Turning tRNA upside down: When aminoacylation is not a prerequisite to protein synthesis

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In the first 20 years since their discovery, the aminoacyl-tRNA synthetases (aaRS) were considered purely for their roles in protein synthesis. Each aaRS links its cognate amino acid to one of the tRNAs bearing the appropriate anticodon trinucleotides before decoding and translation by the ribosome. The accuracy of this aminoacylation reaction depends on the ability of each aaRS to select its cognate substrates from pools of chemically similar amino acid and tRNA substrates, which in turn relies on the presence of highly specific binding sites for both nucleic acids and amino acids. In the original formulation of Crick's adaptor hypothesis, each amino acid and corresponding tRNA was envisioned to match a single cognate aaRS, but this view has largely been overturned (1). Large-scale sequencing efforts revealed that many genomes of extant organisms contain both duplications of full-length tRNA synthetases, as well as aaRS-like proteins, which are evolutionarily conserved fragments reiterating functional domains from many known synthetases (2, 3). Functions have been deduced for only a small fraction of these proteins, and, in most cases, it is the catalytic core domains that have been recruited for a variety of new roles. These include roles in amino acid biosynthesis (4, 5), DNA replication processivity (6), and protein synthesis quality control (7, 8). The paper by Salazar *et al.* (9) in this issue of PNAS adds to this list by describing how the glutamyl-tRNA synthetase (GluRS) paralog YadB attaches glutamate to queuosine, generating a hypermodified nucleoside at the first anticodon position of $tRNA^{Asp}$. This surprising finding provides a further dramatic illustration of the catalytic and RNA recognition versatility of the class I aaRS catalytic fold, and it implies that tRNA modifications might be more widespread than previously anticipated, owing to their chemical lability.

The function of YadB, which lacks the C-terminal anticodon-binding domain of GluRS, had been the subject of speculation for some time. YadB was first observed in the emerging *Escherichia coli* genome but was later found to be relatively widespread in bacterial genomes. Initially, YadB was proposed to be a relic of an ancient transamidation pathway (10). That particular hypothesis postulated that the ancestor of YadB was a GluRS able to attach glutamate to the noncognate substrate $tRNA^{Gln}$. Recent studies confirmed YadB as a structural analogue of the catalytic core of GluRS that has retained the ability to activate glutamate (11), albeit without the need for tRNA binding that characterizes GluRS (12). Closer examination revealed that YadB does not attach activated glutamate to either $tRNA^{Gln}$ or $tRNA^{Glu}$ (11), but instead to $tRNA^{Asp}$ (9, 13). Although the latter report by Dubois *et al.* (13) in this issue of PNAS provided unequivocal evidence for the surprising aminoacylation of $tRNA^{Asp}$, it left unanswered the more vexing question of what function, if any, this apparent heterologous aminoacylation serves *in vivo*.

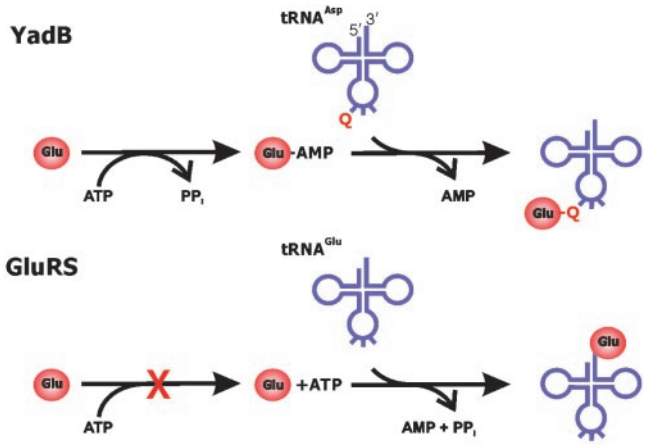

Fig. 1. Pathways used by YadB and GluRS to attach glutamate to tRNA. YadB (upper pathway) first activates glutamate (Glu) in the absence of tRNA to yield glutamyl-adenylate (Glu-AMP) and pyrophosphate (PP_i). Glu-AMP then serves as substrate for the attachment of glutamate to the Q-base at the first anticodon position of $tRNA^{Asp}$. In contrast, GluRS (lower pathway) requires the presence of $tRNA^{Glu}$ for Glu-AMP synthesis and subsequently attaches glutamate to the 3′-terminal adenosine of $tRNA^{Glu}$.

The surprising answer to the question of YadB's function is provided by the work of Salazar *et al.* (9). In addition to confirming what was reported concerning YadB (11, 13), Salazar *et al.* establish a truly surprising fate for the amino acid in YadB aminoacylation: rather than being transferred to the 3′ end of the tRNA, the glutamate becomes attached to the hypermodified nucleoside queuosine (Q) at the first anticodon position, leading to the formation of glutamyl-Q (Fig. 1). They base this remarkable conclusion on three telling observations. First, whereas a typical 3′ aminoacylated tRNA is resistant to treatment with periodate, the YadB aminoacylation product exhibits the same decrease in length as unacylated tRNA upon periodate treatment. Second, use of aspartyl-tRNA synthetase (AspRS) and YadB in concert with pure $tRNA^{Asp}$ substrate produces a product that can be labeled with both radioactive glutamate and aspartate. Third, mass spectroscopy analysis of tRNA hydrolysates provides definitive physical evidence that the glutamate is transferred to queuosine. Because the only position in $tRNA^{Asp}$ that exhibits the Q modification is the wobble base of the anticodon (nucleotide 34), the glutamate modification is restricted to a single nucleotide. Consistent with this hypothesis, YadB is unable to aminoacylate transcripts of $tRNA^{Asp}$, and tRNA prepared from an *E. coli* strain deficient in queuosine synthesis is refractory to modification. Consequently, YadB provides one of the first definitive cases of a "closely

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related" aaRS paralog based on the class I scaffold in which the reaction is distinct from the aminoacylation reaction that provides direct precursors for protein synthesis.

Among the obvious questions springing from this novel observation are why this modification has eluded detection previously, and what is its specific role in bacterial physiology. At present, the former is much more easily addressed than the latter. The lability of the glutamyl-Q linkage in neutral and mildly alkaline solutions virtually guarantees that, under tRNA isolation conditions that promote the formation of deacylated tRNA, the Q34 glutamylation will be lost. When tRNA is isolated under mildly acidic conditions, like those used by Salazar *et al.* (9), the modification is preserved. This finding indicates that the whole question of the methodology of tRNA purification ought to be revisited, because current protocols may remove labile but functionally significant modifications. As for the functional significance of the Q34 modification, Salazar *et al.* provide clues but no definitive answers. Most pertinently, an *E. coli* strain in which YadB has been disrupted genetically is viable under standard growth conditions employing either minimal or rich media. This result suggests that, in keeping with a number of other tRNA modifications, the Q34 glutamylation may exert a subtle effect on translational fidelity that is restricted to a small but functionally significant group of *E. coli* messages. Along these lines, mutants in Q-base synthesis in *Shigella* have been shown to exhibit reduced virulence, a phenotype associated with reduced translation of the virulence factor VirF (14). This phenotype is akin to the requirements for Q-base synthesis in some cancerous cells (15), a fact that was recognized early on. In any event, final assignment of the function of this modification will ultimately require settling the question of whether YadB-mediated glutamylation extends to other Q-base-containing tRNAs, such as $tRNA^{His}$ and $tRNA^{Tyr}$. As with many other tRNA modifications, elucidating the role of glutamyl-Q may now require a more rigorous examination of the physiological consequences of its presence in tRNA (16).

For those with a more focused interest in protein–RNA interactions, YadB is remarkable as the first example of a synthetase-like protein whose enzymological activity is focused on the anticodon of tRNA, as opposed to its acceptor end, or just the cognate amino acid. The ability of YadB to aminoacylate a modified base in the anticodon creates a special structural challenge: how is the tRNA anticodon accommodated in the YadB active site? The recently solved crystal structure of YadB provides some indirect insight, in as much as $tRNA^{Glu}$ could not be docked onto YadB despite its high homology to GluRS (11). Furthermore, YadB contains a distinctly modified version of a zinc cluster used by GluRS for $tRNA^{Glu}$ acceptor stem recognition. These findings indicate how the removal of one RNA binding domain, and alteration of another, effectively excludes the ancestral substrate $tRNA^{Glu}$ while allowing the binding of another tRNA in an alternate conformation. At our present, limited level of understanding, how YadB manages to achieve this clever reversal of RNA specificity remains an attractive research question, and the ability of YadB to interact with other Q-base tRNAs cannot be predicted or excluded on structural grounds alone. Notably, YadB does not appear to share any structural homology with the tRNA-guanine transglycosylase that incorporates queuosine into the wobble position of tRNA (17).

With the finding that YadB encodes a functional tRNA modification activity, the overall role of tRNA synthetase paralogs in cellular physiology is becoming clearer. Essentially, aaRS paralogs appear to fall into two broad functional categories. Enzymes in the first grouping, which includes YadB, YbaK, and the other paralog-based isolated editing domains, all appear to increase the accuracy of interpretation of genetic information. Many of these proteins (including YadB or YbaK) increase translational fidelity through either effects on decoding or by transediting of misacylated tRNA (7, 8). To this category one might also add the B subunit of the mitochondrial DNA polymerase γ, which is structurally related to the α_2 glycyl-tRNA synthetase, and which confers increased processivity in DNA synthesis (6). The second major functional category of tRNA synthetase paralogs includes enzymes that provide a largely biosynthetic role, typically associated with the synthesis of amino acids and cofactors. Examples in this group include the biotin synthetase repressor (18), the asparagine synthetase A (5), and the HisZ regulatory subunit of the HisZ–HisG ATP PRTase (4). Remarkably, the specific evolutionary path taken by each paralog to arrive at these functions appears to be unique.

While most paralogs appear to have retained the original class I or class II core catalytic domain, as well as the ability to bind the cognate amino acid, the retention or loss of catalytic function in each paralog is idiosyncratic. For most paralogs, the simplest model is that they represent a duplicated aaRS that underwent further specialization to a function distinct from the production of aminoacylated tRNA for protein synthesis. In the case of AsnA and HisZ, the phylogenetic data reported suggest that both of the synthetase-like proteins evolved from functional tRNA synthetases. Hence, their evolution to assume functions associated with amino acid biosynthesis was accompanied by loss of specific binding of tRNA (5, 19). The evolution of AsnA also had to include the ability to activate aspartate on the β-carboxylate, as opposed to the α-carboxylate. By contrast, YadB retained the ability to form the adenylate, as well as the ability to carry out aminoacylation. At which stage during the evolution of YadB was the anticodon-binding domain lost? How does YadB maintain specificity despite having a more limited RNA–protein interface than a full-length tRNA synthetase? The structure of YadB certainly raises as many new questions as it answers, and it reiterates the surprising functional diversity of the ancient aaRS family.

Note Added in Proof. Some of the findings of Salazar *et al.* (9) have also been reported elsewhere (20).

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