Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Guiding divisome assembly and controlling its activity

Mary-Jane Tsang and Thomas G Bernhardt

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*.

Addresses
Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, United States

Corresponding author: Bernhardt, Thomas G (thomas_bernhardt@hms.harvard.edu)

Current Opinion in Microbiology 2015, **24**:60–65

This review comes from a themed issue on **Cell regulation**

Edited by **Carol Gross** and **Angelika Gründling**

For a complete overview see the Issue and the Editorial

Available online 28th January 2015

http://dx.doi.org/10.1016/j.mib.2015.01.002

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].

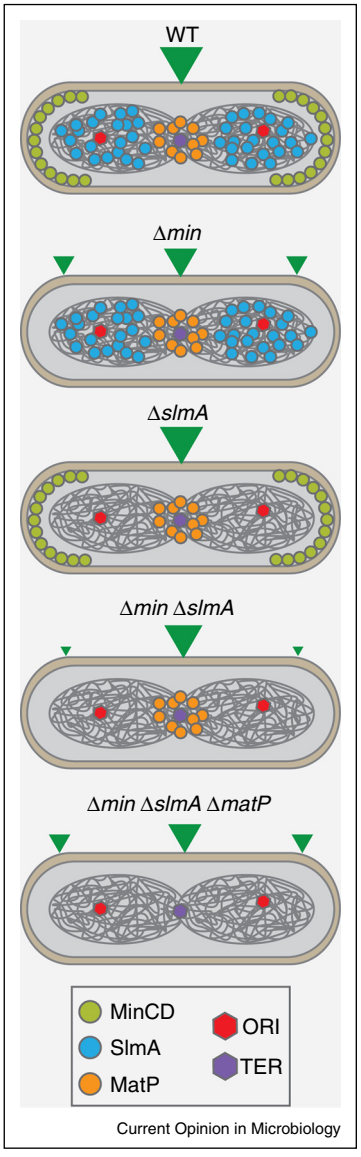
Connecting *Z*-ring formation to the chromosome

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 [12–16,17•]. 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

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.

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 Δ*slmA* Δ*minC* cells divided more accurately at midcell than a single Δ*minC* mutant [29••]. Surprisingly, they also observed a dramatic drop in the number of polar (minicell) divisions displayed by the Δ*slmA* Δ*minC* mutant relative to cells lacking MinC alone, which showed the classic minicell phenotype [29••]. These findings thus suggested that a new positional marker at midcell becomes a dominant feature guiding *Z*-ring assembly when SlmA is inactivated in Δ*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••,33,34,35•]. 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 Δ*slmA* Δ*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 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 also 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••,37–39] 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].