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Curr Opin Microbiol. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:
Curr Opin Microbiol. 2015 April ; 24: 60–65. doi:10.1016/j.mib.2015.01.002.

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Guiding divisome assembly and controlling its activity

Mary-Jane Tsang and **Thomas G. Bernhardt***
Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115

Abstract

Cell division in bacteria requires the construction of two new polar caps for the daughter cells. To constrict the cell membrane and build these new surface layers, bacteria employ a multiprotein machine called the divisome. Over the years, most of the essential division proteins have been identified and localized to the ring-like divisome apparatus. The challenge now is to determine the molecular function of these factors, how they cooperate to bring about the dramatic transformation of the mother cell envelope, and what coordinates their activity with other major cell cycle events. In this review, we discuss recent progress in these areas with an emphasis on results from the model organisms *Escherichia coli* and *Bacillus subtilis*.

Introduction

The bacterial cell cycle culminates with the onset of cell division. The process initiates with the polymerization of the tubulin-like FtsZ protein into a ring structure (the Z-ring) just underneath the cytoplasmic membrane [1,2**]. Following Z-ring assembly, numerous essential and non-essential division proteins are recruited to midcell to form the mature division apparatus called the divisome or the septal ring [3]. Over the years, most, if not all, of the core proteins required for divisome activity have likely been identified [3,4]. A great deal has also been learned about the regulators that control Z-ring positioning to ensure that division takes place at the appropriate location. Despite this progress, major questions remain unanswered. Not all of the factors controlling Z-ring formation are known, including those that coordinate its assembly with the replication and segregation of the chromosome. Also, the precise functions of many core division proteins remain to be determined. Finally, although the steps of divisome assembly have been well characterized, the factors controlling the switch from an assembly phase to active cell constriction remain largely mysterious. This review focuses on recent work that has shed light on these outstanding questions. For a more in-depth overview of cell division, the reader is referred to several excellent reviews [3–6].

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*To whom correspondence should be addressed. Thomas G. Bernhardt, Ph.D., Harvard Medical School, Department of Microbiology and Immunobiology, Armenise Building, Room 302A, 200 Longwood Avenue, Boston, Massachusetts 02115, Phone: (617) 432-6971, Fax: (617) 738-7664, thomas_bernhardt@hms.harvard.edu.

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Connecting Z-ring formation to the chromosome

In the model bacteria *Escherichia coli* and *Bacillus subtilis*, the regulation of Z-ring placement is mediated by two negative regulators: the Min system and the nucleoid [7–11] (Figure 1). The output of the Min system is the FtsZ antagonist MinC, which together with its partner protein MinD, interferes with Z-ring formation [12–17*]. In *E. coli* the MinCD complex oscillates from pole-to-pole [13,14,18], whereas in *B. subtils* it is targeted to both cell poles [19]. However, the end result is the same in both cases; polar Z-ring formation is inhibited, and midcell Z-ring assembly is favored.

The phenomenon of nucleoid occlusion reflects the negative effect of the chromosome on division [9,10]. Division inhibitors that associate with the nucleoid to mediate nucleoid occlusion were identified several years ago: Noc in *B. subtilis* and SlmA in *E. coli* [20,21]. The target of Noc regulation remains unknown. SlmA, on the other hand, directly antagonizes FtsZ assembly [22,23*,24,25*,26,27**]. Irrespective of their precise molecular target, Noc and SlmA share a surprising number of features considering that they belong to different protein families. Both proteins bind to distinct, yet specific, DNA sequences that are broadly distributed around the origin proximal two-thirds of their respective chromosomes, but absent near the replication terminus (Ter region) [22,24,28]. Coupled with the known dynamics of chromosome regions during the replication cycle, this binding site distribution is thought to be one of the possible mechanisms for coordinating chromosome replication and segregation with division [22,24,28] (Figure 1). Mutants defective for the nucleoid occlusion proteins also share the property of being synthetically lethal with Min system inactivation [20,21]. Cells lacking both systems fail to divide in rich medium and form long filamentous cells [20,21]. Interestingly, Z-ring formation is not completely random in these cells. Robust structures were still primarily observed between segregated nucleoids in the cell filaments. It was thus suggested that additional positional queues exist to guide Z-ring formation and position it relative to the chromosome [20,21].

A breakthrough in this area was recently reported by Bailey and co-workers [29**]. Their quantitative study of cell division positioning in Min^- $SlmA^-$ *E. coli* cells grown in minimal medium, a condition previously shown to suppress the synthetic lethal phenotype [21], revealed that *slmA minC* cells divided more accurately at midcell than a singe *minC* mutant [29**]. Surprisingly, they also observed a dramatic drop in the number of polar (minicell) divisions displayed by the *slmA minC* mutant relative to cells lacking MinC alone, which showed the classic minicell phenotype [29**]. These findings t hus suggested that a new positional marker at midcell becomes a dominant feature guiding Z-ring assembly when SlmA is inactivated in *minC* cells. Further investigation implicated the chromosomal terminus organization protein MatP [30,31*] as the potential marker [29**]. This possibility was intriguing because MatP interacts with the ZapB protein, which together with ZapA associates with FtsZ and helps to coalesce the Z-ring structure [32**–35*]. Espeli and co-workers [32**] showed that this network of interactions is important for “anchoring” the Ter chromosomal domain to midcell after it localizes to this region during replication. Bailey and colleagues show that in *slmA minC* cells these interactions can also stimulate Z-ring formation at midcell [29**]. It currently remains to be determined whether the Ter region provides an important guide for Z-ring positioning in wild-type cells or if the connection

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between the Z-ring and the Ter domain simply functions to maintain and/or stabilize the midcell localization of these macromolecular structures. In either case, these two reports [29**,32**] highlight the potential for distinct domains in the chromosome and their associated binding proteins to function as landmarks for the proper organization of cellular processes.

Using outgrowing *B. subtilis* spores as a model, Rodrigues and Harry also recently observed precise midcell Z-ring formation in the absence of Min and nucleoid occlusion [36**]. This finding has led to the proposal that midcell is identified independently of these factors and that Min and Noc may primarily function to ensure the efficient utilization of this site. Although the identity of the factor(s) that determine(s) this positioning is not clear, several previous studies from the Harry laboratory implicate the early stages of chromosome replication in Z-ring formation and positioning [37–39]. Further support for a link between DNA replication and cell division in *B. subtilis* was also recently reported by Arjes and colleagues [40**]. They find that after several mass doublings following division inhibition, the resulting cell filaments are unable to initiate new rounds of replication. Intriguingly, Arjes and colleagues a lso find that cell division is inhibited after several generations following a block in the initiation of DNA replication [40**]. It therefore appears that, contrary to the widely held view in the field, there is an obligatory link between cell division and DNA replication, at least in *B. subtilis*. Although the mechanism of this coupling remains unclear, an exciting possibility is that the factors involved here [40**] are also responsible for the phenomena observed by Harry and co-workers [36**–39] connecting early stages of replication with Z-ring formation.

Controlling divisome activity

In *E. coli*, recruitment of essential divisome components to midcell proceeds via a mostly linear dependency pathway starting with the FtsZ-interacting proteins FtsA and ZipA that anchor the Z-ring to the membrane and ending with the bitopic membrane protein FtsN [41–52]. Because it is the last divisome protein in the recruitment pathway, FtsN has long been though to play a role in the switch from divisome assembly to the constriction phase of division [53]. This idea was reinforced with the demonstration that FtsN joins the divisome in a self-enhancing process involving its small, membrane-proximal, essential domain (^{E}FtsN) and its C-terminal, peptidoglycan (PG)-binding SPOR domain (^{S}FtsN) [54,55]. Based on this observation, it was proposed that cell constriction is driven by a positive feedback loop in which ^{E}FtsN stimulates the synthesis and remodeling of cell wall material by other divisome components to create the recruitment signal for ^{S}FtsN, which brings more ^{E}FtsN to the division site to stimulate more cell wall synthesis, and so on [54] (Figure 2).

In addition to binding FtsZ, FtsA was recently demonstrated to interact directly with the cytoplasmic N-terminus of FtsN (^{N}FtsN) [56**]. This interaction was shown to be important for the initial localization of FtsN to the divisome [57*], suggesting that the FtsA-^{N}FtsN interaction may be responsible for initiating the proposed positive feedback loop that promotes constriction (Figure 2). Clues as to how this process may work have come from the isolation and analysis of *ftsA* mutants in *E. coli* that bypass the normal requirement for

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other essential division proteins [58–60]. The first "bypass mutant" identified was *ftsA*(R286W)* [59]. It was isolated as a suppressor that allowed cells to survive in the absence of the other membrane anchor of FtsZ, ZipA. Subsequent studies indicated that this allele and/or other alleles of *ftsA* were also able to bypass the essential functions of divisome proteins FtsK and FtsN, and also suppress the division defects of certain temperature-sensitive *ftsQ* mutants [58,60,61]. Pichoff and co-workers [62**] recently found that the activity of FtsA bypass variants is likely related to defects in FtsA-FtsA interactions. They used a genetic screen to identify FtsA derivatives with a reduced ability to self-interact as assessed by in vivo assays. Strikingly, this collection of variants included the original FtsA* variant. They subsequently showed that all self-interaction defective variants of FtsA could function as ZipA bypass suppressors [62**]. Furthermore, in addition to bypassing the function of essential division proteins, many of the poorly self-interacting FtsA variants were also shown to promote early cell division [62**], suggesting that a reduction in FtsA-FtsA interactions stimulates division. Importantly, the FtsA-^{N}FtsN interaction described above involves the 1c domain of FtsA [53,56**]. Based on a structural analysis of FtsA polymers [63**], this interaction is likely to interfere with FtsA-FtsA interactions. Thus, when all of the genetic and biochemical studies are taken together, the results point to a competition between FtsA-FtsA and FtsA-^{N}FtsN interactions in the control of constriction initiation with both ^{E}FtsN and monomeric FtsA stimulating the process [3,56**,57*,62**, 64*] (Figure 2). One possible scenario suggested previously [3,62**,64*] is that ZipA disrupts FtsA-FtsA interactions at the Z-ring to generate free FtsA interfaces for the recruitment of downstream divisome proteins like FtsN and the eventual activation of constriction. Thus, FtsA* variants with a reduced capacity to interact bypass ZipA function. Alternatively, or in addition to serving as a recruitment factor for other division proteins, the polymeric status of FtsA and its interaction with FtsN may also serve as a sensor or signal [56**,57*,65] used to monitor the status of divisome assembly and promote constriction only after the machinery is deemed stable enough to successfully complete division. In this case, FtsA may be progressively converted to a reduced polymeric form as the machine assembles, at first spontaneously, but then stimulated by the self-enhanced recruitment of FtsN. This conversion of FtsA would proceed until a threshold level of monomers or small oligomers is achieved, which, together with FtsN, would then somehow trigger a change in other components of the machinery to to stimulate ring closure. How this activation might occur is not clear, but recent results indicate that the FtsQLB subcomplex of the divisome is involved and may receive signals from both FtsA and FtsN in order to activate cell wall synthesis and remodeling at the division site to stimulate constriction [66*,67*].

In addition to its potential role in sensing divisome assembly, the direct connection of FtsA with FtsZ also puts it in position to play a key role in modulating Z-ring activity and/or dynamics during the division cycle. This possible function was highlighted by a recent study of FtsZ polymer dynamics on supported lipid bilayers [68**] that showed dramatic changes in the FtsZ patterns formed depending on whether FtsZ was recruited to the membrane by FtsA or ZipA. Polymer bundles that associated with the membrane surface via an interaction with ZipA were found to form relatively stable patterns [68**]. Polymers brought to the membrane by FtsA, on the other hand, formed rapidly rotating swirls [68**]. These dynamic patterns were shown to result from the ability of FtsA to destabilize the FtsZ polymer

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network [68**]. This observation connects with in vivo results indicating that the FtsA/FtsZ ratio is important for proper division and that too much FtsA can inhibit Z-ring formation [69,70]. Thus, FtsA may, at least initially, promote the formation of a more dynamic, less stable pattern of FtsZ polymers at midcell. An attractive possibility is that changes in FtsA polymerization status brought about through the recruitment of downstream divisome proteins like FtsN may change the effect of FtsA on FtsZ polymer dynamics such that it now stabilizes the Z-ring pattern at midcell and promotes constriction (Figure 2). In support of this possibility, the self-interaction defective FtsA(R286W) derivative has been shown alter the Z-ring to reduce its sensitivity to negative-regulators [59]. However, this variant of FtsA was not found to alter the FtsZ swirl patterns formed in the supported lipid bilayer experiments [68**], suggesting that factors in addition to changes in FtsA self-association status are likely needed to more faithfully reconstitute FtsZ pattern formation at the membrane in vivo. Nevertheless, the possibility that FtsA polymer status controls Z-ring and/or divisome activity remains highly attractive and warrants further investigation.

Conclusions

Most of the straightforward aspects of divisome assembly, such as determining what proteins localize to the structure and when they get there, have been extensively characterized over the last two decades. We now face questions that are much more difficult to solve. What are all of these proteins doing at the division site? How do they work together to transform the cell envelope? What couples their activity to other major cellular processes like DNA replication? As described in this review and the highlighted references, progress in these areas is being made thanks to multidisciplinary efforts that encompass everything from genetic analysis and in vivo imaging to biochemical reconstitutions and structural biology. We look forward to seeing how the mechanistic picture of bacterial cell division evolves as we continue to apply these and other emerging technologies to understand this fundamental process.

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Highlights

- The chromosome-binding protein MatP plays a role in division site positioning
- DNA replication and cell division are obligatorily coupled in *Bacillus subtilis*
- FtsA-FtsA interactions play a critical role in divisome maturation and activation

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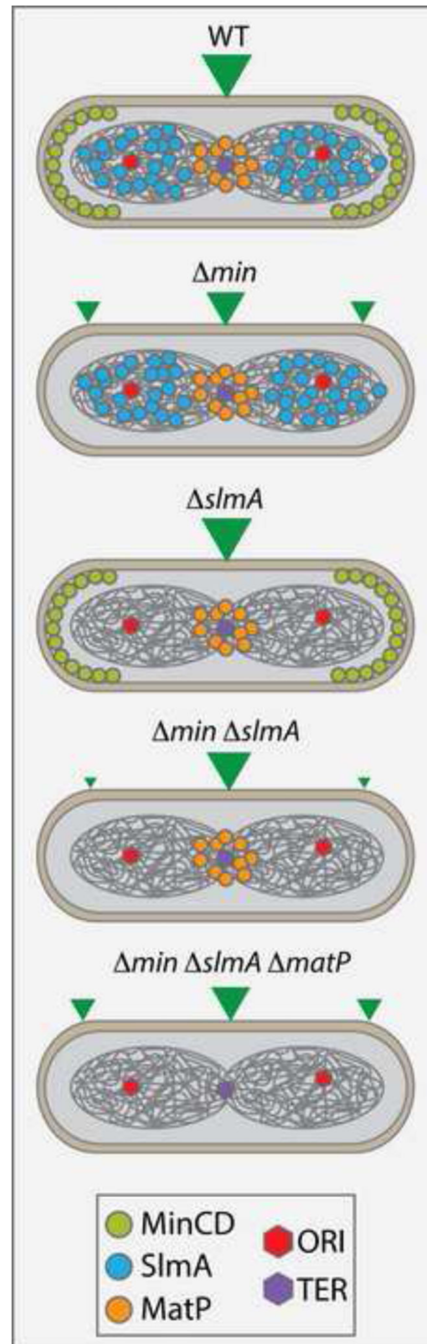

Figure 1. Determinants of division site positioning in *E. coli*

Shown is an illustration summarizing the results of Bailey et al. 2014 [29**] showing that MatP and the Ter macrodomain of the chromosome can serve as a determinant of division site positioning in addition to Min and SlmA. Green triangles indicate possible division sites with their size reflecting preference for a particular site. See text for details.

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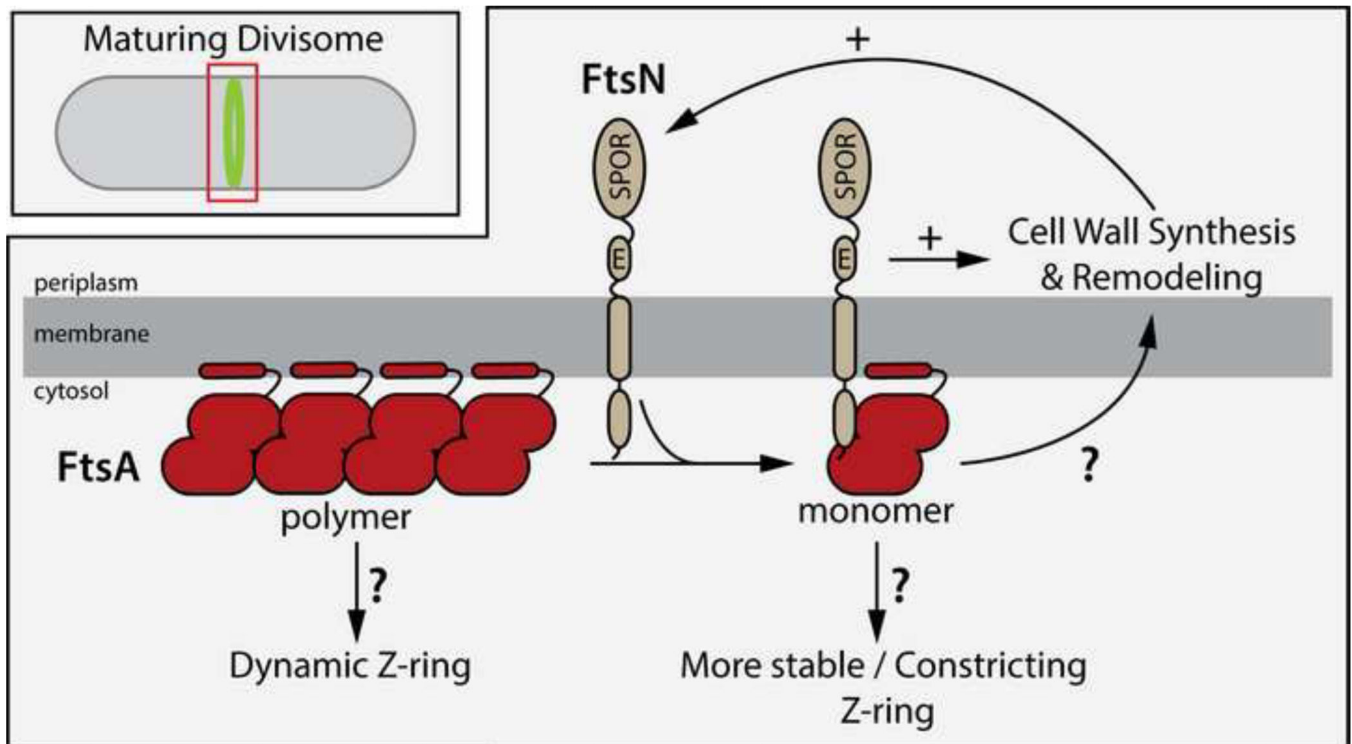

Figure 2. Potential role of FtsA-FtsN interactions in triggering cell constriction

Shown is a diagram depicting potential events occurring at a maturing divisome focusing on the FtsA-FtsN interaction. As described in the text, a growing number of studies suggest the attractive possibility that FtsA serves as a "sensor" of divisome assembly. As late components of the divisome like FtsN are recruited to the structure, they promote the formation of a reduced polymeric form of FtsA. Once a threshold level of this altered FtsA form accumulates at midcell, it may trigger changes in FtsZ polymer dynamics to initiate contraction of the ring in conjunction with the activation of divisome components associated with cell wall synthesis by both FtsA and FtsN. These activities of FtsA and its partner FtsN may coordinate transitions in the Z-ring pattern with cell wall remodeling processes on the other side of the membrane. Although not shown, it has also been proposed that ZipA may promote the formation of FtsA monomers to stimulate the recruitment of downstream divisome proteins like FtsN [3,62,64]. See text for details.