BACTERIAL PHYSIOLOGY

LCP proteins take the final step

In a recent paper in the *EMBO Journal*, Jeff Errington and colleagues describe a new family of bacterial cell wall assembly proteins, the LytR–Cps2A–Psr (LCP) family, members of which are responsible for the final step in the biogenesis of the bacterial cell wall.

The cell wall of Gram-positive bacteria comprises a thick layer of peptidoglycan decorated with anionic polymers, including wall teichoic acids (WTAs). Most enzymes in the WTA synthesis pathway are known and are encoded by the *tag* genes, but the enzyme responsible for the final phosphotransfer reaction that links the WTA polymer to peptidoglycan remained unknown. In rod-shaped bacteria such as *Bacillus subtilis*, the bacterial actin homologue MreB also has an integral role in cell wall synthesis. Kawai *et al.* used a variety of biochemical techniques to identify interacting partners of MreB in *B. subtilis*. Three genes encoding members of the LCP family were identified as being of interest. The genes, *ywtF*, *lytR* and *yuhJ*, which were shown to be located in close proximity to the *tag* genes and to be widely distributed in Gram-positive bacteria, were renamed *tagT*, *tagU* and *tagV*, respectively.

Bacterial two-hybrid analysis confirmed the MreB interaction for TagT and TagU, and the localization of GFP fusions with these two proteins was reminiscent of the distribution of MreB. Membrane potential is required for MreB localization, and perturbation of this potential disrupted the localization of the TagT and TagU fusion proteins, as did deletion of *mreB*. No aberrant phenotype was associated with *tagT*, *tagU* or *tagV* single mutations. The effect of deleting all three genes was investigated using an inducible *tagV* construct in a *tagT* and *tagU* double-mutant background, and it was shown that the absence of all three genes led to loss of rod shape and was lethal. Moreover, the WTA content of cell wall material isolated from the triple mutant was greatly reduced. Together, these results indicate that LCP proteins associate with MreB and are involved in WTA biosynthesis.

To further probe the function of LCP proteins, Kwai *et al.* moved on to structural studies. The crystal structure was solved for the *Streptococcus pneumoniae* LCP protein Cps2A lacking the amino-terminal transmembrane domain (ΔTM-Cps2A). Analysis of the LCP domain revealed the presence of a decaprenylphosphate lipid substrate analogue within a hydrophobic lipid-binding pocket. A co-crystal structure of ΔTM-Cps2A with an octaprenylpyrophosphate lipid product analogue in the binding pocket was then obtained. The residues lining the binding pocket were found to be broadly conserved within the LCP family, and the key residues were identified by mutagenesis. That *B. subtilis* TagT and *S. pneumoniae* Cps2A can function as Mg^{2+}-dependent phosphotranferases was confirmed biochemically.

The authors conclude that LCP proteins are responsible for the attachment of the major anionic polymers to the cell wall and that these proteins could therefore represent new antibiotic targets.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Kawai, Y. *et al.* A widespread family of bacterial cell wall assembly proteins. *EMBO J.* 30 Sep 2011 (doi: 10.1038/EMBOJ 2011.358)