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BACTERIAL PHYSIOLOGY

Wrapping the cell in a CozE shell

Recent work characterizing CozE, a protein that controls the function of the class A penicillin-binding protein PBP1a, sheds new light on our understanding of the synthesis of the bacterial peptidoglycan shell.

Adrien Ducret and Christophe Grangeasse

François Jacob wrote "*le rêve de toute cellule: devenir deux cellules*" (the dream of every cell is to become two cells). In bacteria, most of our knowledge on how this dream comes true, that is, how the cell grows and divides, has been gained through pioneering work performed on the two rod-shaped bacteria, *Escherichia coli* and *Bacillus subtilis*[1]. However, advances in genetics and imaging in bacteria from other phyla have found that although some proteins and mechanisms are widely conserved, species-specific factors and processes are also required to satisfy the specific needs of different cell shapes and modes of life[2]. Writing in this issue of *Nature Microbiology*, Fenton *et al.*[3] characterize a new widely conserved regulatory factor, CozE, that is involved in cell morphogenesis of the bacterial pathogen *Streptococcus pneumoniae* (the pneumococcus).

Over the past five years, *S. pneumoniae* has made its mark as a model to study bacterial cell division and morphogenesis. The pneumococcus grows as an ovo-diplococcal shape in which two rugby-ball-shaped cells are linked by the tip of their new cell poles (Fig. 1a). An explanation of how the pneumococcus achieves this shape remains elusive, as it does not encode some of the well-known cell division and morphogenesis factors found in *E. coli* or *B. subtilis*, such as the actin-like protein MreB or the septum site-positioning Min and nucleoid occlusion systems, among others[4]. However, the pneumococcus does encode other division genes, such as the cell division regulator MapZ, and the serine/threonine protein kinase StkP (refs 5,6). The latter was notably shown to act as a critical regulator of peptidoglycan (PG) synthesis, the main component of the bacterial cell envelope that confers the shape and structure of the cell[7]. The PG mesh is assembled only at mid-cell in the pneumococcus, enabling both cell elongation (peripheral PG synthesis) and cell constriction (septal PG synthesis) (Fig. 1a, inset). The composition and dynamics of the proteins assembling PG inside the division machinery (divisome) are still not well defined, but the pneumococcus encodes two PG polymerases (RodA and FtsW) and a homologue of the lipid flippase MurJ, as well as five penicillin-binding proteins (PBPs)[8]. The two class B PBPs, PBP2x and PBP2b, are essential and probably work together with the two shape, elongation, division and sporulation (SEDS) proteins, FtsW and RodA, to assemble peripheral and septal PG, respectively. By contrast, the three class A PBPs (PBP1a, PBP1b and PBP2a) are non-essential and bi-functional, displaying both transpeptidase and transglycosidase activities (Fig. 1b). According to a recent model, these three PBPs may fill gaps in PG that arise during the cell cycle[9]. However, how the activity of these three PBPs is regulated, coordinated and organized, relative to the SEDS proteins and class B PBPs, is not well understood.

Interestingly, the *pbp1a pbp2a* double deletion is lethal, suggesting that these two class A PBPs fulfil distinct but complementary functions. Fenton *et al.*[3] used this observation as a basis to screen transposons libraries in wild-type (WT), *pbp1a* and *pbp2a* mutant backgrounds for

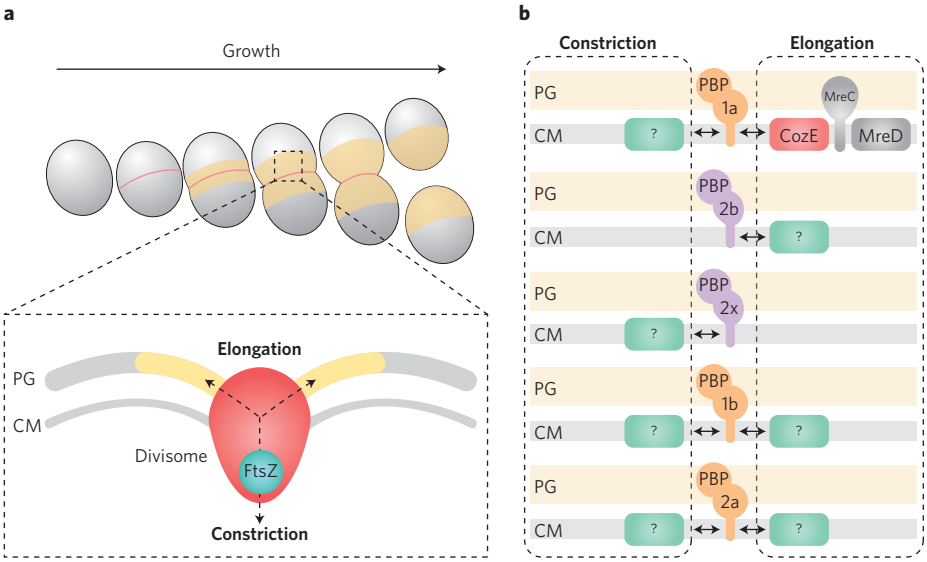

Figure 1 | Cell wall synthesis in *S. pneumoniae*. **a**, In *S. pneumoniae*, both septal and peripheral peptidoglycan (PG) synthesis occur at the division septum at mid-cell (red ring). As the new PG is inserted, the old poles are pushed apart, causing the cell to elongate and creating the new cell halves of the two daughter cells (orange hemispheres). This process is followed by the constriction and formation of the new cell poles. *S. pneumoniae* PG is assembled by a complex web of proteins called the divisome (red oval), including five PBPs. **b**, Class A (harbouring transpeptidase and transglycosidase activities) and class B (encoding only transpeptidase activity) PBPs are shown in orange and purple, respectively. The current model proposes that PBP2b is involved in peripheral synthesis (cell elongation) whereas PBP2x is involved in septal synthesis (cell constriction). PBP1a would participate in both septal and peripheral PG synthesis, while the role of other PBPs, such as PBP1b and PBP2a, in cell elongation and/or cell constriction remains unknown. Given that PBP1a forms a complex with MreC and MreD and is regulated by CozE (red box), new regulators (green boxes) may also influence and coordinate the function of other PBPs. CM, cytoplasmic membrane.

potential regulators of class A PBPs. They identified mutations in *mreC* and *mreD*, two conserved genes required for PG biosynthesis, as well as a new factor called CozE (for coordinator of zonal elongation), whose inactivation was lethal in WT and *pbp2a* mutant strains, but not in the *pbp1a*-deficient background. As CozE homologues are absent in Mollicutes (members of which do not have cell walls), these findings suggest that CozE specifically works with PBP1a in the assembly of the PG mesh. Expression of *pbp1a* in a *pbp1a cozE* double mutant or in a *mreC* mutant strain led to cell defects and eventual lysis, suggesting that MreC and CozE work together to control PBP1a in PG assembly.

To investigate how CozE regulates PBP1a function, the authors tracked the localization of green fluroescent protein (GFP)–PBP1a and PG synthesis in *cozE* and *mreC* mutants. In both mutants, PBP1a and PG synthesis were aberrantly relocated from mid-cell to throughout the cell periphery. This was not seen with a GFP–PBP2a reporter, suggesting that CozE and MreC specifically control the function of PBP1a, probably as part of a complex. Supporting this idea, bacterial two-hybrid and co-immunoprecipitation assays showed that CozE interacts with both MreCD and PBP1a, and imaging found that CozE and MreC were co-dependent on each other for correct septal localization. Altogether, these results demonstrate that PBP1a is part of the complex encompassing MreCD and that CozE specifically controls the localization and thus the function of PBP1a during pneumococcal growth (Fig. 1b).

Together with recent groundbreaking findings regarding how the PG mesh is assembled[8,9], the discovery of CozE changes our view of the PG assembly complex in the pneumococcus and generates new questions. As PBP1a is proposed to be involved in both cell elongation and constriction[10], it would be interesting to determine if CozE-mediated control is specific for PBP1a's peripheral PG synthesis and cell elongation, or if it can also regulate septal PG synthesis and constriction (Fig. 1b). In fact, it remains unclear if two distinct multiprotein machineries (such as those proposed for PG synthesis in *E. coli* and *B. subtilis*) exist in the pneumococcus to regulate septal and peripheral growth, or whether a single finely tuned machine can concomitantly control both elongation and constriction[6]. Here, CozE could be useful as both a tool to further distinguish between the two models and as a potential regulator for favouring cell elongation over constriction. Based on the Fenton *et al.* work, one can also envision that other proteins specifically regulating the other class A (PBP1b and PBP2a) and class B (PBP2x and PBP2b) PBPs may be identified in the near future (Fig. 1b). This work thus paves the way towards a model in which several specific regulators serve crucial roles in generating the critical PG shell that protects and shapes the cell. Given that the pneumococcus (and related Gram-positive cocci) is the causative agent of several invasive diseases with increasing antibiotic resistance[11], identifying CozE homologues and other types of PBP regulators might constitute promising targets to develop new drugs to combat bacterial infections. ❐

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Competing interests

The authors declare no competing financial interests.