Bacterial Cell Division: A Swirling Ring to Rule Them All?

FtsZ, a tubulin homologue and the major constituent of the bacterial Z ring, has been shown to assemble into curved filament bundles, which exhibit GTP-hydrolysis-dependent turnover. Surprisingly, the presence of its membrane adaptor FtsA renders this turnover directional, inducing treadmilling and collective circular motion of filaments *in vitro*.

Petra Schwille

Rings as the symbols of power have fascinated humans of all times, and even inspired some of the most influential works of art, for example, by Wagner and Tolkien. In nature, rings are the obvious constraints of round objects, and in cell biology, contractile rings are assumed to play prominent roles during cell division. In our most naive (and certainly too simplistic) imagination of a dividing cell, such a contractile ring assembles close to the middle and contracts by directional forces, whose true molecular origins have so far not been revealed unambiguously. This is true for the proposed contraction of actomyosin rings in eukaryotic cells, which appear to be much less intuitive than one would assume, considering the involvement of directional motor proteins and the classical sarcomere model (i.e., the concept of actin and myosin filaments sliding against each other). But it is also true for the simpler bacterial cells, where the discovery and formulation of cytoskeletal elements is much more recent. A recent study by Loose and Mitchison [1] delivers new sparks of insight, showing the emergence of dynamic rings *in vitro* from a minimal system of the bacterial divisome.

The Z ring described in many prokaryotes is probably one of the most attractive candidates for a contractile ring, based on its apparent agreement with the simple and intuitive model. Its attractiveness as a potentially archetypical contractile ring results mainly from the protein FtsZ, a tubulin homologue, which has been found to self-assemble into curved filaments and undergo a conformational change upon nucleotide (GTP) binding and hydrolysis, which could be the molecular basis for large-scale contractility [2,3]. In spite of many other important members of the bacterial divisome that have in the last years been described, there is wide agreement on the pivotal function of FtsZ in the assembly of the Z ring, although the actual mechanism during septum constriction has so far remained elusive. This is certainly due to the scales on which Z rings, and other substructures of microorganisms can be observed *in vivo*, which is close to the diffraction limit of standard optical microscopes. Only recently, through the advent of super-resolution light microscopy and electron tomography, have these structures come into focus, potentially invoking a true renaissance of microbiology.

Another approach that has become increasingly popular in fundamental biological studies is cell-free reconstitution of well-defined subsystems, which can be ascribed to the new field of synthetic biology [4], but which reflects in fact another renaissance, namely of biochemistry. True understanding of a biological system on the molecular level will always require protein purification and minimization of assays, in spite of the amazing new tools and protocols to delicately manipulate complex cellular systems. Particularly for the understanding of molecular motors and dynamic cytoskeletal elements, such reconstitution assays have been extremely powerful — ranging from the first observation of microtubule growth and shrinkage, leading to the model of dynamic instability [5], and the study of filament gliding on motor protein carpets [6], to the ATP-driven self-organization of bacterial proteins into travelling waves and oscillations on supported membranes [7,8]. Thus, it seems that only through these kinds of bottom-up approaches, aided by cutting-edge fluorescence microscopy, and assisted by insightful theoretical modeling, will it be possible to ultimately understand contractile forces in cell division.

Much along these lines, the study by Loose and Mitchison [1] reports a fascinating feature of FtsZ when co-reconstituted with one of its native membrane anchors, FtsA, which may bring us a step closer in the quest for the molecular origin of constriction. Although it has already been reported in reconstitution studies that FtsZ forms ring-like structures on surfaces and membranes [9–11], the dynamics reported here show a significant feature not observed so far, namely a collective behavior of FtsZ filaments, seemingly moving directionally on the surface, giving rise to coordinated streams and swirling rings of ca. 1 μm diameter (the circumference of bacterial cells), reminiscent of rapidly moving helical patterns of FtsZ observed in live cells [12]. This behavior shows striking similarities to microtubule gliding assays [6] and large-scale collective effects observed on motor-driven microtubules [13], but with the significant difference that no motor protein is involved that could actively move filaments. However, two types of nucleotides are required to produce this effect: GTP, necessary for FtsZ polymerization and to be turned over before depolymerization, and ATP, in order to promote FtsA binding to FtsZ.

Importantly, Loose and Mitchison show that this directional movement is only observed in the presence of FtsA, and not when other membrane anchors, such as ZipA (the other essential anchoring partner for FtsZ in *Escherichia coli* cells) or a membrane targeting peptide fused to FtsZ are used. In the latter cases FtsZ also organizes into networks of curved filament bundles, but in spite of a constant GTP-hydrolysis-dependent turnover of FtsZ filament fragments, these networks appear static in steady-state. Searching for the distinctive feature between FtsA and the other membrane anchors of FtsZ, they showed that neither a proposed ATPase activity of FtsA, nor the polymerization of FtsA, nor the fact that FtsA binds the membrane transiently, is responsible for the striking collective dynamics, but the fact that FtsA obviously destabilizes FtsZ filaments. The energy for driving the self-organization, however, comes solely from GTP, which is turned over in FtsZ polymerization and depolymerization. Intriguingly, a higher turnover of

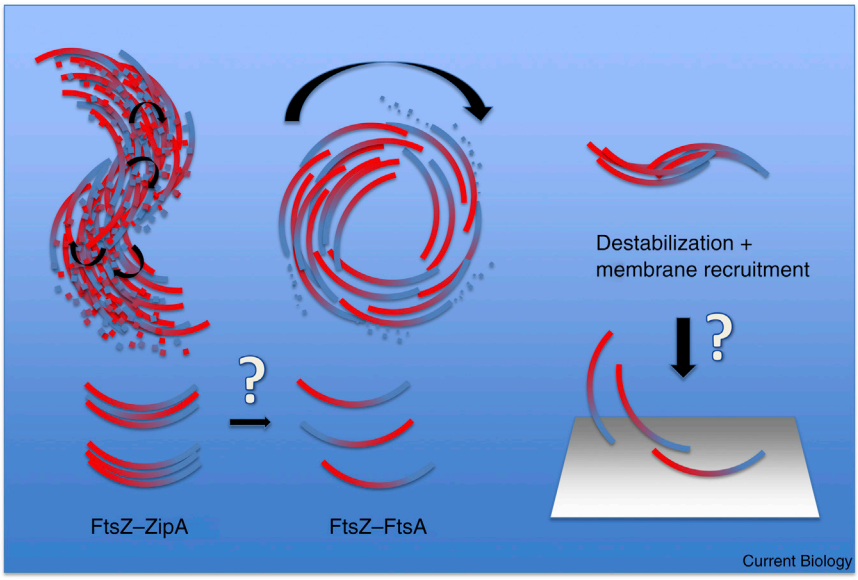

Figure 1. Possible interpretation of FtsA-induced directional dynamics of FtsZ filaments.
As the rate of GTP hydrolysis is not altered, and membrane binding is required for FtsZ fragmentation by FtsA, the predominant effect could be the frustration of lateral contacts. This would allow the treadmilling of single filaments to become significant, and render the turnover non-isotropic (left to middle). Fragmentation of bundles could be aided by a strain on FtsZ filaments when targeted to the membrane (right), explaining the delay in FtsA-induced disassembly.

filament fragments within the bundle, which would result in an increased rate of GTP hydrolysis, could not be observed during the collective behavior.

Only in the presence of FtsA, it seems, does 'treadmilling' of the dynamic FtsZ filaments become significant — in fact, this may be the most remarkable aspect of the paper. Treadmilling is considered one of the most distinctive features of polar filaments, such as actin and microtubules, *in vivo*, creating the illusion of a sliding filament by the balanced turnover of building blocks at the front and at the back in dynamic equilibrium. The emergence of collective streams and swirls with predominant directions is ascribed to several factors, including the polarity of the FtsZ filaments, but also the existence of both positive and negative feedback between FtsZ and FtsA. In their native form, both proteins are mutually dependent on each other in order to be recruited to the membrane, where FtsA then destabilizes FtsZ bundles. But even if FtsA is artificially attached to lipids (as shown in a control experiment), it may still be locally concentrated in the presence of FtsZ filaments. It may reside there for a short while after depolymerization and dissociation of FtsZ filaments, possibly imprinting preferential tracks for new filaments to be recruited to the membrane, thus promoting the emergence of swirls.

Although the authors are careful with regard to the possible implications of their findings, it is of course tempting to speculate about the functional role of the observed directionality of FtsZ polymerization for Z-ring constriction. In other words, how should we interpret the treadmilling? As FtsZ in the Z ring is supposed to assemble into staggered bundles of constantly turned-over filaments [2], the sudden directionality seems to reflect a less isotropic staggering and turnover. Such a rectifying effect could come about by lowering or frustrating the lateral interactions between the filaments (i.e., the bundling), thereby reducing the longitudinal strain on the curved filaments in bundles when attached to the membrane, rendering it more likely for them to, on average, re-align along the same direction of curvature (Figure 1). The fact that membrane recruitment of FtsZ filaments seems to be required for FtsA to execute its destabilizing function (an aspect which is referred to by the authors as a 'delayed negative feedback' of FtsA) seems to support the view that the role of FtsA is to destabilize and shuffle isotropic FtsZ bundles towards protofilaments of corresponding curvature, and thereby rectify both the direction of turnover and the direction of assembly. For stronger membrane attachment of FtsZ monomers, as is the case with ZipA, this effect would be abolished.

Remarkably, the observation of negative regulation of FtsA on FtsZ organization certainly shows an intriguing analogy to recent observations in the actomyosin field, where the depolymerization or severing of actin filaments by myosin motors appear to be an important requirement for actomyosin ring [14] or cortical contractions, as also demonstrated in reconstitution experiments [15,16]. It could be that contraction of the Z ring is also based on an intricate combination of depolymerization and crosslinking [17]. However, many researchers in the field doubt that the contractile forces are brought about by the Z-ring proteins themselves, but rather by the directed insertion of new peptidoglycan material through cell wall synthesis. Along these lines, parallels may be drawn between the swirling rings of FtsZ and the recently observed filaments of MreB spiraling around the cell circumference in super-resolution movies performed on live *E. coli* cells [18,19]. If both FtsZ and MreB were required to guide cell wall synthesis [20], and the number of cell-wall synthesizing enzymes was limiting, homogeneous growth would only be possible through a sequential activity along the cell circumference, in which case directional movement of FtsZ filaments would be required.

Certainly, this study is only at the beginning of cellular reconstitution, and many more experiments will have to follow. In order to understand the functional significance of the observed collective behavior in the minimal system composed of FtsZ and FtsA on a membrane, our attention should in the near future be focused on a more quantitative characterization of the membrane binding dynamics in the ternary FtsZ/FtsA/membrane system, the destabilizing activity of FtsA on FtsZ filaments and filament bundles, and how these reactions are modulated by other division-related proteins (e.g., specific bundling effectors). Taken together, the beautiful phenomenon observed by Loose and

Mitchison leaves us with more new questions than answers yet, which is in fact the best possible outcome of a scientific study.

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Life-History Evolution: At the Origins of Metamorphosis

Metamorphosis is a widespread life history strategy of animals but apart from some model organisms it is poorly characterized. A recent study of moon jellies highlights the similarities and differences between the various types of metamorphosis and illuminates its molecular determinants.

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Because we humans are living on land surrounded by mammals or birds that exhibit life cycles similar to our own, we often do not appreciate that most animals have much more complex life histories. In fact, extant metazoans exhibit a wide variety of life cycles — sometimes incredibly complex ones, especially in parasitic species. The most common strategy is a biphasic cycle with a larva emerging from the egg and a metamorphosis that allows the transformation of the larva into a juvenile that will experience sexual maturation and become an adult. This strategy often involves dramatic morphological, physiological, behavioral and ecological transformations between the larvae and the juvenile [1,2]. Even in some vertebrates, such as teleost fishes, the difference between a larva and a juvenile can be so big that the two forms have sometimes been mistaken as different species [3]. The advantage of such biphasic life history strategies are numerous [2]: for example, larval stages allow for dispersal, as in most marine animals larvae are pelagic forms that take advantage of marine currents to travel far from their site of release. Moreover, this system allows the larva and the juvenile to exploit different ecological niches — most tadpoles, for example, are aquatic herbivores while most frogs are terrestrial carnivores. Finally, biphasic life history strategies allow the larva and the adult to become specialized in different activities: the fly larva (the maggot) is a specialized form to exploit short-term food sources and to grow rapidly. In contrast the adult's main function is to find a mate and to reproduce; in some insects such as mayflies the adult is even unable to feed. The diversity of life history strategies based on this relatively simple system — larva, metamorphosis and juvenile — is enormous, but very little is known about how it originated. A study on the moon jelly *Aurelia aurita* by Fuchs *et al.* [4] in *Current Biology* provides a first and fascinating insight into the evolutionary origin of metamorphosis.

All cnidarians have free-swimming planula larvae that settle and develop into sessile polyps. In anthozoans (corals and sea anemones) these polyps can propagate asexually, but never form medusae. The other groups of cnidarians (called Medusozoa; Figure 1) have sessile polyps, which can either propagate asexually or undergo a transition into a pelagic, free-swimming medusa (jelly) that differentiates gonads and carries out sexual reproduction. The morphology of a medusa is closely related to that of a polyp. In essence, both have a gastrula-shaped body plan with two germ layers (ectoderm and endoderm), an intermediate extracellular matrix (mesoglea) and one opening of the gastric cavity at the oral side. In the medusa, the mesoglea forms an enlarged jelly-like umbrella at the aboral side, which ensures free floating