NOTES

A Mutation in the Decoding Center of Thermus thermophilus 16S rRNA Suggests a Novel Mechanism of Streptomycin Resistance

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A spontaneous kanamycin resistance and capreomycin resistance mutation, A1408G, in the decoding center of 16S rRNA, was identified in the extreme thermophile Thermus thermophilus. Unexpectedly, this mutation also confers resistance to streptomycin. We propose a novel mechanism of streptomycin resistance by which A1408G influences conformational changes in 16S rRNA during tRNA selection.

Ribosomes from the extreme thermophile Thermus thermophilus have proven to be a rich source of structural information regarding the mechanism of tRNA selection during protein synthesis and have provided insights into the mechanism by which antibiotics can disrupt this process (3, 17). This species is also amenable to genetic manipulation (11, 12), and antibiotic resistance mutations in ribosomal protein and rRNA genes are readily isolated (2, 7). The ability to apply both genetics and X-ray crystallography to ribosomes from a single organism creates the exciting possibility of elucidating antibiotic resistance mechanisms at atomic level resolution.

The aminoglycoside antibiotics, including streptomycin and kanamycin, have long been known to cause misreadings of the genetic code, and it is now confirmed from crystallographic analysis of the T. thermophilus 30S subunit that these antibiotics bind at or near the decoding center and in distinct, nonoverlapping sites (3). Indeed, aminoglycosides, including the kanamycins and neomycins, make contact exclusively with 16S rRNA helix 44 independently of the remainder of the 30S subunit (5, 24). In a striking contrast, streptomycin contacts multiple structural elements of the T. thermophilus 30S subunit, including ribosomal protein S12 and 16S rRNA helices 1, 18, 27, and 44 (3). It has been proposed that streptomycin stabilizes a series of intermolecular and intramolecular contacts within the 30S subunit during decoding (16). What is not yet clear from high-resolution structural studies is the mechanism by which some ribosomal mutations confer resistance to streptomycin. A number of mutations occur at positions of A1408, and both these contacts would be lost with the A1408G mutation. At present, there is no direct structural information regarding the interaction of capreomycin and the decoding site, so the mechanism of resistance cannot as yet be deduced. However, direct interaction with the decoding site is strongly implicated by the observation that tuberactinomycins compete with aminoglycosides for ribosome binding and cause misreading (6) and by the tuberactinomycin resistance phenotype of the A1408G mutant (21; this study).

Novel streptomycin resistance phenotype conferred by A1408G. To assess the antibiotic resistance phenotypes of these mutants, we streaked cells from saturated overnight cultures onto plates containing closely spaced concentrations of antibiotics (twofold intervals or smaller) to obtain single colonies. We found that the A1408G mutation confers high-level kanamycin resistance (Table 1). We also observed that the A1408G mutation confers substantial resistance to streptomycin. This result was unexpected, as this mutation in a number of other organisms has been described previously (22), but
Streptomycin resistance has not been reported. Streptomycin resistance is associated most often with mutant alleles of *rpsL*, the gene which encodes ribosomal protein S12 (6), although streptomycin-resistant *rpsD* alleles in *Salmonella enterica* serovar Typhimurium have also been described recently (1). Sequencing of *rpsL* and *rpsD* revealed no secondary mutations in our *Thermus thermophilus* mutants, leading us to conclude that the A1408G mutation alone is responsible for the streptomycin resistance phenotype. This assessment is also supported by the high frequency at which this mutation was identified, consistent with a single mutational event, and the fact that multiple independent isolates carrying the A1408G allele isolated on either kanamycin medium or capreomycin medium all proved to be streptomycin resistant, despite having never been exposed to this drug. We suggest that this previously overlooked phenotype is in some measure the result of the recessive nature of streptomycin resistance (14), combined with the presence of multiple rRNA genes in most bacterial species (19). Interestingly, eukaryotic ribosomes, which are insensitive to streptomycin, naturally carry a G at position 1408 (15).

### Structural basis for streptomycin resistance conferred by A1408G

The mechanism by which A1408G might counter the inhibitory action of streptomycin is not immediately obvious. Streptomycin contacts helix 44 at C1490 and G1491 via backbone interactions and makes no direct contact with A1408, which is situated on the opposite side of the helix (Fig. 1) (3). There is no predicted loss of contact with the drug due to long-range conformational distortion, and a nuclear magnetic resonance solution of an oligonucleotide mimic of 16S rRNA helix 44 bearing an A1408G substitution shows only minor conformational differences from the wild-type structure (15). Further, a G1491A mutation, which we also isolated in selections for capreomycin-resistant *T. thermophilus* mutants, does not confer streptomycin resistance, giving an MIC identical to that of wild-type *T. thermophilus* IB-21 (Table 1). This result is in spite of the fact that G1491 makes direct backbone contact with streptomycin (Fig. 1) (3) and G1491A is predicted to create greater local distortion than A1408G, by creating an A-C mismatch with C1409. A1408 is on the side of helix 44, opposite the universally conserved A1492 and A1493 (Fig. 1). These bases participate directly in codon-anticodon recognition during the tRNA selection process, involving a change from a “tucked-in” conformation to a “flipped-out” conformation (Fig. 1) (16). The flipped-out conformation is also induced by the aminoglycoside paromomycin (3). In the tucked-in conformation, A1492 and A1493 stack within helix 44, and A1493 base-pairs with A1408 (15, 25). A1408G has been shown to increase slightly the thermal stability of an A-site rRNA analog with A1492 and A1493 in the tucked-in conformation over that of a wild-type analog in the same conformation (15). It is our hypothesis that A1408G favors the tucked-in conformation of A1492 and A1493 and that this shift in the conformational equilibrium overcomes the inhibition imposed by streptomycin. Streptomycin affects tRNA selection in part by increasing the affinity for near-cognate aminoacyl-tRNA (9). A mutation negatively affecting the conformational transition of A1492 and A1493 to the flipped-out position that occurs during codon recognition might enhance cognate codon recognition over near-cognate codon recognition to an extent sufficient to overcome this effect of streptomycin. One prediction of our model is that mutants bearing the A1408G allele should display a hyperaccurate phenotype (1, 8). Future experiments will be aimed at assessing the effect of this mutation on translational accuracy.

Streptomycin resistance has also been shown to occur as a result of a mutant allele of *rpsD* encoding ribosomal protein S4 (1). S4 makes no direct contact with streptomycin, and this

![FIG. 1. Three-dimensional arrangements of A1408, G1491, A1492, and A1493 in the *T. thermophilus* 30S ribosomal subunit crystal structure with (left) A1492 and A1493 in the flipped-out conformation and in the presence of streptomycin (PDB 1FJG) (left) (3) and with A1492 and A1493 in the tucked-in conformation (PDB 1J5E) (right) (25). A1408 pairs with A1493 in the tucked-in conformation, and the A1408G mutation confers streptomycin resistance. The figure was rendered with PyMol (4).](http://jb.asm.org/Downloaded from http://jb.asm.org/)

### Table 1. Mutants and their growth rates and the effects of mutations on antibiotic resistance

<table>
<thead>
<tr>
<th>Strain</th>
<th>Doubling time (min)</th>
<th>MIC (µg/ml)</th>
<th>KAN</th>
<th>STR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB-21 (wild type)</td>
<td>46</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>A1408G mutant</td>
<td>57</td>
<td>3,000</td>
<td>500</td>
<td>20</td>
</tr>
<tr>
<td>G1491A mutant</td>
<td>54</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

a KAN, kanamycin.
b STR, streptomycin.
mutation probably exerts its resistance phenotype indirectly by affecting the kinetics or thermodynamics of the open-to-closed conformational transition of the 30S subunit. It is therefore likely that resistance to streptomycin caused by mutations modulating the kinetics and thermodynamics of conformational transitions will be found to be a more common mechanism than was previously predicted.

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REFERENCES