Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

Division site selection in rod-shaped bacteria

Marc Bramkamp and Suey van Baarle

Rod-shaped bacteria often divide with high precision at midcell to produce two equally sized daughter cells. The positioning of the division machinery in *Escherichia coli* and *Bacillus subtilis* is spatially regulated by two inhibitory systems, the nucleoid occlusion and the Min system. The current models suggest that the target of the inhibitory mechanism is the cytoskeletal element FtsZ and that the concerted action of nucleoid occlusion and Min are necessary for correct placement of the division machinery. However, recent advances show that at least the Min system also ensures that division occurs only once in a cell cycle and might also act downstream of FtsZ assembly.

Address
Institute for Biochemistry, University of Cologne, Zülpicher Str. 47, D-50674 Cologne, Germany

Corresponding author: Bramkamp, Marc (marc.bramkamp@uni-koeln.de)

Current Opinion in Microbiology 2009, **12**:1–6

This review comes from a themed issue on
Prokaryotes
Edited by Patrick Viollier

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2009.10.002

Introduction

Cell division is a highly complex cytological process to produce viable progeny. In virtually all cells cytokinesis requires cytoskeletal elements [1••]. In bacteria the tubulin homolog FtsZ is the central cell division protein [2] that assembles into a cytoskeletal scaffold known as the Z-ring. Subsequently, other proteins that drive invagination of cell membrane and synthesis of cell wall material are recruited to the division site [3,4]. It has long been known that cell division in rod-shaped bacteria is restricted to midcell, between the segregated nucleoids [5,6]. In the last decades research has revealed that two inhibitory systems are involved in the spatial regulation of cytokinesis (Figure 1). The Min system has been shown to prevent aberrant cell division close to the cell poles, while nucleoid occlusion (NO) prevents cell division from occurring over the nucleoids [7–9]. Although, the textbook view on division site selection is that the inhibitory effect is due to the combined action of the Min and NO systems on FtsZ, a number of publications have provided evidence that the regulation of cytokinesis by the Min system could also occur downstream of FtsZ assembly [10•,11].

This review will focus on division site selection during vegetative growth in the rod-shaped model organisms *Escherichia coli* and *Bacillus subtilis*. Although the Min system is widespread among bacteria, other regulatory principles have evolved in bacteria whose genomes do not encode prototypical Min/NO systems. An interesting example is the spatial control of division in the vibrioid rod *Caulobacter crescentus* ([12••], and see the review by M Thanbichler in this issue). We emphasize recent discoveries that challenge and extend the classical view of the Min system.

The cytokinetic machinery

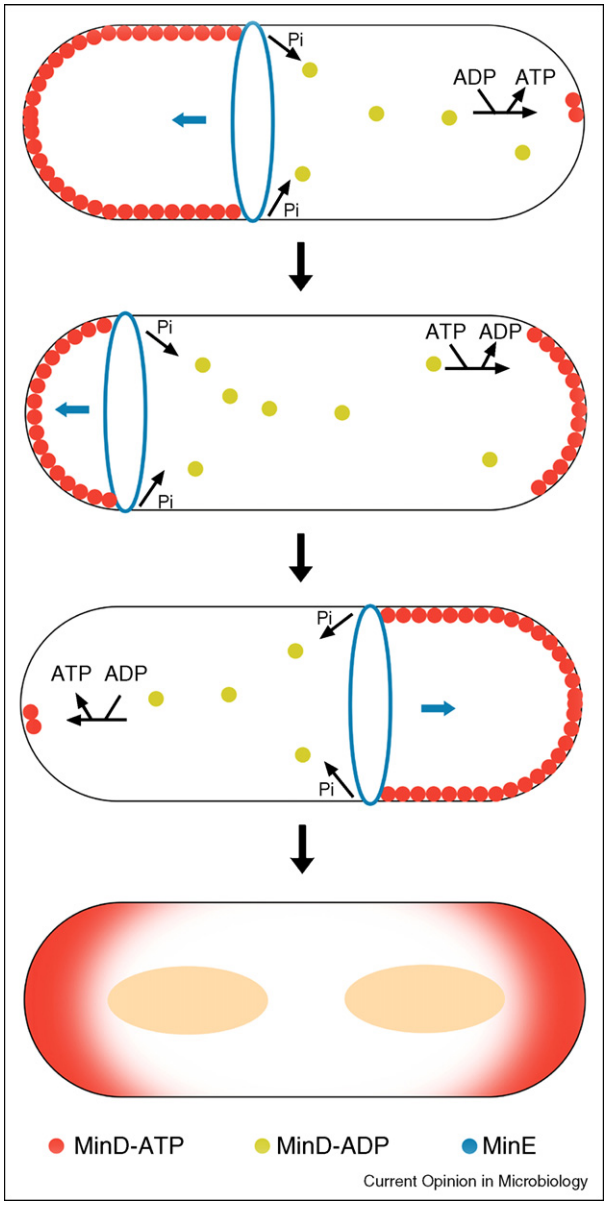
The cytokinetic machinery, or divisome, is highly conserved in bacteria and many of the essential components are found in almost all bacterial cells. The tubulin homolog FtsZ is the first protein to be localized at the incipient division site [2,13]. When bound to GTP, FtsZ assembles into protofilaments that can interact laterally to form the Z-ring. Associated proteins like ZapA and ZipA promote lateral bundling and help the Z-ring to coalesce into a functional ring [14,15]. The Z-ring is associated with the plasma membrane through FtsA [16], which peripherally binds to the membrane via its amphipathic helix [17]. The membrane-bound Z-ring subsequently recruits a set of membrane-integral proteins, as reviewed in detail by [1••,18,19].

Nucleoid occlusion

One of the two identified systems that determine division site selection is the NO system. Although nucleoid occlusion was proposed long ago [7], the actual effector, Noc (*yyaA*) of *B. subtilis* was only identified recently [8]. Although a *noc* null mutation had no obvious phenotype, a conditional deletion with *minD* resulted in a severe division defect with a failure to form functional Z-rings between the nucleoids, while overproduction led to cell elongation. A GFP–Noc fusion protein localized to the nucleoid and *in vitro* studies confirmed the DNA-binding capacities of Noc. When initiation of DNA replication was inhibited by depletion of DnaA, cell division in a *noc* mutant occurred over the nucleoid [8]. Noc is highly homologous to the partitioning protein ParB and in a similar mechanism Noc binds to a palindromic sequence located throughout the chromosome, with the exception of the terminus region [20•]. The transplantation of the palindromic consensus sequence to a multicopy plasmid resulted in a severe block in cell division, adding to the

Please cite this article in press as: Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria, Curr Opin Microbiol (2009), doi:10.1016/j.mib.2009.10.002

Figure 1



Division site selection in *E. coli* is governed by a dynamic Min system. The MinCDE system in *E. coli* is oscillating between the cell poles. MinD-ATP (red spheres) binds to the cell membrane and recruits MinC (not shown). MinE (blue ellipse) binds to MinD and activates ATP hydrolysis and inorganic phosphate (P_i) is released. This reaction leads to effective removal of MinD-ADP (green spheres) and, as passive passengers, MinC and MinE from the membrane. Nucleotide exchange in the cytoplasm reactivates the membrane affinity of MinD and subsequently, MinD-ATP assembles at membrane areas that are far distant from the highest MinE concentration (usually the opposite pole). This simple oscillation generates over time a MinCD gradient which is lowest at midcell. The last cell in the panel should illustrate this gradient (red shading). The replicated and segregated nucleoids are illustrated in this cell as light orange ellipsoids. The nucleoid occlusion factor (SlmA in *E. coli*) is bound to the DNA and leads to the inhibition of efficient Z-ring formation in this area. Together, the dynamic Min system and the nucleoid occlusion allow assembly of a functional cytokinetic ring only at midcell.

notion that nucleoid-associated Noc is indeed able to inhibit cytokinesis [20•]. The NO effect in *E. coli* is carried out by SlmA (synthetic lethal with a defective Min) [9]. SlmA is not related to Noc, but is functionally similar. SlmA has been shown to bind DNA and, as with Noc, depletion of DnaA in the absence of SlmA resulted in cytokinesis across the nucleoid, showing that SlmA is required for an antiguillotine checkpoint. *In vitro* experiments confirmed that SlmA is able to directly bind FtsZ and, interestingly, promote polymer assembly [9]. It may sound paradox that promoting lateral assembly of FtsZ should inhibit division. However, SlmA recruits FtsZ away from the cell membrane and may also compete with other known FtsZ assembly factors, such as ZapA. Hence, SlmA reduces the ability of FtsZ polymers to develop into a functional cytokinetic ring near the nucleoid.

The Min system

The Min system prevents aberrant division at the cell poles [21••,22] and it consists of the actual inhibitor MinC, a membrane-associated ATPase MinD that localizes MinC to the plasma membrane and a topological factor that spatially organizes the inhibitory MinCD complex. Only the topological factor differs between *E. coli* (MinE) and *B. subtilis* (DivIVA). Interestingly, the Min system is also found in chloroplasts [23••]. Chloroplasts contain MinD and MinE; however, a MinC-like protein has not been identified.

The inhibitory MinCD complex

In the absence of any component of the Min system, division close to the cell poles generates an anucleate minicell [24,25]. *In vivo* overexpression and *in vitro* experiments with purified components revealed that MinC is an inhibitor of division [26,27•,28]. The molecular details of MinC action are still not fully understood but a direct effect of MinC on FtsZ has been shown for *E. coli* and *B. subtilis* [27•,28]. Early models suggested that MinC prevents FtsZ polymerization. However, recently it was shown that *E. coli* MinC antagonizes the scaffolding function of FtsZ by inhibiting lateral interactions of FtsZ. This effect can be suppressed by ZapA, a positive factor of lateral FtsZ associations [29•]. *In vitro* studies with *E. coli* MinC showed that the N-terminal and C-terminal domains of MinC inhibit FtsZ assembly via two distinct inhibitory mechanisms [30•,31,32]. The N-terminal domain of MinC is essential for FtsZ binding and has a potent inhibitory effect that is observed even in the absence of MinD, while the C-terminal domain is important for the interaction with MinD. Recent results also revealed that the C-terminal domain is able to inhibit FtsZ *in vivo* [33•]. Interestingly, overexpression of the C-terminal part of MinC displaced FtsA (and/or ZipA), which would prevent recruitment of downstream division proteins [30•]. The structure of MinC clearly supports the idea that MinC is composed of two independent domains

Please cite this article in press as: Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria, Curr Opin Microbiol (2009), doi:10.1016/j.mib.2009.10.002

[34]. Strikingly, a comparison between MinC from *E. coli* and *B. subtilis* reveals that the C-terminal domain is conserved in Gram-negative and Gram-positive bacteria, while the N-terminal part seems to be less conserved. MinC is recruited to the membrane by MinD. MinD is an ATPase that belongs to the ParA/MinD family of proteins [35] and associates with the membrane via a C-terminal amphipathic helix [36]. Membrane association of the MinCD complex is essential for proper FtsZ inhibition. Interestingly, in *E. coli*, a gene product, DicB, of the cryptic prophage Kim, is able to recruit MinC and localize it to the site of septation independent of MinD [37]. Thus, the inhibitory function of MinC can be activated by two different proteins.

The topological determinants, MinE and DivIVA

The inhibitory activity of the MinCD complex must be restricted to the cell poles, which in *E. coli* is carried out by MinE [25,38,39]. The MinCDE complex shows a remarkable oscillation between the poles of *E. coli* cells (Figure 1). Oscillation is driven by MinE which stimulates ATP hydrolysis of MinD. Upon ATP hydrolysis MinD (and hence MinC) is released from the membrane into the cytoplasm [40,41]. Subsequently, cytosolic MinD binds ATP and reassembles at the opposite pole. Protein–protein interactions between MinD and MinE result in the recruitment of MinE to the outer rim of the MinD-covered membrane. MinCDE can also use ectopic poles of branched cells for oscillation, showing that the system is able to detect geometrical cues [42]. Strikingly, the self-organization of the *E. coli* Min system can be reconstituted *in vitro* with the minimal components MinD, MinE, ATP, and a phospholipid membrane [43••]. The self-organization of the Min system in *E. coli* has been analyzed intensively using mathematical modeling [44•,45]. The common idea of all mathematical models is the dynamic instability of protein distribution as a prerequisite for oscillation. Most current models favor a cooperative binding of MinD (in its ATP bound state) to the membrane at sites that are already covered with MinD [44•].

DivIVA is the topological factor in *B. subtilis* [46,47]. Mutations in *divIVA* lead to a dispersed MinCD localization as well as filamentation and minicell formation [48]. GFP fusions to DivIVA revealed that the protein localizes to the cell poles and is recruited late to the assembling divisome where it is then retained, after the divisome is disassembled [4,47]. Strikingly, DivIVA is able to find curved membrane regions even in unrelated cells, such as *E. coli* or even yeast cells [49]. On the basis of these observations it seems likely that DivIVA has the intrinsic capacity to bind and accumulate at negatively curved membranes. However, DivIVA itself was not able to impose negative curvature [50•,51].

In striking contrast to the oscillatory behavior of the Min system in *E. coli*, DivIVA was thought to stably recruit MinCD to the cell poles in *B. subtilis* [48,52]. A report using a functional MinC–GFP fusion provided the first evidence that MinC (and hence MinD) is not static in *B. subtilis*, but rather dynamically relocalized from the old pole to the site of septation [53••]. Later in the division cycle strong accumulation of MinCD was consequently found only at the young poles, suggesting that MinCD ensures that division is not reinitiated at sites of recent septation.

The novel component MinJ

Recently, MinJ, a novel component of the Min system has been identified in *B. subtilis* [10•,54•]. The phenotype of a *minJ* (*yvjD*) null mutant was almost identical to a *divIVA* knockout, with filamentous cells and a significant production of minicells. The *minJ* associated block in division could be suppressed by a simultaneous deletion of *minCD* as is the case in a *divIVA* mutant [10•,54•]. GFP fusions of MinJ localized to the cell poles and to the division site [10•,54•]. The new division site selection protein MinJ was shown to be the missing link in the interaction between DivIVA and MinD. Thus, MinJ acts as an adaptor protein linking the topological factor DivIVA to the inhibitory complex MinCD. A bacterial two hybrid interaction map could be corroborated by *in vivo* localization studies [10•,54•]. Subcellular localization of MinJ depends on DivIVA, while MinJ is necessary for correct localization of MinD and, hence, indirectly for MinC. Unlike the conventional Min proteins, MinJ is a membrane-integral protein that spans the membrane six times. It was shown that the localization of membrane-integral division proteins like FtsL or Pbp2B is facilitated by MinJ and, interestingly, MinJ interacts with several division proteins [10•]. These findings suggest that MinJ connects the membrane-integral part of the divisome with the Min system. This raises the question whether the regulatory function of the Min system only acts on the level of FtsZ polymerization or whether there is also a second regulatory level that acts on the membrane-integral divisome components. In fact the classical view on the Min system was that FtsZ polymers are not present in the vicinity of MinCD. Hence, dispersed MinCD, would suppress Z-ring formation; and lead to filamentation. However, new results with the *minJ* mutant showed that although the inhibitory MinCD complex is dispersed throughout the cell, Z-rings form readily in between the segregated nucleoids (and close to the cell poles) in *B. subtilis* [10•].

EzrA — part of the division site selection system?

Nucleoid occlusion and the Min system are not the only factors that negatively regulate Z-ring formation. Using a temperature-sensitive *ftsZ* allele in *B. subtilis*, the gene locus *ezrA* (extra Z-rings) was identified [55]. EzrA inhibits Z-ring formation and as a result, the concentration of FtsZ needed to form functional Z-rings is lowered in an *ezrA* mutant. Similar to a *minCD* mutant, *ezrA* depleted

Please cite this article in press as: Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria, Curr Opin Microbiol (2009), doi:10.1016/j.mib.2009.10.002

cells exhibit polar Z-rings. In contrast to *minCD* mutants, however, these Z-rings rarely constrict, suggesting that the divisome does not mature at these sites. Unlike MinCD, EzrA is localized throughout the membrane and accumulates at the septum due to binding with FtsZ. The FtsZ inhibitory function was mapped in the C-terminal domain of EzrA [56,57]. Biochemical evidence suggests that EzrA interferes with the GTPase activity of FtsZ [58].

Conclusions

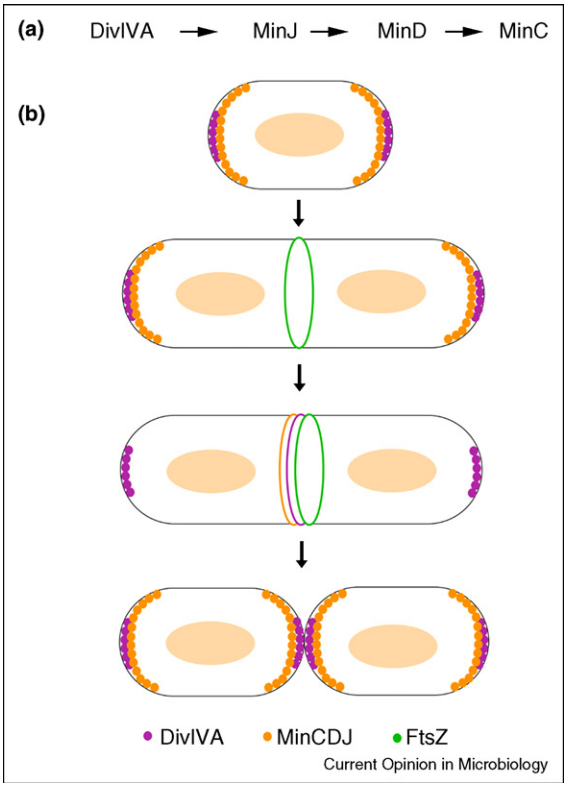
Division site selection in rod-shaped bacteria is regulated by at least two negative regulators of FtsZ assembly, the NO and the Min system. Their combined action defines the site of septation in many rod-shaped bacteria. Recent discoveries have added to the knowledge about the mechanism how the division machinery is spatially regulated and opened up new, unexpected, insights into division site selection.

A revised mode of action for the Min system

An overwhelming amount of publications shows that the inhibitory effect of the Min system is based on a direct effect of MinC on FtsZ. Thus, by spatially organizing MinC towards the cell poles, the central region in between the segregated nucleoids is the only available site for FtsZ to polymerize efficiently into a mature Z-ring which has the potential to recruit all downstream division proteins. However, this model has recently been challenged by different observations. The filamentous phenotype of cells where MinCD is dispersed was thought to be due to uncontrolled action of MinCD throughout the cell [48], inhibiting the formation of Z-rings. However, at least for *B. subtilis*, it has been reported that the cytosolic components of the Z-ring (e.g. FtsZ, FtsA, and ZapA) can assemble in the absence of the topological determinants DivIVA and MinJ, although MinCD is dispersed [10•]. This suggests that MinCD acts on division proteins downstream of the Z-ring assembly, as well. The novel division site selection protein MinJ seems to couple this inhibitory effect of MinCD to the membrane-integral parts of the divisome. Further support for the notion that the Min system can act downstream of FtsZ assembly comes from results that were obtained with *ezrA* mutants. Although Z-rings form with high frequency close to cell poles in an *ezrA* mutant, only a few minicells are observed in these strains, compared to a *minCD* mutant [55], suggesting that MinCD prevents the maturation of Z-rings into functional cytokinetic machines. One report shows that FtsZ-rings do form at regular intervals in *E. coli* filaments generated by MinCD overexpression [11], similar to the results reported for *B. subtilis* [10•].

Furthermore, the current models for Min systems suggested a static model in *B. subtilis* and a dynamic model in *E. coli*. However, recently, it was shown that the Min system in *B. subtilis* is indeed less static than originally supposed [53••] and that the main site of MinCD activity is at the young poles protecting sites of active division from reinitiation of division. Therefore, the Min system ensures that division is only initiated once per cell cycle. How is this activity achieved? We propose a model were the MinCDJ system in *B. subtilis* is dynamically relocalized from the old poles to the divisome during the late steps of divisome assembly (Figure 2). At this stage the divisome is already committed to divide and the FtsZ inhibitory function of MinC cannot be implemented at this stage. However, after completion of septation the

Figure 2



Revised model for division site selection in *B. subtilis*. A summary of the interactions between the division site selection proteins in *B. subtilis* is given in (A). MinJ interacts with DivIVA and MinD, while MinC only interacts with MinD and DivIVA only interacts with MinJ. The order of proteins also shows the dependency for their localization *in vivo*. The revised model for division site selection in *B. subtilis* takes into account that MinC (and presumably MinD and MinJ) is dynamically relocalized to the maturing divisome (B). In newborn cells DivIVA (purple spheres) recruits the MinCDJ complex (orange spheres) to the poles. Note that only MinJ binds directly to DivIVA. Upon polymerization of FtsZ (green spheres) into a Z-ring (green ellipse) at midcell, MinCDJ is recruited away from the cell poles to the site of active division. A reason for the recruitment of the inhibitory MinCDJ complex to the divisome is likely to prevent a new round of division. After completion of septation, MinCDJ is distributed evenly to both poles. The orange ellipses represent the segregated nucleoids, which impose the nucleoid occlusion effect (here executed by the Noc protein) and together with the Min system restrict division to midcell, only.

Please cite this article in press as: Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria, Curr Opin Microbiol (2009), doi:10.1016/j.mib.2009.10.002

MinCDJ system ensures effective disassembly or prevention of immediate reassembly of the division machinery at the young pole.

Acknowledgements

We thank Catriona Donovan, Inga Wadenpohl, and Frank Bürmann for critical comments on the manuscript and fruitful discussions. Work in the author's lab is funded by the Deutsche Forschungsgemeinschaft (DFG). SB was supported by the Max Planck Society and an EU Marie-Curie ADOPT fellowship.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Oliferenko S, Chew TG, Balasubramanian MK: **Positioning cytokinesis**. *Genes Dev* 2009, **23**:660-674.
This excellent review concisely describes the mechanisms that position the division machineries in prokaryotes and eukaryotes.

2. Bi EF, Lutkenhaus J: **FtsZ-ring structure associated with division in *Escherichia coli***. *Nature* 1991, **354**:161-164.

3. Aarsman ME, Piette A, Fraipont C, Vinkenvleugel TM, Nguyen-Disteche M, den Blaauwen T: **Maturation of the *Escherichia coli* divisome occurs in two steps**. *Mol Microbiol* 2005, **55**:1631-1645.

4. Gamba P, Veening JW, Saunders NJ, Hamoen LW, Daniel RA: **Two-step assembly dynamics of the *Bacillus subtilis* divisome**. *J Bacteriol* 2009, **191**:4186-4194.

5. Adler HI, Fisher WD, Cohen A, Hardigree AA: **Miniature *Escherichia coli* cells deficient in DNA**. *Proc Natl Acad Sci U S A* 1967, **57**:321-326.

6. Teather RM, Collins JF, Donachie WD: **Quantal behavior of a diffusible factor which initiates septum formation at potential division sites in *Escherichia coli***. *J Bacteriol* 1974, **118**:407-413.

7. Woldringh CL, Mulder E, Huls PG, Vischer N: **Toporegulation of bacterial division according to the nucleoid occlusion model**. *Res Microbiol* 1991, **142**:309-320.

8. Wu LJ, Errington J: **Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis***. *Cell* 2004, **117**:915-925.

9. Bernhardt TG, de Boer PA: **SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over Chromosomes in *E. coli***. *Mol Cell* 2005, **18**:555-564.

10. • Bramkamp M, Emmins R, Weston L, Donovan C, Daniel RA, Errington J: **A novel component of the division-site selection system of *Bacillus subtilis* and a new mode of action for the division inhibitor MinCD**. *Mol Microbiol* 2008, **70**:1556-1569.
This paper describes the discovery of *minJ* (together with [54•]) and shows evidence that the MinCD complex can act downstream of FtsZ assembly in *Bacillus subtilis*.

11. Justice SS, Garcia-Lara J, Rothfield LI: **Cell division inhibitors SulA and MinC/MinD block septum formation at different steps in the assembly of the *Escherichia coli* division machinery**. *Mol Microbiol* 2000, **37**:410-423.

12. •• Thanbichler M, Shapiro L: **MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter***. *Cell* 2006, **126**:147-162.
This paper describes the identification of a ParA-like protein, MipZ, in *Caulobacter crescentus* that spatially controls septum placement in this vibrioid rod. Using *in vivo* and *in vitro* experiments the direct inhibitory effect of MipZ on FtsZ has been shown.

13. Wang X, Lutkenhaus J: **The FtsZ protein of *Bacillus subtilis* is localized at the division site and has GTPase activity that is dependent upon FtsZ concentration**. *Mol Microbiol* 1993, **9**:435-442.

14. Hale CA, Rhee AC, de Boer PA: **ZipA-induced bundling of FtsZ polymers mediated by an interaction between C-terminal domains**. *J Bacteriol* 2000, **182**:5153-5166.

15. Gueiros-Filho FJ, Losick R: **A widely conserved bacterial cell division protein that promotes assembly of the tubulin-like protein FtsZ**. *Genes Dev* 2002, **16**:2544-2556.

16. Sanchez M, Valencia A, Ferrandiz MJ, Sander C, Vicente M: **Correlation between the structure and biochemical activities of FtsA, an essential cell division protein of the actin family**. *EMBO J* 1994, **13**:4919-4925.

17. Pichoff S, Lutkenhaus J: **Tethering the Z-ring to the membrane through a conserved membrane targeting sequence in FtsA**. *Mol Microbiol* 2005, **55**:1722-1734.

18. Errington J, Daniel RA, Scheffers DJ: **Cytokinesis in bacteria**. *Microbiol Mol Biol Rev* 2003, **67**:52-65.

19. Goehring NW, Beckwith J: **Diverse paths to midcell: assembly of the bacterial cell division machinery**. *Curr Biol* 2005, **15**:R514-526.

20. • Wu LJ, Ishikawa S, Kawai Y, Oshima T, Ogasawara N, Errington J: **Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation**. *EMBO J* 2009, **28**:1940-1952.
Here, it is shown that the nucleoid occlusion protein Noc from *Bacillus subtilis* binds palindromic sequences that are dispersed around the chromosome with the remarkable exception of the terminus region. This highlights that the sequence similarity between Noc and ParB is also reflected by their mode of DNA binding.

21. •• Lutkenhaus J: **Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z-ring**. *Annu Rev Biochem* 2007, **76**:539-562.
This review gives an excellent and comprehensive overview about the *E. coli* Min system.

22. Rothfield L, Taghbalout A, Shih YL: **Spatial control of bacterial division-site placement**. *Nat Rev Microbiol* 2005, **3**:959-968.

23. •• Maple J, Møller SG: **Plastid division: evolution, mechanism and complexity**. *Ann Bot (Lond)* 2007, **99**:565-579.
This is an excellent review about the proteins and mechanisms involved in plastid division.

24. de Boer PA, Crossley RE, Rothfield LI: **Isolation and properties of *minB*, a complex genetic locus involved in correct placement of the division site in *Escherichia coli***. *J Bacteriol* 1988, **170**:2106-2112.

25. de Boer PA, Crossley RE, Rothfield LI: **A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli***. *Cell* 1989, **56**:641-649.

26. de Boer PA, Crossley RE, Rothfield LI: **Central role for the *Escherichia coli minC* gene product in two different cell division-inhibition systems**. *Proc Natl Acad Sci U S A* 1990, **87**:1129-1133.

27. • Scheffers DJ: **The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA**. *FEBS Lett* 2008, **582**:2601-2608.
This article describes details about the polymerization of *Bacillus subtilis* FtsZ and how MinC interferes with the lateral assembly. In our opinion this piece of work is a very careful biochemical analysis and worth reading.

28. Hu Z, Mukherjee A, Pichoff S, Lutkenhaus J: **The MinC component of the division site selection system in *Escherichia coli* interacts with FtsZ to prevent polymerization**. *Proc Natl Acad Sci U S A* 1999, **96**:14819-14824.

29. • Dajkovic A, Lan G, Sun SX, Wirtz D, Lutkenhaus J: **MinC spatially controls bacterial cytokinesis by antagonizing the scaffolding function of FtsZ**. *Curr Biol* 2008, **18**:235-244.
Extensive *in vitro* analysis of the *Escherichia coli* MinC showing that MinC controls the scaffolding function of the Z-ring, rather than simply preventing FtsZ polymerization. On the basis of the results the authors present an interesting mathematical model how the Z-ring coalesces into a condensed, functional cytokinetic ring.

Please cite this article in press as: Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria, Curr Opin Microbiol (2009), doi:10.1016/j.mib.2009.10.002

30. Shen B, Lutkenhaus J: **The conserved C-terminal tail of FtsZ is required for the septal localization and division inhibitory activity of MinC^{C}/MinD**. *Mol Microbiol* 2009, **72**:410-424.
The authors isolated mutants of FtsZ that were resistant to the toxic effects of MinC^{C}/MinD overexpression. Data in this paper show that the C-terminal domain of MinC is necessary for correct localization and function of MinC. Binding of C-terminal MinC to FtsZ displaces FtsA and/or ZipA. Moderate expression of MinC^{C}/MinD cause a division block, but Z-rings were visible, suggesting that MinC^{C}/MinD acts downstream of FtsZ assembly. In summary this report gives evidence that the Min system in *Escherichia coli* can act at different levels during Z-ring maturation.

31. Hu Z, Lutkenhaus J: **Analysis of MinC reveals two independent domains involved in interaction with MinD and FtsZ**. *J Bacteriol* 2000, **182**:3965-3971.

32. Hu Z, Lutkenhaus J: **A conserved sequence at the C-terminus of MinD is required for binding to the membrane and targeting MinC to the septum**. *Mol Microbiol* 2003, **47**:345-355.

33. Shiomi D, Margolin W: **The C-terminal domain of MinC inhibits assembly of the Z-ring in *Escherichia coli***. *J Bacteriol* 2007, **189**:236-243.
Although the inhibitory function of MinC has been attributed to the N-terminal half of the protein, this paper presents evidence that the C-terminal domain is also able to inhibit division in a MinD-dependent manner. Interestingly, the inhibition was at the level of Z-ring disassembly.

34. Cordell SC, Anderson RE, Löwe J: **Crystal structure of the bacterial cell division inhibitor MinC**. *EMBO J* 2001, **20**:2454-2461.

35. Cordell SC, Löwe J: **Crystal structure of the bacterial cell division regulator MinD**. *FEBS Lett* 2001, **492**:160-165.

36. Szeto TH, Rowland SL, Habrukowich CL, King GF: **The MinD membrane targeting sequence is a transplantable lipid-binding helix**. *J Biol Chem* 2003, **278**:40050-40056.

37. Johnson JE, Lackner LL, de Boer PA: **Targeting of ^{D}MinC/MinD and ^{D}MinC/DicB complexes to septal rings in *Escherichia coli* suggests a multistep mechanism for MinC-mediated destruction of nascent FtsZ-rings**. *J Bacteriol* 2002, **184**:2951-2962.

38. Pichoff S, Vollrath B, Touriol C, Bouche JP: **Deletion analysis of gene *minE* which encodes the topological specificity factor of cell division in *Escherichia coli***. *Mol Microbiol* 1995, **18**:321-329.

39. Zhao CR, de Boer PA, Rothfield LI: **Proper placement of the *Escherichia coli* division site requires two functions that are associated with different domains of the MinE protein**. *Proc Natl Acad Sci U S A* 1995, **92**:4313-4317.

40. Raskin DM, de Boer PA: **Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli***. *Proc Natl Acad Sci U S A* 1999, **96**:4971-4976.

41. Hu Z, Lutkenhaus J: **Topological regulation of cell division in *E. coli* spatiotemporal oscillation of MinD requires stimulation of its ATPase by MinE and phospholipid**. *Mol Cell* 2001, **7**:1337-1343.

42. Varma A, Huang KC, Young KD: **The Min system as a general cell geometry detection mechanism: branch lengths in Y-shaped *Escherichia coli* cells affect Min oscillation patterns and division dynamics**. *J Bacteriol* 2008, **190**:2106-2117.

43. Loose M, Fischer-Friedrich E, Ries J, Kruse K, Schwille P: **Spatial regulators for bacterial cell division self-organize into surface waves *in vitro***. *Science* 2008, **320**:789-792.
The dynamic behavior of MinD and MinE from *Escherichia coli* was reconstituted *in vitro* on a phospholipid bilayer. Amazingly, only addition of ATP to the three-partite system was enough to generate waves *in vitro*. The data presented in this article strongly argue for a reaction-diffusion model of the MinD/MinE dynamics.

44. Kruse K, Howard M, Margolin W: **An experimentalist's guide to computational modelling of the Min system**. *Mol Microbiol* 2007, **63**:1279-1284.
The title says it all: if you want a state-of-the-art review about mathematical modeling of the Min oscillation, we recommend reading this review.

45. Howard M, Kruse K: **Cellular organization by self-organization: mechanisms and models for Min protein dynamics**. *J Cell Biol* 2005, **168**:533-536.

46. Cha JH, Stewart GC: **The *divIVA* minicell locus of *Bacillus subtilis***. *J Bacteriol* 1997, **179**:1671-1683.

47. Edwards DH, Errington J: **The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division**. *Mol Microbiol* 1997, **24**:905-915.

48. Marston AL, Thomaides HB, Edwards DH, Sharpe ME, Errington J: **Polar localization of the MinD protein of *Bacillus subtilis* and its role in selection of the mid-cell division site**. *Genes Dev* 1998, **12**:3419-3430.

49. Edwards DH, Thomaides HB, Errington J: **Promiscuous targeting of *Bacillus subtilis* cell division protein DivIVA to division sites in *Escherichia coli* and fission yeast**. *EMBO J* 2000, **19**:2719-2727.

50. Lenarcic R, Halbedel S, Visser L, Shaw M, Wu LJ, Errington J, Marenduzzo D, Hamoen LW: **Localisation of DivIVA by targeting to negatively curved membranes**. *EMBO J* 2009, **28**:2272-2282.
A long standing question was how DivIVA finds the cell poles. Here, evidence for an N-terminal membrane targeting sequence is given. It is further shown that DivIVA preferentially binds to curved membranes, although the protein itself does not impose curvature.

51. Ramamurthi KS, Losick R: **Negative membrane curvature as a cue for subcellular localization of a bacterial protein**. *Proc Natl Acad Sci U S A* 2009, **106**:13541-13545.

52. Marston AL, Errington J: **Selection of the midcell division site in *Bacillus subtilis* through MinD-dependent polar localization and activation of MinC**. *Mol Microbiol* 1999, **33**:84-96.

53. Gregory JA, Becker EC, Pogliano K: *****Bacillus subtilis* MinC destabilizes FtsZ-rings at new cell poles and contributes to the timing of cell division**. *Genes Dev* 2008, **22**:3475-3488.
Results presented in this paper suggest that the MinCD system in *Bacillus subtilis* is not static, as all current models suggested. Using excellent time-lapse analysis and a functional MinC–GFP copy, it became apparent that MinC is not always associated with the poles, but rather travels to the site of septation. Thus, the strongest accumulation of MinC is at the young poles.

54. Patrick JE, Kearns DB: **MinJ (YvjD) is a topological determinant of cell division in *Bacillus subtilis***. *Mol Microbiol* 2008, **70**:1166-1179.
This paper describes the discovery of *minJ* (together with [10•]). Subcellular localization and interaction studies revealed that MinJ acts as an adaptor between DivIVA and MinD.

55. Levin PA, Kurtser IG, Grossman AD: **Identification and characterization of a negative regulator of FtsZ-ring formation in *Bacillus subtilis***. *Proc Natl Acad Sci U S A* 1999, **96**:9642-9647.

56. Haeusser DP, Garza AC, Buscher AZ, Levin PA: **The division inhibitor EzrA contains a seven-residue patch required for maintaining the dynamic nature of the medial FtsZ-ring**. *J Bacteriol* 2007, **189**:9001-9010.

57. Singh JK, Makde RD, Kumar V, Panda D: **A membrane protein, EzrA, regulates assembly dynamics of FtsZ by interacting with the C-terminal tail of FtsZ**. *Biochemistry* 2007, **46**:11013-11022.

58. Chung KM, Hsu HH, Yeh HY, Chang BY: **Mechanism of regulation of prokaryotic tubulin-like GTPase FtsZ by membrane protein EzrA**. *J Biol Chem* 2007, **282**:14891-14897.

Please cite this article in press as: Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria, Curr Opin Microbiol (2009), doi:10.1016/j.mib.2009.10.002