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THE ROLE OF 2′- AND 3′-HYDROXYL GROUPS OF A76 tRNATyr AT THE FIRST STEPS OF TRANSLATION QUALITY CONTROL

M. Yu. RYBAK

Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv;
e-mail: mariia.rybak@gmail.com

Introduction. Translational control is an essential step for the quality and fidelity of protein biosynthesis. Several checkpoints exist to avoid the mistakes of initiation and elongation steps. Aminoacyl-tRNA-synthetases (aaRSs), enzymes which activate amino acids and attach them to cognate tRNAs, can possess editing domains in their structure, therefore protecting cells from including non-proteinogenic amino acids into proteins. Some aaRSs, for example, tyrosyl-tRNA-synthetase (TyrRS), do not have such domains; additionally, TyrRS demonstrate the weakest specificity in recognition of D- and L-Tyr enantiomers. Besides, supplementary trans-editing enzyme – D-aminoacyl-tRNA-deacylase (DTD) – can remove the mistakes of aaRSs. It hydrolyses the ester linkage between D-amino acids and tRNAs. Previously, we have successfully cloned, expressed and purified DTD from Thermus thermophilus. In this work we have analyzed and identified the role of 2′- and 3′-OH groups of A76 tRNATyr as the primary sites for aminoacylation by TyrRS and deacylation by DTD during translation initiation step.

Methods. To address this issue, we applied two biochemical assays with [32P]-labelled tRNATyr substrates: wild type A76 tRNATyr and its 2′- and 3′-deoxyA76 derivatives. We determined the catalytic parameters of these reactions and analyzed data in Origin 9.0.

Results and Discussion. We identified the primary site of D-Tyr attachment to tRNATyr – its 2′-OH group of terminal ribose in A76. L-Tyr bounds similarly to 2′- and 3′-OH groups. DTD catalyzes deacylation specifically from the 3′-OH group and 2′-OH only assists in this hydrolysis.

Conclusions. Our research resulted in the molecular mechanisms of cooperations between tRNATyr, TyrRS, and DTD, representing the studies of D-Tyr involvement in translation process in thermophilic bacteria *T. thermophilus*.

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