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Cell division of *Streptococcus pneumoniae*: think positive!

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Bacterial cell division is achieved by a dynamic protein complex called the divisome. The accurate placement of the divisome, and more specifically that of the tubulin-like protein FtsZ which forms the contractile Z-ring at mid-cell, is finely regulated by different mechanisms tailored to each bacterial class. To give rise to two viable daughter cells with the same genetic heritage and cell shape, *Streptococcus pneumoniae* uses an original system that relies on the membrane protein MapZ. This system is required for identifying the division site as well as positioning the Z-ring at mid-cell. In addition, MapZ undergoes phosphorylation by the serine/threonine kinase StkP and controls the constriction of the Z-ring. Here, we discuss recent advances and concepts of the MapZ system.

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Introduction

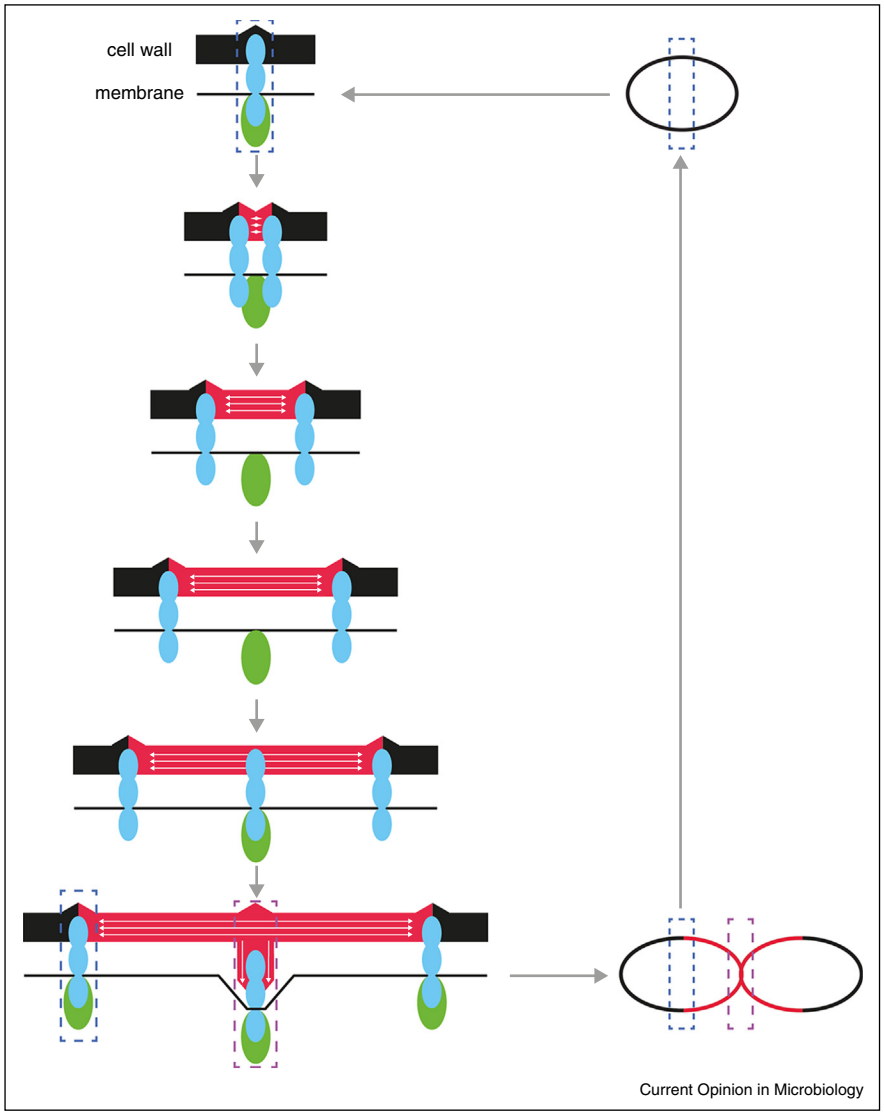
Finding the middle of the cell is a crucial initial step for many bacteria that divide by binary fission [1]. Initially, and thanks to a series of pioneering studies in the model rod-shaped bacteria *Escherichia coli* and *Bacillus subtilis*, two regulatory systems, the Min system and the nucleoid occlusion (NO) system, were shown to favor the polymerization of the tubulin-like protein FtsZ in a ring (the Z-ring) at mid-cell [2–4]. Once assembled, the Z-ring serves as a scaffold for the other cell division proteins to form the divisome [5,6•]. One should however note that the Min and NO systems are likely not the only factors allowing positioning FtsZ at mid-cell. Indeed, the proper selection of the septum site is not completely abolished in cells with compromised Min and NO systems [7–9]. A complementary process selecting the division site remains therefore to be discovered in these two species. A number of bacterial species lack both Min and NO proteins suggesting that alternative systems are required for the selection of the division site [10]. Over the last decade, research on other bacterial models displaying either a particular developmental program and/or dividing asymmetrically have confirmed this statement and new original systems have emerged. In the α-proteobacterium *Caulobacter crescentus*, the placement of the Z-ring at the cell center is coordinated with chromosome segregation by the protein MipZ [11,12]. This protein directly interferes with the polymerization of FtsZ. One should note that these 3 systems act negatively by preventing the assembly of the Z-ring anywhere other than at mid-cell.

Positive regulation of bacterial cell division

It was thus unexpected when the actinobacterium *Streptomyces coelicolor* and soon after in the deltaproteobacterium *Myxococcus xanthus* were found to use processes that do not act negatively but that positively regulate the positioning of FtsZ at mid-cell [13,14]. The protein SsgB recruits FtsZ and stimulates its polymerization during sporulation in *S. coelicolor* while in *M. xanthus*, PomZ localizes before FtsZ at the division site and stabilizes the Z-ring. In both systems, how SsgB and PomZ position themselves at the division site remains unknown. These findings have challenged the view of how the division site is selected and have led to the idea that a variety of mechanisms, not necessarily conserved among the different bacterial clades, could exist.

The same is true for *Streptococcus pneumoniae*, a member of the Firmicutes phylum, which does not possess any of the systems described so far and has evolved its own positive regulatory mechanism to select the division site. The system identified in *S. pneumoniae* relies on a membrane protein called MapZ [15••] (Figure 1) (also named LocZ in) [16•]. The existence of different regulatory systems of

Figure 1



The MapZ system. MapZ (cyan ovals) positions at mid-cell together with FtsZ (green ovals) in newborn cells. Then, the MapZ ring splits into two rings that are pushed by nascent peptidoglycan (red) elongating the cell (white arrows) toward the cell equators of the two future daughter cells. Then, MapZ positions as a third ring at the division septum, followed by (i) the constriction of the Z-ring and the synthesis of the cross wall at the dividing septum (blue dotted rectangle) and (ii) the relocalization of FtsZ at the cell equator with MapZ (purple dotted rectangle). For clarity, StkP and MapZ phosphorylation at the dividing septum have been omitted in the picture.

bacterial cell division, either positive or negative, suggests that they have evolved independently and have likely been acquired or replaced several times during bacterial evolution according to species specificities (*e.g.* developmental process, ecological niche, cell shape) of a given bacterial class. Interestingly, many Firmicutes are also devoid of MapZ reinforcing the hypothesis that alternative mechanisms will be discovered in the near future in this bacterial phylum.

MapZ: an all-in-one positive regulatory system

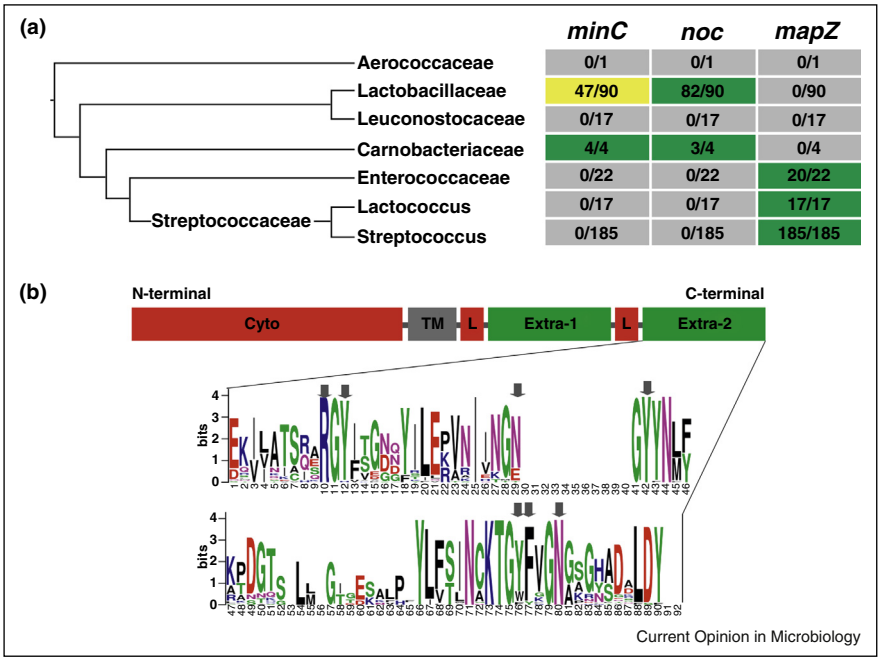
MapZ possesses a localization pattern that varies in the course of the cell cycle (Figure 1). It forms a ring at mid-cell that co-localizes with the Z-ring at the division site in newborn cells [15••,16•]. As the cell elongates, the MapZ-ring splits in two new rings that move apart at the speed of cell elongation and that position at the cell equator (which eventually corresponds to the division site of the daughter cell). As the two MapZ-rings move, a third MapZ-ring positions itself at the constricting division site and persists till constriction of the cell is achieved [15••]. Thus, three MapZ-rings are present in the cell before the cell division process is completed. This localization pattern allows MapZ to fulfil two distinct and essential functions in the cell. On the one hand MapZ serves as a molecular beacon

signaling the position of the division site when positioned at the cell equator (Figure 1). In cells devoid of MapZ, the Z-ring is consequently unable to position at mid-cell and to find the orthogonal division plane. On the other hand, MapZ contributes to the regulation of cell constriction. Indeed, a premature constriction of the Z-ring interfering with chromosome segregation is detected in absence of MapZ (Figure 1) [15••]. The Z-ring structure is also compromised in a significant percentage of cells. MapZ was found to be phosphorylated in the pneumococcal phosphoproteome [17]. Further works showed that it is targeted by the serine/threonine-kinase StkP, a crucial regulator of pneumococcal cell division and morphogenesis [18,19,20•]. Although MapZ phosphorylation does not affect the selection of the division site, an unbalanced ratio between the phosphorylated and non-phosphorylated MapZ forms induces Z-ring shape and constriction defects [15••]. An in-depth survey of complete bacterial proteomes shows that MapZ is conserved only in the two main families of *Lactobacillales*, namely the *Enterococcaceae* and *Streptococcaceae*, and that it never co-occurs with Min and NO systems (Figure 2a). MapZ could therefore represents an all-in-one system encompassing the function of both Min and NO systems and allowing Z-ring positioning at mid-cell but hindering its assembly and constriction over the nucleoid. Although *Carnobacteriaceae* and *Lactobacillaceae* seem to possess the Min and NO systems, one should note that both *Leuconostocaceae* and *Aerococcaceae* lack the three systems identified so far in Firmicutes (Figure 2a). This suggests that alternative mechanisms not yet identified should exist in these bacterial families. It would also be interesting to determine if these alternative mechanisms would be conserved in the other *Lactobacillales* families, and especially *Enterococcaceae* and *Streptococcaceae* and if they might work together with the MapZ system. Indeed, MapZ was not found to be crucial for the cell division process in pneumococcal capsulated strains suggesting that a complementary process works together with MapZ to position the divisome at mid-cell [21].

What regulates MapZ positioning at the cell equator?

The pneumococcus is devoid of MreB homologues. Consequently, cell elongation is not achieved by lateral insertion of peptidoglycan [22]. Rather, the synthesis of the peptidoglycan required for cell elongation and the

Figure 2

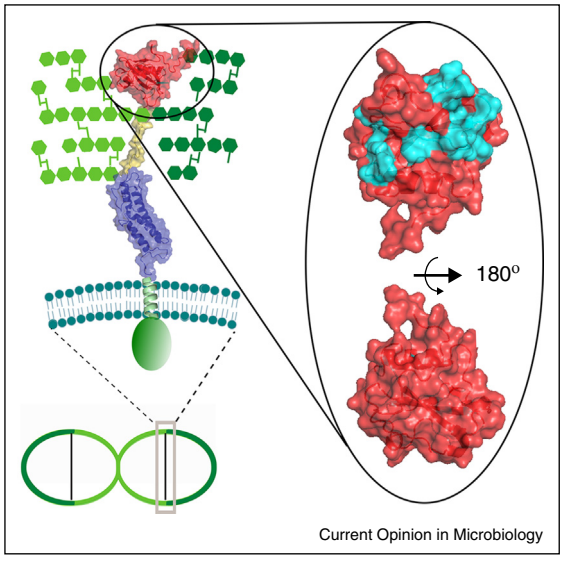


Taxonomic distribution and amino acid conservation of MapZ in Lactobacillales. **(a)** Taxonomic distribution of MinC, Noc and MapZ in the six families composing Lactobacillales. Only full proteomes were considered. The absence and presence of MinC, Noc or MapZ is highlighted in gray and light green, respectively. Yellow indicates that the corresponding proteins are present in a subset of strains of the considered family. Proteome analyses have been performed using BLASTP 2.2.26 on the 336 complete proteomes of Firmicutes available at the NCBI (ftp://ftp.ncbi.nlm.nih.gov). **(b)** Schematic representation of protein domain composition of MapZ in *Enterococcaceae* and *Steptococcaceae*. Cyto, TM, L and Extra stand for cytoplasmic, trans-membrane, linker and extracellular domain, respectively. The amino acid conservation in each protein domain of MapZ is indicated in red (low conservation, average percentage identity $< 30\%$) and green (high conservation, average percentage identity $> 30\%$). Average percentage identities have been calculated with average distances using Seaview [27]. The logo plot of C-terminal domain of MapZ from *Enterococcaceae* and *Steptococcaceae* is also shown. 37 MapZ sequences from *Streptococcaceae* and *Enterococcaceae* have been aligned using MAFFT v7 [28]. Logo plot has been constructed from multiple alignments using WebLogo [29]. Gray arrows point the 7 amino acids shown to be involved in peptidoglycan binding [25••].

synthesis of the cross-wall is organized by the Z-ring itself at the cell center [23,24]. MapZ possesses an extracellular domain that is able to bind nascent peptidoglycan at the division site. It was thus proposed that MapZ, firmly attached to peptidoglycan, is shuttled toward the cell equator by the peptidoglycan elongating the cell [15••]. This view has been recently supported by the characterization of the structure of the extracellular domain of MapZ (Figure 3), composed of two sub-domains separated by a flexible linker [25••]. The sub-domain immediately following the trans-membrane span serves as a pedestal for the C-terminal sub-domain which binds peptidoglycan, anchoring MapZ in the cell wall. MapZ is thus continuously pushed toward the cell equator of the daughter throughout cell elongation and until constriction of the cell begins.

This model of MapZ positioning implies two main conclusions. First, as MapZ localizes at the cell equator before StkP, which itself positions after FtsZ, MapZ phosphorylation should not have an impact on both MapZ and FtsZ positioning at the cell equator. This point has been confirmed experimentally [15••]. Second, cell elongation and consequently MapZ positioning at the cell-equator should be governed by a regulator being part of the divisome of the mother cell and that should switch the synthesis of peptidoglycan required for cell elongation with the one required for cell constriction. An interesting possibility would be that StkP-mediated phosphorylation of MapZ itself could be part of this switch. No clear-cut evidence supports this hypothesis. However, MapZ phosphorylation was shown to influence the constriction of the Z-ring [15••]. The same is true for the kinase activity of StkP which is required for cell constriction [18]. On the other hand, StkP is proposed to act as a kinase-receptor signaling information about the status of the cell wall to its targets inside the divisome [20•,26], including the cell division protein DivIVA which is required for cell elongation [23]. Altogether, a role for StkP together with MapZ in regulating the switch between cell elongation and cell constriction is likely not further from the truth.

Figure 3



Organization of MapZ in the cell wall. The peptidoglycan elongating the mother cell and generating the new two cell halves is shown in light green. Peptidoglycan of the mother cell half is shown in dark green. MapZ is positioned at the cell equator of the daughter cell at the interface between the two types of peptidoglycan. The cytoplasmic domain of MapZ whose structure remains uncharacterized is shown with a green oval. The extracellular domain of MapZ is composed of the subdomain following the trans-membrane span that serves as a pedestal (blue) for the C-terminal subdomain (red) that binds peptidoglycan. The flexible linker between the two is shown in yellow. On the right hand side, magnification of the C-terminal subdomain shows the conservation of surface exposed amino acids (cyan) (R409, Y411, N426, Y430, Y440, L441, N445, K447, T448, Y450, F451, L462, D463 and Y464) on one side of the domain.

Interestingly, the amino acid sequence of the C-terminal subdomains of MapZ is well conserved among MapZ homologues from *Enterococcaceae* and *Steptococcaceae* (Figure 2b). More precisely, the 7 amino acids found to be involved in peptidoglycan binding lie in 4 conserved zones [25••] (Figure 2b). An additional C-terminal fifth zone encompasses the highly conserved LDY motif (Figure 2b). When placed on the MapZ structure, one can observe that these 5 zones define a conserved surface on one side of MapZ C-terminal sub-domains (Figure 3). This suggests that the mode of binding to peptidoglycan as well as the peptidoglycan moiety recognized by MapZ are both conserved among bacterial species.

Positioning of MapZ as a third ring at mid-cell

Two intriguing questions regarding MapZ remain unanswered: firstly, how does MapZ position as a third ring at the constricting septum and secondly, why does this third ring persist at the constricting septum instead of being shuttle toward the cell equator by peptidoglycan synthesis? One possibility would be that the third MapZ ring positions at the constricting septum when cell elongation stops. This would support our hypothesis regarding StkP-mediated phosphorylation of MapZ as being part of the switch between cell elongation and constriction. Another possibility would be that MapZ recognizes a particular composition and/or architecture of the peptidoglycan produced specifically during the early stage of cell elongation. Considering the presence of a flexible linker between the two extracellular subdomains of MapZ, one also cannot exclude that some structural re-arrangements dictate the ability of MapZ to bind peptidoglycan. In this view, one conformational state would be prompt to bind peptidoglycan whereas another conformation would be hampered for peptidoglycan binding and would favor insertion of MapZ as a third ring at the constricting septum. It also suggests that the particular composition and/or architecture of peptidoglycan mentioned above therefore would not be

necessary for MapZ binding. Future works is now necessary to decipher how MapZ is being positioned as a third ring at the mid-cell.

How MapZ positions FtsZ and regulates the Z-ring constriction?

The structural organization of the cytoplasmic domain of MapZ remains unknown. It was shown that the first N-terminal 40 amino acids are sufficient for interacting with FtsZ [15••]. A simple view would be that MapZ acts as a 'sticky stop' that fishes cytoplasmic FtsZ to form a ring at the cell equator. More intriguing is how the interaction between FtsZ and MapZ is then disrupted to allow the two MapZ-rings to migrate toward the cell equator. At the moment, one can only speculate that early components of the divisome, probably not yet characterized, are involved in this crucial step. Another important question is how MapZ regulates the constriction of the Z-ring at mid-cell. Its phosphorylation clearly plays an important function *in vivo*. However, MapZ phosphorylation does not affect either FtsZ polymerization or its GTPase activity [15••]. The impact of MapZ phosphorylation on Z-ring constriction should therefore be indirect and should require yet uncharacterized partners, most likely being part of the divisome. MapZ phosphorylation would therefore modulate either its ability or FtsZ ability to interact with these regulators. In sharp contrast with the extracellular domain of MapZ homologues, the amino acid sequence of the MapZ cytoplasmic domain diverges significantly among bacterial species. Indeed, 28.4% sequence identity is found whereas 59.5% of amino acids are conserved in the C-terminal extracellular subdomain. In addition, the two phosphorylation sites of MapZ in *S. pneumoniae*, namely Thr-67 and Thr-78, are not strictly conserved. This suggests that the mode of action of MapZ, the interactions between MapZ and its cellular partners and/or FtsZ as well as the nature itself of MapZ partners could vary from one strain to another.

Concluding remarks and future directions

Many aspects of the MapZ system remain unknown. Complicated as it may be, future work should provide further insights into this system in the pneumococcus but also in other bacteria. Considering the low sequence conservation of the cytoplasmic domain compared to that of the extracellular domain, one cannot exclude that the recruitment of FtsZ at mid-cell by MapZ is not conserved. This is also true for the other positive regulatory systems identified in other bacteria, like SsgB and PomZ for which we still do not understand how they find and mark the division site. One can also speculate that new positive regulatory mechanism will be uncovered in species that are devoid of the systems identified so far. At that moment, it will be of particular interest to decipher how these mechanisms have emerged and evolved and what are the evolutionary driving forces that have led to such a diversity of systems in bacteria.

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