Changes in the mutual orientation of tRNA and 23S rRNA at the peptidyl transferase center of the ribosome detected by crosslinking of a photoreactive transition-state analog

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Abstract

Dynamic interactions between the amino acid acceptor end of tRNA and the ribosome underlie the synthesis of successive peptide bonds at the peptidyl transferase center (PTC) of the 50S ribosomal subunit. Photo-crosslinking of the 3′ -terminal nucleotide of tRNA, which is adjacent to the attached amino acid or peptide, to components of the 50S subunit has proven to be a sensitive means for identifying specific protein and RNA segments in close proximity to the site of peptide bond formation. I have used this approach to follow changes in the position of the tRNA during peptide bond formation using several photoreactive tRNA-derived ligands. Three new photoreactive tRNA derivatives have been synthesized for use as probes of the PTC of the ribosome. In two of these derivatives, the 3′ adenosine in position 76 of yeast $tRNA^{Phe}$ has been replaced by either 2-azidodeoxyadenosine or 2-azido-2′ -*O*-methyladenosine, while in a third the 3′ -terminal 2-azidodeoxyadenosine of the tRNA is joined to puromycin via a phosphoramidate linkage to generate a photoreactive transition-state analog. All three derivatives bind to the P site of 70S ribosomes with affinities similar to that of unmodified $tRNA^{Phe}$ and can be crosslinked to components of the 50S ribosomal subunit by irradiation with near UV light. Yeast $tRNA^{Phe}$ containing 2-azidoadenosine, $[2N_3A76]tRNA^{Phe}$, typically crosslinks to the N-terminal sequence of protein L27 as well as to nucleotides U2506 and U2585 of the 23S rRNA. While the photoreactive transition-state analog, $[2N_3dA76]tRNA^{Phe}$-p-Puro, crosslinked the same components as $[2N_3A76]tRNA^{Phe}$, the distribution of crosslinks is altered significantly. The crosslinking to nucleotide U2506 is strongly reduced, and two new crosslinked nucleotides A2450 and A2602 were detected. Characteristic differences in the crosslinking patterns suggest that these

tRNA derivatives can be used to follow subtle changes in the position of the tRNA relative to the components of the PTC. ^

Subject Area

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