BACTERIAL PHYSIOLOGY

Flipping out over MurJ

Assembly of the peptidoglycan sacculus requires the precursor lipid II to be translocated or 'flipped' across the cytoplasmic membrane into the periplasm for subsequent insertion into the growing sacculus. The identity of the flippase has long been debated, but Sham *et al.* now show that the inner-membrane protein MurJ mediates this event in *Escherichia coli*.

To investigate flippase activity *in vivo*, the authors developed a sensitive assay in which the toxin colicin M (ColM) was used to specifically cleave flipped lipid II in the periplasm, which generates a soluble product (PP-M_{pep5}-G) that is detectable by high-performance liquid chromatography. To determine whether MurJ functions as the flippase, the authors examined a MurJ-inactivated mutant and found that, compared with wild-type bacteria, the mutant produced only negligible amounts of PP-M_{pep5}-G, which would be insufficient to support bacterial survival. This result suggests that inactivation of MurJ protects lipid II from ColM cleavage either by blocking lipid II translocation or by interfering with ColM import and/or activity. The

authors showed that removal of the outer membrane, which provides ColM with direct access to flipped lipid II in the periplasm, failed to restore PP-M_{pep5}-G production in the MurJ mutant, which confirms that MurJ is essential for the translocation of lipid II.

An *in vitro* study previously proposed that another inner-membrane protein, FtsW, functions as the flippase in *E. coli*. However, Sham *et al.* found that lipid II translocation was relatively unperturbed in a mutant lacking FtsW, which suggests that FtsW is not essential for flippase activity *in vivo* or has only a minor contribution. This study provides compelling *in vivo* evidence that MurJ functions as the lipid II flippase, so it will be interesting to see whether these findings will put an end to the flippase controversy.

Christina Tobin Kährström

ORIGINAL RESEARCH PAPER Sham, L.-T. *et al.* MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. *Science* **345**, 220–222 (2014)

FURTHER READING Typas, A. *et al.* From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nature Rev. Microbiol.* **10**, 123–136 (2012)

MurJ is essential for the translocation of lipid II