Exposing a chink in the armor of methicillin-resistant *Staphylococcus aureus*

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Antibiotics have provided arguably the greatest benefit to human health among developments of the past century, and so the emergence of widespread antibiotic resistance is now a grave threat to 21st-century health (1). Among antibiotic-resistant organisms, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged early and presents one of the most serious challenges, a situation compounded by the extension of its range from being primarily a nosocomial problem to include community-acquired and livestock-associated spread (2, 3). Novel strategies to combat MRSA are therefore urgently needed, and so it is notable that, in PNAS, Brown et al. (4) have identified a surprising chink in the bacterial armor that may allow the development of novel therapeutic strategies to target MRSA.

β -Lactam antibiotics such as methicillin target the penicillin-binding proteins that form the peptide cross-links that provide structural integrity to the peptidoglycan, the uniquely bacterial polymer of the cell wall. MRSA strains emerge when methicillin-susceptible *S. aureus* (MSSA) acquires the *mecA* gene through lateral gene transfer on the staphylococcal chromosomal cassette *mecA*, which encodes a low-affinity penicillin-binding protein, PBP2a, which is insensitive to penicillin and to many other β -lactam antibiotic agents (3, 5). However, the robustness of the MRSA phenotype is modulated by many other aspects of the MSSA strain background that acquires *mecA* (6). The complexity of the MRSA phenotype thus offers hope that drug interactions and other interventions can be devised that will allow the restoration of an MSSA phenotype to MRSA strains (7–9). The work of Brown et al. (4) identifies a significant target that may be exploited to allow resensitization of MRSA.

Teichoic Acid Biosynthesis Affects the MRSA Phenotype

Gram-positive bacteria such as *S. aureus* lack outer membranes but have relatively thick cell walls. In addition to the peptidoglycan component, these cell walls also typically contain a covalently integrated cell wall polymer that, in many cases, is a teichoic acid (TA) (10, 11). TAs are structurally diverse polyanionic polymers that include alditol-phosphates in their repeating units. In *S. aureus*, the TAs are

polymers of ribitol-phosphate that are decorated with several substituents including β -O-*N*-acetyl-D-glucosamine (β -O-GlcNAc) (4). Building on their earlier observation that inhibition of the first step in TA biosynthesis resensitized MRSA to β -lactams (9), Brown et al. (4) elegantly define the precise aspect of TA structure that modulates this susceptibility.

As part of their ongoing investigations of the TA biosynthesis pathway, Brown et al. (4) screened the genetic loci associated with TA biosynthesis in the *S. aureus* genome for glycosyltransferases that might

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be involved in TA decoration. By using an in vitro assay facilitated by their ability to synthesize the poly(ribitol-phosphate) substrate, they identify one candidate glycosyltransferase as a β -O-GlcNAc transferase capable of modifying the TA backbone. Additional biochemical characterization confirmed the specificity of the reaction, allowing the gene encoding the glycosyltransferase, *SAOUHSC_00228*, to be redesignated *tarS*. Generation of mutant strains lacking *tarS* confirmed its functionality as a TA β -O-GlcNAc transferase.

By using a TA null mutant (9) in comparison with a panel of *S. aureus* strain MW2 mutants lacking specific decorations of the TA, Brown et al. (4) are able to dissect out the contribution of different TA structural features to various phenotypes associated with TA function. Most strikingly, they convincingly demonstrate that it is the TarS-mediated β -O-GlcNAc modification of the TA that influences the MRSA phenotype. The TA null mutant and *tarS* deletion mutant showed decreased minimum inhibitory concentrations (i.e., increased sensitivity, typically at least fourfold) to a panel of six β -lactam antibiotics. As expected, complementation with *tarS* reversed this increase in sensitivity, whereas complementation with an inactivated mutant *tarS* allele did not. Moreover, complementation with the gene

(*tarM*) encoding the *S. aureus* enzyme that modifies the TA with α -O-GlcNAc or a gene encoding a *Bacillus subtilis* β -O-glucosyl transferase confirmed that the effect of TA modification on the MRSA phenotype was specific to the β -O-GlcNAc modification.

Reversing Resistance can Prolong Antibiotic Utility

The work of Brown et al. (4) thus strongly implicates TarS as a potential new target, inhibition of which could resensitize MRSA to β -lactam antibiotic agents. Restoration of antibiotic sensitivity to resistant strains, thereby reviving the effectiveness of an established antibiotic agent, is an attractive proposition given the well-documented “innovation gap” in the development of novel antibiotic agents (1). The utility of this approach is already evidenced by the well-established use of β -lactamase inhibitors to allow the continued use of β -lactam antibiotic agents in situations in which strains would be otherwise resistant to β -lactams through the production of β -lactamases (12).

The mechanism by which TarS modulates the MRSA phenotype is also of interest, especially as the precise functions of TA still remain elusive (11). Whereas TA null mutants are defective in cell division (4, 9), *tarS* and *tarM* mutants (and a *tarS tarM* double mutant) of *S. aureus* MW2 are not (4). Brown et al. (4) plausibly speculate that modification of *S. aureus* TA with β -O-GlcNAc may influence its ability to act as a scaffold for cell envelope associated proteins, and that this directly or indirectly modulates PBP2a activity. However, it is also likely that TA modifications with sugars and/or D-alanine will impact on the biophysical properties of the peptidoglycan matrix by affecting its packing and influencing the overall cell wall milieu. In this regard, it is notable that a recent study shows that the D-alanine modification of membrane-anchored lipoteichoic acid (LTA), which is directly comparable to D-alanine modification of TA, affects the packing density of the cell wall of group B *Streptococcus* (13). Intriguingly, D-

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alanine modification of TA and LTA by the Dlt system (11) appears to have variable influence on the sensitivity of *S. aureus* strains to β -lactam antibiotics, as deletions in this pathway restored the susceptibility to some β -lactams but not others (4). Moreover, whereas Brown et al. (4) report a fourfold increase in methicillin susceptibility of a *dltA* mutant of *S. aureus* MW2, Nakao et al. (14) have reported that a transposon insertion mutation in the *dlt* operon of a different MRSA strain decreased methicillin susceptibility eightfold. As these data imply that there may be strain-specific variations in the response to changes in cell envelope

properties, it is important to emphasize that the effect of *tarS* deletion on the MRSA phenotype was further confirmed by Brown et al. (4) by deletion of *tarS* in four other MRSA strains.

No Longer "Secondary" Cell Wall Polymers

Despite their considerable contribution to the bulk physical properties of the cell walls of Gram-positive bacteria (and the energetic expense inherent in their biosynthesis), TAs and related polymers were initially somewhat dismissed as "secondary" cell wall polymers. Now that situation is changing, and there is a growing mo-

mentum to studies into their biosynthesis and their contributions to bacterial physiology (10, 11, 15). Further collaborations between biochemists, biophysicists, geneticists, microbiologists, and structural biologists will be needed to unravel the fine details of TA function. It is an exciting prospect that improved understanding of the biosynthesis of TA and of membrane-anchored LTA (16) may identify attractive targets for the development of novel antimicrobial interventions. By dissecting out the roles and consequences of the tailoring modifications of its TA, Brown et al. (4) uncover a potentially valuable Achilles heel of *S. aureus*.

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