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BACTERIAL PHYSIOLOGY

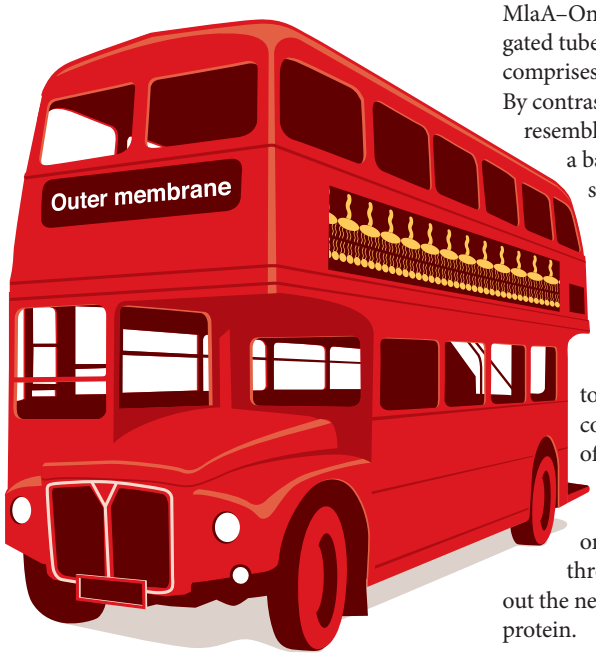
Shuttling lipids across bacterial membranes

> insights into phospholipid and lipoprotein trafficking across the bacterial membranes

The cell envelope of Gram-negative bacteria comprises the inner and outer membranes, which are separated by the hydrophilic periplasm. The outer membrane functions as a selective barrier that prevents the entry of many small molecules into the cell and consists of an asymmetric bilayer comprising phospholipids, lipopolysaccharide (LPS) and lipid-anchored lipoproteins. Two new studies now reveal novel structural and mechanistic insights into phospholipid and lipoprotein trafficking across bacterial membranes.

Ekiert, Bhabha *et al.* provide the mechanism and structural basis of phospholipid trafficking across the periplasmic space, which was, until now, poorly understood. In particular, they identified members of the mammalian cell entry (MCE) protein family as mediators of lipid transport in double-layered bacterial membranes. Using a combination of biochemistry, X-ray crystallography and electron microscopy techniques, the authors obtained mechanistic insights and detailed structural information for three MCE proteins of *Escherichia coli* — MlaD, YebT and paraquat-inducible protein B (PqiB).

MlaD forms a homo-hexameric ring that has a central hydrophobic pore and directly binds to phospholipids as part of the MlaFEDB transporter complex in the inner membrane. As the complex associates with MlaC, which is a lipid-binding protein that is located in the periplasmic space, the authors hypothesize that this interaction could facilitate the transport of lipids between the inner and outer membranes. Consistent with this model, the authors also reported a direct and specific interaction between MlaC and the outer membrane complex MlaA–OmpF. YebT forms an elongated tube-like barrel structure that comprises seven stacked MCE rings. By contrast, the structure of PqiB resembled that of a syringe, with a barrel consisting of three stacked rings connected to a narrow needle-like tube. Single-particle cryo-electron microscopy revealed that these structures form homo-hexameric complexes that are anchored to the inner membrane and could span the entire width of the periplasmic space, potentially enabling the direct transport of lipids or hydrophobic molecules through their channels, without the need for a periplasmic shuttle protein.



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In a second study, Grabowicz and Silhavy identified a possible novel alternative pathway for the trafficking of outer membrane lipoproteins. According to the prevailing paradigm, the LolABCDE pathway transports lipoproteins to the outer membrane. The periplasmic chaperone LolA extracts lipoproteins from the LolCDE complex at the inner membrane and delivers them to the outer membrane receptor LolB, which catalyses their insertion into the outer membrane. Until now, each component of this system was considered essential. However, the authors reveal that whereas the LolCDE complex is vital, LolA and LolB are not mechanistically essential for trafficking. They showed that LolA and LolB instead prevent the toxic accumulation of outer-membrane-targeted lipoproteins in the inner membrane. In agreement with this, LolA and LolB could be bypassed in strains that lacked non-essential lipoprotein substrates that might be toxic if mislocalized by activating envelope stress responses. Thus, these findings suggest that an alternative LolA-independent and LolB-independent trafficking pathway exists that receives substrates from the LolCDE complex at the inner membrane and delivers them to the outer membrane.

Taken together, these studies provide novel mechanistic insights for lipid and lipoprotein transport across bacterial membranes.

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ORIGINAL ARTICLES Ekiert, D. C., Bhabha, G. *et al.* Architectures of lipid transport systems for the bacterial outer membrane. *Cell* **169**, 273–285 (2017) | Grabowicz M. & Silhavy T. J. Redefining the essential trafficking pathway for outer membrane lipoproteins. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1702248114 (2017)