Chapter 5
Structure, Biosynthesis, and Function of Teichoic Acids and Related Cell Wall Glycopolymers in the Gram-positive Cell Envelope

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5.1 Introduction

The cell wall of most Gram-positive bacteria contains two types of structural components (Fig. 5.1). Peptidoglycan (PG), which is a highly conserved component, represents the first and almost omnipresent polymer. The second is represented by additional cell wall glycopolymers (CWGs), which are very variable between species and even between individual strains. Peptidoglycan has the well-studied function of imparting mechanical stability on the bacterial cell, whereas the function of the CWGs is still elusive. Most Gram-positive bacteria contain two types of CWGs: (1) the covalently PG-anchored polymers (P-CWG) and (2) membrane glycolipid-anchored polymers (M-CWG) (Fischer 1988; Neuhaus and Baddiley 2003; Weidenmaier and Peschel 2008). There are bacterial species such as *Bacillus subtilis* that even contain three or four different types of CWGs. Cell wall glycopolymers are composed of repeating units formed by one or more sugar building blocks and, in many cases, additional non-sugar residues such as phosphate, alanine, succinate, pyruvate, choline, or mycolic acides, to name but a few. If the polymer backbone contains diester-linked phosphate groups the CWG is usually named teichoic acid (TA), according to James Baddiley's nomenclature from the 1950s (Armstrong et al. 1959). Most TA exhibit zwitterionic properties because of the negatively charged phosphate groups and additional D-alanine residues on the repeating units, which have free positively charged amino groups. Some Gram-positive bacteria produce CWGs without phosphate groups; hence their polymers are uncharged as in many actinobacteria or certain bacilli, or they are anionic because their repeating units contain uronic acid, pyruvyl, or succinyl groups (Delmas et al. 1997; Greenberg et al. 1996; Powell et al. 1975; Schäffer and

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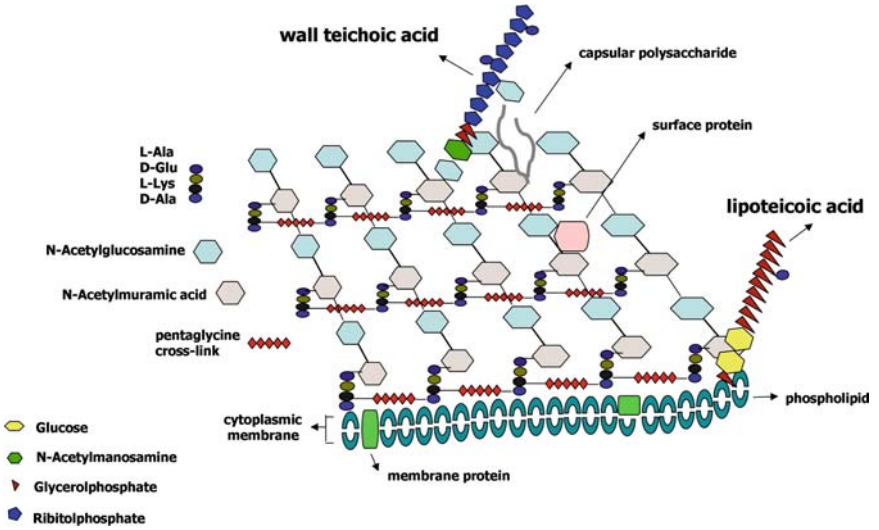

Fig. 5.1 Molecular structure of the cell wall of *S. aureus,* composed of peptidoglycan, teichoic acids, proteins, and capsular polysaccharide. With permission (Kohler et al. 2009b)

Messner 2005; Soldo et al. 1999; Ward 1981). This review will focus on TA-like CWGs with special emphasis on TA structures, biosynthetic pathways, and functions. Other types of CWGs will be mentioned briefly.

5.2 Teichoic Acid Structures

5.2.1 Wall Teichoic Acid and Other Peptidoglycan-Anchored Polymers

Peptidoglycan-anchored polymers are usually attached to PG via more or less conserved linkage units. In most cases these linkage units start with *N*-acetylglucosamine (GlcNAc), which is linked to the *N*-acetylmuramic acid of the PG via a phosphodiester bond. The GlcNAc can be connected to further sugars of the linkage unit or it is connected directly to the CWG (Araki and Ito 1989; Naumova and Shashkov 1997). Up to 50% of the entire cell wall mass is formed by TA of the P-CWG type, which are referred to as wall teichoic acids (WTA) (Fig. 5.2). Since WTA composition is eminently diverse its structure differs profoundly between species or even strains (Endl et al. 1983; Fischer et al. 1993; Naumova and Shashkov 1997; Potekhina et al. 1993). The WTA of *Staphylococcus aureus* is particularly well studied. The linkage unit consists of one GlcNAc residue, one *N*-acetylmannosamine and two glycerolphosphate (Gro-P) residues. The WTA repeating units contain sugars of various sizes ranging from trioses to hexoses.

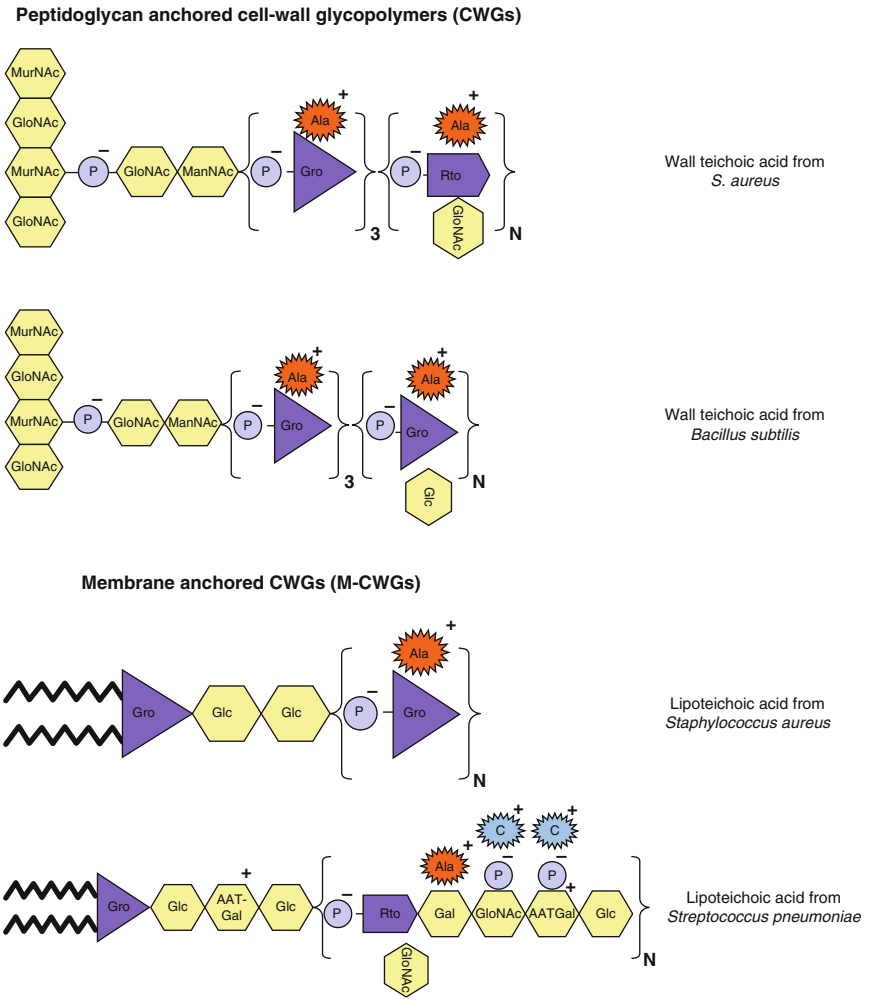

Fig. 5.2 Selected WTA and LTA structures. Trioses, peptoses, and hexoses are shown as triangles, pentagons, and hexagons, respectively. Fatty acids are shown as zigzag lines. Non-glycosyl residues: Ala, D-alanine; C, choline; P, phosphate; Glycosyl residues: AATGal, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; Gro, glycerol; ManNAc, *N*-acetylmannosamine; Rto, ribitol. With permission (Kohler et al. 2009b)

Often these sugars are reduced to their corresponding polyols like glycerol or ribitol. Most *S. aureus* strains produce a WTA polymer, which is formed by up to 40 repeating units of ribitolphosphate (Endl et al. 1983). In *Staphylococcus epidermidis* and other staphylococcal species simpler WTA structures can be found. Here Gro-P units form the entire polymer and are not only found in the linkage unit. A more complicated WTA structure with repeating units composed of

glycerolphosphate occurs, e.g., in *Staphylococcus hyicus* (Endl et al. 1983). Among the known polymers, the most complex WTA-like molecule has been found in *Streptococcus agalactiae*. This polymer is extremely complex and branched. It is composed of different types of repeating units, which form the individual branches of the molecule (Sutcliffe et al. 2008). Notably, this polymer represents the species-specific antigen for serological differentiation of streptococci according to Rebecca Lancerfield (Lancefield and Freimer 1966). The repeating units of the WTA molecules are usually further substituted with a diverse set of additional sugars and D-alanine (Neuhaus and Baddiley 2003), other amino acids such as glutamate or lysine (in certain Actinobacteria) (Potekhina et al. 1993; Shashkov et al. 2006), or phosphocholine (*S. pneumoniae*) (Fischer 2000). The negative charge, which derives from the phosphate groups, is neutralized to a large extent by the incorporation of D-alanine (Neuhaus and Baddiley 2003).

A less well studied group of so-called "secondary cell wall polymers" are the anionic P-CWGs, which lack phosphate. This group includes the teichuronic acids (TUA), which are distinguished by the presence of uronic acid residues in their repeating units (e.g., in many bacilli and micrococci) (Soldo et al. 1999; Ward 1981) and pyruvylated polymers (e.g., in *Bacillus anthracis* and *B. cereus*) (Choudhury et al. 2006; Leoff et al. 2007; Schäffer and Messner 2005). When grown under phosphate limitation *B. subtilis* expresses TUA instead of WTA. The linkage unit of *B. subtilis* TUA is similar to that of the WTA. It consists of GlcNAc and glucuronic acid (Soldo et al. 1999). Recently the pyruvylated *B. anthracis* P-CWG has been characterized. It consists of repeating units formed by several hexoses (Choudhury et al. 2006). Many actinobacteria produce uncharged, often branched P-CWG, especially those bacteria with an outer membrane-like mycolic acid layer. For instance, *Mycobacterium tuberculosis* has a branched arabinogalactan polymer, which connects PG and the mycolic acid membrane via the covalently bound mycolic acid (Brennan 2003; Takayama et al. 2005).

5.2.2 *Lipoteichoic Acid and Other Membrane Glycolipid-Anchored Polymers*

Membrane glycolipid-anchored polymers such as lipoteichoic acids (LTA) are attached to the cytoplasmic membrane by linking the polymer to glycolipids. In *S. aureus* the lipid anchor consists of a diglycosylated diacylglycerol (Fischer 1988). In *S. aureus,* as in many other Gram-positive bacteria, the backbone is formed by Gro-P repeating units. As a likely consequence of the unique biosynthetic pathway, the structures of LTA are usually less diverse than those of WTA (see below) (Fischer 1994). In *Streptococcus pneumoniae* a very complex LTA structure can be found. Its repeating units contain phosphocholine and they are identical to those of pneumococcal WTA (Fischer et al. 1993). At the 2-hydroxy group of the glycerol most LTA polymers are substituted with D-alanine and additional sugars as in WTA.

In many actinobacteria, M-CWGs are produced without phosphate in the repeating units. Because of the substitution with succinyl groups such polymers are often anionic (Delmas et al. 1997; Greenberg et al. 1996; Powell et al. 1975). In many mycolic acid-producing bacteria uncharged, branched lipoarabinomannans (LAM) can be found (Briken et al. 2004; Sutcliffe 2005).

5.3 Biosynthesis of Wall Teichoic Acid and Lipoteichoic Acid

Wall teichoic acid and LTA usually rely on profoundly different biosynthetic pathways and precursor molecules (Fig. 5.3), although they exhibit structural similarity. It has been difficult to identify pathways and biosynthetic genes by comparative genomics, probably because of the enormous structural diversity of P-CWGs; this notion implies that most bacterial species require different sets of genes. In *B. subtilis 168* only 13 genes are required for the rather simple Gro-P WTA (Qian et al. 2006) whereas *S. agalactiae* is supposed to depend on more than 120 genes for the synthesis of the extremely complex WTA-like polymer (Sutcliffe et al. 2008). In any case, certain principles are widely conserved among P-CWG-producing bacteria, such as the use of a C_{55} lipid carrier during the assembly process, the enzymes mediating the first steps of linkage unit biosynthesis, and the allocation of biosynthetic genes in clusters. In *B. subtilis* and *S. aureus* most knowledge has been gathered as outlined in the section below (Bhavsar and Brown 2006; Xia and Peschel 2008).

5.3.1 Wall Teichoic Acid Biosythesis in Bacillus subtilis and Staphylococcus aureus

The involvement of nucleotide-activated precursor molecules such as CDP-glycerol, CDP-ribitol, and UDP-GlcNAc has been demonstrated using sophisticated in vitro reconstitution studies of certain WTA-biosynthetic steps (Baddiley 1989; Bracha et al. 1978; Brooks et al. 1971; Nathenson et al. 1966). Biosynthesis is initiated on the undecaprenyl pyrophosphate lipid carrier (C_{55}), which is also used for PG or capsular polysaccharide biosynthesis, at the inside of the cytoplasmatic membrane (Anderson et al. 1972). In *B. subtilis* 168 the first gene cluster involved in biosynthesis of WTA–*tagABCDEFGH*– has been identified by the laboratory of Dimitri Karamata by analysis of temperature-sensitive mutants (Pooley and Karamata 1994). In the last couple of years large-scale sequencing projects and comparative genomics have been used to identify further genes such as *tagO* and the more complex WTA gene clusters of *B. subtilis W23* and *S. aureus*, both of which produce ribitolphosphate (Rbo-P) WTA (Qian et al. 2006). As outlined below, biochemical studies with crude enzymatic preparations derived from temperature-sensitive mutant strains or with recombinant enzymes produced in *Escherichia coli* led to functional predictions for most of

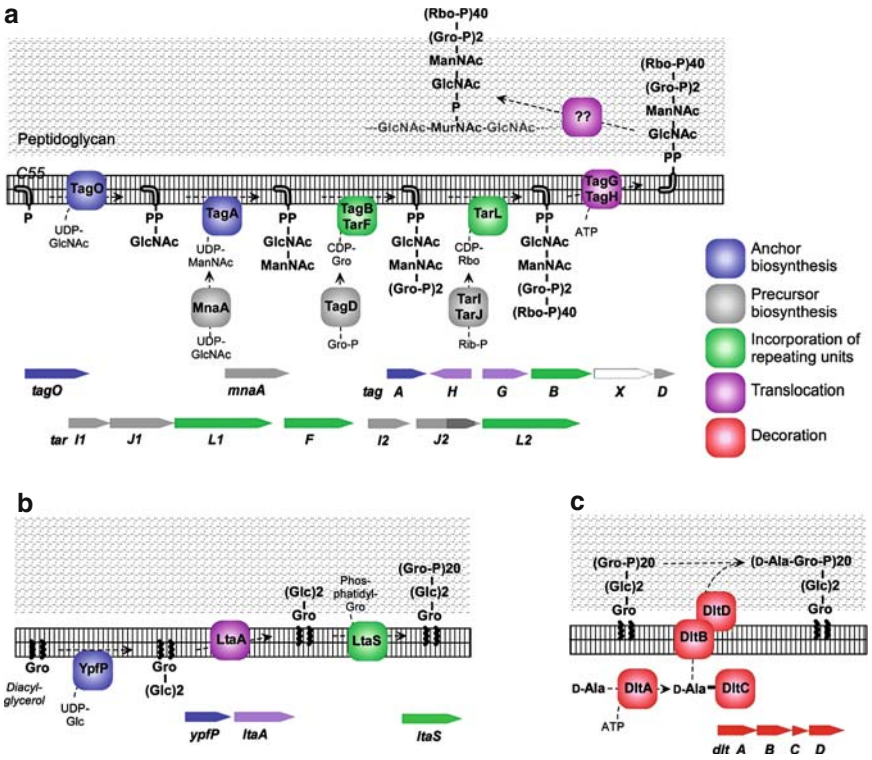

Fig. 5.3 Pathways of *S. aureus* wall teichoic acid (WTA) biosynthesis (**a**), lipoteichoic acid (LTA) biosynthesis (**b**), and D-alanine incorporation into LTA and WTA (**c**). CDP-Gro, cytidyldiphosphate-glycerol; CDP-Rbo, cytidyldiphosphate-ribitol; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Gro, glycerol; Gro-P, glycerolphosphate; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetyl muramic acid; Rbo-P, ribitol phosphate; Rib-P, ribulose-5-phosphate; UDP-Glc, undecaprenylphosphate-glucose; UDP-GlcNAc, uridine-5′-diphosphate-glucose; UDP-GlcNAc, uridine-5′-*N*-acetylglucosamine; UDP-ManNAc, uridine-5′-*N*-acetylmannosamine. With permission (Kohler et al. 2009b)

the WTA-biosynthetic gene products. Fig. 5.3 shows CWG-biosynthetic steps, which can be divided into four groups:

1. The synthesis of linkage units that usually have conserved structures and connect the polymers with PG or the membrane belong to the first group of WTA-biosynthetic enzymes (Araki and Ito 1989). Hence, an easy identification of CWG gene clusters is feasible since the genes involved, *tagO* and *tagA*, are well conserved among many Gram-positive bacteria (Ginsberg et al. 2006; Weidenmaier et al. 2004). TagO transfers GlcNAc phosphate to C_{55} phosphate (Soldo et al. 2002a) and TagA adds a ManNAc unit using UDP-ManNAc precursors (Ginsberg et al. 2006).
2. The generation of special nucleotide-activated precursor molecules such as UDP-ManNAc (ManA) (Soldo et al. 2002b), CDP-glycerol (TagD) (Park et al.

1993), or CDP-ribitol (TarI, TarJ) (Pereira and Brown 2004) is mediated by the second group of enzymes. Due to the interaction with the nucleotides, many of these genes share conserved domains.

3. Incorporation of the preformed repeating units into CWGs is mediated through the third group of enzymes, which includes both priming and polymerizing enzymes. The Gro-P WTA polymer of *B. subtilis 168* depends on the primase TagB adding the first repeating unit to the C_{55}-bound linkage unit and the polymerase TagF, which adds the additional Gro-P units (Ginsberg et al. 2006). In *S. aureus* Rbo-P WTA biosynthesis the situation is more complicated. Here the TagB reaction is followed by the addition of only one additional Gro-P unit mediated by the TarF enzyme (Brown et al. 2008). Subsequently, the TarL polymerase synthesizes the RboP polymer (Brown et al. 2008; Pereira et al. 2008). No Rbo-P primase seems to be involved in *S. aureus*, whereas in *B. subtilis W23* such an enzyme has been implicated in Rbo-P WTA biosynthesis (TarK) (Bhavsar and Brown 2006). The major challenge in studying enzyme functions is that three genes which are involved in Rbo-P generation and incorporation seem to be duplicated (Qian et al. 2006). Recent studies indicate that the same types of reaction are mediated by the two TarL enzymes, albeit leading to WTA of different chain length and electrophoretic migration. Apparently *S. aureus* can control WTA structure according to bacterial density and environmental changes, since one of the *tarL* genes (also been named *tarK*) is regulated by the *agr* quorum sensing system (Brown et al. 2008).
4. The fourth group consists of proteins which mediate the transfer of the WTA polymers to the outer membrane leaflet (TagG, TagH, forming an ABC transporter) (Lazarevic and Karamata 1995) and the transfer from C_{55} to the acceptor molecules (responsible proteins still unknown).
5. Decoration of WTA/LTA repeating units with sugars, D-alanine, choline, pyruvate or other residues is mediated by further proteins, which are allocated into group v. For WTA with more complex hexose-containing repeating units, such as the minor WTA of *B. subtilis* (Freymond et al. 2006) or the branched WTA-like polymer of *S. agalactiae* (Sutcliffe et al. 2008), different biosynthetic pathways have been proposed which only share *tagO* and (in many cases) *tagA* genes with those described above.

5.3.2 *Lipoteichoic Acid Biosynthesis in Staphylococcus aureus*

The Gro-P repeating units of LTA are derived from a major constituent of bacterial membranes, namely the phospholipid phosphatidylglycerol, but not from a nucleotide-activated precursor (Glaser and Lindsay 1974). Lipoteichoic acid is not polymerized on C_{55} but directly on the glycolipid serving as the membrane anchor for LTA, which is a second major difference between LTA and WTA biosynthesis (Fischer 1988; Koch et al. 1984). Different genes have been implicated in glycolipid biosynthesis of *S. aureus* and *S. agalactiae*, as the glycolipids differ between species

(Doran et al. 2005; Kiriukhin et al. 2001). Lipoteichoic acid is linked to diglycosyldiacylglycerol in *S. aureus*. This lipid is generated by the YpfP enzyme, which adds two glucose residues from UDP-glucose to diacylglycerol (Jorasch et al. 1998, 2000; Kiriukhin et al. 2001). A membrane protein encoded by the *ltaA* gene, which is necessary for efficient LTA biosynthesis, is thought to be a flippase that translocates the glycolipid from the inner to the outer leaflet of the cytoplasmic membrane (Grundling and Schneewind 2007b). Recently, the LTA polymerase LtaS has been discovered by Angelika Gründling and Olaf Schneewind. It utilizes Gro-P units from phosphatidylglycerol to synthesize the LTA polymer at the outer surface of the cytoplasmic membrane (Grundling and Schneewind 2007a). Lipoteichoic acid biosynthesis seems to be a rather conserved process since one or several *ltaS*-related genes are found in most LTA-producing bacteria. Amazingly, deletion of *ypfP* does not block biosynthesis of LTA but leads to synthesis of LTA whose polymer is attached to diacylglycerol (Fedtke et al. 2007; Kiriukhin et al. 2001). For unknown reasons *ypfP* mutants produce unaltered or strongly reduced amounts of the altered LTA compared to the wild-type strains depending on the *S. aureus* strain background (Fedtke et al. 2007). In conclusion, far fewer genes are required for LTA biosynthesis compared to WTA biosynthesis. Nevertheless, highly complex LTA polymers, which are most probably synthesized in a C_{55}-dependent fashion, are produced by bacteria such as *S. pneumoniae* (Draing et al. 2006; Fischer et al. 1993).

5.3.3 *Incorporation of D-Alanine into Wall Teichoic Acid and Lipoteichoic Acid*

A very constant trait of most TA polymers is the modification with D-alanine, whereas most other WTA and LTA components are variable. These substituents seem to be absent in TA molecules without polyol constituents in the repeating units such as the minor WTA of *B. subtilis* (Freymond et al. 2006). The *dltABCD* genes responsible for D-alanine activation and incorporation into WTA and LTA are highly conserved and always seem to form an operon (Neuhaus et al. 1996; Neuhaus and Baddiley 2003), which is in accord with the high prevalence of D-alanylation. Teichoic acid net charge is profoundly affected by D-alanine modification. Accordingly, disruption of the *dltABCD* genes has crucial consequences regarding resistance to antimicrobial peptides, adhesion to host cell receptors and biofilm formation (Weidenmaier and Peschel 2008). The *dlt* operons of *S. aureus* and *S. epidermidis* are controlled via the ApsXRS (also named GraXRS) regulatory system in response to antimicrobial peptide challenge and cell wall stress (Herbert et al. 2007; Kraus et al. 2008; Li et al. 2007). D-Alanine is incorporated after biosynthesis of the TA polymers is completed. D-Alanine can be repeatedly incorporated into a given molecule, since the D-alanine esters are rather labile and are easily lost (Koch et al. 1985). Four proteins (DltA, -B, -C, -D) are required for transfer of D-alanine into TAs. These four proteins form a pathway that includes

activation of D-alanine in the cytoplasm (DltA), linkage to a D-alanine carrier protein (DltC), translocation, and incorporation of D-alanine into TAs (DltB, DltD) (Neuhaus and Baddiley 2003). Hydrolysis of ATP is required for the first DltA-catalyzed step. DltA is homologous to the activating domains of peptide synthetases (Heaton and Neuhaus 1992; Neuhaus et al. 1996). Similarly to the biosynthesis of non-ribosomally synthesized peptides or fatty acids, D-alanine is activated and transferred to a dedicated carrier protein. All these pathways involve intermediates linked to the phosphopantetheine prosthetic groups of the carrier proteins by energy-rich thioester bonds. Transferring the D-alanine into TAs is the last step, which is less well understood. DltB, an integral membrane protein, and DltD, a membrane-tethered hydrophilic protein, seem to be required (Debabov et al. 2000). Thus Dlt proteins represent promising targets for blocking D-alanylation through inhibitory compounds. Recently, it has been reported that bacteria became more susceptible to cationic antimicrobial molecules such as defensins by specifically inhibiting DltA (May et al. 2005). Accordingly, such inhibitors seem to be very efficient in clearing bacterial infections in vivo (Escaich et al. 2007).

5.4 Roles of Wall Teichoic Acid and Lipoteichoic Acid in Bacterial Physiology

Very important roles of WTA and LTA-like polymers for bacterial integrity and fitness are suggested, since bacterial cells commit considerable amounts of energy and genetic information to their biosynthesis and because of the universal presence of CWGs in Gram-positive bacteria. Recently, more detailed characterization of CWG functions has become feasible through the availability of defined mutants lacking CWG (D'Elia et al. 2006; Weidenmaier et al. 2004) or exhibiting altered CWG structures (Doran et al. 2005; Kristian et al. 2005; Peschel et al. 1999). Wall teichoic acid has been shown to be dispensable for viability of *S. aureus* and *B. subtilis* (D'Elia et al. 2006; Weidenmaier et al. 2004). When grown under laboratory conditions these mutants displayed only minor defects. In contrast LTA is indispensable in *S. aureus* at normal temperature (Grundling and Schneewind 2007a) but seems to be dispensable at low temperatures below 30°C (Oku et al. 2008). Amazingly, in vitro growth behavior is not affected by a strongly reduced LTA content in the cell envelope (Fedtke et al. 2007). Accordingly, many of the functions assigned to CWGs appear not to be essential. Many of those functions are supposed to be critical only in certain instances such as exposure to environmental stresses or to host defense factors (Weidenmaier and Peschel 2008). In fact, many reports suggest critical functions of WTA and LTA in protecting the cell envelope from penetration by harmful molecules such as host defense molecules, bacteriocins and antibiotics. This protective function may be either direct by blocking pores and cavities between PG layers or indirect by attachment of outer protection layers such as S-layer proteins (e.g., *B. anthracis*) (Mesnage et al. 2000) or mycolic acids

(e.g., *M. tuberculosis*) (Brennan 2003). Mutants lacking D-alanine in WTA and LTA are more susceptible to cationic antimicrobial peptides (Peschel et al. 1999, 2000; Peschel and Sahl 2006). Moreover, *S. aureus* WTA contributes to lysozyme resistance as well as to resistance against antimicrobial fatty acids (Bera et al. 2007; Kohler et al. 2009a, b). On the other hand, WTA can also increase susceptibility for harmful molecules such as certain phages that use WTA as a receptor (Lopez et al. 1982; Park et al. 1974; Wendlinger et al. 1996) or the human antimicrobial defensin hBD3 and secretory group IIA phospholipase A2 (Koprivnjak et al. 2008).

Certain bacterial proteins are non-covalently anchored by CWG to the cell wall, e.g. in *S. pneumoiae* where many virulence factors are anchored to the phosphocholine residues of the CWG (Bergmann and Hammerschmidt 2006). Furthermore, *B. anthracis* and relatives attach their S-layer proteins to the bacterial surface by the use of pyruvylated CWG (Mesnage et al. 2000). Autolysins of *S. aureus* and other bacteria show a high affinity for WTA and LTA (Bierbaum and Sahl 1987; Giudicelli and Tomasz 1984) but it is proposed that autolysins bind to the cell wall independently of CWG, since *S. aureus* mutants with reduced LTA exhibited no reduced amounts of autolysins (Fedtke et al. 2007). Wall teichoic acid and LTA play a profound role in controlling autolysin activity by interactions that may involve CWG-bound bivalent cations; however, these interactions are only partially understood. It is assumed that LTA interacts with components of the membrane-bound cell division machinery and contributes to its proper placement or regulation, because depletion of LTA leads to bacterial cells with distorted shapes and division sites in *S. aureus* (Grundling and Schneewind 2007a).

Important roles in shaping the ionic milieu in the cell wall have been proposed for the zwitterionic and anionic CWG types that have ion-exchanger-like properties. In particular, TAs have high affinities for magnesium ions and are regarded as magnesium ion storage molecules (Heptinstall et al. 1970). Surface-exposed CWGs are highly hydrophilic, which has a strong influence on the physicochemical properties of bacterial cell surfaces. A strong impact on biofilm formation on biomaterials has been observed in *S. aureus* and *Enterococcus faecalis* mutants with altered CWGs. Furthermore these mutants are attenuated in virulence in animal models (Fabretti et al. 2006; Fedtke et al. 2007; Gross et al. 2001; Kristian et al. 2003). CWGs can play a second role in biofilm formation when they are released by the bacteria to form parts of the biofilm matrix, which protects bacterial cells and glues them together (Sadovskaya et al. 2004; Vinogradov et al. 2006). The precise mechanism by which CWGs are shed from the cell wall or membrane anchors is still unknown.

5.5 Lipoteichoic Acid and Interactions with Host Cell Receptors

Bacteria colonizing or infecting animal hosts (Fig. 5.4) appear to depend on CWGs, which play various crucial roles in microbe–host interactions. A number of recent studies indicate that WTA and LTA can shape the entire infection process of

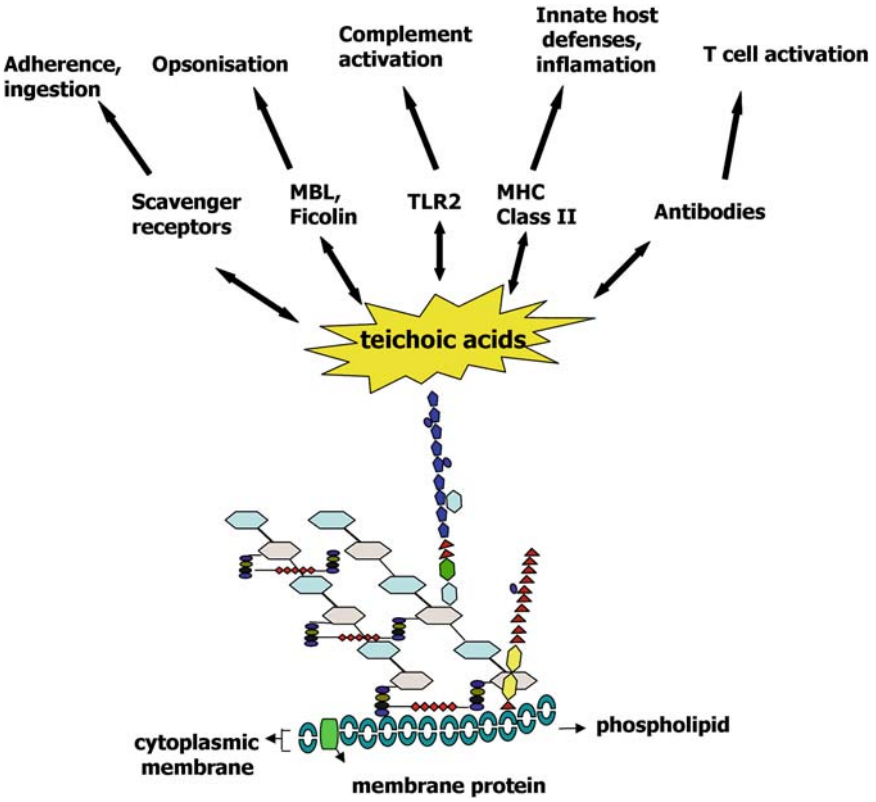

Fig. 5.4 Interaction of LTA and WTA with host molecules. Scavenger receptors, mannose-binding lectin, and ficolins interact with different CWGs, which leads to binding and internalization by host cells or complement activation. Some M-CWGs seem to elicit proinflammatory responses through TLR2. MBL, Mannose-binding lectin; MHC, major histocompatibility complex; TLR2, Toll-like receptor 2. With permission (Kohler et al. 2009b)

S. aureus from initial colonization to activation of innate immunity and to recognition by the adaptive immune system (Weidenmaier and Peschel 2008). Binding to epithelial and endothelial cells has been abrogated in *S. aureus* mutants lacking WTA. Furthermore, these mutants have lost the ability to colonize the nose in animal models (Weidenmaier et al. 2004, 2008) or to leave the bloodstream and infect subendothelial tissues in endovascular infections (Weidenmaier et al. 2005b). Similar impacts on bacterial host cell binding have been observed upon altering TA structure by disrupting the D-alanylation pathway in *S. aureus* (Weidenmaier et al. 2004, 2005a), *Streptococcus pyogenes* (Kristian et al. 2005) and *Listeria monocytogenes* (Abachin et al. 2002) or by altering LTA membrane anchoring in *S. agalactiae* (Abachin et al. 2002). There is evidence for direct binding of WTA to as yet unidentified receptors on epithelial and endothelial cells. Of interest, these interactions seem to contribute to *S. aureus* host cell attachment to a similar extent

as the staphylococcal adhesion proteins (Weidenmaier et al. 2008), which bind, e.g., keratin, fibronectin, or fibrinogen (Foster and Hook 1998; Mongodin et al. 2002; Navarre and Schneewind 1999). Wall teichoic acid-mediated binding can be inhibited by polyinosinic acid, an established inhibitor of scavenger receptors (SR), indicating that SR-like receptors play a major role in the WTA-mediated staphylococcal binding to host cells (Weidenmaier et al. 2008). Accordingly, some members of the SR family that have been identified on mammalian cells have been shown to bind purified CWG and intact *S. aureus* cells. SCARA5 and LOX1, which are expressed by airway epithelial and endothelial cells, respectively, are candidate receptors for WTA-mediated binding (Jiang et al. 2006; Shimaoka et al. 2001).

While SRs appear to mediate bacterial attachment, other host receptors have been shown to stimulate inflammatory processes upon CWG binding. Pathogen-Associated Molecular Pattern (PAMP) molecules such as LTA, mycobacterial LAM and other M-CWG activate the innate immune system via Toll-like receptor 2 (TLR2) (Hermann et al. 2002; Hoebe et al. 2005; Sugawara et al. 2003; Tapping and Tobias 2003). LTA-mediated TLR2 activation seems to require co-receptors such as TLR6, CD14, LBP, and CD36 (Chavakis et al. 2002; Han et al. 2003; Henneke et al. 2005; Hoebe et al. 2005). However, many of the commonly used M-CWG preparations have been shown to be contaminated with lipopeptides, which account for a large percentage of the proinflammatory activity (Hashimoto et al. 2006, 2007). Thus, the proinflammatory potency of M-CWG is still a matter of debate. Nevertheless, synthetic LTA analogs seem to stimulate TLR2 (Deininger et al. 2003). Lipoteichoic acid and LAM also have been shown to bind to soluble C-type lectins such as mannose-binding lectin (MBL), L-ficolin, and the lung surfactant proteins A and D (Ferguson et al. 1999; Lynch et al. 2004; Polotsky et al. 1996, 1997; Sidobre et al. 2000; van de Wetering et al. 2001). The lectin-initiated complement pathway is activated by MBL and L-ficolin upon binding to CWG on the bacterial surface, which leads to bacterial opsonization and release of chemotactic complement split products (Endo et al. 2007; Takahashi et al. 2007).

For many decades CWGs such as *S. aureus* TA have been well-known targets for antibodies (Kumar et al. 2005; Verbrugh et al. 1981; Verhoef et al. 1983). Accordingly, CWGs have been considered as vaccination targets. Indeed it has been shown that vaccination with enterococcal LTA induced the production of protective, opsonic antibodies (Theilacker et al. 2006). Furthermore, promising results were obtained by passive vaccination with a humanized monoclonal antibody targeting staphylococcal LTA (Weisman 2007). Traditionally glycopolymers have been regarded as T cell-independent antigens. There is increasing evidence that zwitterionic glycopolymers can lead to activation of T cells through processing of ingested CWG by antigen-presenting cells and subsequent presentation via MHC class II molecules (Kalka-Moll et al. 2002; Mazmanian and Kasper 2006). Most detailed studies of this new pathway are available for *Bacteroides fragilis* capsular polysaccharides. Recent studies have led to the suggestion that the equally zwitterionic *S. aureus* WTA might serve as a T cell-independent antigen (McLoughlin et al. 2006; Tzianabos et al. 2001). Although these studies are still in their infancy,

CWGs should increasingly be regarded as vaccine candidates with the capacity to elicit immunological memory, if this new concept can be confirmed. Mycobacterial LAM has been shown to stimulate T cells restricted to the MHC-like molecule CD1, which is known to present certain lipid antigens, which reveals another way how CWGs might stimulate specific T cells (Prigozy et al. 1997). It is tempting to speculate that M-CWGs represent more general substrates for CD1 presentation than previously thought.

5.6 Conclusions

After the discovery of TA and other CWGs in the 1960s, these polymers represented a field of active research. However, subsequently the chemical, biochemical, and genetic basis of CWG biosynthesis was studied by only a small group of scientists. Certain CWGs have only recently received increasing attention within the scientific community, since these polymers turned out to be of pivotal importance in microbe–host interaction. Moreover, CWG chemistry and biology has been put on the list of major scientific challenges, since there is a desperate need for new antimicrobial target structures. Recently promising studies have revealed the suitability of CWGs as targets for new antibiotics or vaccines (May et al. 2005; Mikusova et al. 1995; Theilacker et al. 2004). A broader view of the diversity and variability of CWG structures can be achieved in the near future now that improved glycochemical methods are available. The availability of genomic and metagenomic databases represents a valuable basis for predicting CWG biosynthetic pathways by bioinformatics methods. Predicted enzyme functions can be confirmed with the help of in vitro reconstitution of biosynthetic steps, which has recently yielded major scientific progress. Moreover, this progress will assist the use of the reactions in high-throughput screening programs in the search for new antibiotics. In *B. subtilis* the WTA biosynthetic enzymes seem to form a membrane-associated complex, which indicates that CWG biosynthesis is a highly organized process (Formstone et al. 2008). It can be assumed that the CWG and PG biosynthetic machineries are coordinated in sophisticated ways, maybe in cooperation with cytoskeletal elements and with the cell division apparatus.

Structural features of CWGs might be correlated with certain functions in cell wall physiology or host interaction with the help of the increasing availability of defined mutants. The proinflammatory capacity of M-CWGs and the potential of CWGs to activate specific T cells upon processing and presentation in MHC class II or CD1 molecules are the major open questions which need to be approached. Furthermore, many host receptors recognizing and binding CWGs remain to be identified. Because of cell- and species-specific differences in expression of CWG-binding molecules, it is tempting to speculate that the enormous diversity of CWG structures plays a role in bacterial cell and host tropism.

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