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Probing the Peptidyl Transferase Center of Ribosomes Containing Mutant 23S rRNA with Photoreactive tRNA

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Abstract
There is strong crystallographic evidence that the 23S rRNA is the only catalytic entity in the peptidyl transferase center. Various mechanisms for the catalysis of peptidyl transfer have been proposed. Recently, attention has been given to the idea that the 23S rRNA simply acts to position the tRNA for spontaneous peptidyl transfer and that chemical catalysis may play only a secondary role. Conserved nucleotides U2585 and U2506 are thought to be involved in positioning the 3' ends of A- and P-site substrates based on the crystallographic evidence, and because mutagenesis at these sites severely impairs peptide bond formation. In this study, pure populations of ribosomes with either U2585A or U2506G mutations in the 23S rRNA were analyzed to test the hypothesis that substitutions at nucleotides U2585 and U2506 in the peptidyl transferase center impair peptide bond formation by altering the position of the 3' end

of P-site tRNA relative to the 23S rRNA. Pure populations of mutant or wild-type ribosomes were obtained by an affinity tagging system and probed with 32P-labeled [2N3A76]tRNAPhe to determine how the 3' end of tRNA interacts with the ribosomal proteins and 23S RNA at the peptidyl transferase center. Some of the data for the ribosomes with a G at position 2506 are consistent with a model suggested by Schmeing and coworkers in which nucleotide U2506 breaks from its original wobble base pair with nucleotide G2583 during A-site tRNA binding and swings towards the 3' end of P-site tRNA, while nucleotide U2585 simultaneously moves away from the 3' end of P-site tRNA.

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