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Advances in understanding *E.coli* cell fission

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Abstract

Much of what we know about cytokinesis in bacteria has come from studies with *Escherichia coli*, and efforts to comprehensively understand this fundamental process in this organism continue to intensify. Major recent advances include in vitro assembly of a membrane-tethered version of FtsZ into contractile rings in lipid tubules, in vitro dynamic patterning of the Min proteins and a deeper understanding of how they direct assembly of the FtsZ-ring to midcell, the elucidation of structures, biochemical activities and interactions of other key components of the cell fission machinery, and the uncovering of additional components of this machinery with often redundant but important roles in invagination of the three cell envelope layers.

Introduction

Cytokinesis in bacteria is driven by a complex ring-shaped organelle variously referred to as divisome, septasome, FtsZ ring, or septal ring (SR) [1–3]. For this review, I'll use Z-ring (ZR) to indicate the mostly cytoplasmic intermediate structure that assembles first, and septal ring (SR) to indicate more mature forms of the organelle (Fig. 1).

The mature, constriction-competent, SR in *E.coli* is now known to contain over two dozen different protein components. Ten of these (FtsA, B, I, K, L, N, Q, W, Z, and ZipA) are essential to the cell constriction process, and can be considered to form the core of the apparatus. Cells lacking any of the core proteins fail to constrict and display the classical lethal division phenotype where they elongate into very long filaments with a commensurate number of evenly spaced nucleoids and non-functional ZR or SR structures before eventually dying. The number of known non-essential protein components of the SR has increased steadily in the last few years. Though none of these are individually required for cell fission and viability, many have overlapping roles in important aspects of cell constriction and their study is required for a satisfactory understanding of the whole process.

Though this review is focused on progress in our understanding of the cell fission process in *E.coli* in the last two years, I'll freely refer to excellent recent progress with *Bacillus subtilis*, *Caulobacter crescentus* and other organisms, especially when particularly relevant to the *E.coli* system

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Z-ring assembly

FtsZ plays a key role in cytokinesis of most prokaryotes and plastids, and is the likely ancestor of eukaryotic tubulin. Construction of the SR starts with the accumulation of FtsZ in a ring-like arrangement on the cytoplasmic face of the inner-membrane (IM) at the future site of cell constriction (Fig. 1, stage I). This involves the GTP-dependent homopolymerization of FtsZ into linear protofilaments and a means to tether these to the IM. FtsZ has no intrinsic affinity for phospholipid membrane and assembly of a Z-ring in *E.coli* cells minimally requires the presence of either FtsA or ZipA. Both FtsA and ZipA are membrane-associated and interact with a small domain (C-core) at the extreme C-terminus of FtsZ [1,4,5].

One profound development in the last two years was the recreation of Z-ring assembly in a minimal in vitro system by Erickson and coworkers [6**]. They smartly bypassed the requirement for a separate membrane-associated partner (e.g. FtsA or ZipA) by replacing the tail of FtsZ with YFP and the C-terminal amphiphatic membrane-targeting sequence (MTS) of MinD (see below). Remarkably, when mixed with liposomes and GTP, this FtsZ-YFP-MTS chimera somehow ended up in the lumen of some multilamellar phospholipid tubules where it then readily formed membrane-associated rings. Moreover, bright rings were associated with clear indentations of the liposome wall, implying they exerted a constrictive force [6**]. These results greatly clarified a number of issues, and showed that GTP, a membrane-tether, and a curved membrane surface are sufficient for FtsZ to spontaneously form ZRs that are at least partially functional.

One next hurdle will be to assemble Z-rings in vitro using native FtsZ and its natural partners, including FtsA, ZipA, and ZapA. Besides helping to tether FtsZ to the IM, FtsA and ZipA are normally both required for further maturation of the ZR to an SR as well (Fig. 1, stage II). ZipA is a bitopic membrane protein (N-out) with a large cytoplasmic domain that binds and bundles FtsZ protofilaments in vitro and helps to stabilize ZRs in vivo. While ZipA appears restricted to the γ -proteobacteria, FtsA is much better conserved and implicated in several important aspects of SR formation and function. FtsA belongs to the actin/Hsp70 superfamily of ATPases, and binds the IM peripherally via a C-terminal amphiphatic MTS [1–3]. Membrane binding is critical to FtsA function but its MTS can be replaced with a heterologous one or, remarkably, with a regular trans-membrane helix, indicating that any reversibility in membrane association is not a crucial property of FtsA [7,8*]. Studies on *E.coli* FtsA have long been frustrated by the purified native protein showing little biochemical activities. Significant progress, therefore, is the recent demonstration that a purified hypermorphic variant, FtsA* (R286W), stimulates curvature and de-polymerization of FtsZ protofilaments in an ATP-dependent fashion [9**]. In vivo, FtsA* stimulates cell fission at reduced cell mass, and also has the remarkable property of supporting efficient cell division in the complete absence of ZipA [10,11]. The FtsZ depolymerizing activity of FtsA* suggests a role in Z-ring constriction during active cell envelope invagination (Fig. 1, stage III), but seems less compatible with the role of FtsA in ZR assembly and with the ability of FtsA* to compensate for the absence of a protein (ZipA) that normally stabilizes Z-rings in vivo and FtsZ polymers in vitro. Evidently, FtsZ depolymerization by FtsA must be tightly regulated in the cell, and future work on FtsA* may well elucidate how this is accomplished [9**].

The ZapA and ZapB proteins also associate with early Z-ring assemblies. Unlike FtsA and ZipA, these proteins are not essential and *E.coli* single mutants show only modest phenotypes [12–15*]. ZapA is well conserved, and like ZipA, binds and bundles FtsZ polymers in vitro, and promotes the assembly and stability of the ZR in vivo [16–18*]. The recently discovered ZapB appears restricted to the γ -proteobacteria. It is a small and abundant protein that forms antiparallel coiled-coil dimers that readily polymerize into

filaments in vitro [15*]. Initially suspected to interact with FtsZ directly, it primarily associates with the Z-ring via ZapA instead [14*]. Remarkably, fluorescent fusions to ZapB accumulate in a slightly smaller ring than ZapA or FtsZ itself, indicating that ZapB molecules form or decorate some structure that extends from the Z-ring far enough into the cytoplasm to be detectable by light microscopy [14*]. The purpose of such a structure is unclear, but it is possible that a cytoplasmic barrier forms before actual closure of the IM septal pore and that this may be advantageous.

Positioning of the Z-ring

Proper fission at midcell requires accurate placement of the Z-ring along the cell axes. One remarkable observation with the FtsZ-YFP-MTS chimera was that it spontaneously formed 'closed' rings in lipid tubules [6**], arguing that positioning of the ZR along the short axis can be largely determined by cell geometry and inherent properties of FtsZ itself.

Lateral positioning of the ZR is determined by two negative regulatory systems, Min and NO (nucleoid occlusion). NO counteracts assembly of Z-rings in the close vicinity of chromosomes and is mediated by DNA-binding proteins SlmA in *E.coli* [19] and by Noc in *B.subtilis* [20]. Noc binds to ~70 chromosomal sites that are conspicuously absent from the chromosomal terminus, and placing Noc binding sites in this region delays cell division, indicating Noc helps coordinate ZR assembly with progression of chromosome replication [21**]. Interestingly, it is likely only the DNA-bound form of Noc that is able to counteract ZR assembly, but how it does so still needs to be determined [21**]. *E.coli* SlmA interacts directly with FtsZ, but the precise mechanism by which it inhibits Z-ring assembly also needs clarification [19].

The Min system in *E.coli* is a dominant determinant of lateral positioning of the ZR, and its absence leads to frequent fission near a cell pole and the consequent release of chromosome-less minicells. It comprises the MinC, D, and E proteins and all three rapidly oscillate from one cell end to the other in a coordinated and interdependent fashion. Typically MinC, MinD, and a portion of MinE accumulate along the membrane at one cell end as another portion of MinE accumulates more sharply in a MinE-ring that appears to cap the Min polar zone and prevent it from extending past midcell. The MinE ring then moves poleward as the Min polar zone shrinks until most protein is released from that cell end and all three proteins reassemble on the membrane at the opposite end. MinD is a founding member of the MinD/ParA/Soj Walker A cytoskeletal ATPases (WACA) and is central to the Min system. Upon ATP binding, MinD forms a sandwich dimer and engages the IM via its C-terminal amphiphatic MTS. It then recruits additional MinD.ATP, forming larger oligomers/polymers on the membrane surface, as well as MinC and MinE homodimers from cytoplasmic pools [4,5,22,23].

MinC/MinD.ATP complexes (MinC/MinD) interfere with Z-ring assembly, and the mechanism has recently been further clarified. MinD binds to the C-terminal domain of MinC (MinC^C) and the MinC^C/MinD moiety of the complex in turn binds the C-core domain of FtsZ. This interaction itself interferes with Z ring assembly to some extent as: i) MinC^C/MinD competes with FtsA and ZipA for binding FtsZ polymers, and ii) MinC^C reduces lateral interactions between the polymers [24**,25*]. Importantly, binding of MinC/MinD to the C-core of FtsZ also positions the N-terminal domain of MinC (MinC^N) near its substrate. MinC^N is sufficient to inhibit Z ring assembly when overexpressed by itself in vivo, and it shortens FtsZ polymers in vitro [24**]. Unlike the SOS-induced division inhibitor SulA [26*], MinC^N does not interfere with polymerization per se, but is now proposed to specifically weaken those longitudinal bonds in FtsZ filaments that harbor GDP in the FtsZ subunit interface via an interaction with helix-10 of FtsZ [24**,27*].

Pole-to-pole oscillation of the Min proteins is critical as it ensures that the time-integrated concentration of membrane-associated MinC/MinD is highest at the cell ends and lowest at the desired site for SR assembly and fission (midcell). MinE promotes release of MinC/MinD complexes from the membrane both by competition with MinC for binding MinD and, importantly, by stimulating MinD to hydrolyze bound ATP. Oscillation results from the interplay of the interactions between MinD with ATP, itself, membrane, and MinE, and this has been extensively modeled and reviewed [4,22,28]. Most models predicted it would be possible to recapitulate MinD/MinE oscillation in minimal *in vitro* systems, and two groups reported important strides towards that end [29**,30**]. Both demonstrate spontaneous ATP-dependent dynamic patterning of MinD and MinE on the surface of planar lipid bilayers and that, as predicted by most models, pattern dynamics involves rapid cycling of the proteins on/off the membrane. Traveling waves of MinD/E as seen in the first study [29**] are in the second study [30**] often preceded by spatially homogenous oscillations and followed by the formation of dynamic ‘amoebas’ of a MinD-rich zone ringed by a MinE-rich border. The dimensions and dynamics of ‘amoebas’ most closely resemble a surface projection of MinD/E patterns seen *in vivo* [30**]. Notably, the latter study also argues against bulk diffusion coefficients of MinD and MinE as critical determinants in generating surface patterns as is assumed in many current models for MinD/E oscillation. Obviously, our understanding of Min oscillation is still too limited, and more refined models that consider direct interaction of MinE with phospholipids [31,32], effects of membrane potential on MinD-membrane binding [33*], effects of MinD/E on phospholipid surface properties [34–36], and possible biochemical timing mechanisms [30**] are needed. A solid understanding of MinD/E oscillation is important not only for understanding Z-ring placement, but also for our understanding of other WACA class cytomotive systems, and for our understanding of biological pattern formation in general.

In the absence of Min and NO, FtsZ still displays a modest bias for accumulation in-between segregated nucleoids, indicating the existence of a third system that contributes to positioning of the Z-ring [19,20]. Work with outgrown spores suggests that initiation of chromosome replication at *oriC* somehow helps control ZR positioning in *B.subtilis* in a Noc-independent and, possibly, positive fashion [37*]. This is an enticing prospect, but much needs to be learned before the effects can be fully understood.

Maturation of the Z-ring to a Septal ring

Temporally, there is a substantial delay between assembly of the Z-ring and recruitment of the later assembling components [38,39]. During this interval, the Z-ring directs cylindrical murein synthesis at midcell [2,40,41]. How the Z-ring does so, and what signals it to begin further maturation to an SR, are still pressing questions. Maturation involves recruitment of other SR proteins in an ordered fashion (Fig. 1, stage II). Structures of SR proteins [15*, 42*–45*], and detailed information on the multitude of interactions between them [46–51*] continue to emerge, helping to construct a picture of how components fit together in the constriction-competent organelle. This goal is also getting more challenging, however, as the number of known components of the division apparatus continues to climb. Besides ZapB [15*], recently identified components in *E.coli* include MurG [41,52], MtgA [53], FtsP [45*,54], YmgF [55], NlpD [56*], DacA [57], the SPOR-domain proteins DamX, DedD, and RlpA [58*,59], and the Tol-Pal complex [60,61*]. How each of the SR proteins joins the complex is still unclear for many. Though it is likely that specific protein-protein contacts often contribute, local substrate availability [57,62] or transient forms of murein generated at constriction sites [58*,59,63*] are proposed to provide important topological cues for localization of some of the SR proteins.

One intriguing example of the latter is FtsN, a core component whose accumulation at the SR appears to coincide with initiation of the constriction process [63*,64]. A major

localization determinant is its periplasmic C-terminal SPOR domain that is now thought to specifically recognize a transient form of septal murein [58*,59,63*,65]. However, initiation of constriction and generation of the SPOR localization substrate depends on a small periplasmic juxta-membrane domain of FtsN, leading to a model in which self-enhanced accumulation of FtsN at the SR helps to trigger and sustain the constriction process (Fig. 1, stage III) [58*,63*]. Despite progress, the exact essential activity of FtsN is still unclear. One reasonable model is that the small essential domain of FtsN promotes the onset of constriction by allosterically modulating the periplasmic murein synthase activities of PBP3 (FtsI), MgtA, and/or PBP1A and PBP1B [47,53,58*,63*]. However, the fact that production of a mutant form of FtsA can rescue division in the complete absence of FtsN (albeit inefficiently) suggests that the signal for septal murein synthesis may more directly emanate from the Z-ring on the cytoplasmic side of the membrane [58*,66].

Cell constriction

Once constriction ensues (Fig. 1, stage III) the SR needs to accomplish a number of tasks in a coordinated manner: i) IM invagination and fission, ii) septal murein synthesis, iii) septal murein splitting, and iv) Outer membrane (OM) invagination and fission.

The ability of FtsZ assemblies to deform membrane surfaces *in vitro* [6**,67**] has provided strong support for the idea that IM invagination during cell constriction is one of the jobs of the Z-ring portion of the SR. How the ZR exerts force on the membrane is the subject of much modeling activity and debate, but I'll defer to an excellent recent review on the topic [68]. One key experimental observation has been the *in situ* visualization of FtsZ polymers at the division site of *Caulobacter crescentus* cells by cryo-EM tomography, indicating they are unexpectedly sparse and solitary [69]. Whether this is true for other bacteria is unclear but, at least for *C. crescentus*, this result argues against lateral association of FtsZ polymers playing a decisive role in force generation. Bending of individual FtsZ polymers could provide a driving force [68,69]. Recent evidence that the bending direction of an FtsZ polymer is fixed relative to its long axis and also determines the direction of membrane deformation *in vitro* provides compelling support for this idea [67**].

Septal murein synthesis requires the transpeptidase PBP3 (FtsI) and at least one other synthase, likely PBP1A, PBP1B, and/or Mgt [47,53,70]. How septal murein is laid down is still poorly understood, but the simplest model is that it is added perpendicular to the cylindrical murein in a relatively thick layer that subsequently needs to be carefully split from the OM-proximal side in order to shape the two nascent cell poles and to allow OM invagination [71,72]. Splitting of septal murein is accomplished by murein hydrolases, most prominently the three murein amidases AmiA, B, and C [71,73,74**]. The LytM-domain proteins EnvC and NlpD are as critical for proper septal murein processing as the amidases, but how they act has been unclear [56*,75]. Recent *in vitro* and *in vivo* evidence convincingly shows that EnvC directly stimulates the murein amidase activities of AmiA and AmiB while NlpD stimulates that of AmiC [74**]. This work provides an important step in understanding how potentially lethal enzyme activities are controlled to perform precision surgery on the murein sacculus without compromising its structural integrity. The work also adds to other recent examples of murein synthase or hydrolase activities being controlled by protein regulators [47,76**] and increased efforts in this area can be expected.

Invagination of the OM was long thought to be a rather passive process as tethering of the OM to the murein sacculus by abundant murein-binding OM proteins such as Lpp and OmpA would inevitably cause the OM to move inwards as the septal murein was split into new poles during the constriction process. It is now clear, however, that efficient OM invagination requires the action of the Tol-Pal complex [60,61*], previously implicated in OM integrity and the uptake of colicins and ssDNA phages [77]. All components of the

complex, comprising three IM proteins (TolQAR), one periplasmic protein (TolB) and the OM lipoprotein Pal, accumulate at the SR near the time of constriction initiation and mutants lacking the intact complex display a substantial delay in OM invagination [60,61*]. It is proposed that Tol-Pal forms a sub-complex of the SR that uses proton motive force to establish transient transenvelope connections that draw in the OM as the IM and murein layers invaginate [60].

Finally, the septal pore in the IM must close to compartmentalize the cytoplasm and a similar closure of the OM must occur to compartmentalize the periplasm and finalize the separation of daughter cells. We know precious little about either membrane fission/sealing event. SpoIIIE is required for septal membrane fission during endospore formation in *B.subtilis*, but only if the pore entraps chromosomal DNA [78*]. This could also be true for the analogous DNA translocase FtsK during division in *E.coli*, but it is clear that FtsK-independent mechanisms of membrane fission must be operative as well [79*,80]. Whether OM septal pore closure is a spontaneous or controlled event is not known.

Conclusions

Progress in the last two years has been quite exhilarating and the pace of new discoveries is likely to accelerate further. The field is clearly benefiting from increased activities by both new laboratory leaders and by established labs across disciplines that bring valuable expertise in imaging, biophysical, biochemical, and theoretical approaches. Efforts to reconstitute parts of the division machinery and its regulators with purified components in vitro will increase, as will efforts to understand its precise molecular composition and molecular architecture. Much remains to be learned, not least about how all the activities of septal ring proteins are controlled to achieve an efficient and coordinated invagination of the three envelope layers, or how the whole constriction process is coordinated with other cell cycle events, such as chromosome replication and cell elongation/growth. Based on the last few years, answers to these and many other interesting questions will emerge sooner than one might think.

Acknowledgments

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[80], it is clear that FtsK-independent mechanism(s) of inner-membrane scission must exist. [PubMed: 20033058]

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Figure 1.

Schematic representation of septal ring assembly in *E. coli*. Indicated are three stages in development of the septal ring, known protein components, and the approximate step at which they become associated with the apparatus. Proteins that are essential for viability are underlined. Proteins assembling at the cytoplasmic face of the inner membrane are in blue, trans-membrane inner-membrane proteins are in black, periplasmic proteins in orange, and outer-membrane (lipo-) proteins are in purple. Regulators of Z-ring positioning are in red. Some septal ring components (listed in box) were left out of the recruitment pathway for clarity, or because pertinent information is missing.