Microbiology

# Bacterial envelope built to a peptidoglycan tune

Michaël Deghelt & Jean-François Collet

How a bacterium coordinates the assembly of its outer layers, and couples the formation of this envelope to cell growth and division, is not fully understood. Assessing the role of peptidoglycan molecules provides some answers. See p.953

A classification system in use since the end of the nineteenth century groups bacteria as either Gram-positive or Gram-negative. This sorting system depends on the level of microbial permeability to an exterior dye, a characteristic influenced by the properties of structures that form bacterial outer layers and are termed the cell envelope. This envelope has many essential roles in enabling bacteria to function and survive. On page 953, Mamou *et al.*[1] report evidence that sheds light on a long-standing mystery about how the cell envelope of Gram-negative bacteria is constructed.

Gram-negative bacteria have a particularly complex cell envelope that contains an inner and an outer membrane (Fig. 1). Between these two membranes is a space, called the periplasm. This contains a thin mesh of peptidoglycan, which consists of chains of sugars (glycans) connected by short peptides.

The composition of the outer membrane is unusual. Most biological membranes contain a bilayer of phospholipid molecules, whereas the outer membrane of Gram-negative bacteria is an asymmetric bilayer with phospholipids for the inner layer and lipopolysaccharide molecules making up the outer layer[2].

The proteins embedded in the outer membrane also exhibit striking properties. Similar to those found in the outer membrane of organelles such as mitochondria, these proteins arise from a structure called a β-sheet, which folds into a barrel shape (termed a β-barrel). These folded proteins often contain a central pore, and some such proteins enable nutrients to enter the cell. All the components of the various envelope layers are made in the

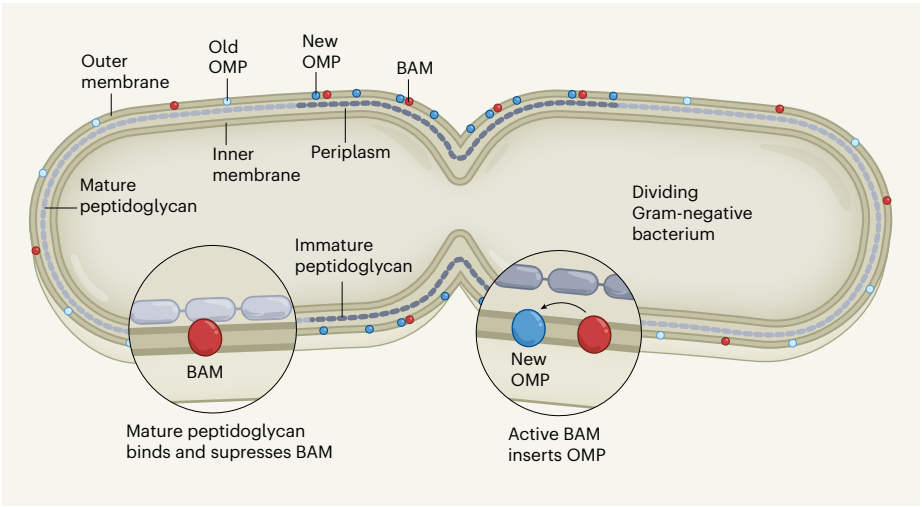


**Figure 1 | A mechanism that coordinates bacterial growth.** Bacteria in the Gram-negative group have inner and outer membranes, and between these membranes is a layer called the periplasm, which contains the molecule peptidoglycan. Mamou *et al.*[1] reveal how the addition of proteins to the outer membrane depends on the status of the adjacent peptidoglycan. An outer membrane protein (OMP) is inserted through the action of the protein complex BAM. The authors show that mature peptidoglycan binds to and inhibits BAM, whereas immature peptidoglycan doesn't bind or interfere with BAM, thus enabling OMP insertion. This system results in the spatial coordination of new OMP insertion at sites of peptidoglycan synthesis, such as in the mid-cell region of a dividing cell.

cytoplasm or the inner membrane, and complex molecular machineries transport, target and deliver these components to sites where new envelope material is made.

The machineries that assemble the outer membrane and the peptidoglycan must coordinate their actions in time and space for harmonious cell growth and a successful cell-division process. Thus, the many proteins that act as builders in envelope assembly must, like orchestral musicians, work in synchrony and follow the same tempo so that outer-membrane growth keeps pace with peptidoglycan production. Yet despite a wealth of information on the properties of these builders, how this process is coordinated has remained an enduring mystery.

Mamou and colleagues report their discovery of a molecular link between peptidoglycan maturation and outer membrane assembly, providing a long-sought explanation of how these two processes are coordinated. The authors began by focusing on a protein complex called BAM; this is the machinery that inserts β-barrel proteins into the outer membrane[3]. Mamou *et al.* sought to clarify where β-barrel insertion occurs on the cell surface.

Some studies report preferential insertion at the mid-cell region[4–6]; others indicate that insertion occurs throughout the cell surface[7,8]. Mamou *et al.* confirmed that the core component of BAM — a protein called BamA, which is itself a β-barrel protein — clusters into small islands that are evenly distributed on the cell surface. Studying two β-barrel substrates of BAM to track β-barrel insertion, the authors determined that these two proteins predominantly appear on the surface at sites of cell division in the mid-cell region and, to a lesser extent, on the long axis of cells.

Although BAM is uniformly distributed, β-barrel insertion occurs only in a specific pattern at particular sites, indicating that BAM activity is spatially controlled in a manner that depends on the cell cycle. How might this happen? The authors got a hint from their observation that the patterns of β-barrel insertion corresponded to sites where, as had been previously reported[9,10], peptidoglycan assembly occurs. The authors confirmed this connection using a fluorescence microscopy approach.

This result prompted Mamou *et al.* to test for interactions between BAM components and peptidoglycan fragments. The authors used two kinds of fragment: disaccharide pentapeptide chains (associated with maturing peptidoglycan) and disaccharide tetrapeptide chains (generated from disaccharide pentapeptide chains and found in mature peptidoglycan). Excitingly, the authors found that BAM interacts with disaccharide tetrapeptides, but not with disaccharide pentapeptides, and that this interaction inhibits BAM activity. Their results demonstrate that the cell-wall composition drives β-barrel patterning in the outer membrane of the bacterium *Escherichia coli*, revealing that BAM activity is controlled by the maturation of the adjacent peptidoglycan layer. Thus peptidoglycan maturation is the maestro of the peptidoglycan–BAM orchestra.

**"Mamou and colleagues' work will contribute to the global effort to find new antibiotics."**

By revealing one of the first known molecular mechanisms by which processes in the formation of the cell envelope are coordinated, this study provides a crucial step forwards in our understanding of how this complex macromolecular structure is assembled. Further detailed molecular dissection of this mechanism is necessary. This work also raises many intriguing questions to be addressed in the future. It is tempting to speculate that other essential machineries for envelope formation, such as those that insert lipopolysaccharide and lipoproteins into the outer membrane, might also be controlled by peptidoglycan maturation. The active forms of these machineries might also be mainly located at division sites, which could represent a hotspot for cell-envelope formation.

Mamou *et al.* focused on *E. coli*, but also provide data indicating that the same strategy controls envelope assembly in other types of bacterium (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Future research should explore whether this strategy is evolutionarily conserved in bacteria that have different modes of growth and division.

Destabilizing the outer membrane is an attractive strategy for generating antibacterials, and scientists are actively searching for BAM-targeting compounds[11–14]. There is little doubt that, by providing crucial molecular insights into how BAM activity is controlled, Mamou and colleagues' work will contribute to the global effort to find new antibiotics.

**Michaël Deghelt** and **Jean-François Collet** are at WELBIO – Walloon Excellence in Life Sciences and Biotechnology, 1200 Brussels, Belgium, and also at the de Duve Institute, Université Catholique de Louvain, Brussels.
e-mail: jfcollet@uclouvain.be

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