On the Source of the Ingression Force during Bacterial Cytokinesis

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Abstract | In order for bacteria to replicate, they need to fully constrict their membrane(s) and cell wall inwards, forming two separate cells. How the ingression force required for membrane constriction is generated, has been debated for several decades. The two most likely candidates for force generation are FtsZ and peptidoglycan synthesis. FtsZ has long been the most promising of the two, showing a capability to constrict vesicles on its own *in vitro*. However, various studies in recent years have demonstrated that PG synthesis is in fact the more probable force generator, able to perform cell division independent of FtsZ. This essay will explain the history behind both models and discuss their validity.

Introduction

One could say that the ultimate "goal" of a bacterium is to multiply itself. To do so, most bacteria rely on a cell division process called binary fission[1]. The concept of binary fission seems very simple: the cell needs to double its entire mass and subsequently cut itself in the middle to produce two identical daughter cells. However, this entire process has to be precisely regulated by the cell in both time and place, making the underlying mechanisms quite complex. It is therefore not surprising that dozens of different proteins have been identified that contribute in the control and execution of binary fission in model organisms such as *Escherichia coli* and *Bacilus subtilis*. Even after decades of research, many questions about the coordination and precise role of these various proteins still remain[2]. It is of great importance to understand bacterial cell division in full detail, not in the least because it may provide us with promising targets for the development of new antibiotics[3]

The question that will be central in the current essay, will be that of the so called 'source of the force'. In order to divide themselves, bacteria need to apply an ingression force on their cell membrane(s), leading to constriction at the middle of the cell. How and where this force is exactly generated, however, remains unclear[4]. Several ideas have been proposed in the past thirty years or so, including pulling by an internal protein complex called the 'Z-ring', pushing by the synthesis of additional peptidoglycan (PG) and pushing by the production and incorporation of extra phospholipids in the cell membrane. In this essay, the validity of these different propositions (mainly the first two) will be discussed to see what's the most convincing source of the ingression force according to current research. But first, in order to fully appreciate the different proposals, a more elaborate overview will be given of the mechanisms governing cytokinesis in bacteria.

The Bacterial Divisome

Although the exact details of binary fission may vary between bacterial species, the various conceptual stages must always be the same. These are: (i) the doubling of the cell mass, including replication and segregation of the nucleoid, (ii) the localization of early division proteins to the middle of the cell, (iii) the recruitment of late division proteins by the early division proteins, (iv) application of constriction force and synthesis of the inward septum and (v) cell separation leading to two identical daughter cells[4-5]. A schematic overview of these steps is shown in figure 1.

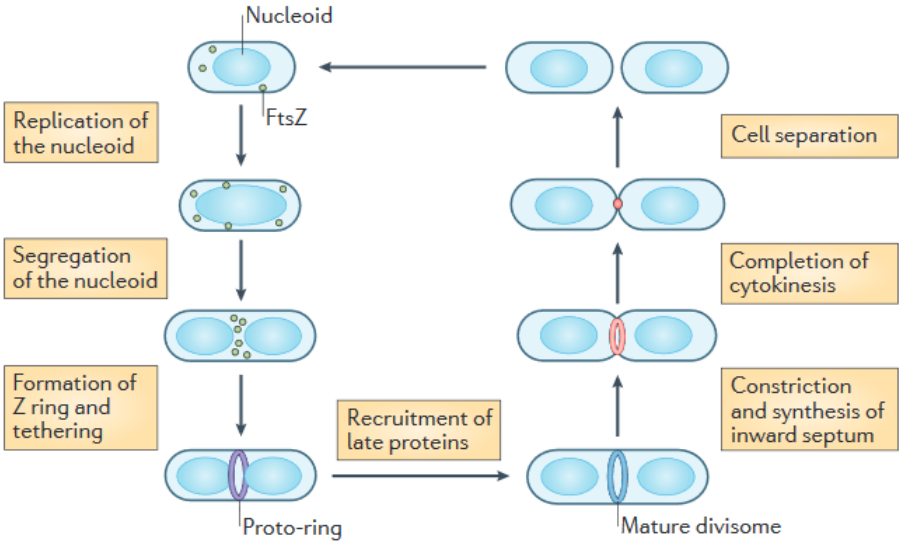

Figure 1. A schematic overview of binary fission. All five steps mentioned in the main text are shown here. The early division proteins, mainly FtsA and FtsZ, together form the tethered Z ring in the middle of the cell. Only after recruitment of additional proteins is it called the divisome. Picture taken from Haeusser and Margolin[6].

Of all the dozens of proteins involved in binary fission, only a few seem absolutely essential and even fewer seem universal among the bacterial kingdom. The undisputed champion in ubiquity is the highly conserved bacterial tubulin homologue FtsZ. It is present in virtually all eubacteria known[7]. The protein's crystal structure was first solved in 1998[8]. Later research showed that FtsZ can be divided in five distinct domains, of which the central, large N-terminal domain is the GTPase and of which a small C-terminal peptide is implicated in the binding of membrane-associated division proteins[9]. FtsZ is the first protein localizing to the division site and is important for the recruitment of later division proteins[10]. This localization is regulated by both positive and negative spatial regulators. Negative regulation is mostly caused by nucleoid occlusion and the Min system, while positive regulators are highly variable between species[6]. Once FtsZ has been localized to the membrane at mid-cell, it has to be tethered to the membrane by a different protein. The most ubiquitous membrane tether for FtsZ is the actin homologue FtsA, although other proteins that can replace its role as a membrane tether do exist[11]. Binding FtsZ to the cell membrane is not the only function of FtsA however, since it is thought to be involved in the recruitment of the majority of late division proteins[12]. Also, recent studies have shown that it may regulate the activities of other membrane tethers in *E. coli*[13].

In the presence of GTP, FtsZ monomers can polymerize into single-stranded protofilaments. These protofilaments then associate with each other and form the early Z-ring. This Z-ring formation is assisted by several FtsZ associated proteins[14]. Most probably, the filaments form a patchy ring consisting of discontinuous, loosely associated FtsZ protofilaments[15-16]. However, there is also data, derived from electron cryomicroscopy and cryotomography, that suggests a continuous Z-ring[17]. This debate may be of importance to us in the context of the contractile force, since an overlapping ring provides a possible mechanism of membrane contraction in which the membrane tethered FtsZ filaments slide over each other to form ever closer circles. This will be discussed in greater detail later on. The proto Z-ring, either patchy or continuous, is then able to recruit further downstream division proteins. As mentioned before, especially FtsA plays an important role in this regard. Whether these later stage division proteins localize to the Z-ring in a hierarchical fashion or whether they form a large protein complex prior to localizing to the Z-ring is not fully clear, even in the best studied case of *E. coli*[18].

So what exactly are the roles then, of these late stage division proteins? As always, the precise answer may vary from bacterium to bacterium species, but these proteins often include scaffolds for other division proteins, DNA translocases[19], inhibitors of PG synthesis[20], activators of PG synthesis and membrane invagination[21], and PG synthetases themselves. However, it has been suggested that only a few division proteins are absolutely essential for binary fission, namely: FtsZ, a membrane tether, a PG synthetase and a protein that is capable of connecting this PG synthetase to the Z-ring (FtsA can also fulfil this role)[18]. Such a simplified model is shown in figure 2. Since FtsZ and FtsA have been discussed before, and since many of the other late stage division proteins don't seem to be essential, only the PG synthetases will be further elaborated on.

The primary players in PG synthesis are the so called penicillin binding proteins (PBPs). PBPs are a diverse class of transpeptidases and glycosyl transferases of which several are involved in cell division. In *E. coli*, PBP3 (also known as FtsI), PBP1a and PBP1b are all important during cytokinesis. For other bacterial species, alternative members of the PBP family may be involved instead[22]. During cell division in *E. coli*, FtsI associates with the PG glycosyltransferase FtsW, probably via interaction with PBP1a and lipid II[23]. Together, this complex synthesizes new PG at mid-cell.

The major take-away of all this, is that PG synthesis is intrinsically connected to the Z-ring. This means that for proper placement of the PG layer during cell division, a functional Z-ring is required. The relevance of this connection for the rest of the story, is that it is challenging to study the two systems (the Z-ring and PG synthetase) independently. So although they will now be discussed separately, keep in mind that in the living cell, these two systems are strongly linked.

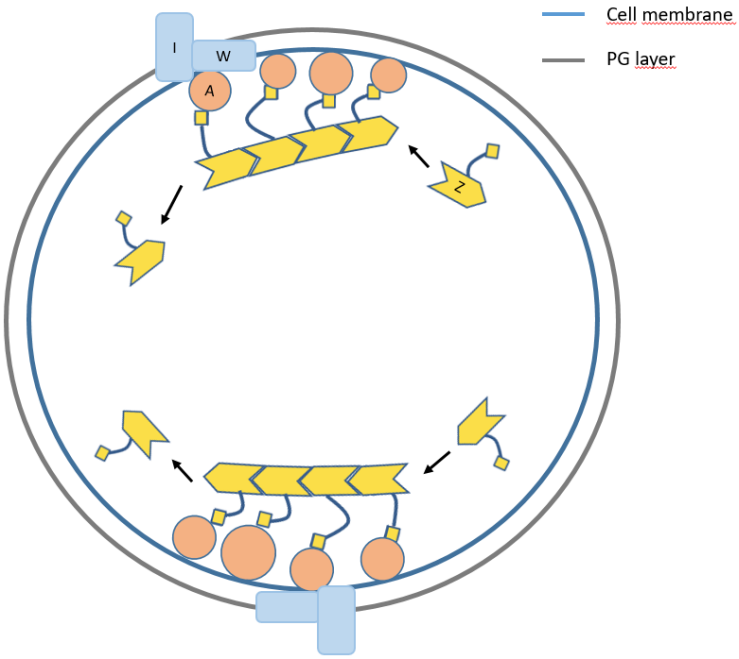

Figure 2. A minimalistic model of the essential division proteins, depicted in a Gram-positive bacterium. Shown here, are all the necessary elements for binary fission (FtsZ, a membrane tether, PG synthetase and something to connect the synthetase to the Z-ring). Note that this model is no representation of the divisome in any particular bacterial species. These essential elements are usually accompanied by various protein scaffolds and regulators to control the timing and placement of the divisome formation. FtsI and FtsW are taken here as the PG synthetases, since they fulfil this role in *E. coli*. Picture is loosely based on Du and Lutkenhaus[18].

FtsZ Driven Cytokinesis

It has been known for almost three decades that, during cytokinesis, FtsZ is capable of ring formation at mid-cell. However, it was at first unclear whether this ring merely played a role in guiding or controlling septal PG biosynthesis, or that it might hold some additional function as well[24]. Later suggestions included a model in which the Z-ring facilitated the binding of some unidentified motor protein (analogous to eukaryotic cytokinesis), and a model in which the protein's main function was the generation of a constriction force[25]. In this last model, it was suggested by Erickson that FtsZ could undergo a conformational change, driven by GTP hydrolysis. If the Z-ring is attached to the membrane (via FtsA or any other membrane tether), while its FtsZ monomers are primarily in their straight confirmation, one could imagine a constriction force being generated by gradually inducing the curved conformation of the FtsZ monomers. Very little of this was backed by experimental evidence however, so this model of constriction driven by conformational change of FtsZ was highly speculative. In the years thereafter, researchers started to show that FtsZ protofilaments could indeed adjust to a curved conformation, strengthening Erickson's original hypothesis[26-27].

This proposition was further elaborated on after the new Cryo-EM observation that the Z-ring in *Caulobacter crescentus* was not a continuous ring, but in fact consisted of a series of short, interrupted filaments (approximately 100 nm in size)[28]. Protofilaments were found in both straight and curved conformations, often containing abrupt kinks in them. These results lead to the idea that several of these small protofilaments could anchor to the cell membrane and hydrolyze GTP, thus assuming a curved conformation. This would decrease the diameter of the division plane by slightly pinching the membrane inwards. The protofilaments would then depolymerize and exchange GDP for GTP, after which the cycle can repeat itself. PG synthesis only serves to preserve the changes made by forming a stabilizing layer behind the constricting membrane. The model provided an adequate explanation as to how FtsZ could constrict the very last stages of cell division, in which the size of the division plane is only a few nanometers across. This was difficult to imagine with an actual Z-ring, which could hardly be stable after being bent so severely. Protofilaments, on the other hand, could consist of just a few monomers and still constrict the membrane through these last stages of cell division.

Keep in mind, however, that this entire hypothesis resulted merely from the observation of straight

and curved FtsZ protofilaments in living cells. Even though the derived model seems quite convincing in the sense that it provides a general mechanism for the broad span of cell division, there are several problem with it. First, finding curved protofilaments does in no way prove that the conformational changes actually cause the cell membrane to bend. It would still be just as plausible to assume that the protofilaments bend in response to membrane constriction, so they can follow its curvature. This could be necessary if the main function of the protofilaments is to guide other division proteins, such as FtsI and FtsW. Secondly, it would seem that a short protofilament could simply roll 90 degrees and curve in the already existing plane of the membrane[29]. This is possible due to the fact that FtsZ is attached to the membrane tether via its C-terminal peptide, which is at the end of a flexible linker. Thirdly, it may be possible that FtsZ protofilaments do indeed cause membrane constriction, but not by inducing a conformational change. Instead, sliding of protofilaments and increasing their amount of lateral bonds could also provide the contractile force on the cell membrane, by decreasing the circumference and thus constricting the Z ring[17]. However, theoretical models have shown that this mechanism of force generation is probably invalid and that – if FtsZ is indeed the main force generator – it is more likely to be generated by conformational changes of the protofilaments[30-31]. Also, this kind of force generation would require a continuous Z ring, which is not always the case: at least not in the early stages of constriction[32].

Although there was no lack of mathematical models at that time showcasing that FtsZ could in theory generate a contractile force, it was clear that more empirical evidence was needed to demonstrate that it could actually do so in a living cell. Probably one of the most important landmark papers in this regard, was published by Erickson's group over ten years ago[33]. In this study, a fluorescently tagged FtsZ's C-terminal peptide was replaced with an amphipathic helix. This way, they were able to produce an FtsZ molecule that could anchor itself into the membrane, removing the need for FtsA or any other membrane tether. This modified FtsZ protein was mixed with large multilamellar vesicles in the presence of GTP. By some unknown mechanism, FtsZ ended up inside these vesicles, where they spontaneously assembled into Z-rings (again, probably consisting of multiple layers of protofilaments). Constrictions were often found at the sites of these Z-rings; roughly five times more often than at sites where no Z-ring was present. Once the vesicles were depleted of GTP, these constrictions abruptly relaxed again. The Z rings were always situated perpendicular to the axis of the tube, once again indicating that the rings themselves probably generate the constriction force. Experiments were repeated with these same modified FtsZ proteins. Except this time, the membrane tether was applied to the outside of the vesicles[34]. Interestingly, bulges were formed at the sites where FtsZ protofilaments associated with these extravesicular membrane anchors. FtsZ proved capable of bending the membrane not only inwards, but in the opposite direction as well. This further supported the idea of bending protofilaments as the primary source leading to constriction.

It is important to note, however, that constriction was never fully completed in any of these vesicles in either experiment, no matter the amount of GTP present. Yes, some constriction force generated by FtsZ alone was positively shown in these vesicles, but whether this is truly the main motor driving constriction forward during cell division *in vivo*, still remained to be seen. A vesicle doesn't represent the living cell in terms of turgor pressure, size and structural support provided by the PG layer. At the very least, FtsZ seemed to need some help finishing the job.

It took several years before a protein membrane tether was implemented in the vesicular constriction system, partly due to the fact that *E. coli* FtsA is hard to work with *in vitro*[35]. For this reason, an FtsA mutant that was amenable to *in vitro* experiments was taken instead and it was added together with FtsZ to large vesicles, in a similar procedure as described above[36]. The organization of the Z-rings differed from those observed in the absence of FtsA, as may be expected given the role of FtsA as ligand for FtsZ. More interestingly though, was that this time, the FtsZ-FtsA system was occasionally able to accomplish complete constriction. So complete vesicle collapse seemed to rely on an

interaction between FtsZ and FtsA. This was true not only for FtsA, but worked for another membrane tether called ZipA as well[37]. The collapsing event was completely blocked when FtsZ interaction with its tether was inhibited, indicating again that FtsZ needs some kind of anchor in order to transmit its generated force to the membrane. A different *in vitro* study simultaneously found that polymerization of FtsA inside membrane vesicle could also cause membrane constriction[38]. This may explain why the FtsZ-FtsA system earlier could cause complete membrane septation, as opposed to FtsZ system lacking any membrane tethering protein. The problem, though, is that the observed FtsA-caused shrinkage was dependent on ATP, which was not present in the FtsZ-FtsA system. Still, it seems plausible that FtsA plays an important role in this regard, especially since it was shown that FtsZ is not present at the divisome during the last stages of constriction *in vivo*[39]. Other divisome proteins, including FtsA and FtsI, were shown to remain at the division site after FtsZ was already disassembled. These studies emphasize the fact that FtsZ is – at least – not the only actor in membrane constriction.

So how exactly would this kind of FtsZ dependent force generation work on a structural level? Insights came from crystallography studies, analyzing FtsZ protofilaments derived from a wide range of bacterial species[40]. It was found that FtsZ formed shorter and more strongly curved protofilaments in the presence of GDP as compared to GTP. This was because, when bound to GTP, FtsZ monomers were found to be in the so called T3 "T" state. In this state, monomers were able to form longitudinal interactions with each other. Upon hydrolysis of GTP, intermolecular interactions on one side of these monomers were lost. These now GDP-bound monomers could now only form interactions with each other at the side that would *in vivo* be facing away from the membrane. The protofilament thus bends in a sort of hinge-opening mechanism, as the authors of the study called it. Figure 3 shows a schematic overview of this hydrolysis mediated structural transition. It was again suggested that this transition itself is the force generator of membrane constriction. A notion which was later also reinforced by molecular dynamics simulations, which was based on both *in vitro* and *in vivo* parameters[41]. However, the same problem encountered earlier still seems to stand: if the linker connecting FtsA and FtsZ is flexible, why wouldn't the protofilaments simply roll over and follow the curvature of the membrane? The membrane is shown to be straight in figure 3, but this is obviously not the case in a living cell. This problem hasn't been solved up to this day. Very recent 3D modelling

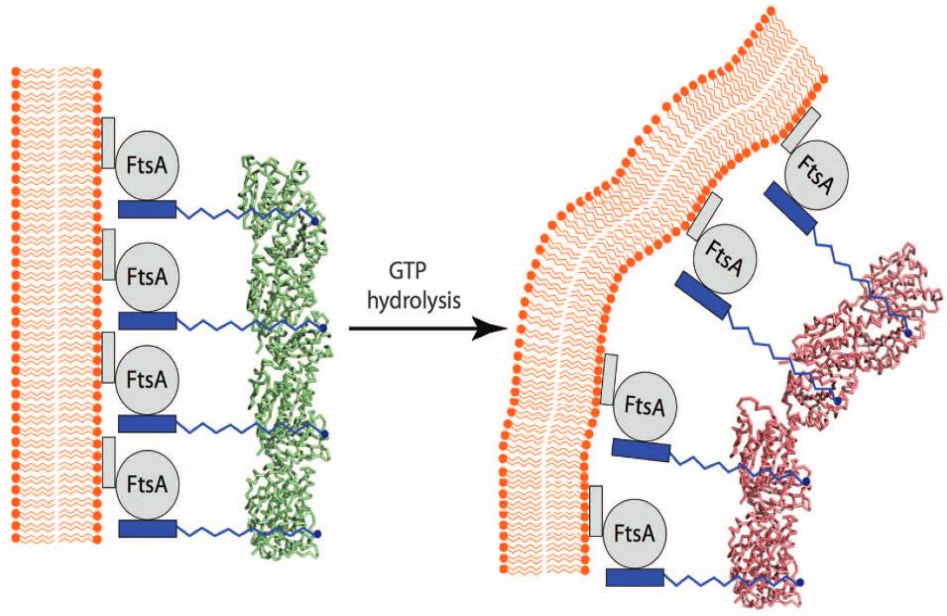

Figure 3. Schematic representation of the often proposed structural conformation of FtsZ protofilaments being the force generator during cell division. The four monomers shown left are all in the T3 "T" state, since they are bound to GTP. The second monomer from the bottom is then hydrolyzed, causing a hinge-opening motion of the protofilament in its middle. Figure taken from Li et al[40].

studies that aimed to simulate FtsZ force generation, simply assumed that filament rolling was prevented by a rigid linker formed by several FtsZ-binding proteins[42]. However, no such linker has ever been found *in vivo*.

So, thus far it seems quite convincing that FtsZ can form protofilaments that are capable of undergoing a conformational change and that this is – together with a membrane tether – sufficient for membrane constriction *in vitro*. To further assess whether this FtsZ-generated membrane constriction observed in vesicle systems bears any relevance to the living cell, it remains important to quantify its force. It was estimated on paper that the Z ring could generate a force in the order of several pN[43]. An analysis of several *in vitro* studies showed this force rather to be in the order of several tens of pN[44]. More recently, a new *in vitro* assay allowed researchers to study these estimates more precisely[45]. They observed forces generated by the Z ring of only around 2 pN in strength. Note however, that they observed a deformation mechanism other than the conformational change of FtsZ: namely torsional stress by treadmilling of the protofilaments (treadmilling will be further discussed in the next section). Even a few pN is more than enough force to constrict a vesicular membrane, as was already demonstrated in the earlier FtsZ-FtsA vesicle system experiments. But, as briefly mentioned before: a vesicle is no cell. Whether the same force is also sufficient to drive cytokinesis *in vivo* mainly depends on the turgor strength inside the cell, and determining this strength is no easy task[46]. Turgor pressure may differ greatly between Gram-positive and Gram-negative bacteria. In Gram-positive species it is probably a lot higher, suggesting that mechanisms aside from FtsZ force generation are definitely required here for full membrane constriction. Erickson himself, however, proposes the idea that the Z ring might ignore turgor pressure, because it takes place within the high pressure environment of the periplasm. This would require the space between the (inner) cell membrane and the cell wall to be isoosmotic, something which is well known for Gram-negative bacteria, but still debated for Gram-positive bacteria. But even for Gram-negative species, it would seem that constriction of the outer membrane would nonetheless have to fight against the inner turgor pressure. The aforementioned simulation experiments also found that turgor pressure is not easily overcome and requires a larger curvature difference between the membrane and the protofilaments[42].

PG Synthesis Driven Cytokinesis

Another likely candidate of force generation during bacterial cytokinesis, is the production of septal PG, pushing on the cell membrane from the outside. Aside from a few exceptions, virtually all bacterial species possess a PG cell wall around their (inner) membrane. Therefore, if true, PG synthesis may offer an almost universal mechanism for force generation in the bacterial kingdom (or even broader than that, since PG synthesis has also been found to push during cytokinesis in yeast[47]). The idea is nothing new. However, compelling evidence to support the model has been found only in the last couple of years. This is partly due to the difficulty of uncoupling PG synthesis from the formation of the Z ring. As explained before, the two systems are intertwined: PG synthetases are recruited by the early divisome. So a minimal *in vitro* division system – such as that with FtsA-FtsZ – cannot be built with just PG synthesis.

Even so, early observations showing that PG synthesis may be essential at least in some bacteria, were discovered already more than ten years ago[48]. In these studies, bacterial L forms (known for their lack of a PG cell wall) were found to possess a small amount of PG synthesis. When this last bit of PG synthesis was blocked, cells were unable to divide, suggesting that PG synthesis is essential for cell division in *E. coli*. However, it was not shown whether this PG synthesis was needed to generate a force that pushed the membrane inwards, or to stabilize the deformed membrane which was previously constricted by the Z ring. These finding were contradicted by later studies though, that showed that no PG synthesis (or FtsZ) at all was needed for division in these L form bacteria[49]. L form division will

be discussed in more detail later on.

In the years thereafter, the focus in the field of bacterial division lay mainly on FtsZ driven constriction. Until four years ago, when a study was published in which researchers showed that cell wall synthesis – and not FtsZ – limited the rate of septum closure in *E. coli*[50]. Mutating several FtsZ properties, such as its GTPase activity and Z ring characteristics, did not significantly alter the progress of cytokinesis. For example, bacterial strains that had a low Z ring density, showed both an increase and a decrease in the division rate. In contrast, a strong correlation was found between cell growth and septum closure rate in several strains, suggesting that both processes are governed by the same mechanism: cell wall synthesis. This corresponds nicely to an earlier modeling study that showed that bacterial growth and division are tightly coupled[51]. The proposition that PG synthesis drives cytokinesis was further supported by the observation that an altered FtsI activity did in fact curb septum closure[50]. Additionally, a different study around the same time managed to produce – via several mutations – an *E. coli* strain that completely lacked FtsZ, but was nonetheless capable of efficient growth[52]. Shortly after these initial studies, researchers used cryo-EM to investigate the different contributions of FtsZ and PG synthetases to the initiation of cell division[53]. It turned out that the presence of FtsZ-FtsA at mid-cell was insufficient for the initiation of membrane constriction *in vivo*. Instead, cell division was triggered upon the localization of FtsI and its allosteric activator at mid-cell.

Just as during the earlier discussion of FtsZ driven membrane constriction, mathematical models can provide a powerful method for illustrating the various forces that contribute to cytokinesis. One such model, also published in the same year as the aforementioned PG studies, confirms that PG synthesis is the most probable driver of membrane constriction[54]. Figure 4, which was taken from this study, shows that no constrictive force from FtsZ is required at the rate of PG synthesis observed in *C. crescentus* and *E. coli*. So what would the role of FtsZ then be, if PG synthesis generates the force required for membrane constriction? It was already shown that FtsZ is important in recruiting downstream PG synthetases, but why would it need to form such highly dynamic polymers? And how exactly would it guide cell wall formation?

Answers came first from the observation that FtsZ protofilaments *in vitro* tend to polymerize at one end and depolymerize at the other end[55]. Such a treadmilling model (as shown earlier in figure 2) could explain the observed directionality of the reorganizing protofilament network. More recent experiments confirmed this model and showed that treadmilling was dependent on the GTPase activity of FtsZ[56]. It was found that the treadmilling dynamics were responsible for a uniform spatial distribution of PG synthesis along the division site. This could also explain why the PG synthesis

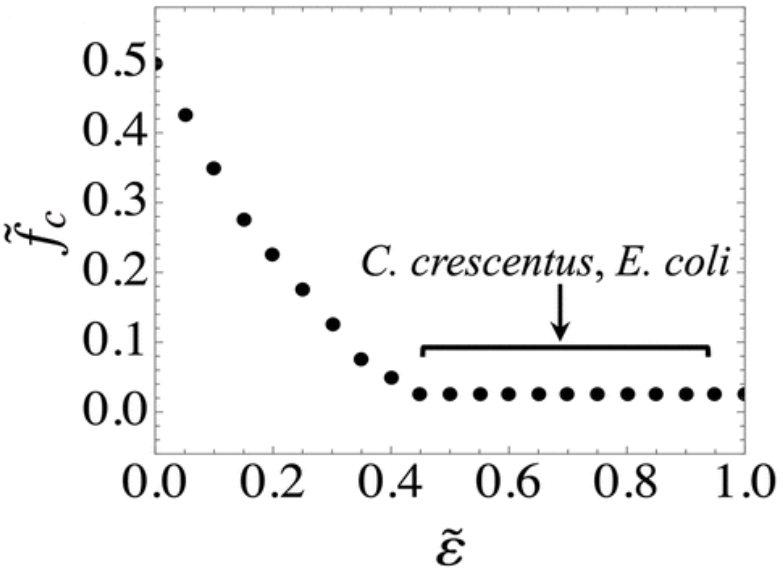

Figure 4. Constrictive force f_c of FtsZ protofilaments required for full membrane constriction as a function of chemical potential ε of PG synthesis. For the constrictive force, a numerical estimate of 8-80 pN is taken. For PG synthesis, an estimate of >12 nN μm^{-1} is taken. From this graph, it follows that no constrictive force is needed to complete cytokinesis if the chemical potential is higher than 0.4. This is calculated to be true for species such as *C. crescentus* and *E. coli*. Graph taken from Banerjee et al[54].

study described before did not find that an impaired GTPase activity diminished cell division[50]: GTPase dependent treadmilling governs the distribution, but not the rate of PG synthesis in *E. coli*. Besides controlling the spatial distribution, FtsZ treadmilling may also influence the composition of the synthesized cell wall. A different study – performed around the same time – confirmed the *in vivo* treadmilling behavior of FtsZ and its tight coupling to several PG synthetases[57]. Here, however, it was found that treadmilling did in fact control the rate of PG synthesis in *B. subtilis*: a lower FtsZ-FtsA velocity lead to a decreased total amount of PG production. It would be interesting to see if an impaired GTPase activity of FtsZ does indeed alter cell division rate in *B. subtilis*. This observed effect might reflect on the different needs of PG synthesis in Gram-negative and Gram-positive species. Since the cell wall in the latter bacteria is much thicker, this may require a more highly regulated PG synthesis. Or perhaps Gram-negative bacteria have extra difficulty in organizing PG synthesis, since they also need to couple it to their outer membrane insertion. Later studies showed an important role for the different *E. coli* membrane tethers in this coupling of FtsZ protofilaments with PG synthetases[58].

The exact role of FtsZ treadmilling is still debated though. It was recently found in the Gram-positive species *S. aureus* that treadmilling is only required at the onset of cell division, and is dispensable later in the process[59]. So even in Gram-positive bacteria – where FtsZ treadmilling was thought to fully control the rate of PG synthesis – pathways other than FtsZ treadmilling could also be responsible for the recruitment of various PG synthetases.

Extraordinary Cases

So far, mostly standard model strains of *E. coli* and *B. subtilis* have been considered in the discussion. The major exceptions were the FtsZ and cell wall lacking L form bacteria (although, as briefly discussed, they might still possess some PG). Assuming that they have indeed completely lost both possible force generators, how could these bacteria possibly divide? One suggestion is that these cells over-synthesize phospholipids, leading to excess membrane production[60]. The proposed mechanism is shown in figure 5. This model was supported by the observation that two genetic changes linked with membrane production were needed for L form growth in *B. subtilis*. In normal bacteria, excess membrane production could possibly generate an inward pushing force, since the rigid cell wall will force the excess membrane in only one direction. But although several suggestions have been made that this kind of force generation could cause membrane invagination, evidence of its occurrence in living cells is lacking[46]. Excess membrane production may also help explain how primitive cells were able to divide early in evolution, when both FtsZ and a PG cell wall were not yet present.

Some other interesting cases are the groups of *Planctomycetes* and *Chlamydia*. Both these bacterial groups completely lack FtsZ[61-62]. They do undergo binary fission however, via a form of polarized cell division. The exact details may differ between the various species, but it seems that they do all utilize some of the division proteins normally associated with FtsZ. And, importantly, both synthesize PG into their cell walls[63-64]. It's intriguing that they independently evolved an FtsZ-independent division system, indicating that the central role of FtsZ may not be so irreplaceable. It's possible that other proteins have taken up the task of recruiting PG synthetases, as reflected by the earlier explained observation that FtsZ treadmilling was not essential in the later stages of cell division in *S. aureus*[59].

Lastly, there are also bacteria that possess FtsZ, but no cell wall. These *Mycoplasmas* as they are called, are slow growing and very small organisms: tiny even by the standards of bacteria[65]. Turgor pressure within these bacteria is minimal[46], so it is likely that FtsZ protofilaments are sufficient here

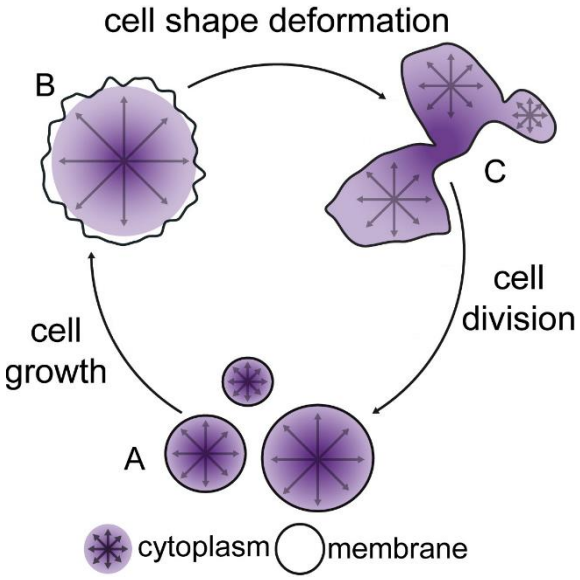

Figure 5. Proposed division mechanism for L form bacteria that lack both FtsZ and a PG cell wall. In this model, the primary force is instead generated by excess membrane production. An overabundance of surface area in relation to volume would lead to random deformations in the cell shape of these growing bacteria. This may push the membrane inwards, occasionally resulting in complete scission events. If some of these progeny cells contain a full genome and the correct protein machinery required for cell growth, they may be able to mature and repeat the cycle. Although this mechanism seems too unregulated to be the sole driver of cell division in normal bacteria, it may provide yet another force source that can contribute to the entire orchestra that steers membrane constriction. Picture taken from Mercier et al[60].

for generating the force for complete membrane collapse, just as it was the case with the *in vitro* vesicle systems. FtsZ being the primary force generator in *Mycoplasmas* may also help explain why these bacteria have a reduced growth rate, since the *S. aureus* study proposed that the initial FtsZ dependent constriction step was a slow process[59]. It is also possible that excess membrane production plays a role in force generation in *Mycoplasma* cell division, but this is entirely speculative.

Conclusion

All in all, it seems that FtsZ protofilaments are definitely capable of generating a force, but that this is not the main driver of membrane constriction in most bacterial species.

The various FtsZ-FtsA systems capable of full vesicle collapse *in vitro* nicely show that these proteins can induce such a constrictive force. However, required forces for full membrane constriction *in vivo* are probably of a few orders of magnitude higher. So – with the exception of a few special cases such as *Mycoplasmas* – PG synthesis is most likely responsible for the main driving force needed for cytokinesis in fast growing cells such as *E. coli*. Both *in silico* and *in vivo* studies have shown that PG synthesis may generate a force of ample strength to drive cell division and that it is plausible that it actually does so in a living cell.

What then the exact role of FtsZ is in membrane constriction, remains debatable. It is clearly somehow involved in the recruitment and guidance of PG synthetases, likely by treadmilling across the circumference of the cell membrane. Many have also suggested that FtsZ is involved in the initial force generation during the early stages of cell division. However, studies showing that FtsZ is not present at the onset of cytokinesis, question this proposition. So if FtsZ does not generate the initial constrictive force, and it is not the primary driver during cell division, what then is the function of the FtsZ protofilament bending *in vivo*? A possibility is that its ability to conform to a curved state is purely functional in signal transduction. It could also function to localize the FtsZ protofilaments to the membrane position with the lowest circumference, thus guiding PG synthetases to the correct site. Some of the most important challenges that remain are to further elucidate these potential roles of FtsZ and the way it may transmit signals to the cell wall components.

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