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GUEST COMMENTARY

Novel Links between Antibiotic Resistance and Antibiotic Production▽

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Streptomyces coelicolor is the best-developed model system for an enormous family of filamentous soil bacteria. One reason for the interest in these organisms is that they produce numerous secondary metabolites, many of which are employed clinically as antibiotics. Most streptomycetes produce several biologically active secondary metabolites; *S. coelicolor* produces at least four. Not surprisingly, they also possess resistance genes for the antimicrobial molecules they produce; often these are linked to and are coregulated with the antibiotic biosynthesis genes. As our understanding of secondary metabolism advances, it is becoming increasingly clear that the relationship between antibiotic production and resistance is more complicated than expected. For example, the *S. coelicolor* genome encodes proteins that are similar in sequence and mechanism to those that confer clinical resistance to vancomycin (7, 8). This was a surprise because *S. coelicolor* does not produce vancomycin or, indeed, any glycopeptide antibiotics. More recently, environmental isolates of *Streptomyces* spp. have been described that harbor enzymatic resistance mechanisms for antibiotics that are semisynthetic or wholly synthetic and, presumably, have never existed in nature (5, 17). Where did the selective pressure for these resistance mechanisms come from? In addition to this apparent disconnect between biosynthesis and resistance, antibiotic production appears to be controlled by a regulatory network of truly Byzantine proportions: to date at least 18 genes have been shown to influence antibiotic production in *S. coelicolor*—a subset of these also control sporulation (3). Clearly, bacteria have devoted a great deal of evolutionary time to developing antibiotic resistance mechanisms and the regulatory apparatus for controlling for antibiotic production. In this issue of the *Journal of Bacteriology*, and in a companion article published in *Molecular Microbiology* (15), Kenji Nishimura and coworkers in Kozo Ochi's laboratory report the elucidation of the mechanism of type II streptomycin resistance (12). Their discoveries strongly reinforce the suspicion that there is much to learn about the relationship between antibiotic resistance and biosynthesis.

Streptomycin, a secondary metabolite produced by several *Streptomyces* strains, was introduced as a therapeutic agent in the early 1940s and proved spectacularly successful against a number of serious infections. Sadly, however, it went on to set the pattern for clinical resistance to antibiotics. By 1946, resistant strains had been reported, and by the early 1950s, clinical resistance was so widespread that the antibiotic began to fall into disuse, supplanted as a miracle cure-all by newer drugs.

In *S. coelicolor*, two categories of streptomycin-resistant mutants have been characterized. Type I mutants are resistant to high concentrations of the antibiotic, and type II mutants are resistant to much lower concentrations. Both mechanisms are specific to streptomycin; neither confers resistance to other antibiotics. This pattern of distinct high and low resistance has been reported for other bacteria (6, 11). An odd effect of both types of *strR* mutations on *S. coelicolor* is that they bring about the overproduction of the secondary metabolite actinorhodin, a polyketide that is otherwise unrelated to streptomycin (9, 13, 16). Indeed, *strR* mutations can overcome the effects of mutations in genes such as *relA*, *relC*, and *brgA* that, on their own, impair actinorhodin production (16).

Type I resistance is brought about by mutations in the *rpsL* gene, which encodes the S12 protein of the 30S subunit of the ribosome (16). The mechanism responsible for type II resistance was first demonstrated to be genetically distinct from that of type I resistance in 1948 (6) but eluded molecular characterization until now. Nishimura and coworkers (12) have demonstrated its association with the gene *rsmG* in *S. coelicolor* and its orthologue *gidB* in *Escherichia coli*, *Mycobacterium tuberculosis*, and other species (15).

This work linking *rsmG* to streptomycin resistance is of interest for technical reasons as well as biological ones. Genetic mapping is challenging in *S. coelicolor* and has apparently proven to be particularly difficult in this case. Reasons for this may be that the *rsmG* mutant phenotype is a relatively weak one and that most bacteria throw off type II streptomycin-resistant mutants at a relatively high frequency. The authors therefore made use of chip technology (1) recently developed for *S. coelicolor*, in which the entire genome sequence is arrayed in overlapping oligonucleotides. The arrays are interrogated by annealing them to wild-type and mutant chromosomal DNA, and the result is the straightforward identification of point mutations, inser-

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tions, or deletions within the mutant genome. In this case, the authors were able to show that a type II mutant had a sequence change in the *S. coelicolor* gene SCO3885, which they went on to rename *rsmG* for *r*RNA *s*mall subunit *m*ethyltransferase (12). This technology would likely be applicable to many organisms. For example, the identification of mutations that confer resistance to the diarylquinolone drug R207910 necessitated the nearly complete sequencing of three *Mycobacterium* sp. genomes (2). While high-throughput DNA sequencing grows increasingly efficient and affordable, it still requires a significant computational effort that could be avoided by this array technology.

The *rsmG* gene encodes a highly conserved *S*-adenosylmethionine (SAM) binding protein and is found in all sequenced bacterial genomes. In spite of this high degree of conservation, the gene is nonessential: a deletion mutation confers type II streptomycin resistance and, in *S. coelicolor*, the overproduction of actinorhodin. This phenotype is associated with the loss of a specific 16S rRNA methylation at G518 in *S. coelicolor* (12) or G527 in *E. coli* (15), a residue that is found within the "530 loop" of the 16S rRNA and which interacts directly with streptomycin (4). Mutations in the *M. tuberculosis* orthologue of *rsmG*, *gidB*, were found to be tightly associated with type II streptomycin resistance in a large collection of clinical isolates (15).

Previous work from the Ochi laboratory and others (10, 14, 16) demonstrated that type II mutants of *S. coelicolor* express SAM synthetase at higher levels than congenic wild-type strains (14). Consistent with this, they show here that an *rsmG* deletion mutant exhibits increased SAM synthetase activity late in the growth cycle and that this could be correlated with enhanced transcription of the SAM synthetase-encoding gene *metK*. An additional feature of this mutant is that translation was greatly enhanced in stationary phase cells relative to that a wild-type strain. This, however, was not caused by the elevated level of SAM synthetase or SAM levels as overexpression of *metK* from a high-copy-number plasmid did not confer enhanced translation in a wild-type strain. Enhanced translational efficiency may therefore be linked to the absence of 16S rRNA methylation. Overexpression of *metK* did, however, give rise to the overproduction of actinorhodin, as reported previously (10, 14). These phenomena are summarized in Fig. 1. What is most striking is the fact that in addition to changing the ribosome's sensitivity to streptomycin, modification by RsmG seems to lower actinorhodin and SAM production through transcriptional effects. Somehow, the status of the ribosome is influencing the transcription of *metK* and *actII*-ORF4, the pathway-specific activator of the actinorhodin biosynthetic genes.

In addition to addressing a 60-year-old question in antibiotic resistance, this work raises significant questions (12). It would appear that all bacteria, including the soil bacterium *S. coelicolor*, which may well share its habitat with streptomycin producers, encode a methyltransferase that makes them more sensitive to streptomycin. What do they gain from this? Loss of RsmG-mediated modification of the ribosome increases SAM synthetase production, translation efficiency during stationary phase, and remarkably, the production of the polyketide actinorhodin. How do the pathway-specific and pleiotropic antibiotic regulators identified in *S. coelicolor* contribute to this regulation? What is the significance of this chemical genetic interaction between the two types of streptomycin resistance and actinorhodin production, and does this sort of interaction extend to other antibiotics? This work is clearly an important step toward addressing these questions.

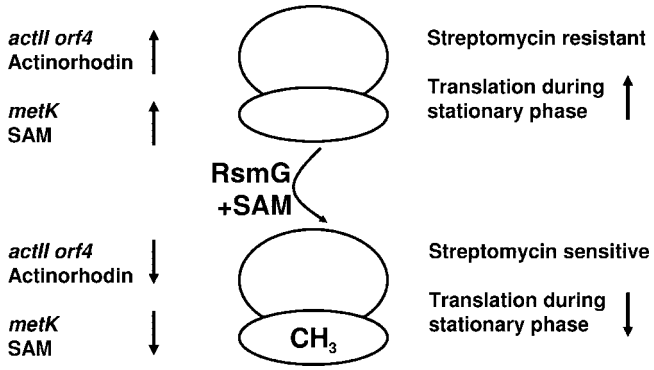

FIG. 1. Summary of the known biochemical, transcriptional, translational, and resistance effects of the RsmG methyltransferase.

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