**Title: A beacon for bacterial tubulin**

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The discovery of the highly-conserved bacterial FtsZ protein several decades ago hallmarked the beginning of our understanding of how a bacterial cell undergoes binary fission. This prokaryotic precursor of tubulin self-polymerizes to form the cytokinetic ring, called the Z ring, at the division site, which has been believed to mark the earliest step in cell division. An outstanding question in the field is, how is the FtsZ-ring positioned correctly to ensure equal partitioning of DNA into the newborn cells? While various models for how this occurs have been proposed, the mechanisms are far from fully resolved. On page xxx of this issue1, Fleurie *et al*. make the novel revelation that in the human pathogen, *Strepotococcus* *pneumonia*,the Z ring is positioned by the MapZ protein, which acts as a beacon to identify the site of division. They also show that phosphorylation of MapZ has an important function in the regulation of division.

Bacterial cells divide by forming a septum composed of cell envelope layers (cell wall and membranes) which then splits down the middle to produce two newborn cells. The FtsZ-ring recruits at least 20 bacterial division proteins to the division site, leading to subsequent FtsZ-ring constriction and cytokinesis2,3. Until recently, cell division had only been intensively studied in a few well-known, non-pathogenic bacterial species, such as *Escherichia coli* and *Bacillus subtilis*. Decades of research on these rod-shaped bacteria led to the canonical model that division site placement in bacteria is solely regulated by a combination of two systems, known as Min and nucleoid (chromosome) occlusion. These systems allow division only at midcell by preventing FtsZ-ring formation at all other positions in the cell4. However, several pathogenic and non-pathogenic bacteria do not have the Min or nucleoid occlusion systems (some have one but not the other). Furthermore, even in these bacteria that have both systems, Z rings can form at midcell with the same precision in their complete absence, indicating that the signpost for the division site remains elusive5,6. More recent studies on FtsZ-ring positioning mechanisms in a diverse range of other bacteria have uncovered novel negative and positive signalling systems acting on FtsZ-ring assembly7. In their paper, Fleurie *et al*. uncover a new mechanism for division site positioning in bacteria. They found that in the ovococcus (oval-shaped), *Streptococcus pneumonia*, an organism that does not have a Min or nucleoid occlusion system, the MapZ protein localizes to the division site and acts as a beacon for the FtsZ-ring. It is the first protein shown to function in positioning the division site in this group of organisms.

Fleurie and collaborators demonstrated that deletion of the *mapZ* gene, misplaces the FtsZ-ring and the division septum, which are normally positioned at the cell centre (midcell) in this organism. Using time-lapse and a type of super resolution microscopy, known as 3D-structured illumination microscopy (3D-SIM), they showed that the MapZ protein precedes the FtsZ-ring in localizing to midcell. What is particularly intriguing is the demonstration that once both the MapZ-ring and FtsZ-ring locate to midcell, the MapZ-ring splits into two and moves to the two future division sites, while the FtsZ-ring stays at midcell. FtsZ then subsequently follows the MapZ-rings to these future sites. Then both rings close at the midcell site to complete cytokinesis.

How do the MapZ-rings migrate to future division sites? This relates to the distinct mode of cell wall synthesis in these oval-shaped cells. To increase in cell size, cell wall synthesis in Streptococci begins at the division site at midcell and moves in both directions towards the future division sites, creating two new cell halves8. This is in contrast to elongation in rod-shaped cells which involves cell wall synthesis all along the long axis of the cell. The authors show convincingly that MapZ-ring migration in these oval-shaped cells is the result of cell elongation. They included an elegant approach of sequential labelling of two different fluorescently-labelled cell wall substrates (D-alanine derivatives) and fluorescently-labelled MapZ in live *S. pneumonia* cells. The dependence on cell wall synthesis for MapZ localization was further supported by demonstrating that MapZ binds to the cell wall material, peptidoglycan, and that specific inhibition of cell wall synthesis using the antibiotic vancomycin delocalized MapZ.

What remained to be shown is direct evidence that MapZ actually functions to beacon FtsZ to the division site. A direct interaction between FtsZ and MapZ was demonstrated, and shown to be dependent solely on the N-terminal 41 residues of MapZ. Crucially, deletion of this region from MapZ still allowed MapZ septal localization but FtsZ was delocalized. These results were a little complicated in that *mapZ* mutants often form misshapen cells, however careful inspection of FtsZ-ring positioning in mapZ mutant cells that have a normal shape confirmed these results. Further site-directed mutagenesis of the N-terminal 41 residues of MapZ to test its ability to localize FtsZ, and corresponding compensatory mutations in FtsZ will certainly be worthwhile future studies.

MapZ (Midcell Anchored Protein Z) was first identified as Spr0334, a known target of the *S. pneumonia* StkP kinase, which is crucial for septum assembly, cell shape and localization of cell wall synthesis9. Fleurie *et al*. show that phosphorylation of MapZ occurs at two threonine residues (residues 67 and 78), not in the region of MapZ that directly interacts with FtsZ. And non-phosphorylated MapZ still interacts with FtsZ, and does not affect Z ring positioning. However the phosphorylation state of MapZ appears important for another regulatory role for MapZ, possibly in the splitting, stability and constriction of the Z ring.

How does all this come together? MapZ is predicted to have a single trans-membrane anchor linking a cytoplasmic N-terminal domain and an extracellular C-terminal domain. The extracellular domain binds peptidoglycan localizing MapZ to the division site, and the N-terminal domain beacons FtsZ to the division site. As cell wall synthesis occurs, MapZ remains attached to arrive at the new cell equators of the daughter cells. The phosphorylation/dephosphorylation of MapZ by Stk/PhpP is proposed to regulate cytokinesis (FtsZ-ring constriction) via another division protein that is a substrate for these proteins, not FtsZ. What’s for sure is that division site positioning in bacteria has evolved a surprising level of diversity – perhaps a consequence of the diversity of lifestyle, cell shape and mode of cell wall synthesis.

FtsZ-rings also formed aberrant, non-ring structures in *mapZ*-deleted cells. The authors suggest that the accompanying aberrant cell wall synthesis and the abnormal morphology of cells is a consequence of the abnormal FtsZ-ring structures. Perhaps it is the other way around, and it would be worthwhile testing this, particularly with the accumulating support for peptidoglycan structures (so-called “piecrusts”) proposed to mark the future FtsZ-ring assembly site in some bacteria10. This may answer the burning question of how MapZ is localized to the division site in the first place. In determining just what IS the first step of bacterial cell division, only time will tell.

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