete Z rings. Most important was the assumption that protofilaments 3 or more subunits long are tethered to the membrane, and limited to a 100-nm–wide zone in the middle of the cell. This produces a 1,000-fold increase in the concentration of the

oligomers in this zone, relative to the 3-dimensional cytoplasm. The other 2 new features are annealing, which produces longer protofilaments, and cyclization, which is essentially annealing of the ends when the protofilament has encircled the cell. Annealing is greatly enhanced by the 1,000-fold increase in concentration of the tethered protofilaments. The enhanced concentration and annealing of membrane-tethered protofilaments should be considered in any model of Z-ring assembly. A strength of this model is that it predicts rapid assembly of Z rings, which are long protofilaments making a closed circle. However, the model needed an "unidentified signal" to proceed to constriction. Specifically, it needed a mechanism to substantially reduce assembly of monomers onto protofilaments, while maintaining vigorous cyclization. The mechanisms suggested in the article do not seem attractive, but it is worth thinking about a mechanism for sequestering monomers that could apply both in vivo and in liposomes.

Ghosh and Sain (38) proposed a model that is a combination of lateral bonds and bending, but where bending is the primary source of force production. Unlike most models, where all protofilaments are tethered to the membrane, and lateral bonds are in the plane of the membrane, this model proposes that lateral bonds connect the protofilaments perpendicular to the membrane, in the radial direction. The Z ring would be a flat annulus of 3–9 protofilaments, where only the outer layer would be tethered to the membrane. This model ignored the subunit entropy and therefore underestimates the cooperative effect of multiple lateral bonds. An additional problem is posed by the need to accommodate lateral bonding to the decreasing radius. If lateral bonds are optimized for connecting straight protofilaments, they will have a spacing of 4.3 nm on each side. However, if a 5-nm–thick protofilament is curved to R = 50 nm, the subunit spacing will be 4.3 nm through its center, but will be 4.5 and 4.1 nm on its outer and inner edges, respectively. This would mean that if a lateral bond were made between a given pair of subunits in adjacent protofilaments, the next pair of subunits would be displaced 0.4 nm and the next pair 0.8 nm from their preferred docking positions. Thus, lateral bonding that worked for straight protofilaments would fail completely as they curved to smaller radii. This problem would apply to any model proposing lateral bonds in the radial direction.

Allard and Cytrynbaum (20) proposed a "hydrolyze and bend" model based on the simple model that the protofilament interface is straight for GTP and bent 22° for GDP. They used the flexural rigidity of Mickey and Howard, corresponding to Lp = 2.8 μm, and found that this could generate a substantial bending force. The Z ring was modeled as an assembly of short protofilaments connected by lateral bonds. FtsZ entered the Z ring only as whole protofilaments preassembled in the cytoplasm and left only by dissociation of single subunits from ends of protofilaments. This would seem to avoid the entropy problem, but a part of the model did apparently involve breaking lateral bonds and sliding. "Under the influence of the curvature-induced constriction force, weak lateral bonds are broken, filaments slide past each other and reconnect." As detailed above, even for weak lateral bonds, if there are more than 3, the ensemble becomes much too strong to break.

This model introduced 1 novel concept that has long posed a problem for "hydrolyze and bend" models. The problem is that GDP-FtsZ is known to assemble very weakly, suggesting that hydrolysis should lead to fragmentation of protofilaments before it could generate a bending force. Allard and Cytrynbaum proposed that hydrolysis in the middle of a protofilament does not lead to fragmentation. GDP subunits dissociate rapidly, but can only do so when they are at the end. This proposition involves the still mysterious mechanism of cooperative assembly, which requires that fragmentation at a GTP interface in the middle of a protofilament is highly unfavorable, relative to removing a subunit from the end. Since we don't yet understand why fragmentation is inhibited at GTP interfaces, we might as well extend the mystery to GDP interfaces. Miraldi et al. (43) also proposed that GDP interfaces in the middle of a protofilament, surrounded by high affinity subunits, would be locked in a high affinity conformation and not lead to fragmentation.

Allard and Cytrynbaum (20) predicted a constriction force of >8 pN for their hydrolyze and bend model, which is the force suggested to overcome cell wall elasticity and turgor forces (40). The ring was modeled as an intact circular structure, and it is not clear what would happen if there were small or larger gaps.

Li et al. (14) suggested a form of hydrolyze and bend that could match their cryo-EM tomography. Their image reconstructions showed the Z ring of *Caulobacter* to be an irregular arrangement of short protofilaments, with no obvious contacts or connections to each other. They proposed "iterative pinching," in which each short protofilament generates a localized bending force on the membrane. A single bending protofilament would tend to pull the membrane inward at its ends, but this would be balanced by an outer push on the membrane at the middle of the protofilament. It is not yet clear how this could generate an overall constriction force, but this would seem a fruitful area for future modeling. This model, with multiple gaps between the short protofilaments, raises again the question of how any model can accommodate gaps.

Cooperative Assembly of Protofilaments 1-Subunit Thick. Many cytoskeletal filaments assemble cooperatively, meaning that (*i*) the kinetics of initial assembly shows a lag phase for nucleation, and (*ii*) at steady state, there is a critical concentration (Cc) below which no polymer is formed, and above which all additional subunits go into polymer, leaving an equilibrium with monomers at a concentration Cc.

Cooperative assembly of actin is well understood and explained by its 2-stranded helical structure and the 2 types of bonds between subunits: Longitudinal bonds connect subunits in the long-pitch helical protofilaments, and diagonal bonds connect subunits between helices (32). Removing a subunit from the end involves breaking 1 longitudinal and 1 diagonal bond, whereas fragmentation involves breaking these plus 1 more longitudinal bond. Fragmentation is 10^7 less favorable (in terms of K_A) than end dissociation (32).

For a protofilament 1-subunit thick, there is only 1 type of bond, and fragmentation should be equivalent to removing a subunit from the end. This isodesmic assembly should be dominated by fragmentation/annealing and should not show nucleation nor a Cc (9). FtsZ protofilaments are 1-subunit thick, as confirmed by quantitative scanning transmission EM (9, 25). Nevertheless, FtsZ assembly showed both a Cc and a weak dimer nucleus (25). This posed an enigma that has challenged biophysicists.

Gonzalez et al. (24) suggested that the appearance of cooperativity might be produced by the formation of circles, when the intermediate curved protofilament grew long enough to form a closed circle. They suggested reasonable values for isodesmic assembly of long, curved filaments, followed by a much more favorable step of closing the circle. This model can explain one aspect of nucleated assembly, the Cc. However, it has 2 problems. First, the model does not explain the need of a weak dimer nucleus to fit the kinetics of initial assembly. Second, in most assembly conditions, protofilaments average only 30 subunits long, even after minutes at steady state. They rarely reach the 150 subunits needed to make a 200-nm diameter circle. Experimentally, circles have been reported under special solution conditions, but are not found by EM under most assembly conditions.

A more attractive approach to solving the enigma was proposed recently by 3 independent groups (21, 26, 37, 43), the article of Miraldi et al. giving the most detailed thermodynamic analysis. The basis for these models is to propose that FtsZ subunits have a low and a high affinity conformation. The low affinity conformation is favored for monomers and the high affinity one in oligomers. A straightforward pathway for the conformational change would be to propose that when a subunit forms an interface with another

subunit on its top, its own bottom interface becomes transformed to a high affinity conformation. Miraldi et al. (43) showed that this pathway does not work. When one writes down all of the equations leading to a simple oligomer, the assumed independent variables are no longer independent. However, Miraldi et al. proposed a ore elaborate conformational change that achieved the appearance of cooperativity, including a weak dimer nucleus and a Cc. The key was to propose that the conformational change simultaneously switched both the top and bottom interface from low to high affinity.

This model seems to work, but it pushes the question to a new level. What is the nature of the conformational change that could substantially and simultaneously alter the affinities of both the top and bottom interface? Typical protein–protein bonds involve formation of a tightly fitting interface between rigid, preformed surfaces of the 2 subunits (34). There are a number of crystal structures of FtsZ from different species and in several nucleotide states, and they all have the same conformation (17). The Löwe group has looked especially for conformational changes that might be caused by GTP or GDP, and they did not find any (17). According to the model of Miraldi et al., the known X-ray structure probably corresponds to the low affinity conformation. The problem now is to propose a rearrangement of the side chains on the upper and lower interfaces that would make them fit better together and give a high-affinity conformation. This is not obvious. Explaining the conformational change, or devising another way to generate cooperativity, remains a major challenge for the field.

Conclusion—Lessons and Questions

I will end by listing some of the insights from the current models that have seemed most important to me, and some questions raised. (*i*) If lateral bonds exist, they are probably nonreversible. Disassembly of subunits can occur at the ends of protofilaments, but whole protofilaments are unlikely to dissociate by breaking lateral bonds. (*ii*) EM has shown protofilament bundles under a variety of conditions, but these seem to involve irregular weak association rather than a regular pattern of specific lateral bonds. (*iii*) Tethering protofilaments to the membrane results in a large increase in effective concentration, which should enhance even weak lateral association, and also annealing. (*iv*) The suggestion that protofilaments may not fragment at internal GDP interfaces is an attractive idea and a simple extension of the models of cooperative assembly. (*v*) Bending protofilaments still seems the most attractive mechanism for generating the constriction force, but the relation of bending to GTP hydrolysis is much more complicated than previously thought or yet modeled. The moderately curved protofilament conformation may play an important role in constriction. (*vi*) Because FtsZ assembles contractile Z rings in liposomes all by itself, a good model should not invoke any switch or signal between assembly and constriction. (*vii*) Finally, the hypothetical switch of FtsZ subunits from low to high affinity conformations to produce cooperative assembly is in serious need of a structural model.

ACKNOWLEDGMENTS. Supported by National Institutes of Health grant no. GM66014.

1. Erickson HP (1997) FtsZ, a tubulin homolog, in prokaryote cell division. *Trends Cell Biol* 7:362–367.
2. Erickson HP, Taylor DW, Taylor KA, Bramhill D (1996) Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers. *Proc Natl Acad Sci USA* 93:519–523.
3. Lu C, Reedy M, Erickson HP (2000) Straight and curved conformations of FtsZ are regulated by GTP hydrolysis. *J Bacteriol* 182:164–170.
4. Horger I, Velasco E, Mingorance J, Rivas G, Tarazona P, Velez M (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.
5. Horger I, Velasco E, Rivas G, Velez M, Tarazona P (2008) FtsZ bacterial cytoskeletal polymers on curved surfaces: The importance of lateral interactions. *Biophys J* 94:L81–L83.
6. Lan G, Daniels BR, Dobrowsky TM, Wirtz D, Sun SX (2009) Condensation of FtsZ filaments can drive bacterial cell division. *Proc Natl Acad Sci USA* 106:121–126.
7. Osawa M, Anderson DE, Erickson HP (2008) Reconstitution of contractile FtsZ rings in liposomes. *Science* 320:792–794.
8. Chen Y, Erickson HP (2005) Rapid in vitro assembly dynamics and subunit turnover of FtsZ demonstrated by fluorescence resonance energy transfer. *J Biol Chem* 280:22549–22554.
9. Romberg L, Simon M, Erickson HP (2001) Polymerization of FtsZ, a bacterial homolog of tubulin. Is assembly cooperative? *J Biol Chem* 276:11743–11753.
10. Lu C, Stricker J, Erickson HP (1998) FtsZ from *Escherichia coli*, *Azotobacter vinelandii*, and *Thermotoga maritima*—quantitation, GTP hydrolysis, and assembly. *Cell Motil Cytoskel* 40:71–86.
11. Feucht A, Lucet I, Yudkin MD, Errington J (2001) Cytological and biochemical characterization of the FtsA cell division protein of *Bacillus subtilis*. *Mol Microbiol* 40:115–125.
12. Anderson DE, Gueiros-Filho FJ, Erickson HP (2004) Assembly dynamics of FtsZ rings in *Bacillus subtilis* and *Escherichia coli* and effects of FtsZ-regulating proteins. *J Bacteriol* 186:5775–5781.
13. Stricker J, Maddox P, Salmon ED, Erickson HP (2002) Rapid assembly dynamics of the *Escherichia coli* FtsZ-ring demonstrated by fluorescence recovery after photobleaching. *Proc Natl Acad Sci USA* 99:3171–3175.
14. Li Z, Trimble MJ, Brun YV, Jensen GJ (2007) The structure of FtsZ filaments in vivo suggests a force-generating role in cell division. *EMBO J* 26:4694–4708.
15. Pichoff S, Lutkenhaus J (2005) Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Mol Microbiol* 55:1722–1734.
16. Geissler B, Elraheb D, Margolin W (2003) A gain-of-function mutation in ftsA bypasses the requirement for the essential cell division gene zipA in *Escherichia coli*. *Proc Natl Acad Sci USA* 12:12.
17. Oliva MA, Trambaiolo D, Lowe J (2007) Structural insights into the conformational variability of FtsZ. *J Mol Biol* 373:1229–1242.
18. Gittes F, Mickey B, Nettleton J, Howard J (1993) Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J Cell Biol* 120:923–934.
19. Mickey B, Howard J (1995) Rigidity of microtubules is increased by stabilizing agents. *J Cell Biol* 130:909–917.
20. Allard JF, Cytrynbaum EN (2009) Force generation by a dynamic Z-ring in *Escherichia coli* cell division. *Proc Natl Acad Sci USA* 106:145–150.
21. Dajkovic A, Lan G, Sun SX, Wirtz D, Lutkenhaus J (2008) MinC spatially controls bacterial cytokinesis by antagonizing the scaffolding function of FtsZ. *Curr Biol* 18:235–244.
22. Romberg L, Mitchison TJ (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.
23. Mingorance J, Tadros M, Vicente M, Gonzalez JM, Rivas G, Velez M (2005) Visualization of single *Escherichia coli* FtsZ filament dynamics with atomic force microscopy. *J Biol Chem* 280:20909–20914.
24. Gonzalez JM, 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 USA* 102:1895–1900.
25. Chen Y, Bjornson K, Redick SD, Erickson HP (2005) A rapid fluorescence assay for FtsZ assembly indicates cooperative assembly with a dimer nucleus. *Biophys J* 88:505–514.
26. Huecas S, Llorca O, Boskovic J, Martin-Benito J, Valpuesta JM, Andreu JM (2008) Energetics and geometry of FtsZ polymers: Nucleated self-assembly of single protofilaments. *Biophys J* 94:1796–1806.
27. Popp D, Iwasa M, Narita A, Erickson HP, Maeda Y (2009) FtsZ condensates: An in vitro electron microscopy study. *Biopolymers* 91:340–350.
28. Michie KA, Monahan LG, Beech PL, Harry EJ (2006) Trapping of a spiral-like intermediate of the bacterial cytokinetic protein FtsZ. *J Bacteriol* 188:1680–1690.
29. Srinivasan R, Mishra M, Wu L, Yin Z, Balasubramanian MK (2008) The bacterial cell division protein FtsZ assembles into cytoplasmic rings in fission yeast. *Genes Dev* 22:1741–1746.
30. Erickson HP, Pantaloni D (1981) The role of subunit entropy in cooperative assembly. Nucleation of microtubules and other two-dimensional polymers. *BiophysJ* 34:293–309.
31. Jencks WP (1981) On the attribution and additivity of binding energies. *Proc Natl Acad Sci USA* 78:4046–4050.
32. Erickson HP (1989) Cooperativity in protein–protein association: The structure and stability of the actin filament. *J Mol Biol* 206:465–474.
33. Doty P, Myers GE (1953) Thermodynamics of the association of insulin molecules. *Discuss Faraday Soc* 13:51–58.
34. Chothia C, Janin J (1975) Principles of protein–protein recognition. *Nature* 256:705–708.
35. Chatelier RC (1987) Indefinite isoenthalpic self-association of solute molecules. *Biophys Chem* 28:121–128.
36. Horton N, Lewis M (1992) Calculation of the free energy of association for protein complexes. *Prot Sci* 1:169–181.
37. Lan G, Dajkovic A, Wirtz D, Sun SX (2008) Polymerization and bundling kinetics of FtsZ filaments. *Biophys J* 95:4045–4056.
38. Ghosh B, Sain A (2008) Origin of contractile force during cell division of bacteria. *Phys Rev Lett* 101:178101.
39. Northrup SH, Erickson HP (1992) Kinetics of protein–protein association explained by Brownian dynamics computer simulation. *Proc Natl Acad Sci USA* 89:3338–3342.
40. Lan G, Wolgemuth CW, Sun SX (2007) Z-ring force and cell shape during division in rod-like bacteria. *Proc Natl Acad Sci USA* 104:16110–16115.
41. Drew DA, Koch GA, Vellante H, Talati R, Sanchez O (2009) Analyses of mechanisms for force generation during cell septation in *Escherichia coli*. *Bull Math Biol* 71:980–1005.
42. Surovtsev IV, Morgan JJ, Lindahl PA (2008) Kinetic modeling of the assembly, dynamic steady state, and contraction of the FtsZ ring in prokaryotic cytokinesis. *PLoS Comput Biol* 4:e1000102.
43. Miraldi ER, Thomas PJ, Romberg L (2008) Allosteric models for cooperative polymerization of linear polymers. *Biophys J* 95:2470–2486.