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Editorial

SFB 766: 12 years of research on the bacterial cell envelope

The cell envelope is an intricate multilayer that surrounds the cytoplasm and defines the boundary of bacteria. In Gram-negative bacteria it comprises the inner and outer membrane that are separated by a peptidoglycan layer while Gram-positive bacteria possess a single membrane enclosed by a thick peptidoglycan sheet. The cell envelope ensures bacterial survival by serving as a semi-rigid and selective permeability barrier: It confers mechanical stability and limits influx of harmful substances and at the same time facilitates uptake of nutrients. Given the essential function of the cell envelope, maintenance of its integrity during growth and division is pivotal, which is achieved by continuous remodeling and passage of building blocks across the cytoplasmic membrane.

Forming the interface between bacterium and immediate environment, the cell envelope is the key site for biological processes involved in the interaction with the surrounding. These processes are mediated by a diverse array of proteins that are associated with or incorporated into the cell envelope. Prominent examples of these proteins or assemblies thereof are sensing and signaling systems, adhesins and secretion systems, which enable bacteria to interact with the extracellular milieu, perceive and respond to external changes and stresses as well as to cause infections. Furthermore, with regard to pathogen-host interaction, cell wall components themselves such as peptidoglycan and wall teichoic acids are critical determinants of innate immune responses to an infection. Moreover, the cell envelope and in particular the cell wall and its biosynthesis is a major target of antimicrobial agents. Penicillin, which inhibits the formation of peptidoglycan cross-links, is used in the clinic for more than 75 years and is still a widely prescribed antibiotic despite the emergence of resistance. However, the cell envelope remains center staged in the combat against antimicrobial resistance.

Regarding the intricate biology of the cell envelope a multitude of questions remain, answers to which will provide insights into fundamental cellular processes with important implications for human health. Building on a history of successful research on the cell envelope in Tübingen, scientists from the University of Tübingen, the University Hospital Tübingen and the Max-Planck-Institute for Developmental Biology initiated a collaborative research center (CRC or, abbreviating the German term Sonderforschungsbereich, SFB) that was financed by the German Research Foundation since 2007 (CRC/SFB 766) to address open questions in the field (Braun, 2015). This special issue summarizes recent findings gained by scientists financed during the third funding period of the SFB 766 (2015–2019).

Cell division of the filamentous Gram-positive bacteria of the genus *Streptomyces* is restricted to the reproductive state, *i.e.* sporulation, and does not take place during vegetative growth. Peptidoglycan synthesis occurs only at the hyphal tips and during sporulation. Thus, *Streptomyces* are ideal model organisms to study the coordination and function of individual proteins involved in division and peptidoglycan synthesis as the deletion of genes encoding these proteins is not lethal. Sporulation of *S. coelicolor* involves the *Streptomyces* spore wall synthesizing complex (SSSC) consisting not only of peptidoglycan and glycopolymer synthesis proteins but also of two eukaryotic like serine/threonine kinases. These kinases are required for proper sporulation by regulating SSSC activity (Vollmer et al., 2019).

Bacteria are capable of recovering and recycling their own cell wall components using distinct pathways. The hydrolase MurQ of *E. coli* is necessary for the recovery of the sugar MurNAc. However, *murQ* is absent from the majority of Gram-negative bacteria, which utilize unmodified MurNAc to synthesize peptidoglycan thereby bypassing the first stages of cell wall biosynthesis. As a consequence, these bacteria are resistant against fosfomycin, an inhibitor of a reaction leading to MurNAc synthesis. Furthermore, Gram-positive bacteria were shown to recycle their own peptidoglycan fragments and wall teichoic acids (WTA) during nutrient limitation (Mayer et al., 2019a).

WTA and capsular polysaccharides (CP) are important factors in the pathogenesis of *Staphylococcus aureus* infection by enabling the bacteria to avoid host immune defense mechanisms amongst others. Biosynthesis of WTA and CP is tightly controlled by a complex regulatory network involving various transcription factors and two-component systems. The differential expression of WTA and CP genes ensures optimal use of cell wall precursors and leads to alterations of the composition of the bacterial surface, which likely plays a vital role in the course of *S. aureus* infections (Keinhörster et al., 2019).

Adenylyl cyclases play a key role in prokaryotic and eukaryotic cell physiology by generating the second messenger cyclic AMP. The activity of mammalian transmembrane adenylyl cyclases is mainly regulated by G proteins. However, in addition to the transmembrane domains of adenylyl cyclases serving as anchors, they might have receptor function. This was studied by constructing chimeric bacterial proteins comprising an adenylate cyclase catalytic domain fused to a membrane receptor domain with known ligands. The results suggest that adenylate cyclase transmembrane domains act as receptors (Finkbeiner et al., 2019).

The cyanobacteria *Nostoc punctiforme* and *Anabaena* sp. display a multicellular life style. The bacteria grow as filaments composed of differentiated cells that serve distinct functions and rely on intercellular communication and nutrient flux. Focused ion beam milling and electron cryotomography revealed that the nanopores observed in the septum between adjacent cells provide the pass-through for septal junctions. These cell junctions are gated and can disconnect the cytoplasm of neighboring cells in response to stress (Kieninger et al., 2019).

Streptomycetes form hyphae that are compartmentalized by peptidoglycan cross walls. The bacteria utilize a novel TraB-dependent mechanism for conjugation occurring in two steps. In the first stage, close contact between lateral walls of donor and recipient hyphae results in

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the formation of a pore allowing intermycelial transfer of the plasmid. Notably, this is followed by a second step in which the plasmid is spread within the recipient hyphae across the compartments separated by septal cross walls (Thoma et al., 2019).

Plants are continuously exposed to a variety of microorganisms such as bacteria and fungi. Similar to the human and animal immune system, the ability to sense microbial oligosaccharides plays a critical role in plant immunity. For this, plants make use of hydrolytic enzymes of the chitinase family that generate degradation products of bacterial peptidoglycan and fungal chitooligosaccharides, which are recognized by lysine motif (LysM)-containing receptors to initiate an adequate host response (Schlöffel et al., 2019).

The cyanobacterium *Anabaena* sp. PCC7120 grows in filaments consisting of cells that perform oxygenic photosynthesis. The lack of fixed nitrogen induces the differentiation of some of these cells into anaerobic nitrogen-fixing heterocysts. The complex development of heterocysts is dependent on several tripartite efflux pumps and ATP-binding cassette (ABC) transporters (Shvarev and Maldener, 2019).

Common survival strategies of Gram-negative bacteria include the secretion of effector proteins or toxins *via* designated secretion systems into the extracellular milieu or into host cells. Pore-forming secretins that allow passage of substrates across the outer membrane are conserved components of diverse types of secretion systems. Targeting of secretin monomers to the outer membrane and their oligomerization is highly regulated and different secretin assembly pathways have evolved in bacteria (Natarajan et al., 2019).

To ensure survival and proliferation during infection, many Gram-negative bacteria employ the type III secretion system for toxic effector protein delivery into host cells. The type III secretion system, also termed the injectisome, is a complex multi-protein apparatus embedded in the cell envelope. The development of techniques such as *in vivo* photo-crosslinking allows for a detailed insight into injectisome assembly and the physical interaction of its components, which is an important basis for the design of agents that interfere with type III secretion system activity (Singh and Wagner, 2019).

Autotransporter proteins are a widespread family of β-barrel proteins that are integrated into the outer membrane of Gram-negative bacteria and mainly serve as virulence factors. Biogenesis of autotransporters and exposure of their passenger domain on the cell surface requires the β-barrel assembly machinery. The passenger domain of the autotransporter adhesins invasin and YadA of *Yersinia enterocolitica* binds to integrin receptors promoting internalization and extracellular matrix proteins, respectively. Host-cell adhesion is of central importance for effector translocation by the type III secretion system. In fact, autotransporter adhesins are involved in rendering certain host cells susceptible to type III secretion system dependent intoxication. Furthermore, autotransporter proteins as well as proteins involved in outer membrane biogenesis represent potential novel therapeutic targets (Bohn et al., 2019; Leibiger et al., 2019).

The bacteriophage-related type VI secretion system has been observed to display a random or polar localization inside bacteria. Similarly, the ATPase ClpV-1 of the *Burkholderia* type VI secretion system 1 localizes to a discrete focus at random sites inside the bacteria, whereas the ATPase ClpV-5 of the type VI secretion system 5 is confined to the pole. Localization studies of ClpV-1 and ClpV-5 revealed that different mechanisms govern the localization of T6SS ATPases (Lennings et al., 2019).

The antibiotic daptomycin is effective against methicillin resistant *Staphylococcus aureus* (MRSA). However, the mode of action of daptomycin and the development of resistance are not well understood. Mutational analysis of the multiple peptide resistance factor (MprF), a phospholipid flippase of *S. aureus*, provided novel insights into the mechanisms underlying daptomycin resistance (Ernst and Peschel, 2019).

The actinomycetes *Amycalotopsis balhimycina* and *Microspora* sp. PTA-5024 produce antibiotics that interfere with peptidoglycan synthesis. Diverse peptidoglycan modifications have been detected to be crucial for the resistance in these bacteria such as the insertion of amide residues into peptidoglycan precursors and alternative crosslinks within the nascent peptidoglycan. The findings illustrate that in addition to the canonical strategies other mechanisms exist that contribute to self-resistance of actinomycetes producing cell wall targeting antibiotics (Unsleber et al., 2019).

Antibiotic acyldepsipeptides (ADEPs) exhibit high antibacterial efficacy by over-activating ClpP, the proteolytic core unit of the Clp protease in bacteria. One of its substrates is the FtsZ protein involved in cell division. While for example extended treatment of bacteria with low ADEP concentrations is very effective, intermediate ADEP concentrations and incubation times did not inhibit the ability of bacteria to resume division. The findings enhance knowledge required to optimize ADEP-based treatment of infections (Mayer et al., 2019b).

Only few classes of chemical structures interact specifically with proteins of the cell envelope. The integral membrane protein MraY is an essential component of the peptidoglycan biosynthesis pathway, which is inhibited by several natural nucleoside antibiotics. One of these are caprazamycins, that are active against *Mycobacterium tuberculosis* MraY *in vitro*. The generation of structurally modified caprazamycins advanced understanding of the effect of distinct structural features on MraY inhibiting activity (Wiker et al., 2019).

Finally, the determination of protein structures is central to understanding cell envelope-related processes at the molecular level. For instance, protein fibers associated with the cell envelope frequently contain coiled-coil domains that are characterized by a high structural diversity (Hernandez Alvarez et al., 2019). Furthermore, a structure-function analysis of an enzymatic complex in *S. aureus*, which amidates the peptidoglycan precursor molecule lipid II will be summarized in this special issue (Noldeke and Stehle, 2019).

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