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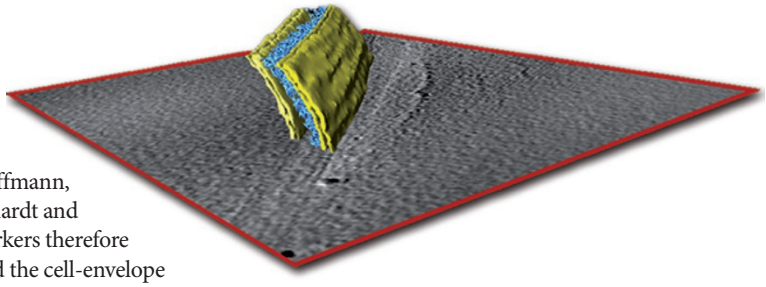
BACTERIAL PHYSIOLOGY

Double trouble



Three-dimensional structure of a section from the cell envelope of *Mycobacterium bovis* bacillus Calmette–Guérin derived by cryo-electron tomography. The inner membrane (left) and the mycobacterial outer membrane (right) are highlighted in yellow. The cell-wall polymers (arabinogalactan and peptidoglycan) that bind the mycolic acids are displayed in blue. The cell envelope is 35 nm thick. Figure kindly provided by Christian Hoffmann and Harald Engelhardt, Max Planck Institute of Biochemistry, Martinsried, Germany.

A clearer picture of the mycobacterial cell envelope has been revealed by Hoffmann, Engelhardt and colleagues using cryo-electron tomography (CET). In *Proceedings of the National Academy of Sciences USA*, they report that, in contrast with the current model, the outer layer of this envelope is a morphologically symmetrical lipid bilayer. Mycobacteria are therefore protected by two lipid bilayers — the cytoplasmic membrane and the newly named 'mycobacterial outer membrane' — which means double trouble for the development of drugs against pathogenic strains.

Mycobacterial cell walls provide an exceptional permeability barrier and contribute to their high intrinsic drug resistance. This fact, together with the medical importance of *Mycobacterium tuberculosis*, means that their structure has been extensively studied. Mycobacterial cell walls contain extractable lipids as well as long-chain mycolic acids that are covalently attached to peptidoglycan through an arabinogalactan network. In the current model, the cell-wall lipids form an asymmetric bilayer of considerable thickness, but this has never been proven directly using microscopy or other approaches.

Hoffmann, Engelhardt and co-workers therefore studied the cell-envelope structure of *Mycobacterium smegmatis* and *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) using CET and frozen-hydrated intact cells. They showed that the native structure of cell envelopes is accurately preserved in CET using the reference organism *Escherichia coli*, and then revealed that mycobacterial cell envelopes have a multilayered structure. In cryo-electron tomograms, the outer layer could be seen as a lipid bilayer that was approximately 8 nm thick, which is only 15% thicker than the cytoplasmic membrane. The authors named this bilayer the mycobacterial outer membrane to differentiate it from the outer membrane of Gram-negative bacteria.

As membrane bilayer structures have not previously been rendered visible in tomograms of intact cells, the authors reproduced their results using ultra-thin frozen-hydrated cryosections of *M. smegmatis* and BCG cells. In addition to confirming the thinner lipid-bilayer structure of the mycobacterial outer membrane, micrographs of the vitreous cryosections showed that this bilayer is morphologically symmetrical.

Hoffmann, Engelhardt and colleagues have therefore proposed a considerably revised model of the mycobacterial cell envelope. In their model, the mycobacterial outer membrane is a morphologically symmetrical lipid bilayer of reduced thickness, which satisfies their data. However, it also fits with other experimental findings and provides a "molecular explanation for the existence of outer membrane proteins and periplasmic proteins ... in mycobacteria."

Rachel Smallridge

ORIGINAL RESEARCH PAPER Hoffmann, C. *et al.* Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. *Proc. Natl Acad. Sci. USA* **105**, 3963–3967 (2008)