Chapter 1
The Bacterial Cell Wall and Membrane—A Treasure Chest for Antibiotic Targets

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Abstract Astonishing progress has been made in recent years to understand the structural complexity and functions of the biosynthetic pathways of the bacterial and archaeal envelopes. This progress has prompted me to assemble the present book that provides a detailed overview and the state-of-art of the respective research field. Ideally, the book will provide students and advanced scientists an up to date picture of the different parts of the bacterial and archaeal cell envelope and enable them to understand their functional roles.

Keywords Antibiotic targets · Biosynthetic pathways · Conformational cycles · Multicomponent complex assembly · Regulatory interactions · Substrate movement in transporters

Outer Membrane

We will start off in Chap. 2 with the outer membrane and its outer exposed layer, the lipopolysaccharide (LPS). Here, tremendous progress has been made in unraveling the biosynthetic pathway. In this pathway, Lipid A with core sugars is flipped across the inner membrane catalyzed by MsbA. The flipped lipid A then binds to $LptB_2FG$ and is transferred to LptA and LptC that together forms a bridge across the periplasm. At the outer membrane, LptED receives the LPS precursor and can implant it most likely through a lateral gate allowing it to integrate into the outer leaflet of the outer membrane. An atomic structure of all the participating proteins is now available. This sets the basis for the understanding the molecular events that occur along the pathway and for further investigating the required conformational movements in these proteins which are driven by ATP hydrolysis within LptB.

The next Chap. 3 is focusing on lipoproteins which are found mostly in the inner leaflet of the outer membrane but also in the outer leaflets of both membranes to fulfill their many functional roles. Lipoproteins are synthesized in the cytoplasm with

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a signal peptide that is cleaved off by a unique signal peptidase (termed SPase II) after its transport across the inner membrane by the Sec translocase. The cysteine residue at the N-terminus of the mature protein is modified by three fatty acids that are recognized by LolCDE complex. Lipoproteins destined to the outer membrane are released from LolCDE and transferred to LolA, a periplasmic carrier protein. LolA then moves across the periplasm and delivers the lipoprotein to LolB at the outer membrane for its insertion into the inner leaflet. Although there is a clear distinction between the biosynthetic pathways of lipoproteins and lipopolysaccharides, common principles of their transport are evident. In both pathways, ABC transporter components are involved in releasing the substrate from the inner membrane and bind their substrates in the periplasm for transfer onto carrier components.

Porins, the main proteins of the outer membrane, are discussed in Chap. 4. A major focus is on the unique regulation of the expression of the porins by stress response circuits involving two component systems and anti-sense RNA molecules. The biosynthesis and folding of the β-barrel porins by the BAM complex is discussed that is currently a topic of ongoing research. A further, very important aspect of the porins is their involvement in multidrug resistance. Interestingly, distinct transcriptional regulation systems offer possibilities to manipulate the sensitivity to antibiotics that can also lead to resistance.

Periplasm

We then move to the periplasm (Chap. 5), which is filled with a sponge-like structure of the peptidoglycan. Although the chemical structure and the major enzymes involved in peptidoglycan synthesis has been known for a long time, a more detailed picture has emerged in analyzing the peptidoglycan from different species, including Gram-positive bacteria. There is a variety among the cross-linking peptides and the modifications that occurs within the glycan strands. In addition, the usual 4-3 cross-linking can be replaced by 3-3 crosslinks. This diversity has a big impact on whether antibiotics kill certain bacteria or develop resistance. Moreover, it has been noticed that pathogenic strains are enriched in such modifications, which may contribute to the survival of the bacteria in host organisms.

The biosynthetic machineries for peptidoglycan synthesis are the elongasome and divisome that coordinate transpeptidase and glycosyltransferase activities but consist of specific components. The details of how septation is regulated and how the two new daughter sacculi that are formed are controlled by the temporal and spatial amidase (Ami ABC) activities is currently investigated.

The chaperones Skp and SurA play an essential role for escorting the outer membrane proteins to the outer membrane after they leave the Sec translocon at the periplasmic side of the inner membrane. In Chap. 6 the mechanistic details of Skp and how it binds various client proteins are discussed including the important role of the flexible arms of the Skp trimer that holds onto the client protein. SurA has, as some other periplasmic proteins do, a prolyl-isomerase activity domain. However,

the functional relevance of this activity is still unknown. The SurA domain that is involved in binding to the client proteins does this in a clamp-like fashion.

Preproteins and prolipoproteins, which are processed by signal peptidases, are discussed in Chap. 7. SPase II removes the signal peptide of prolipoproteins and was detected after the discovery of globomycin from *Actinomyces* that inhibits the peptidase. In contrast, SPase I cleaves the signal peptides of periplasmic and outer membrane proteins and is important for the release from the inner membrane allowing them to reach their destination. Recent research has led to the finding that arylomycins are potent inhibitors of signal peptidases. These arylomycins are promising candidates for a new class of antibiotics.

Inner Membrane

The many different proteins of the inner membrane are represented here by transporters, proteins translocases and respiratory complexes. We start in Chap. 8 with the phosphotransferase PTS systems which are central for sugar uptake in bacteria. The predominant function the PTS systems have in bacterial metabolism is reflected by the many regulatory interactions of their components. The phosphorylation cascade and network is a beautiful example of a simple signal transduction system in cells. The coupling of the phosphorylation cascade to transport sugar across the membrane is a mechanistically challenging problem but the novel structural information of the maltose and the diacetylchitobiose transporters from *Bacillus cereus* has now shed light on this. Intriguingly, the substrates are moved through the membrane by an "elevator mechanism" involving topological rearrangements of the transmembrane helices of the transporter. This mechanism of substrate movement is distinct from the rocking switch mechanism found in LacY.

The secondary active transporters are found with 3 different protein folds and are discussed in Chap. 9. LacY and other members of the major facilitator superfamily (MFS) are organized as two symmetric 6-helical bundles that bind the substrates in a central cavity between the two bundles. The rocking movement of the bundles switches LacY from an outwards open to inwards open state, which is used to transport the lactose. Only the protonated LacY can bind the substrate sugar at the periplasmic face and is released after deprotonation at the cytoplasmic face of the membrane. In contrast to MFS, the LeuT and NhaA folds are organized pseudo-symmetrically with interwound 5-helical bundles. In addition, structural flexibility has been observed for NhaA after substrate binding, which lead the authors to propose that transport occurs also here by an elevator transport mechanism.

A wide variety of respiratory complexes present in different bacteria is covered in Chap. 10. Starting with aerobic systems in *E. coli* that operate to establish a proton gradient across the inner membrane and following with respiration of mainly marine bacteria, that generate a sodium ion gradient are discussed. The most fascinating feature of the respiratory complexes is the electron transfer among the individual modules each containing electron binding cofactors. The aerobic and anaerobic res-

piratory chains act as redox loops and are coupled to proton (or sodium) pumps. Bacteria show a wide variety of different respiration systems. Nevertheless, some of these systems could serve as antibiotic targets depending on their essential function in specific environments.

Newly synthesized membrane proteins are inserted by the multi-subunit Sec translocase which operates to translocate unfolded protein chains. In Chap. 11 the details of targeting the ribosome nascent chain or the preprotein chaperone complex to the Sec translocase are presented as well what is known about the movement of the protein chain through SecY and how the movement is powered by conformational cycles of the SecA ATPase. The roles of the YidC insertase alone, or of YidC together with Sec to insert proteins are discussed. Folded proteins, in particular metallo-proteins are translocated by the TAT system which might operate in a concerted action of TatA oligomers surrounding and translocating the client protein in a yet unknown mechanism.

Pili

Since pili are exposed on the bacterial cell wall they are primary antigenic targets and are also involved in biofilm formation. In addition, pili are important for the adherence of bacteria to eukaryotic cells and often involved in the invasion into these cells. Because of their importance in pathogenic infections there is a major medical interest to control the invasion process by compounds that interact with pili or even inhibit pilus formation. Chapter 12 gives an overview on the different pili systems known, starting with the chaperone-usher (CU) system of Gram-negative bacteria. Their assembly pathway at the periplasmic face of the outer membrane is an impressive example of protein folding events leading to consecutive subunit interactions. The folding mechanisms of donor-strand-exchange and the donor-strand-complementation between the subunits lead to subunit pairing and filament formation, respectively. Different from the pili that are assembled by the CU system are the type IV pili, which are involved in the twitching motility but also in cell adhesion, phage and DNA uptake. They are anchored to the inner membrane and to the outer membrane as multi-component complexes that also control filament extension and retraction reactions. New cryo-EM and crystal structures of several components of the type IV pili provide now the first structural details.

The amyloid fibrillar structure of Curli and Fab pili is another fascinating and ongoing area in protein folding research. The pili subunits are transported from the periplasm through a β barrel in the outer membrane and presumably assemble into an amyloid cross- β architecture by a mechanism supported by a chaperone on the extracellular surface.

Different from the pili involved in adhesion are the conjugative pili of the secretion system T4SS. These pili support DNA uptake and DNA delivery into recipient cells in natural competence and bacterial conjugation. The T4SS conjugative pili consist of a multi-component structure that is anchored in the outer and inner membrane

forming a continuous structure from the cytoplasm to the extracellular surface. The conjugative pili are retractile and lead to an intimate donor-recipient cell contact before the DNA is transported through the membranes of the mating cells.

Actinobacteria and Archaea

The cell walls of the Gram-positive Actinobacteria and of archaea are of great interest because of their differences to the well-studied Gram-negative cell wall. In addition, research on the pathogenic Mycobacteria and Corynebacteria has made substantial advances in our knowledge of the actinobacterial cell walls during recent years. Although most of the Actinobacteria have the monodermic Gram-positive cell wall architecture, Mycobacteria and Corynebacteria have evolved a diderm cell envelope. Interestingly, the outer membrane of Mycobacteria, the mycomembrane, is rich in mycolic acids especially in the inner leaflet of the outer membrane bilayer. Another component is the mycocerosyl lipid also present in the outer leaflet which is presumably transported by the ß-barrel protein LppX during its biosynthesis. These components lead to the dense and waxy character of the mycomembrane. Together with a complex capsule structure, which is composed of secreted polysaccharides, a thick electron transparent zone surrounds the mycobacterial cells. The unique properties of the actinobacterial cell wall pose many evolutionary questions such as how they have been developed but they also provide a multitude of promising new chemotherapeutic targets.

Archaea have the capacity to develop an astonishing variety of cellular envelopes. For example, monodermic and didermic cell walls are found in archaea. The didermic cell walls are similar to those of Gram-negative although there are differences in their biochemical composition. Most common among most archaea is the existence of a surface layer (S-layer) consisting of highly glycosylated proteins arranged in 2-dimensional crystal layers. The S-layers contribute to the extreme heat and osmotic stability of the archaeal cells.