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Mini Review

Pathways and roles of wall teichoic acid glycosylation in *Staphylococcus aureus*

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ABSTRACT

The thick peptidoglycan layers of Gram-positive bacteria are connected to polyanionic glycopolymers called wall teichoic acids (WTA). Pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, or *Enterococcus faecalis* produce WTA with diverse, usually strain-specific structure. Extensive studies on *S. aureus* WTA mutants revealed important functions of WTA in cell division, growth, morphogenesis, resistance to antimicrobials, and interaction with host or phages. While most of the *S. aureus* WTA-biosynthetic genes have been identified it remained unclear for long how and why *S. aureus* glycosylates WTA with α - or β -linked N-acetylglucosamine (GlcNAc). Only recently the discovery of two WTA glycosyltransferases, TarM and TarS, yielded fundamental insights into the roles of *S. aureus* WTA glycosylation. Mutants lacking WTA GlcNAc are resistant towards most of the *S. aureus* phages and, surprisingly, TarS-mediated WTA β -O-GlcNAc modification is essential for β -lactam resistance in methicillin-resistant *S. aureus*. Notably, *S. aureus* WTA GlcNAc residues are major antigens and activate the complement system contributing to opsonophagocytosis. WTA glycosylation with a variety of sugars and corresponding glycosyltransferases were also identified in other Gram-positive bacteria, which paves the way for detailed investigations on the diverse roles of WTA modification with sugar residues.

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Introduction

The Gram-positive bacterium *Staphylococcus aureus* is one of the most successful opportunistic pathogens causing severe infections worldwide. The natural niche of *S. aureus* are the nares of healthy humans and *S. aureus* nasal carriage was shown to be a major risk factor for acquiring invasive infections (von Eiff et al., 2001).

Successful colonization of human nares and infection of tissues requires efficient mechanisms to counteract human defense systems and efficient attachment to human cells. One of the most important *S. aureus* nasal colonization factors is the cell wall-bound wall teichoic acid (WTA) (Weidenmaier et al., 2004), a cell surface polymer produced by most Gram-positive bacteria including major human pathogens such as *Streptococcus pneumoniae*, *Listeria monocytogenes* or *Enterococcus faecalis* (Denapajta et al., 2012; Fiedler, 1988; Geiss-Liebisch et al., 2012). Interestingly, WTA structures are highly diverse among Gram-positive bacteria and are often strain or species-specific (Neuhaus and Baddiley,

2003; Weidenmaier and Peschel, 2008). In most *S. aureus* clones including highly virulent strains such as USA300 and MW2 the WTA polymer consist of 11–40 ribitol-phosphate (RboP) repeating units substituted with D-alanine (D-ala) and N-acetylglucosamine (GlcNAc). The WTA polymer is covalently linked to the 6-OH group of N-acetylmuramic acid residues in peptidoglycan via a disaccharide consisting of GlcNAc-1-P and N-acetylmannosamine followed by two glycerol-phosphate (GroP) units (Weidenmaier and Peschel, 2008) (Fig. 1A). In addition to WTA *S. aureus* and most other Gram-positive bacteria produce membrane-bound lipoteichoic acids (LTA) whose backbone is composed of GroP repeating units (Reichmann and Grundling, 2011).

Major functional roles of *S. aureus* WTA were elucidated during the last couple of years using defined mutants with targeted deletion of WTA biosynthesis genes such as the *dltABCD* operon responsible for the D-alanylation of WTA (Peschel et al., 1999). D-ala residues on WTA (and LTA) contribute to resistance to cationic antimicrobial peptides such as defensins or cathelicidins (Collins et al., 2002; Peschel et al., 1999) and to glycopeptide antibiotics such as vancomycin or teicoplanin (Peschel et al., 2000). Moreover, deletion of *tagO* encoding the first enzyme of the WTA biosynthesis pathway revealed that WTA is dispensable for *S. aureus* viability under laboratory conditions (Weidenmaier et al., 2004) but affects several critical cellular processes including autolysis

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Fig. 1. *S. aureus* WTA biosynthesis and known WTA glycosylation functions. (A) WTA biosynthesis pathway in *S. aureus*. After anchor synthesis, priming, and polymerization TarM and TarS glycosylate the WTA polymer, which is subsequently exported across the cytoplasmic membrane (TagGH), alanylated (DltABCD), and covalently linked to the peptidoglycan via LcpA (sa1195), LcpB (sa0908) and LcpC (sa2103). (B) WTA biosynthesis gene clusters of *S. aureus*. WTA glycosyltransferases *tarM* and *tarS*, a putative glycosyltransferase *tagX* and the WTA D-alanylation gene cluster are indicated (coloured). The location of a transposase (SAB0902c) within the *tarM* gene cluster present in the bovine *S. aureus* isolate RF122 (*) and genes with unknown functions are indicated (?). (C) Known functions of WTA α- and β-glycosylation in *S. aureus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Vergara-Irigaray et al., 2008), cell division (Brown et al., 2012; Campbell et al., 2011), localization of penicillin binding protein 4 (PBP4) (Atilano et al., 2010), and survival at high temperatures (Vergara-Irigaray et al., 2008). Remarkably, *S. aureus* WTA contributes to biofilm formation (Vergara-Irigaray et al., 2008), adhesion to epithelial (Weidenmaier et al., 2004) and endothelial cells, and is important for severe invasive infections such as endocarditis (Weidenmaier et al., 2005). *S. aureus* WTA elicits robust antibody responses in humans and activates the human complement system via both the classical and the mannose-binding lectin pathway leading to opsonophagocytosis (Jung et al., 2012; Kurokawa et al., 2013; Park et al., 2010). How variation of WTA structure may affect the interaction of *S. aureus* with the innate and adaptive immune systems remains to be investigated.

During the last 60 years *S. aureus* increasingly acquired resistance genes to counteract extensive antibiotic treatment (Chambers and Deleo, 2009). Of note, WTAs are exposed at the *S. aureus* cell surface and they are required for PBP2a-mediated β-lactam antibiotic resistance (Campbell et al., 2011; Farha et al., 2013; Maki et al., 1994). Moreover, *S. aureus* mutants lacking WTA are susceptible to antimicrobial fatty acids from human skin (Kohler et al., 2009) and WTA contributes to *S. aureus* lysozyme resistance (Bera et al., 2007) suggesting a general protective role of WTA against antimicrobials. Because of its importance *in vivo* and in resistance to β-lactam antibiotics *S. aureus* WTA represents a very attractive target for the development of new antibiotics. Recently several promising novel compounds have been developed

that inhibit TagO or the WTA transporter TagGH thereby rendering methicillin-resistant *S. aureus* (MRSA) susceptible to β-lactams (Campbell et al., 2011, 2012; Farha et al., 2013; Suzuki et al., 2011; Wang et al., 2013). Thus, WTA inhibitors could become useful for blocking *S. aureus* adhesion to host cells or for sensitizing MRSA to β-lactams.

Interaction with the human host during colonization or infection or the role of WTA in resistance towards antimicrobials are probably important reasons why *S. aureus* is synthesizing such a polymer. From an evolutionary point of view WTA seems to have a crucial function because WTA serve as adsorption receptor for many staphylococcal phages (Chatterjee, 1969; Park et al., 1974) that are largely responsible for horizontal gene transfer in *Staphylococcus*.

Although most of the *S. aureus* enzymes required for WTA biosynthesis have been elucidated and many functions have been attributed to WTA in the past years, it remained a mystery for decades how does *S. aureus* glycosylate WTA. Of note, early studies revealed strain-specific anomeric configurations (α- and β) of the WTA GlcNAc substitutions (Endl et al., 1984) suggesting that *S. aureus* may encode two different WTA glycosyltransferases.

In silico analysis of WTA glycosyltransferases in *S. aureus* genomes

Most of the genes and enzymes involved in the initiation, (*tagOA*, *mnaA*), priming (*tagDB*), polymerization (*tarI/J/L/F*), transport

(*tagGH*), and D-alanylation (*dltABCD*) of *S. aureus* WTA were identified and studied in genetic and/or *in vitro* reconstitution approaches (Swoboda et al., 2010; Xia et al., 2010a). Recently the ligation of WTA from lipid-linked WTA precursors to peptidoglycan was proposed to be accomplished by a new family of enzymes named LCP proteins (Chan et al., 2013; Dengler et al., 2012; Kawai et al., 2011) (Fig. 1A and B). However, it remained unknown until recently, which genes encode the WTA glycosyltransferases in *S. aureus* (Fig. 1B).

S. aureus NCTC8325-4 encodes ca. 20 putative glycosyltransferases and interestingly, two putative glycosyltransferases are co-localized within well studied WTA biosynthesis gene clusters namely *tarS* and *tagX* (Brown et al., 2012; Swoboda et al., 2010; Xia et al., 2010a) (Fig. 1B). In contrast, the WTA glycosyltransferase *tarM* (Xia et al., 2010b) does not co-localize with WTA gene clusters but is adjacent to genes encoding putative ABC transporters and proteins of unknown function (Fig. 1B). Remarkably *TarM* does not exist in every *S. aureus* genome and seem to be strain specific (see below).

The two unrelated enzymes *TarM* and *TarS* glycosylate WTA of *S. aureus*

Evidence for WTA glycosylation by β -GlcNAc in *S. aureus* dates back to the 1960s and 1970s. WTA β -GlcNAc transferase activity was demonstrated in strain Copenhagen (Nathenson and Strominger, 1963) while a phage-resistant mutant 52B2 was found to lack any detectable WTA GlcNAc transferase activity (Shaw et al., 1970). By screening a transposon mutant library derived from *S. aureus* strain RN4220, a mutant that was resistant to serogroup B phages was isolated and named K6. Sequencing analysis revealed that the transposon had disrupted a gene (SACOL1042), which encodes a putative glycosyltransferase. The gene product was named *TarM*. Based on the facts that mutant K6 that bears a transposon insertion in *tarM* lacked GlcNAc on WTAs and *tarM* complemented K6 mutant synthesizes α -O-GlcNAc on WTA, it was concluded that *TarM* is a WTA α -O-GlcNAc transferase (Xia et al., 2010b). Surprisingly, unlike transposon mutant K6, a targeted *tarM* deletion mutant (*S. aureus* RN4220 Δ *tarM*) was still phage-susceptible and nuclear magnetic resonance (NMR) elucidation revealed the presence of β -O-GlcNAc substituted WTA in this strain further suggesting *S. aureus* encodes a second WTA GlcNAc transferase, potentially *tagX* or *tarS* (Brown et al., 2012). However, combined deletion of *tarM* and *tagX* did not alter phage susceptibility (Xia et al., unpublished data) and no *in vitro* WTA glycosyltransferase activity was found for *TagX* (Brown et al., 2012). When the other putative β -glycosyltransferase gene *tarS* of mutant K6 was sequenced it was found to be truncated because of a point mutation. Moreover, *TarS* glycosylated RboP WTA polymers *in vitro* and deletion of both *tarM* and *tarS* resulted in complete absence of GlcNAc strongly suggesting *tarS* encodes the second *S. aureus* WTA glycosyltransferase (Brown et al., 2012).

Thus, *TarM* and *TarS* represent the RboP WTA α - and β -O-GlcNAc transferases, respectively, of *S. aureus* (Fig. 1A and B).

Functional roles of WTA glycosylation in *S. aureus*

While the discovery of *TarM* and *TarS* clarified the molecular basis of WTA glycosylation several questions remained unanswered: (i) Why is *S. aureus* glycosylating its WTA, (ii) are there different functions for α - or β -O-GlcNAc residues linked to WTA, and (iii) how is WTA glycosylation regulated? Several early studies already suggested that WTA GlcNAc may serve as an adsorption receptor for serogroup B phages such as Φ 52A (Chatterjee, 1969; Park et al., 1974). However, *S. aureus* mutants lacking either α - or

β -O-GlcNAc remained susceptible to most of the *S. aureus* phages while only the complete loss of WTA glycosylation resulted in phage resistance (Brown et al., 2012; Xia et al., 2010b, 2011; Xia and Wolz, 2013) (Table 1 and Fig. 1C). Thus, serogroup B phages require WTA glycosylation for efficient adsorption and infection but do not distinguish between different anomeric configurations of the GlcNAc residues on WTA. Similar observations were made for horizontal transfer of *S. aureus* pathogenicity islands (SaPIs) by SaPI particles formed by helper phage proteins indicating that SaPI particles adopt the receptor specificities of helper phages and do not distinguish between different WTA GlcNAc configurations (Winstel et al., 2013). Remarkably, similar WTA structures enable horizontal gene transfer among Gram-positive pathogenic bacteria, even across long phylogenetic distances (Winstel et al., 2013) (Table 1 and Fig. 1C).

MRSA mutants lacking WTA were found to be highly susceptible to β -lactam antibiotics, such as methicillin (Campbell et al., 2011; Farha et al., 2013). In contrast to MRSA mutants lacking either the D-alanine or α -O-GlcNAc substitutions on WTA, which were still resistant to most β -lactam antibiotics the lack of β -O-GlcNAc residues rendered MRSA susceptible to β -lactams (Brown et al., 2012) (Table 1 and Fig. 1C). The reasons for the essential role of *TarS* in β -lactam resistance remain elusive. The WTA β -O-GlcNAc may serve as a scaffold for the cell wall synthesis complex or cell wall-associated proteins that directly or indirectly modulate PBP2a activity (Brown et al., 2012). Along this line there is evidence that PBP2a and PBP4 interact with WTA (Atilano et al., 2010; Qamar and Golemi-Kotra, 2012). However, it is also possible that WTA glycosylation affects the physicochemical properties of the peptidoglycan matrix by influencing the cell wall milieu (Sutcliffe, 2012).

Mutants lacking β -O-GlcNAc were also more susceptible to lysostaphin, which cleaves the pentaglycine crossbridges of *S. aureus* peptidoglycan, than the parental strains (Brown et al., 2012) suggesting that WTA β -O-GlcNAc interferes with binding of lysostaphin to peptidoglycan (Fig. 1C). In contrast, several other critical processes that depend on WTA such as autolysis, biofilm formation, and cell division were not affected in mutants lacking WTA glycosylation (Brown et al., 2012) (Fig. 1C). Interestingly, there are first indications that *S. aureus* can modulate the relative amounts of α - and β -glycosylation depending on environmental conditions. *tarS* but not *tarM* expression levels were strongly upregulated by oxacillin treatment, which underscores the critical role of *TarS* in β -lactam resistance (Brown et al., 2012).

Human sera contain high levels of antibodies directed against the β -GlcNAc residues of *S. aureus* WTA indicating that these modifications can be dominant antigens in staphylococcal infections (Kurokawa et al., 2013). Moreover, human MBL was found to bind to glycosylated WTA and activated the lectin pathway of complement (Kurokawa et al., 2013) (Table 1 and Fig. 1C).

It remains unclear, which *S. aureus* regulation system controls the WTA glycosylation pattern and if there are further conditions by which *tarM* or *tarS* expression is affected. Thus, WTA glycosylation plays crucial roles in the susceptibility of *S. aureus* to phages, β -lactam antibiotics, lysostaphin and interaction with the human immune system.

tarM is absent in several *S. aureus* clonal complexes

While *tarS* was found in nearly all so far sequenced *S. aureus* genomes *tarM* is absent in several strains (Table 2). In particular *tarM* seems to be an ancient feature of *S. aureus* because the very early branching *S. aureus* clonal complex (CC) 75, sequence type (ST) 1850 strain MSHR1132 bears both, *tarS* and *tarM* in its genome (Holt et al., 2011) (Table 2). Interestingly, *tarM* is absent in several healthcare-associated MRSA strains such as N315, Mu50, Mu3, JH1

Table 1
WTA structures and known WTA glycosylation functions in various Gram-positive bacteria.

Species/strain	WTA type ^{a,b} (glycosylation)	Phenotype of WTA glycosylation-deficient mutants (reference)
<i>S. aureus</i> RN4220	RboP (α, β-GlcNAc)	Phage resistance; increased lysostaphin and β-lactam susceptibility in MRSA (Brown et al., 2012; Xia et al., 2010b, 2011); decreased SaPI particle susceptibility (Winstel et al., 2013); no MBL mediated complement activation, no anti-WTA IgG binding, no C3 and C4 deposition (Kurokawa et al., 2013)
<i>S. epidermidis</i> ATCC14990	GroP (α-Glc, α-GlcNAc)	No mutants described
<i>B. subtilis</i> 168	GroP (α-Glc)	Phage resistance (Allison et al., 2011; Young, 1967)
<i>B. subtilis</i> W23	RboP (β-Glc)	unknown
<i>L. monocytogenes</i> Serotype 1/2a	RboP (α-GlcNAc, α-Rha)	No binding of CDBP35 ^d (Eugster et al., 2011), phage resistance (Tran et al., 1999)
<i>L. monocytogenes</i> serotype 4b	RboP (Glc, Gal)	Phage resistance (Cheng et al., 2008); attenuated virulence in gastrointestinal listeriosis (mice) (Faith et al., 2009)
<i>L. grayi</i> ATCC27840	RboP (GlcNAc)	No mutants described
<i>E. faecalis</i> V583 ^c	GalNAc-RboP (α-Rha) (WTA 1); Glc-GalNAc-RboP (α-Glc) (WTA 2)	No mutants described
<i>E. faecium</i> U0317	GalNAc-GroP (absent)	No WTA glycosylation
<i>S. pneumoniae</i>	AATGal-Glc-RboP-GalNAc-GalNAc (GalNAc ^e & P-Cho)	No mutants described; phosphocholine moieties contribute to virulence (Gehre et al., 2009; Kharat and Tomasz, 2006)

^a WTA repeating units (bold) and WTA glycosylation () are indicated.
^b Ribitol-phosphate (RboP), glycerol-phosphate (GroP), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), glucose (Glc), Rhamnose (Rha), Galactose (Gal), 2-acetamido-4-amino-2,4,6-trideoxygalactose (AATGal) and phosphorylcholine (P-Cho) are indicated.
^c Strain V583 synthesizes two different WTAs.
^d CDBP35, cell wall binding domain of *Listeria* phage endolysin PlyP35.
^e A recent study by Gisch et al. (2013) suggest no GalNAc modification on LTA and potentially on WTA because of identical repeating units of LTA and WTA.

(all CC5) or certain ST398 strains (Table 2). As indicated in Table 2 there was no correlation between the presence or absence of *tarM* and the MRSA status but the absence of *tarM* seems to be a conserved feature of certain STs or even of several closely related STs.

In addition, there was no correlation of *tarM* with host specificity, e.g. human or bovine origin (e.g. USA300 or RF122, respectively; both bear *tarM*) (Table 2). It can be assumed that the loss of *tarM* happened repeatedly but only rarely during *S. aureus* evolution.

Table 2
Presence of the *tarM* and *tarS* genes in available *S. aureus* genomes.

<i>S. aureus</i> strain ^a	<i>tarS</i> ^b	<i>tarM</i> ^{b,c}	MRSA status	Sequence type	Clonal complex
MW2*	+	+	MRSA	1	1
MSSA476*	+	+	MSSA	1	1
TCH70	+	+	MRSA	1	1
N315*	+	–	MRSA	5	5
Mu50*	+	–	MRSA	5	5
Mu3*	+	–	MRSA	5	5
A5937	+	–	MRSA	5	5
A6224	+	–	MRSA	5	5
JH1*	+	–	MRSA	105	5
04-02981	+	~	MRSA	225	5
USA300*	+	+	MRSA	8	8
NCTC8325*	+	+	MSSA	8	8
RN4220	+	+	MSSA	8	8
Newman*	+	+	MSSA	8	8
TW20*	+	+	MRSA	239	8(239)
COL*	+	+	MRSA	250	8
H0-5096-0412*	+	~	MRSA	22	22
TCH60	+	+	MRSA	30	30
MN8	+	+	MSSA	30	30
C101	+	+	MSSA	30	30
BTN1260	+	+	MSSA	30	30
MRSA252*	+	+	MRSA	36	30
A9635	+	–	MSSA	45	45
MSHR1132	+	+	MSSA	1850	75
RF122*	+	+	MSSA	151	151
PS187	–	–	MSSA	395	395
S0385*	+	~	MRSA	398	398
71193	+	~	MSSA	398	398
DR10	+	–	MSSA	398	398

^a Only fully annotated genomes are indicated (*).
^b Presence (+) or absence (–) of *tarS* or *tarM* genes are indicated.
^c Truncated *tarM* variant is indicated (~).

Currently it is mysterious where *tarM* came from during *S. aureus* evolution and if *tarM* is mobile. *tarM* may have been acquired via horizontal gene transfer since *tarM* is flanked by a transposase possibly contributing to mobility e.g. in the bovine *S. aureus* isolate RF122 (Table 2 and Fig. 1B). In addition, it remains unclear if there is any advantage of bearing or losing *tarM* and if α -glycosylated WTA is beneficial for certain *S. aureus* lineages during colonization or infection or for susceptibility to certain phages.

Role of WTA glycosylation in other Gram-positive bacteria

Most of the Gram-positive bacteria have WTAs of highly variable composition and structure at their cell surfaces. Even among staphylococcal species WTA structures are quite diverse (Endl et al., 1984). While *S. aureus* WTA is composed of RboP units most of the coagulase-negative staphylococci (CoNS) produce GroP WTAs or WTA polymers with complex composition containing e.g. hexoses in the WTA backbones (Endl et al., 1984). Most WTAs of CoNS are glycosylated with sugars other than GlcNAc that is found in most *S. aureus* (Endl et al., 1984) (Table 1). Thus, bearing *tarM* and *tarS* and especially β -O-GlcNAc WTA glycosylation seems to be a specific feature of *S. aureus* raising the question why CoNS use different glycosylation patterns and which genes are involved. Of note early studies revealed that certain *S. aureus* produce WTA structures other than the common RboP polymer, e.g. *S. aureus* strains 187 and 406, which were found to produce GroP WTA substituted with α -O-linked N-acetylgalactosamine (GalNAc) (Endl et al., 1984). Recently such a GroP type WTA structure was verified in *S. aureus* strain PS187 by using NMR spectroscopy (Winstel et al., 2013). It seems that those *S. aureus* strains with GroP WTA may be more related to CoNS in terms of cell envelope structure than to classical *S. aureus* strains.

WTAs produced by other Gram-positive bacteria are in some instances similar to *S. aureus* WTA but most were shown to be highly diverse (Neuhaus and Baddiley, 2003; Weidenmaier and Peschel, 2008). Extensive investigations elucidated WTA structure, function, and biosynthesis in *Bacillus subtilis*. *B. subtilis* strain 168 produces GroP WTA substituted with α -linked glucose (Glc) while strain W23 produced RboP WTA modified with β -Glc (Brown et al., 2012; Neuhaus and Baddiley, 2003; Swoboda et al., 2010) (Table 1). Decades ago it was shown that glucosylated WTA were required for phage adsorption in *B. subtilis* 168 (Young, 1967) (Table 1) and the TagE protein (also called GtaA and RodD) of *B. subtilis* 168 was proposed as the WTA glucosyltransferase based on the indirect genetic evidence (Honeyman and Stewart, 1989; Mauel et al., 1989). Recently, TagE was characterized and demonstrated to be a GroP WTA α -glucosyl transferase (Allison et al., 2011). Thus, WTA glycosylation in *B. subtilis* and *S. aureus* share similar functions such as phage adsorption and infection. In addition TarQ was recently found to act as a β -O-glucosyl transferase in *B. subtilis* strain W23 producing RboP WTA substituted with β -Glc (Brown et al., 2012).

Human pathogens from the genus *Listeria* including certain serotypes of *L. monocytogenes* or *L. grayi* produce WTA with RboP repeating units identical to those found in *S. aureus* (Fiedler, 1988). Often their WTA glycosylation pattern is also shared with *S. aureus* but additional sugars such as rhamnose or Glc were described (Fiedler, 1988) (Table 1). Studies on WTA glycosylation in *Listeria* revealed functions related to those of *S. aureus*. WTA glycosyl residues of *L. monocytogenes* serve as adsorption receptors for different *Listeria* phages (Cheng et al., 2008; Tran et al., 1999) and can contribute to virulence, e.g. to gastrointestinal listeriosis in mice (Faith et al., 2009) (Table 1). In agreement with these findings the cell wall binding domain (CBD) of *Listeria* phage endolysin PlyP35 was found to recognize the GlcNAc residues on WTA (Eugster et al., 2011) (Table 1). However, this property does not seem to be a common feature of all *Listeria* phage endolysins (Eugster and

Loessner, 2012). WTA was also shown to restrict the access of certain phage endolysin CBDs to the peptidoglycan of *L. monocytogenes* (Eugster and Loessner, 2012). In contrast *Enterococcus* spp. WTAs have a very complex structure, which is distinct from that of *S. aureus* or *Listeria*. Of note, *E. faecalis* or *E. faecium* produce different WTAs with hexose-containing repeating units (GalNAc-RboP glycosylated with α -linked rhamnose or Glc-GalNAc-RboP glycosylated with α -linked Glc, both WTAs found in *E. faecalis* V583 whereas non-substituted GalNAc-GroP, type WTA was found in *E. faecium* U0317) (Bychowska et al., 2011; Geiss-Liebisch et al., 2012) (Table 1). Although some functions of *Enterococcus* WTAs including evasion from the lectin pathways of the human complement system have recently been described (Geiss-Liebisch et al., 2012) it still has to be elucidated how and why enterococci glycosylate their WTA. Similar to enterococci streptococci such as *Streptococcus pneumoniae* synthesize very complex polymers with identical repeating units in WTA and LTA, which are glycosylated with GalNAc (Denapate et al., 2012) (Table 1) although recent studies on *S. pneumoniae* LTA suggest no any glycosylation, possibly its WTA was not glycosylated either (Gisch et al., 2013). Additionally *S. pneumoniae* teichoic acids bear unusual phosphocholine residues that play crucial roles in *S. pneumoniae* virulence (Gehre et al., 2009; Kharat and Tomasz, 2006). Recent studies on *Lactobacillus plantarum* WTA revealed strain-specific WTA types, including both GroP and RboP WTA polymers substituted with diverse α -D-glucosyl residues (Tomita et al., 2012). Interestingly, *L. plantarum* seems to be capable of WTA-switching between WTA types with different repeating units ('alditol switching') (Bron et al., 2012; Tomita et al., 2013). Thus, although most Gram-positive bacteria produce WTA, structure and glycosylation patterns of the polymers are highly diverse. Further investigation on the diversity of WTA and characterization of mutants with altered WTA will disclose more functions of WTA glycosylation.

Perspectives

While the *S. aureus* WTA biosynthesis pathway including the WTA glycosylation process is largely known now (Brown et al., 2012; Xia et al., 2010b) it remains mysterious why *S. aureus* glycosylates its WTA with GlcNAc in two different anomeric configurations. So far only a few functional roles of *S. aureus* WTA glycosylation were identified and the elucidation of staphylococcal WTA glycosylation paves the way for dissecting the protein-carbohydrate interactions in *Staphylococcus*-host interaction. Especially the function of α -O-GlcNAc-modified WTA and the significance of strain-dependent presence or absence of *tarM* remains unclear. Thus, the elucidation of mechanisms regulating *S. aureus* WTA glycosylation especially under *in vivo* conditions will help to understand why two different enzymatic pathways exist and how they may contribute to fitness or virulence. Moreover, exploring if and which WTA glycosyl residues are expressed *in vivo* will be important for vaccination approaches with WTA-specific opsonic antibodies.

During the last few years *S. aureus* WTA biosynthetic enzymes became promising targets for novel antibiotics because MRSA strains become highly susceptible to β -lactam antibiotics when lacking WTA or WTA β -O-GlcNAc (Brown et al., 2012; Campbell et al., 2011; Farha et al., 2013). Crystal structures of WTA biosynthesis proteins as reported recently for *S. epidermidis* TagF (Lovering et al., 2010), *S. aureus* TagD (Fong et al., 2006), or *B. cereus* DltA (Osman et al., 2009) will help in the rational design of new antimicrobial drug.

It is still mysterious why the various Gram-positive bacteria are using completely different WTA biosynthesis pathways and why so many different WTA structures exist. Amazingly a common feature of various phages infecting distantly related Gram-positive bacteria

is their requirement for WTA as adsorption receptor. Since phages are probably crucial vehicles for horizontal gene transfer of virulence and resistance genes the elucidation of the underlying 'WTA 'glycocode' will enable to predict the likeliness of future phage-mediated horizontal gene transfer events, and to better understand the evolution of Gram-positive bacteria.

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