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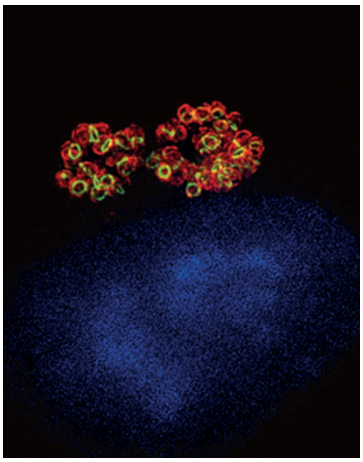
BACTERIAL PHYSIOLOGY

Chlamydiae play by their own rules

> the amoeba symbiont *Protochlamydia amoebophila* and the human pathogen *Chlamydia trachomatis,* do synthesize peptidoglycan

Most bacteria possess a peptidoglycan sacculus (composed of a disaccharide backbone crosslinked by pentapeptide chains), which is crucial for cell division, maintaining cell shape and resisting osmotic stress. Whether the obligate, intracellular Chlamydiae contain this structure has long been debated, but two studies now show that two different *Chlamydia* species, the amoeba symbiont *Protochlamydia amoebophila* and the human pathogen *Chlamydia trachomatis*, do synthesize peptidoglycan.

Some Chlamydiae encode a complete peptidoglycan biosynthesis pathway, and pathogenic strains are sensitive to peptidoglycan-targeting β-lactam antibiotics; however, attempts to detect and purify peptidoglycan from these bacteria have failed. Pilhofer *et al.* used electron cryotomography to examine the cell envelope of two deeply rooting Chlamydiae, *P. amoebophila* and *Simkania negevensis*. They found that only *P. amoebophila* contained detectable peptidoglycan, and high-pressure liquid chromatography combined with mass spectrometry showed that the muropeptides of purified sacculi contained an unknown modification that is absent in peptidoglycan from other Gram-positive and Gram-negative bacteria. Imaging of fluorescent D-amino acid incorporation confirmed the synthesis of peptidoglycan *in vivo*, and treatment of infected amoebae with an inhibitor of peptidoglycan synthesis resulted in the formation of enlarged *P. amoebophila* cells and reduced infection rates. Together, these findings confirm that this *Chlamydia* species can synthesize a unique type of peptidoglycan, which has an important role during infection and in maintaining cell shape.



Infected mouse fibroblast cells (nucleus in blue) show that peptidoglycan (green) is synthesized by *Chlamydia trachomatis* cells (red). Image courtesy of E. Kuru, Indiana University, USA.

Liechti *et al.* developed a novel *in vivo* peptidoglycan labelling approach that used D-Ala–D-Ala dipeptide analogue probes, to which modified fluorophores were attached by click chemistry. Using this method, the authors detected the presence of peptidoglycan in *C. trachomatis*, and found that most of the labelled peptidoglycan localized to the septum of dividing cells, which is consistent with the requirement for peptidoglycan synthesis prior to cell division. Treatment of infected mouse fibroblasts with inhibitors of peptidoglycan synthesis led to the production of fewer *C. trachomatis* cells, which were enlarged, had aberrant cell shapes and were unable to divide. Furthermore, the dipeptide analogues were shown to rescue the *C. trachomatis* growth inhibition that was induced by an inhibitor of the dipeptide ligase, which confirms that they can be used as substitutes for the natural dipeptide.

Together, these studies provide the strongest evidence so far that Chlamydiae possess peptidoglycan. However, although they seem to play by the peptidoglycan rules, Chlamydiae are still unique, as they are the only example of bacteria that contain functional peptidoglycan but lack the cytoskeletal protein FtsZ. Considering that septal peptidoglycan synthesis is orchestrated by FtsZ, these findings challenge the prevailing view that FtsZ is essential in bacteria that possess peptidoglycan.

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ORIGINAL RESEARCH PAPERS Pilhofer, M. *et al.* Discovery of chlamydial peptidoglycan reveals bacteria with murein sacculi but without FtsZ. *Nature Comms.* **4**, 2856 (2013) | Liechti, G. W. *et al.* A new metabolic cell-wall labelling method reveals peptidoglycan in *Chlamydia trachomatis*. *Nature* http://dx.doi.org/10.1038/nature12892 (2013)