Toward the Pathway of *S. aureus* WTA Biosynthesis

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Wall teichoic acid (WTA) contributes profoundly to the virulence of *Staphylococcus aureus*. The successful in vitro reconstitution of poly-ribitolphosphate WTA biosynthesis using recombinant enzymes sheds new light on WTA enzymology and paves the way for developing new antibiotics that target WTA biosynthesis, as discussed in Brown et al. in a recent issue of *Chemistry & Biology*.

In contrast to Gram-negative bacteria, which have an outer membrane, Gram-positive bacteria are less well protected from small harmful molecules that can easily penetrate trough the peptidoglycan cell wall. This seems to be a major reason for incorporation of additional cell wall glycopolymers (CWG) into the envelopes of most Gram-positive bacterial species (Weidenmaier and Peschel, 2008). Because of their surface-exposed nature, CWG represent important targets for anti-infective vaccines, antibiotics, and diagnostics. The best-studied CWG are probably the teichoic acids, which are distinguished from other types of CWG by the presence of phosphate in the polymer backbone. The peptidoglycan-attached teichoic acids (WTA) are highly variable in structure and occur as rather simple glycerolphosphate (GroP) polymers, e.g., in *Bacillus subtilis* 168, or with rather complex combinations of building blocks (Weidenmaier and Peschel, 2008). WTA composed of ribitolphosphate (RboP) in addition to GroP building blocks is produced by important human pathogens such as *Staphylococcus aureus, Staphylococcus saprophyticus,* and *Listeria monocytogenes*. WTA has been shown to be dispensable for viability of *S. aureus* but to contribute profoundly to *S. aureus* host-cell binding, immune evasion, and virulence (Weidenmaier et al., 2004; Peschel et al., 1999). Therefore, WTA biosynthesis represents an interesting target for new antibiotics against notoriously antibiotic resistant bacteria such as *S. aureus*.

WTA is covalently liked to peptidoglycan through a linkage unit that usually consists of a GlcNAc-ManNAc disaccharide. The *S. aureus* WTA polymer contains 2 or 3 units of GroP followed by ~40 units of RboP, which are further modified with GlcNAc and D-alanine residue (Weidenmaier and Peschel, 2008) (Figure 1A). In vitro WTA polymerase studies from the 1960s demonstrated the involvement of CDP-glycerol and CDP-ribitol precursors in WTA polymerization. Subsequently, several WTA biosynthetic genes were identified in *B. subtilis* 168 and shown to be clustered (*tagABDEFGH)* (Pooley and Karamata, 1994) or encoded separately (*tagO*) (Soldo et al., 2002). TagO was suggested to initiate WTA biosynthesis by transferring GlcNAc–phosphate to the lipid carrier undecaprenylphosphate. Using recombinant *B. subtilis* enzymes, the functions of TagA in ManNAc incorporation into the linkage unit, TagD in CDP-glycerol generation, TagB in addition to the first GroP, and TagF in GroP polymerization could be established (Ginsberg et al., 2006; Park et al., 1993; Bhavsar and Brown, 2006). Comparative genomics revealed a similar organization of several WTA biosynthetic genes in *S. aureus* (Qian et al., 2006; Figure 1A) and enabled the generation of a *S. aureus tagO* mutant, which was devoid of WTA, thereby demonstrating that WTA is dispensable for viability but required for *S. aureus* colonization and infection (Weidenmaier et al., 2004). However, the rather complex composition of *S. aureus* WTA was reflected by the presence of an additional gene cluster (*tarFIJL*), which had previously also been identified in a *B. subtilis* strain (W23) producing poly-RboP WTA. While TarIJ could be shown to be responsible for CDP-ribitol generation (Pereira and Brown, 2004), it remained unclear which genes were responsible for RboP polymerization. This question was further complicated by the obvious duplication of the *tarIJL* genes (Figure 1A). Corresponding biochemical studies proved to be very challenging because of the lipophilic nature of the lipid carrier-bound substrates. One of the major advancements in the field was the recent chemoenzymatic synthesis of a lipid carrier analog with a farnesyl chain, which was less hydrophobic than the C_{55} undecaprenyl group. This enabled the in vitro reconstitution of early steps in *B. subtilis* WTA biosynthesis (Ginsberg et al., 2006).

In the January 2008 issue of *Chemistry & Biology*, Brown et al. describe the in vitro reconstitution of four main intracellular steps of *S. aureus* WTA biosynthesis downstream of TagO using purified recombinant enzymes (Figure 1B; Brown et al., 2008). Their data confirm that TagA and TagB mediate the same steps in *S. aureus* WTA biosynthesis as in *B. subtilis* 168. Most importantly, they demonstrate that TarF is responsible for adding a second GroP to the linkage unit and that TarL2 represents the RboP polymerase. Their data further demonstrate that *S. aureus* WTA contains only two GroP units instead of three, as previously reported, and that no additional RboP primase is required. Another important aspect of the study is the finding that only one of the two *tarL* genes (*tarL2*) of the duplicated *tarIJL* gene cluster is active, while the other one is either not expressed or defective. This result is in accord with the previously observed dispensability of *tarL1* (Bae et al., 2004).

Most of the enzymes involved in the early steps of WTA biosynthesis are conserved and organized in similar gene clusters in many Gram-positive genomes. Accordingly, they have been given the same names (*tagO, tagAHGBXD*) in many publications and in genome annotations. In some recent publications, among them

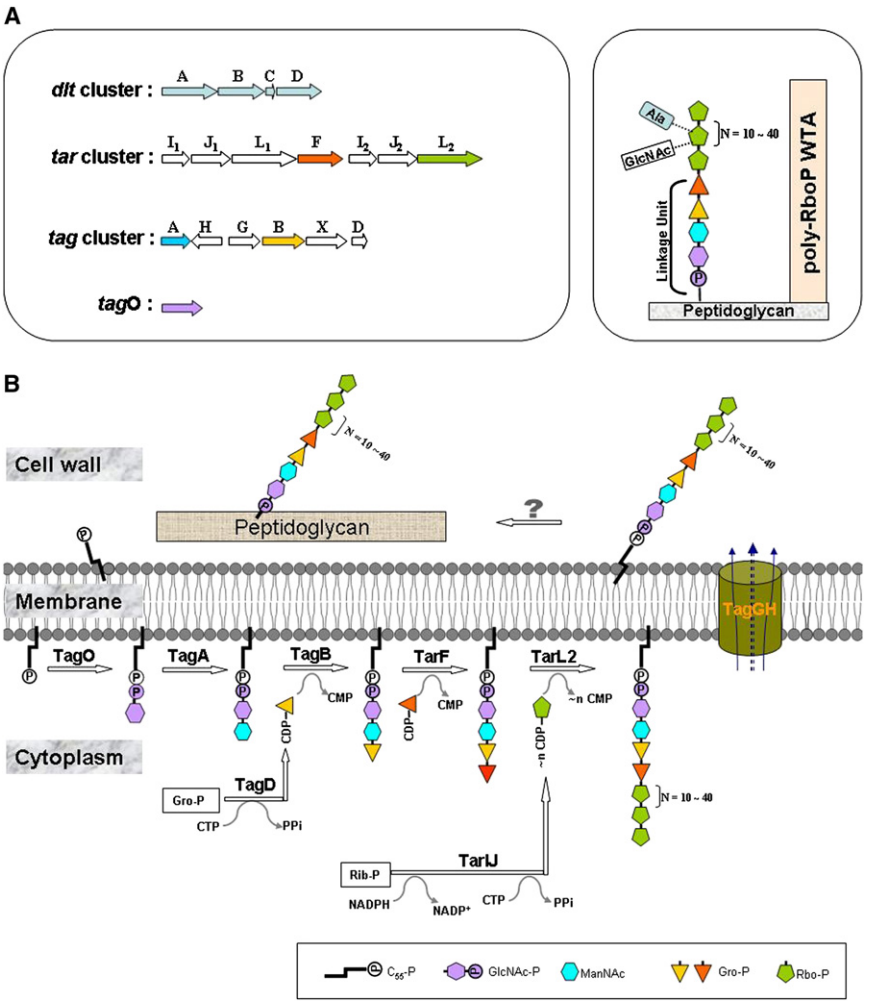

Figure 1. Genes and Enzymes Involved in *S. aureus* WTA Biosynthesis

(A) WTA gene clusters of *S. aureus* NTCT8325 (left) and schematic WTA structure (right) are shown. The *tag* and *tar* genes are involved in WTA backbone biosynthesis, while the *dlt* operon is responsible for teichoic acid D-alanylation. The *tagA, tagB, tarF*, and *tarL2* genes and WTA building blocks transferred by the corresponding enzymes as characterized in the reference paper (Brown et al., 2008) are marked in blue, yellow, red, and green, respectively.

(B) Pathway of poly-RboP WTA biosynthesis in *S. aureus.* The unknown enzyme transferring the polymer to peptidoglycan is indicated by a question mark. C55-P, undecaprenyl phosphate; GlcNAc-P, N-acetylglucosaminephosphate; ManNAc, N-acetyl-mannosamine; Rib-P, ribulose-5-phosphate; Gro-P, glycerolphosphate; RboP, ribitolphosphate.

the one discussed here, they were renamed as "*tarO*…" in bacteria incorporating ribitol into their WTA. In order to better convey relevant information and reduce confusion, we suggest keeping the original "*tag*" names for the initial highly conserved steps and using "*tar*" only for the late *tarFIJL* genes, which are specific for poly-RboP WTA and are often located in a separate gene cluster.

While most of the intracellular WTA biosynthetic steps seem to have been elucidated by now, understanding of the last steps is still in its infancy and represents a challenge for future studies. Evidence for a role of TagGH in WTA translocation to the outer leaflet of the cytoplasmic membrane is only very indirect (Lazarevic and Karamata, 1995). Moreover, the enzyme eventually transferring WTA to peptidoglycan precursors is still unknown. While critical steps of WTA biosynthesis have been reconstituted in vitro, it remains a challenge to employ these assays for high-throughput inhibitor screening approaches. Of note, the quest for inhibitors of the D-alanylation pathway has recently yielded very promising results (Escaich et al., 2007). In addition to the biochemical issues, important questions remain concerning the roles of CWG such as WTA and their enormous structural variability in bacterial physiology and host interaction.

REFERENCES

Bae, T., Banger, A.K., Wallace, A., Glass, E.M., Aslund, F., Schneewind, O., and Missiakas, D.M. (2004). Proc. Natl. Acad. Sci. USA *101*, 12312–12317.

Bhavsar, A.P., and Brown, E.D. (2006). Mol. Microbiol. *60*, 1077–1090.

Brown, S., Zhang, Y.H., and Walker, S. (2008). Chem. Biol. *15*, 12–21.

Escaich, S., Moreau, F., and Vongsouthi, V. Soulama. C, Malacain, E., Prouvensier, L., Gerusz, V., Denis, M, Saccomani, M., and Oxoby, M. (2007). ICAAC Conference 2007. F2–958.

Ginsberg, C., Zhang, Y.H., Yuan, Y., and Walker, S. (2006). ACS Chem. Biol. *1*, 25–28.

Lazarevic, V., and Karamata, D. (1995). Mol. Microbiol. *16*, 345–355.

Park, Y.S., Sweitzer, T.D., Dixon, J.E., and Kent, C. (1993). J. Biol. Chem. *268*, 16648–16654.

Pereira, M.P., and Brown, E.D. (2004). Biochemistry *43*, 11802–11812.

Peschel, A., Otto, M., Jack, R.W., Kalbacher, H., Jung, G., and Götz, F. (1999). J. Biol. Chem. *274*, 8405–8410.

Pooley, H.M., and Karamata, D. (1994). In Bacterial Cell Wall, J.-M. Ghuysen and R. Hakenbeck, eds. (Amsterdam, The Netherlands: Elsevier Science B.V.), pp. 187–197.

Qian, Z., Yin, Y., Zhang, Y., Lu, L., Li, Y., and Jiang, Y. (2006). BMC Genomics *7*, 74.

Soldo, B., Lazarevic, V., and Karamata, D. (2002). Microbiology *148*, 2079–2087.

Weidenmaier, C., Kokai-Kun, J.F., Kristian, S.A., Chanturyia, T., Kalbacher, H., Gross, M., Nicholson, G., Neumeister, B., Mond, J.J., and Peschel, A. (2004). Nat. Med. *10*, 243–245.

Weidenmaier, C., and Peschel, A. (2008). Teichoic acids and related cell wall glycopolymers - key mediators in Gram-positive bacterial physiology and host interactions. Nat. Rev. Microl., in press.