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Glu-Q-tRNA^{Asp} synthetase coded by the *yadB* gene, a new paralog of aminoacyl-tRNA synthetase that glutamylates tRNA^{Asp} anticodon

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Abstract

Analysis of the completed genome sequences revealed presence in various bacteria of an open reading frame (ORF) encoding a polypeptide chain presenting important similarities with the catalytic domain of glutamyl-tRNA synthetases but deprived of the C-terminal anticodon-binding domain. This paralog of glutamyl-tRNA synthetases, the YadB protein, activates glutamate in the absence of tRNA and transfers the activated glutamate not on tRNA^{Glu} but instead on tRNA^{Asp}. It has been shown that tRNA^{Asp} is able to accept two amino acids: aspartate charged by aspartyl-tRNA synthetase and glutamate charged by YadB. The functional properties of YadB contrast with those of the canonical glutamyl-tRNA synthetases, which activate Glu only in presence of the cognate tRNA before aminoacylation of the 3′-end of tRNA. Biochemical approaches and mass spectrometry investigations revealed that YadB transfers the activated glutamate on the cyclopenthene-diol ring of the modified nucleoside queuosine posttranscriptionally inserted at the wobble position of the anticodon-loop to form glutamyl-queuosine. Unstability of the ester bond between the glutamate residue and the cyclopenthene-diol (half-life 7.5 min) explains why until now this modification escaped detection. Among *Escherichia coli* tRNAs containing queuosine in the wobble position, only tRNA^{Asp} is substrate of YadB. Sequence comparison reveals a structural mimicry between the anticodon-stem and loop of tRNA^{Asp} and the amino acid acceptor-stem of tRNA^{Glu}. YadB, renamed glutamyl-Q-tRNA^{Asp} synthetase, constitutes the first enzyme structurally related to aminoacyl-tRNA synthetases which catalyzes a hypermodification in tRNA, and whose function seems to be conserved among prokaryotes. The discovery of glutamyl-Q-tRNA^{Asp} synthetase breaks down the current paradigm according to which the catalytic domain of aminoacyl-tRNA synthetases recognizes the amino acid acceptor-stem of tRNA and aminoacylates the 3′-terminal ribose. The evolutionary significance of the existence of an aminoacyl-tRNA synthetase paralog dedicated to the hypermodification of a tRNA anticodon will be discussed.

Keywords: YadB; Glu-Q-RS; Queuosine; Glutamyl-tRNA synthetase; tRNA aminoacylation; tRNA hypermodification; Paralog

Contents

Abbreviations: aaRS, aminoacyl-tRNA synthetase with aa for amino acid (for individual aaRSs, aa is given in the three letter code: e.g. GluRS for glutamyl-tRNA synthetase); Asn synthetase, asparagine synthetase; EF-Tu, elongation factor Tu; Glu-Q, glutamyl-queuosine; Glu-Q-RS, glutamyl-queuosine-tRNA^{Asp} synthetase; GoA, glutamol-adenosine monophosphate; ORF, open reading frame; Q, queuosine; TGT, tRNA-guanine transglycosylase.

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1. Paralogs of aminoacyl-tRNA synthetases

Aminoacyl-tRNA synthetases catalyze the attachment of aa on the 3′-end of tRNA. These enzymes are modular and contain at least two distinct domains, the catalytic core forming aminoacyl-adenylate by activation of the carboxyl group of the cognate aa with ATP, and the tRNA anticodon-binding domain. Many aaRSs contain domains which increase catalytic efficiency and specificity by emphasizing tRNA recognition or by promoting correction of wrong end-products such as the editing domain in the catalytic core able to promote pre- and post-transfer proofreading by hydrolyzing wrong aminoacyl-adenylate or incorrectly charged aa-tRNA. Most aaRSs contain additional domains probably acquired during evolution that are not directly implicated in tRNA aminoacylation and whose function remains unknown in most cases [1–6].

Genomic analysis reveals open reading frame (ORF) encoding proteins, which exhibit high similarities with domains of aaRSs [5,7]. Until now, only editing and catalytic domains have been characterized as single ORF in the three kingdoms of life. Three freestanding editing domains have been characterized namely AlaX, ProX and ThrX. The ProX protein from *Clostridium sticklandii* [8] and YbaK from *Heamophilus influenzae* [9] exhibit high sequence similarity with the editing domain of ProRS and deacylate Ala-$tRNA^{Pro}$, AlaX is an homolog of the editing domain of AlaRS of *Methanosarcina barkeri* and *Sulfolobus solfataricus* and hydrolyzes Ser-$tRNA^{Ala}$ and Gly-$tRNA^{Ala}$ [8], ThrX of *S. solfataricus* a paralog of the editing domain of ThrRS hydrolyzes Ser-$tRNA^{Thr}$ [10]. Since some bacterial ProRSs and most archaeal ThrRSs are deprived of the editing domain, it has been suggested that AlaX, ProX and ThrX catalyze proofreading of mischarged aa-tRNAs in trans [8]. This hypothesis is reinforced by the fact that editing domains isolated from aaRSs and expressed independently conserve their hydrolytic function [9,11].

Five proteins, homologous to catalytic domains of aaRSs, and expressed in prokaryotes were characterized and their function investigated. All are implicated in other functions than tRNA aminoacylation. Interestingly, some of them, like aaRSs, activate aa and form an aminoacyl-adenylate, which is then used in a second reaction, most generally in aa metabolism. HisZ, a paralog of the catalytic core of HisRS, is present in various bacteria. In *Lactobacillus lacti* it constitutes a subunit of HisG the first enzyme of the His biosynthetic pathway [12]. The archaeal Asn synthetase (AsnAR synthetase) a paralog of AsnRS is structurally and functionally related to bacterial Asn synthetase A [13,14]. This enzyme catalyzes formation of Asn by amidation of the activated β-carboxyl group with ammonia [13]. The Cys-GlcN-Int ligase of *Mycobacterium smegmatis* an enzyme structurally and functionally related to CysRS forms the mycothiol AcCys-GlcN-Ins from cysteinyl-adenylate that is also the intermediate of tRNA cysteinylation by CysRS [15]. The biosynthetic pathway of val-