Point of VIEW

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Targeting Wall Techoic Acid Biosynthesis: An *in Vivo* Based High-Throughput Screen for Small Molecule Inhibitors

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ABSTRACT Identification of uncommon targets for inhibition, such as virulence factors, represents an emerging approach for combating the problem of antibiotic resistance among bacteria. Unfortunately, the lack of effective systems for the discovery and evaluation of inhibitors for such targets has considerably slowed progress. A recent article in *ACS Chemical Biology*, however, details the development of an *in vivo* based high-throughput screening strategy for identification of small molecule inhibitors of wall techoic acid biosynthesis.

The development of antibiotic resistance among bacteria such as *Staphylococcus aureus* has not only heightened the need for continued development of novel therapeutic agents but has also triggered interest in identifying uncommon targets for inhibition (*1*). One such approach centers on targeting the virulence of bacteria or rather the means by which they effectively infect hosts (*2*). This method is greatly aided by the prevalence of molecules that exist on bacterial surfaces to serve primarily as virulence factors. These factors include but are not limited to O-antigen, capsular polysaccharide, exopolysaccharide, and wall teichoic acid (WTA). Unfortunately, attempts at *in vitro* reconstitution of the systems that produce these molecules have been riddled with difficulties, thus limiting the potential for high-throughput inhibitor screening. Nonetheless, a recent article in *ACS Chemical Biology* by Swoboda *et al.* (*3*) describes a significant advance in streamlining identification of inhibitors toward such virulence factors. Specifically, they report an *in vivo* based high-throughput screen for identifying inhibitors of WTA biosynthesis in *S. aureus*.

Much like the biosynthetic pathways of many other polysaccharides, the WTA biosynthetic pathway contains genes that are termed *conditionally essential*. In essence, these genes encode enzymes that are essential for growth unless the initiating nonessential genes for polymer synthesis are deleted. For example, previous studies have shown that the first gene *tarO* in the WTA biosynthetic pathway is non-essential *in vitro*, whereas its downstream genes are vital unless polymer initiation is prevented (*4, 5*). Swoboda *et al.* (*3*) have accordingly exploited this fact, noting that an inhibitor for the product of one of these conditionally essential genes should impact cell growth only if *tarO* is functional. This enabled them to then design a high-throughput screening strategy in which a library of small molecules was applied to both the wildtype *S. aureus* strain and its isogenic Δ*tarO* strain. Given that *tarO* is functional only in the wildtype strain, Swoboda *et al.* (*3*) screened 55,000 small molecules, looking for those that inhibit growth of the wildtype *S. aureus* while having no effect on the Δ*tarO* strain. Screening ultimately revealed a potent WTA biosynthesis inhibitor, named 1835F03, that exhibited a minimum inhibitory concentration (MIC) of 3 μM in wildtype *S. aureus* (Figure 1).

Using previously developed *in vitro* assays, candidates for the target of this molecule were then quickly narrowed to either the two-component ABC transporter (TarGH) or the unknown ligase of this pathway (*6*). A series of very elegant experiments were then performed to clearly identify the target of 1835F03. First, the ligase was ruled out through demonstration that overexpression of TarGH leads to a concomitant increase in the MIC. Verification

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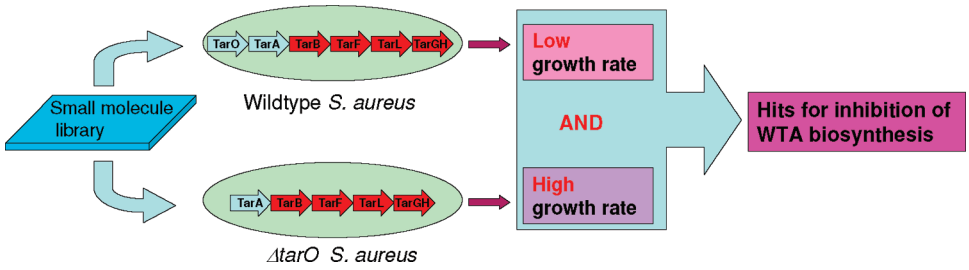

Figure 1. High-throughput screening strategy for WTA biosynthesis inhibitors in *S. aureus*. A library of small molecules is added to both wildtype and Δ*tarO S. aureus* strains. *Only* if the compound results in a low growth rate for wildtype and high rate for the corresponding deletion strain is it considered a HIT (in this paper, optical density is employed to monitor the growth rate).

then proceeded through complementation of the transporter in *S. aureus* with *Bacillus subtilis* 168 TagGH. As expected from the fact that *B. subtilis* 168 proved resistant to the inhibitor, complementation yielded a *S. aureus* strain that was also resistant. Finally, resistant mutants were selected that maintained resistance following termination of treatment with the inhibitor. Two possibilities existed for how these mutants had survived. The mutations may have occurred in the early non-essential genes, or they occurred in the target for 1835F03. Through use of *S. aureus* bacteriophage that utilize WTAs as receptors (*7*, *8*) mutants which definitively did not possess WTAs on the cell surface were selected for targeted sequencing. Of these mutants, all were found to be mutated within *tarG* (Figure 2).

Although no specific inhibitors for WTA biosynthesis have been previously discovered, this elegant study demonstrated a successful *in vivo* based high-throughput screen for such inhibitors of *S. aureus*, including methicillin-resistant *S. aureus*. Subsequent biochemical and genetic studies identified TarG as the molecular target among a series of conditionally essential enzymes. While identification of such an inhibitor, and its molecular target, represents an impressive feat in itself, the potential generality of this strategy as a means to identify inhibitors of other bactoprenol dependent biosynthetic pathways will likely lead to this work being seen as a landmark paper in antibiotic identification.

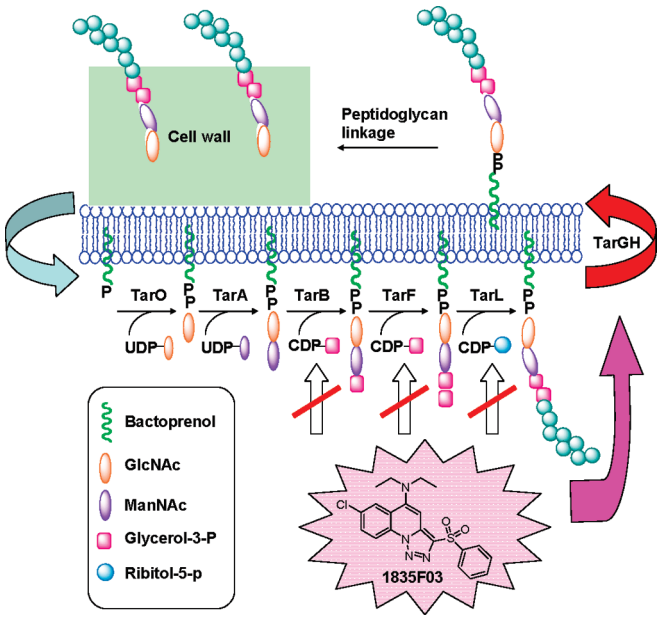

Figure 2. *Staphylococcus aureus* WTA biosynthetic pathway and the molecular target of the inhibitor 1835F03. Bioassays using *in vitro* reconstituted TarBDFI and L excluded them as possible targets (*6*). Heterologous complementation and analysis of selected resistant mutants led to identification of TarG as the target for 1835F03.

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