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Editorial overview: "All in all, it is not just another brick in the wall": new concepts and mechanisms on how bacteria build their wall

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The staining procedure developed by the Danish microbiologist Christian Gram in 1884 allows bacteria to be divided into two basic groups, Gram-positives and Gram-negatives. It then took decades for scientists to understand that how bacteria respond to the staining method depends on the structure of the envelope that separates them from the external environment. It is now textbook knowledge that whereas Gram-positives are surrounded by a thick peptidoglycan layer, which forms a large wall between the cytoplasmic membrane and the environment, the envelope of Gram-negatives is triple-layered, with a thin murein sacculus laying within a compartment delimited by two concentric membranes, the inner and outer membrane. Based on the number of membranes, Gram-positive and Gram-negative bacteria are also often referred to as monoderm or diderm bacteria, respectively. However, it has also become apparent that some bacteria belonging to the class of Actinobacteria, such as *Mycobacteria* spp., or bacteria belonging to the class of Negativicutes or Halanaerobiales, do not conform to the Gram-positive and Gram-negative 'stereotypes'. Despite being phylogenetically embedded within the Gram-positives, these bacteria are triple-layered and diderm since they possess an inner and an outer membrane. Undoubtedly, we have come a long way since H.R. Perkins wrote in a review article in 1963 that 'Somewhere in the neighborhood of its surface, each bacterial cell has a structure which can be regarded as its boundary wall.' We have gained a detailed understanding of the different structures making up the boundary wall and intense research over the past decades also has led to the identification of many of the key factors involved in the assembly of the bacterial envelopes. However, new factors or additional functions of known factors are still being discovered. Furthermore, the molecular mechanisms at play leading to the assembly of the complex bacterial envelope structures remain only partly understood.

In this special issue of *Current Opinion in Microbiology*, an overview on the latest insights into the molecular mechanisms that underpin the assembly of the envelope of bacterial cells is provided, with a special focus on newly identified factors, how they are regulated and how their activities are coordinated. In addition, how resources needed for cell wall synthesis processes and central metabolism are shared and cell wall components recycled will also be addressed.

Perhaps the most studied structure in the bacterial cell envelope is the peptidoglycan layer. While biochemically quite a simple structure,

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de Duve Institute in Brussels. The research interests of the group include the molecular mechanisms by which Gram-negative bacteria in general and *Escherichia coli* in particular assemble and protect their cell envelope, with a special focus on the biogenesis and function of lipoproteins and on factors involved in protein folding and repair.

composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid glycan residues with attached and crosslinked peptide chains, the coordination and regulation of the enzymes required for its assembly and regulated degradation remains a fascinating area of research, full of surprises and new discoveries. Ducret and Grangeasse discuss the most recent advances in the understanding of the mechanisms involved in peptidoglycan assembly in Firmicutes, a large phylum of mainly monoderm bacteria that encompasses *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pneumoniae*. A short description of the key enzymes involved in peptidoglycan assembly and remodeling is presented, and this is followed by a discussion of newly identified factors and regulatory mechanisms. This review highlights the wealth of new insights gained in the peptidoglycan field in the span of a few years. Important recent discoveries concern the exquisite regulation of penicillin binding proteins (PBPs). Pazos and Vollmer focus in their review on the finetuning of the activity of Class A PBPs, which are bifunctional enzymes combining a glycosyltransferase and transpeptidase activity. They discuss the importance and roles of these enzymes, the proteins they interact with as well as their function. This is also done in the light of the recent discovery that the monofunctional class B PBPs, which have only transpeptidase activity, work together with SEDS (shape, elongation, division and sporulation) proteins to form a novel type of glycosyltransferase/transpeptidase pair. It is clear that recent technological developments had a major impact on how we understand peptidoglycan synthesis, as described by Hernandez and Cava. In their article, these authors cover some of the most recent technical advances in the field, with a particular focus on the development of a method allowing for a high-throughput biochemical analysis of the peptidoglycan structure suitable for the investigation of large mutant strain collections as well as state-of-the-art imaging approaches. They explain how these new methods allow studying the spatio-temporal dynamics and mechanical properties of the peptidoglycan with a much higher level of detail.

Not only peptidoglycan synthesis is a highly coordinated process, its synthesis is integrated with the synthesis of other cell envelope components as well as other cellular processes such as the cell division process. Less appreciated and studied is the need for sharing resources between central bacterial metabolism and cell envelope synthesis processes. By considering the key metabolic junctions involved in the synthesis of precursors required for the assembly of the different envelope components as well as central bacterial metabolism, Sachla and Helmann provide a different perspective on the coordination of cell envelope assembly processes. In their article, they highlight how the assembly of the envelope requires a sharing of limited resources between competing cellular pathways. They also explain how bacteria can acquire some resources from recycling parts of their cell envelope components or from their neighbors and highlight some extreme cases in which symbiotic partners share the burden of synthesizing enzymes required for envelope assembly. In some bacteria with a multicellular lifestyle, such as Cyanobacteria, cells differentiate into mutually dependent cell types. In such systems, resources do not only need to be shared within cells, but also between cells; in these cases, the cell envelope presents a formidable barrier for cell-to-cell communication and resource sharing. The way this problem has been solved in Cyanobacteria is by the construction of proteinaceous structures known as septal junctions that traverse the septal cell wall and mediate intercellular communication. In the article by Kieninger and Maldener, the proteins that play a role in cell wall remodeling and cell–cell communication in these microorganisms is reviewed. The recently revealed *in situ*

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architecture of the septal junctions and their mechanism of gating is also discussed. Remodeling and recycling of the cell envelop components is also addressed by Abrahams and Besra in their review on *Mycobacterium tuberculosis* (*Mtb*). As mentioned, *Mycobacteria* such as *Mtb* do not fit the Gram-positive or Gram-negative stereotypes, as the mycobacterial cell wall contains several unique molecular structures, including a mycobacterial outer membrane. The authors present the mechanisms and pathways involved in the synthesis of the main mycobacterial cell envelope components, with a particular focus on aspects of remodeling and recycling. Since *Mtb* and in particular drug resistant strains cause serious public health problems, the authors also highlight untapped opportunities that the new discoveries in the area of cell envelop synthesis and recycling present for the development of novel antibiotics against this pathogenic bacterium.

In contrast to the mycobacterial outer membrane, which is composed to a large part of mycolic acid, the outer membrane in prototype Gram-negative bacteria is an asymmetric lipid bilayer with lipopolysaccharides (LPS) in the outer leaflet and phospholipids in the inner leaflet. How hydrophobic molecules such as LPS and phospholipids are extracted from the inner membrane and are transported across an aqueous compartment — the periplasmic space — to reach and form the outer membrane has been a longstanding question in the field. In particular in terms of the transport of LPS, much progress has been made in recent years. Wilson and Ruiz provide a thorough overview of the Lpt system, which forms a membrane-to-membrane bridge transporting LPS molecules to the cell surface. Also briefly summarized are the current data and models of how phospholipids are transported to the outer membrane; a process about which little is known. Beside LPS and phospholipids, a number of proteins are embedded in the outer membrane of Gram-negative bacteria. These outer membrane proteins usually contain amphipathic β-strands arranged in an antiparallel β-sheet that folds into a barrel. Membrane insertion of these proteins depends on the essential β-barrel assembly machinery (BAM). Tomasek and Kahne explain how new data from biochemical and structural studies have shed new light on the function of the BAM complex. On the basis of this recent work, they propose a detailed molecular model for β-barrel protein insertion and assembly in the outer membrane. Other important proteins that are found attached to bacterial membranes are lipoproteins. These are globular proteins anchored to a membrane by a lipid moiety. Lipoproteins carry out important functions in cellular processes such as envelope biogenesis, stress sensing, virulence, and cell division. El Rayes, Rodriguez-Alonso and Collet review new insights into lipoprotein biogenesis and discuss new functions carried out by lipoproteins. Also highlighted is the recent identification of lipoproteins on the surface of *Escherichia coli* and other bacterial models.

Undoubtably an important function of the outer membrane is its barrier function. It not only helps to prevent toxic molecules from entering bacterial cells, but it also allows Gram-negative and other diderm bacteria to secrete but still retain proteins within the periplasmic space and hence within the cell envelope. On the other hand, secreting proteins into the environment through the envelope of a diderm bacterium provides its challenges. Monoderm Gram-positive bacteria face the opposite challenge; while proteins destined for the outside environment only need to cross a single membrane, retaining proteins within the cell envelope requires additional signals, proteins and protein domains and active retention mechanisms. An elegant solution to this problem is the sortase enzyme-mediated covalent attachment of surface proteins to the peptidoglycan layer in Gram-positive bacteria. Much of the pioneering work in this area was performed by Olaf Schneewind and his group. Bhat, Nguyen, Das and Ton-That present a historic overview to the field, highlighting the tour the force experiments leading to the identification of the sortase-enzyme, and our current understanding of the basic mechanisms involved in sortase-mediated cell wall anchoring of surface proteins. The authors also cover up to date information on the sortase-mediated pilus assembly process in several bacterial models and the most recent advances in the field, such as proposed signalling functions of cleaved and membrane embedded surface protein fragments. Monoderm Gram-positive bacteria lack LPS and an outer membrane, but they produce a range of secondary cell wall polymers. While some of the proposed parallels in the function of LPS and cell wall polymers might be debatable, similarities in the mechanism of their synthesis in particular with regards to their decoration with glycosyl groups clearly exist. Rismondo, Gillis and Gründling present a brief up-to-date overview of the biosynthesis pathways of teichoic acids and more complex polysaccharides cell wall polymers in Firmicutes bacteria. This is followed by a detailed discussion of recent work on enzymes constituting multi-component transmembrane glycosylation systems, required for the decoration of the secondary cell wall polymers.

Clearly, there is no sign of a slowing down in exciting new discoveries that are made in the field of bacterial cell envelope assembly, and undoubtedly several of these findings will find their way into textbooks. But there is of course also an important, more applied, side to this work. Many successful antibiotics target the cell envelope, a compartment which is more easily accessible from the outside and containing many essential components that are unique to bacteria. In nature,

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bacteria will compete with others for access to scare resources in order to grow. To gain the upper hand, many microbes produce compounds and enzymes that will prevent the growth of or kill competing bacteria. Page and Walker focus in their review on antibacterial compounds and enzymes that exist in nature and that target the cell envelope. Some mechanisms that have been validated in the clinics are covered. However, the authors mostly highlight recent discoveries of novel antibacterial agents, focusing on peptide-based natural products, leaving us with much needed hope that some of them will make it to the clinic.

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