432h Biochemical Characterization of Arma, a 16s Rrna Methyltransferase Which Confers Resistance to Aminoglycosides

Grace F. Liou, Marc Galimand, and Patrice Courvalin

Aminoglycosides are a medically important class of antibiotics which are used to treat serious infections. Recently there have been reports of bacterial aminoglycoside resistance due to methylation of 16S rRNA, a mechanism previously observed only in aminoglycoside producers. The first such enzyme identified in a clinical strain, ArmA (Galimand et al., 2003. Antimicrob. Agents Chemother. 47: 2565-71), belongs to the growing Agr (aminoglycoside resistance) family of closely related 16S rRNA methylases. ArmA was overexpressed in E. coli and purified to homogeneity. Methylation of rRNA by ArmA was established in vitro, and N-methylation at position G1405 was determined by a modified primer extension protocol involving borohydride and aniline treatment with specific cleavage at 7-methylguanine. Methylation rates of 16S rRNA, 30S subunit, and 70S ribosome demonstrated specificity for the 30S subunit, implicating the role of ribosomal proteins in recognition by ArmA. Finally, we have compared the binding of an aminoglycoside to unmethylated and methylated ribosomes, establishing the key link between in vitro methylation and in vivo high-level resistance to 4,6-disubstituted deoxystreptamines. More worrisome, the interspecies transfer of the armA gene has been found to result from conjugation and transposition. This combination accounts for the documented wide-spread dissemination of armA throughout Europe and Asia.