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A minimalist glutamyl-tRNA synthetase dedicated to aminoacylation of the tRNA^{Asp} QUC anticodon

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ABSTRACT

***Escherichia coli* encodes YadB, a protein displaying 34% identity with the catalytic core of glutamyl-tRNA synthetase but lacking the anticodon-binding domain. We show that YadB is a tRNA modifying enzyme that evidently glutamylates the queuosine residue, a modified nucleoside at the wobble position of the tRNA^{Asp} QUC anticodon. This conclusion is supported by a variety of biochemical data and by the inability of the enzyme to glutamylate tRNA^{Asp} isolated from an *E.coli* tRNA-guanosine transglycosylase minus strain deprived of the capacity to exchange guanosine 34 with queuosine. Structural mimicry between the tRNA^{Asp} anticodon stem and the tRNA^{Glu} amino acid acceptor stem in prokaryotes encoding YadB proteins indicates that the function of these tRNA modifying enzymes, which we rename glutamyl-Q tRNA^{Asp} synthetases, is conserved among prokaryotes.**

INTRODUCTION

Aminoacyl-tRNA synthetases (aaRSs) constitute a family of essential and ubiquitous enzymes which synthesize 20 families of aminoacyl-tRNAs (aa-tRNAs) that are used by ribosomes for translation of mRNAs into proteins. These enzymes are modular proteins composed of functional units always containing a catalytic domain that activates amino acids before their transfer to tRNA and additional domains which include, in most cases, an anticodon-binding domain (1). As a consequence of this structural organization, the anticodon-binding domain alone binds the tRNA anticodon whereas the catalytic domain exclusively accommodates the accepting arm of tRNA and ensures aminoacylation exclusively at its 3′-end (2).

The accepted scheme of evolution of aaRSs proposes that the catalytic domains are derived from ancient enzymes that activated the amino acid and could transfer them to an acceptor RNA (3). The ability of minihelices derived from modern tRNAs (4,5), including tRNA^{Asp} (6), to be aminoacylated supports this scheme. Both molecules co-evolved by fusion of additional modules. A two-domain L-shaped tRNA emerged from the primitive tRNA by addition of the anticodon stem–loop, while aaRSs evolved by acquiring additional modules, including the anticodon-binding domain, which enhanced specificity for binding of the cognate full-length tRNA and efficiency of its aminoacylation (7). Despite the strong functional interdependence existing between the catalytic core and the anticodon-binding domain, some aaRSs still display functional reminiscences of the ancestral enzyme, since they can aminoacylate acceptor minihelices. However, depriving a tRNA of its anticodon branch or an aaRS of its anticodon-binding domain has effects ranging from a change in specificity of the reaction to a severe drop in aminoacylation efficiency and, often, its abolition (6,8). With regard of these findings, it came as a surprise to see that in sequences emerging from whole genome sequencing projects, a vast number of prokaryotes display open reading frames encoding pieces and even modules of aaRSs (9). Among these truncated aaRSs, six are composed of only the catalytic core of an aaRS. Unexpected functions have been assigned to those which have been studied so far, such as involvement in amino acid metabolism (reviewed in 10) or regulation of translation (11). However, they are all incapable of performing tRNA aminoacylation (10,12).

Escherichia coli encodes a protein, YadB, sharing 34% identity with the catalytic core of glutamyl-tRNA synthetase (GluRS) but deprived of the anticodon-binding domain (13). Unexpectedly, this mini-GluRS is able to aminoacylate tRNA. In contrast to the other GluRSs, YadB activates Glu in the absence of tRNA and, more strikingly, does not transfer the activated Glu to tRNA^{Glu} but to tRNA^{Asp} (14). We here provide a body of evidence indicating that this truncated

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GluRS attaches the activated Glu to the queuine (Q base) in the wobble position of this tRNA. A comparison of the sequences of $tRNA^{Glu}$ and $tRNA^{Asp}$ and modeling of the YadB–$tRNA^{Asp}$ complex suggests that aminoacylation by YadB is mediated by a structural mimicry between the $tRNA^{Asp}$ anticodon stem and loop and the $tRNA^{Glu}$ acceptor helix.

MATERIALS AND METHODS

General

Thin layer chromatography (TLC) cellulose plates (20 × 20 cm^2) and TSK HW-65 S were from Merck, hydroxyapatite CHT5 and UNO-Q1 columns from Bio-Rad and DEAE-cellulose DE52 from Whatman. L-[^{14}C]Asp (207 mCi/mmol), L-[^{14}C]Glu (238 mCi/mmol), L-[^{3}H]Glu (43 Ci/mmol) and [γ-^{32}P]ATP (5000 Ci/mmol) were from Amersham. Sodium periodate and dithionitrobenzoate were from Sigma, unfractionated *E.coli* tRNA and nuclease P1 from Boehringer, venom phosphodiesterase (VPH) from Worthington, apyrase from Sigma, T4 polynucleotide kinase from USB Corp. and RNase T2 was prepared by a classical procedure (15). *Escherichia coli* YadB was purified as described (14) and *E.coli* AspRS was a gift from Dr G.Eriani (IBMC, Strasbourg). The *E.coli* SJ1505 strain *tgt*$^-$ was a gift from Drs K.Reuter and H.Kersten (Erlangen, Germany).

Purification of *E.coli* $tRNA^{AspQ34}$ and $tRNA^{AspG34}$

Escherichia coli strains overexpressing native $tRNA^{AspQ34}$ and queuosine-lacking $tRNA^{AspG34}$ were gifts from Drs F.Martin and G.Eriani (IBMC, Strasbourg) (16). The tRNAs were extracted from overexpressing cells after overnight culture, using phenol saturated with 50 mM sodium acetate buffer pH 5.0, 0.1 mM $MgCl_2$ and 0.1 mM EDTA, followed by chloroform extraction and ethanol precipitation. Dried tRNA was dissolved in sodium acetate pH 5.0, adjusted to 2.5 M ammonium sulfate and precipitated on a 100 ml TSK HW-65 S column. Elution was performed with a linear gradient from 2.5 to 1.5 M ammonium sulfate in sodium acetate pH 5.0. The fractions containing $tRNA^{Asp}$ were pooled, adjusted to 2 M ammonium sulfate and loaded on a 2 ml DEAE-cellulose column before washing with 20 ml of water followed by elution with 1.5 M NaCl. tRNA, precipitated with ethanol and redissolved in 50 mM Tris–HCl pH 7.5 was loaded on a UNO-Q1 column equilibrated with the same buffer and eluted with a linear gradient from 0 to 1 M NaCl. Pure $tRNA^{AspQ34}$ and $tRNA^{AspG34}$ (accepting capacities 32 nmol/mg) were finally obtained by chromatography on a hydroxyapatite CHT-5 column and elution with a linear gradient from 10 to 200 mM potassium phosphate pH 6.8. *Escherichia coli*, *Thermus thermophilus* and yeast $tRNA^{Asp}$ transcripts were obtained as described (17).

Periodate oxidation of *E.coli* tRNA

One milligram of unfractionated *E.coli* tRNA was incubated for 20 min at room temperature in 1 ml of potassium phosphate buffer pH 6.8 containing 0.3 mM dithionitrobenzoate, precipitated with ethanol and treated for 30 min in the dark with 0.1 M sodium periodate in 0.25 ml of 50 mM sodium acetate buffer pH 5.0. After addition of 0.5 M KCl and 5% glycerol, the tRNA was ethanol precipitated, dialyzed, incubated for 3 h on ice in 0.3 ml of 0.1 M dithiothreitol, precipitated, redissolved in water and treated for 20 min with 0.1 M $NaBH_4$ in 0.5 ml of water. The tRNA precipitate was dissolved in 0.l ml of water and dialyzed. In some experiments, $tRNA^{Asp}$ was aspartylated or glutamylated, respectively, by AspRS or YadB prior to periodate oxidation and after oxidation, then deacylated by incubation for 30 min at 37°C in 1.8 M Tris–HCl pH 8.0.

Snake venom phosphodiesterase digestion of *E.coli* tRNA

An aliquot of 0.1 mg of unfractionated tRNA was incubated for 20 min at room temperature with 10 µg snake venom phosphodiesterase in 0.5 ml of 50 mM Tris–HCl pH 8.0. After phenol and chloroform extraction, the tRNA was precipitated with ethanol and dissolved in water.

Aminoacylation reactions

Standard reaction mixtures of 50–250 µl for determination of plateaus or initial rates of aminoacylation contained 100 mM Na HEPES buffer pH 7.2, 30 mM KCl, 10 mM $MgCl_2$, 25 µM L-[^{14}C]Asp (270 c.p.m./pmol) or L-[^{14}C]Glu (330 c.p.m./pmol), 0.1 mg/ml bovine serum albumin, 0.5 mg/ml unfractionated *E.coli* tRNA or 1–2 µM pure $tRNA^{AspQ34}$ or $tRNA^{AspG34}$ and appropriate concentrations of *E.coli* AspRS or YadB. The K_m for $tRNA^{AspQ34}$ was determined in the presence of 10 µM L-[^{3}H]Glu (2000 c.p.m./pmol) and 0.03–0.3 µM $tRNA^{AspQ34}$ and the K_i for $tRNA^{AspG34}$ with the concentration fixed at 0.18 µM. Reactions were conducted at 37°C and the radiolabeled aa-tRNA formed after incubation times ranging from 1 to 40 min was determined in 20 µl aliquots (18).

Analysis of the nucleotide content of $tRNA^{AspQ34}$ and $tRNA^{AspG34}$

One microgram of tRNA was digested for 2 h at 37°C with 0.2 U RNase T2 in 3 µl of 50 mM sodium acetate buffer pH 4.5. The nucleotides were 5′-^{32}P-labeled by incubation for 30 min at 37°C in the presence of 5 µCi (5 µl) [γ-^{32}P]ATP and 25 U T4 polynucleotide kinase. After 15 min treatment with 0.1 µg apyrase at 37°C, the 3′-phosphate of the nucleotides was dephosphorylated by incubation for 30 min at 37°C with 0.75 µg nuclease P1. One-third of the mix was analyzed by 2-dimensional TLC on cellulose plates developed with isobutyrate/ammonia/water (50/28/9) in the first dimension and HCl/isopropanol/water (15/70/15) in the second dimension and the nucleotides were revealed by autoradiography (19).

Docking of $tRNA^{Asp}$ into YadB

The coordinates of YadB, $tRNA^{Asp}$ and the GluRS-adenylate analog employed for docking are those deposited in the PDB, entries 1NZJ, 1EFW and 1N78. The loop 224–238, missing in the electron density map of YadB, has been modeled from that of *T.thermophilus* GluRS (1N78). The adenylate analog (GoA) and position of loop 224–238 were kept as provided by the rigid body superposition of YadB with GluRS complexed to a Glu-adenylate analog (1N78). The docking of $tRNA^{Asp}$ into YadB was performed with the Turbo-Frodo program (20) by manual rigid body rotation/translation; only two exposed sidechain conformations were modified.

RESULTS AND DISCUSSION

YadB does not aminoacylate the 3′ accepting end of tRNA^{Asp}

We have previously shown that YadB aminoacylates pure *E.coli* tRNA^{Asp} as efficiently as AspRS (14). Similar aminoacylation plateaux are obtained when pure tRNA^{Asp} is charged by each enzyme (80% by AspRS and 75% by YadB; Fig. 1A). Surprisingly, aminoacylation with both enzymes results in a plateau of 155% of charged tRNA, suggesting that both Asp and Glu acylate tRNA^{Asp} (Fig. 1A). The extent of total aminoacylation is independent of whether tRNA is charged first by AspRS or YadB. We confirmed that the two amino acids are attached to tRNA by TLC analysis after extraction of the aa-tRNA followed by hydrolysis of the ester bonds (Fig. 1B). Thus YadB can glutamylate an aspartylated tRNA^{Asp} (Asp-tRNA^{Asp}) and, conversely, AspRS can aspartylate a glutamylated tRNA^{Asp} (Glu-tRNA^{Asp}). An immediate interpretation could be that the two OH groups of the terminal adenosine are acylated with Asp and Glu, respectively, similarly to that reported for *T.thermophilus* PheRS able to catalyze the attachment of two Phe residues on the 3′-end of tRNA^{Phe} (21). However, for structural considerations, we felt this interpretation unlikely and favored attachment of the Glu residue on another part of the molecule.

To verify the latter possibility, we treated unfractionated *E.coli* tRNA with snake venom phosphodiesterase that hydrolyzes single-stranded RNAs starting from the 3′-OH end and thus removes the CCA end and the discriminator base of the tRNA molecules (Fig. 2A and B). As expected, *E.coli* tRNA^{Asp} lost its ability to be aspartylated by AspRS, but fully retained its capacity to be glutamylated by YadB. (Fig. 2A and B).

So far, all known aaRSs aminoacylate their cognate tRNA by forming an ester bond between the activated α-COOH group of the amino acid and the 2′- or 3′-OH group of the ribose *cis*-diol of the tRNA terminal adenosine (2,22). Periodate oxidation by opening the ribose ring deprives tRNA of its accepting capacity. However, aminoacylation protects the *cis*-diol of the terminal adenosine against oxidation and thus protects the tRNA against inactivation (23). Therefore, to test whether glutamylation of tRNA^{Asp} by YadB proceeds by esterification of a OH group, we oxidized unprotected and protected *E.coli* tRNA^{Asp} with periodate and analyzed their remaining accepting capacities. Figure 2C shows that YadB, like AspRS, is unable to charge periodate oxidized tRNA^{Asp}. However, tRNA^{Asp} protected by glutamylation with YadB prior to periodate oxidation is still aminoacylated by YadB, but not by AspRS. Likewise, tRNA^{Asp} aspartylated by AspRS prior to periodate oxidation retains its aspartylation ability, but is no longer glutamylatable by YadB (not shown).

Altogether, the obtained data demonstrates that aminoacylation by YadB does not occur at the terminal adenosine of tRNA^{Asp} and should take place at another place on the tRNA^{Asp} molecule. This conclusion is supported by a previous result showing that the mischarged tRNA^{Asp} cannot bind the elongation factor EF-Tu (14), despite the capability of a canonical Glu-tRNA^{Asp}, i.e. when charged on the 3′-end aa-tRNA, to bind the factor (24).

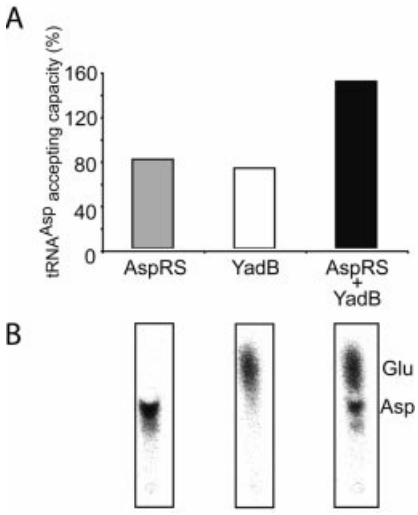

Figure 1. Analysis of the amino acid accepting capacity of *E.coli* tRNA^{Asp} when aminoacylated by AspRS, YadB and by both. (**A**) Extents of pure tRNA^{Asp} charging with *E.coli* AspRS (gray bar), YadB (white bar) or AspRS and YadB (black bar). (**B**) The amino acids acylating tRNA^{Asp} are identified by TLC as described (10).

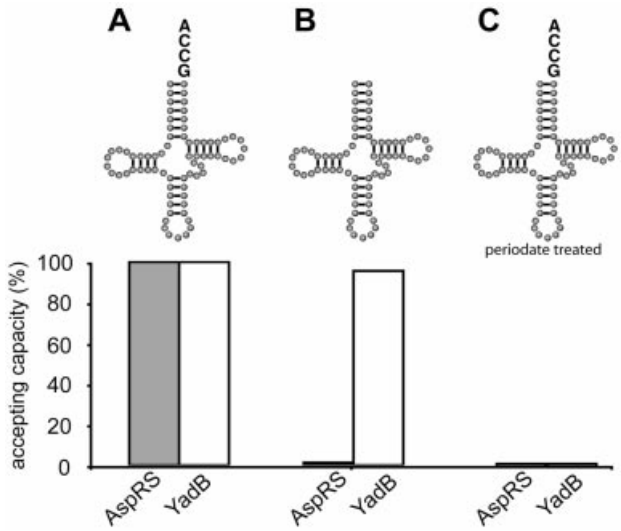

Figure 2. Protection of tRNA^{Asp} amino acid accepting capacity by *E.coli* AspRS or YadB catalyzed aminoacylation prior to periodate oxidation. (**A**) Extent of tRNA^{Asp} aspartylation by AspRS (gray bars) and glutamylation by YadB (white bars). (**B**) The same experiment with tRNA^{Asp} lacking its single stranded 3′ terminus. (**C**) Same experiments with tRNA^{Asp} treated with periodate.

YadB aminoacylates the queuosine located in the first position of tRNA^{Asp} anticodon

Analysis of the chemical structure of the modified nucleosides present in *E.coli* tRNA^{Asp} (25) reveals that the queuosine (Q) in the first position of the anticodon (position 34 of tRNA) possesses a cyclopentenyldiol ring containing a *cis*-diol that could possibly mimic the 2′- and 3′-OH groups of the ribose of the terminal adenosine (26–28) (Fig. 3A). Introduction of queuosine into tRNA is achieved by a complex cascade of enzymatic reactions involving tRNA-guanine transglycosylase (TGT), which results by its exchange with G34 (29).

To check whether glutamylation of tRNA^{Asp} by YadB is dependent on the presence of queuosine we first attempted to glutamylate the *E.coli* tRNA^{Asp} transcript deprived of modified nucleotides. Table 1 shows that pure *E.coli* tRNA^{Asp} is fully charged by YadB, whereas the transcript, deprived of post-transcriptional modifications but instead containing G34, is not charged by YadB. Likewise, *Saccharomyces cerevisiae* and *T.thermophilus* tRNA^{Asp} deprived of Q (30,31) are fully aspartylated but not charged by YadB.

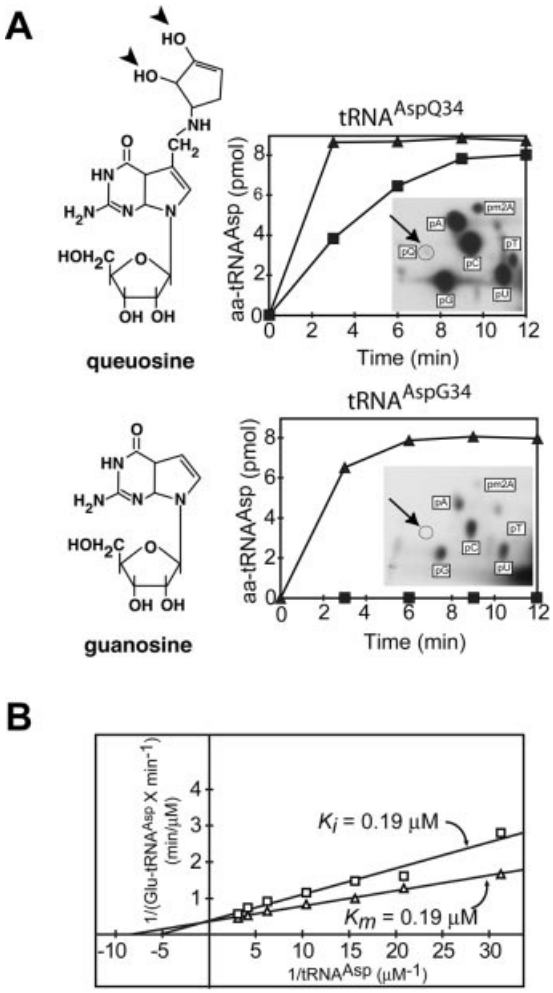

Figure 3. Amino acid accepting capacities of *E.coli* native tRNA^{AspQ34} and queuosine-lacking tRNA^{AspG34}. (**A**) Kinetics of aminoacylation of tRNA^{AspQ34} (top) and tRNA^{AspG34} (bottom) by *E.coli* AspRS (black triangles) and YadB (black squares). (Insets) 2-Dimensional TLC analysis of the 5′ phosphate nucleosides present in the two tRNAs (the arrows indicate the positions where pQ migrates). The structures of Q and G are shown at the left with the arrows indicating the OH groups of the cyclopentenyldiol ring that are the putative Glu acceptors on Q. (**B**) Lineweaver–Burk plots allowing determinations of the K_m (open triangles) of YadB for native tRNA^{Asp} and K_i (open squares) of tRNA^{AspQ34} for aminoacylation of native tRNA^{Asp} by YadB.

To unambiguously prove that YadB aminoacylates the queuosine of *E.coli* tRNA^{Asp}, we purified a variant of it containing all modified nucleosides except Q34 (tRNA^{AspG34}) and compared its glutamylation capacity with that of the wild-type tRNA^{Asp} (tRNA^{AspQ34}). tRNA^{AspG34} was purified from an *E.coli tgt*$^-$ strain, unable to introduce Q into tRNAs. As expected, both tRNAs are completely aspartylated by *E.coli* AspRS (Fig. 3A). However, while YadB fully aminoacylated tRNA^{AspQ34}, this enzyme was unable to glutamylate tRNA^{AspG34} (Fig. 3A). As confirmed by 2-dimensional TLC analysis of tRNA hydrolysates, the two tRNA variants differ only in the absence of Q in tRNA^{AspG34} (Fig. 3A, insets). A further confirmation that Q34 is the acceptor of the activated Glu was obtained by inhibition of glutamylation of wild-type tRNA^{Asp} in the presence of tRNA^{AspG34}. Figure 3B shows that tRNA^{AspG34} competitively inhibits charging of tRNA^{AspQ34} by YadB, which displays the same affinity for both tRNAs, as indicated by the kinetic constants (K_m for tRNA^{AspQ34} and K_i for tRNA^{AspG34} of 0.19 μM). Since both tRNAs bind with the same affinity to YadB, whereas the protein charges only the species containing Q, it is concluded that glutamylation by YadB occurs on Q.

What are the structural elements that promote aminoacylation of the *E.coli* tRNA^{Asp} anticodon by YadB?

In *E.coli*, tRNA^{Asp}, tRNA^{Asn}, tRNA^{His} and tRNA^{Tyr} contain Q in the wobble position of their anticodons (32), yet only Q34 of tRNA^{Asp} is glutamylated by YadB (14). This indicates that Q is not solely responsible for the recognition of tRNA^{Asp}. In fact, as shown above, it does not contribute to the affinity of tRNA^{Asp} for YadB.

To identify potential determinants responsible for the interaction of tRNA^{Asp} with YadB, we compared the tRNA^{Asp} and tRNA^{Glu} sequences from *E.coli*. This comparison revealed a striking mimicry between the tRNA^{Asp} anticodon stem and loop and the tRNA^{Glu} acceptor arm (Fig. 4A). The first four base pairs of the tRNA^{Glu} acceptor arm are identical to the first four base pairs of the tRNA^{Asp} anticodon stem, while the discriminator G73 residue and C74 of tRNA^{Glu} align with G39 from the tRNA^{Asp} anticodon stem and C38, the last nucleotide of the anticodon loop, respectively.

Expanding the comparative analysis to all tRNA^{Glu} and tRNA^{Asp} species of bacteria encoding YadB genes, we observed partial conservation of the sequence mimicry (Fig. 4A). Interestingly, the sequence conservation in the

Table 1. Aspartylation and glutamylation of tRNA^{Asp} of various origins possessing either G34 or Q34

Enzyme	Extent of charging (%)						
	E.coli tRNA^{Asp}			*T.thermophilus* tRNA^{Asp}		*S.cerevisiae* tRNA^{Asp}	
	AspG34[a]	AspG34	AspQ34	AspG34[a]	AspG34	AspG34[a]	AspG34
AspRS	37	85	85	100	100	75	100
YadB	n.c.	n.c.	93	n.c.	n.c.	n.c.	n.c.

AspRS and YadB were from *E.coli*. n.c., not charged.
[a]tRNA^{Asp} transcript; other tRNAs were modified tRNA^{Asp} versions.

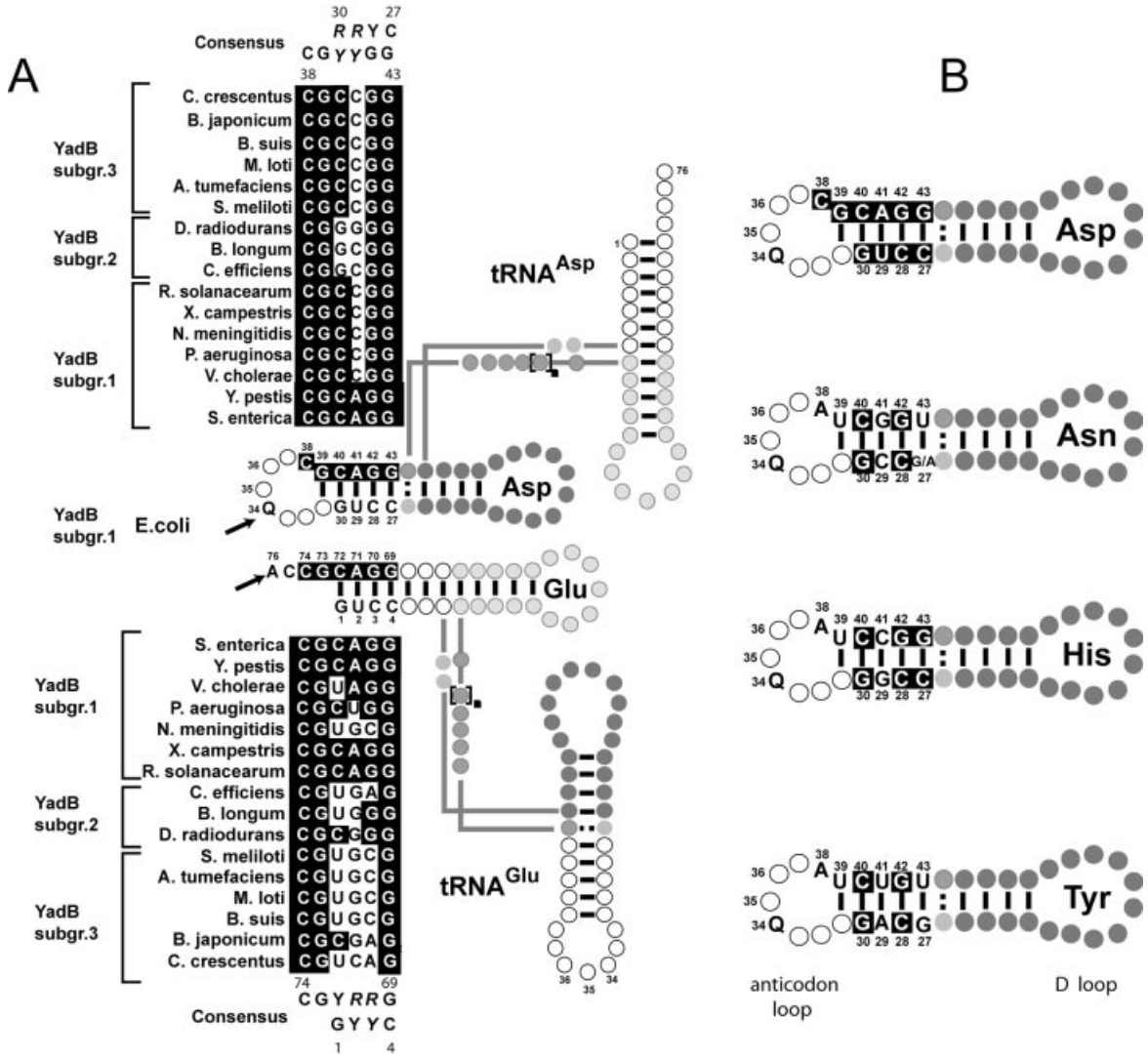

Figure 4. Sequence comparison of $tRNA^{Glu}$ acceptor arm and $tRNA^{Asp}$ anticodon stem and loop from bacteria encoding YadB and expansion of the comparison to $tRNA^{Asn}$, $tRNA^{His}$ and $tRNA^{Tyr}$ anticodon stems and loops. tRNAs from 17 bacterial species were compared. (**A**) *Escherichia coli* $tRNA^{Asp}$ and $tRNA^{Glu}$ are presented in a 2-dimensional projection of their L-shaped architecture. Nucleotides targeted by the comparison are displayed and the amino acid-accepting nucleotides (Q34 and A76) indicated by an arrow. Numbering of nucleotides is according to Sprinzl *et al*. (32). For each bacterial species $tRNA^{Asp}$ anticodon stem and loop positions (base pairs 27–43 to 30–40 and nucleotides 39 and 38) were compared to the corresponding positions of the $tRNA^{Glu}$ acceptor stem (base pairs 4–69 to 1–72 and nucleotides 73 and 74) from the same species. When nucleotides are the same as in $tRNA^{Asp}$ and $tRNA^{Glu}$ from *E.coli* tRNAs, they are displayed in white on a black box. For clarity, only nucleotides 43–38 from $tRNA^{Asp}$ and 69–74 from $tRNA^{Glu}$ are displayed. Consensus sequences of the 38–43 stretches in $tRNA^{Asp}$ and 74–68 stretches in $tRNA^{Glu}$ are given together with the consensus of the complementary residues (30–27 and 1–4 stretches). Residues in italic are partially conserved; R stands for purine and Y for pyrimidine. The sequences displayed are ranked according to the YadB classification (14). (**B**) 2-Dimensional representation of *E.coli* $tRNA^{Asp}$ and the consensus sequences in the anticodon branch of the 17 bacterial species. When a nucleotide is the same as its counterpart from the consensus sequence of $tRNA^{Glu}$ it is displayed in white on a black box.

two tRNAs follows the phylogenetic classification of the YadB proteins in three structure-based subgroups (14). Thus complete mimicry between the two tRNAs is found in the two γ-proteobacteria, *Yersinia pestis* and *Salmonella enteritica*, that are most closely related to *E.coli* with respect to their YadB proteins (Fig. 4A). Mimicry between $tRNA^{Asp}$ and $tRNA^{Glu}$, as found in *E.coli*, becomes looser in bacteria, where the divergence between YadB proteins increases, but interestingly high mimicry exists between tRNAs of organisms with YadB proteins from the same subgroup.

Altogether, this comparative analysis indicates that four nucleotides from the 5′-side of the anticodon stem–loop of the $tRNA^{Asp}$ species are conserved (namely C38 from the anticodon loop as well as G39, G42 and G43 from the anticodon stem) and three are present in a 6 nt sequence stretch from the 3′-terminus of the corresponding $tRNA^{Glu}$ species (namely single-stranded C74 from the CCA terminus and discriminator residues G73 and G69). This sequence mimicry with the anticodon branch of $tRNA^{Glu}$ is never found in $tRNA^{Asn}$, $tRNA^{His}$ and $tRNA^{Tyr}$ that possess Q in position 34 but in which G39 and C38 are always replaced by U39 and A38 (Fig. 4B). Likewise, no such mimicry is found between eukaryotic $tRNA^{Asp}$ and $tRNA^{Glu}$ species. In conclusion, this suggests that those residues from the consensus highlighted in

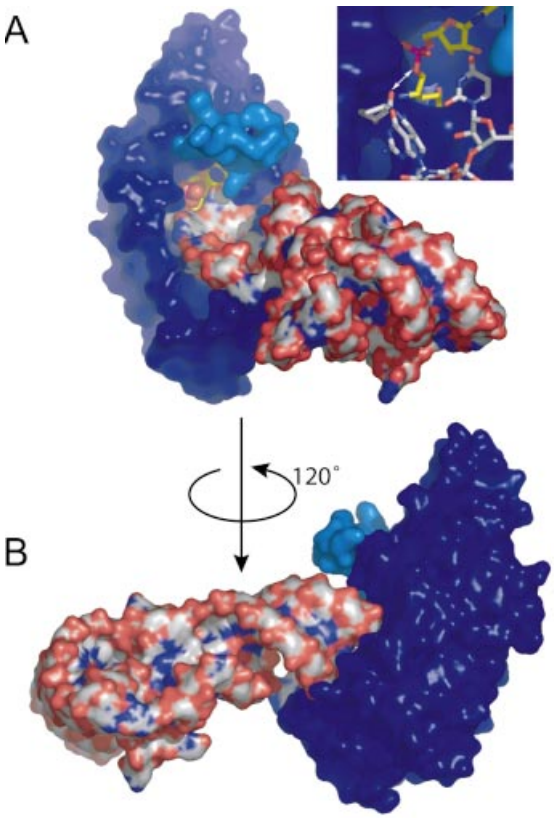

Figure 5. A tentative model of the complex of *E.coli* $tRNA^{Asp}$ with cognate YadB. (**A**) The YadB molecular surface is displayed in dark blue, the loop in lighter blue and adenylate in sphere representation. The docking procedure was based on the constraint that the reactive group, the cyclopentenyldiol ring of Q34, like the ribose from A76, should be close to the Glu–adenylate phosphate–Glu scissile bond. The Q34 cyclopentenyldiol ring was therefore used as a pivot around which the tRNA rigid body moved. The $tRNA^{Asp}$ is displayed as an atom color coded molecular surface. (Inset) Close-up of the interaction illustrating the vicinity of the adenylate donor molecule and the acceptor group, Q34 of $tRNA^{Asp}$. Adenylate and the $tRNA^{Asp}$ domain with Q base are displayed in stick representation. (**B**) Rotation of ~120° around a vertical axis and ~30° clockwise in the plane.

Figure 3, especially C38, G39, G42 and G43, are critical for recognition of $tRNA^{Asp}$ by YadB and, conversely, their non-conservation in $tRNA^{Asn}$, $tRNA^{His}$ and $tRNA^{Tyr}$ contribute to the rejection of these Q-containing tRNAs by YadB proteins.

To gain a deeper insight into the structural elements that could promote $tRNA^{Asp}$ anticodon recognition and aminoacylation by YadB, the minimal GluRS, we constructed a docking model of *E.coli* YadB and *E.coli* $tRNA^{Asp}$ (Fig. 5). By aligning the two similar helical parts of the $tRNA^{Asp}$ anticodon stem (residues 24–45) and $tRNA^{Glu}$ acceptor stem (without $_{73}GCCA_{76}$) we observed that in $tRNA^{Asp}$ Q34 protrudes in a loop, while in $tRNA^{Glu}$ the GCCA stem residues fold back in the opposite direction. As a consequence, the amino acid accepting OH groups of $tRNA^{Asp}$ (Q34) and $tRNA^{Glu}$ (A76) are presented in different conformations. Therefore, simple docking of $tRNA^{Asp}$ to YadB, without structural deformation of the anticodon loop, is not compatible with 'classical' tRNA binding in the catalytic domain of a class I synthetase. However, the tRNA could be fitted with only the ~30–38 segment in the groove (Fig. 5). This position is correct with regard to shape complementarity and to reactive group closeness (Fig. 5, inset). Note that the interacting surface between YadB and $tRNA^{Asp}$ is quite large in the tentative docking model: 1100 $Å^2$ of YadB (~10% of the total surface) are shielded from solvent in the complex. In this model the interacting protein surface is slightly reduced as compared to the complexes involving class I GluRS in which 1600 $Å^2$ are shielded from the solvent (33).

Functional and evolutionary considerations

Data reported here break down the current paradigm underlying all the research on tRNA recognition and aminoacylation by aaRSs, namely that the catalytic domain of synthetases recognizes the amino acid branch of tRNAs and attaches the activated amino acid via an ester bond to their 3′-CCA_{OH} termini. All the literature on structural and functional biology in the field has been exclusively based on this paradigm. Therefore, it was a great surprise to discover that a protein closely mimicking the catalytic domain of *E.coli* GluRS, namely the YadB protein from *E.coli*, recognizes the anticodon branch of a tRNA and attaches an amino acid on a modified base from the tRNA anticodon. Interestingly, YadB has the ability to activate Glu (14), although without the assistance of $tRNA^{Glu}$, as is the case of canonical GluRSs (34), but also the unexpected ability to interact with the distal extremity of *E.coli* $tRNA^{Asp}$ and to acylate the cyclopentenyldiol ring of the Q base post-transcriptionnally inserted at position 34 of the anticodon. This ring, with two *cis*-diol residues, mimics the structure of the 3′-terminal ribose of tRNA and thus Glu is attached to the Q base via an unstable ester bond (half-life 7.5 min) (14). This explains why the presence of a glutamylated Q base (which can be abbreviated as Glu-Q) in *E.coli* has escaped identification up to now. Docking of the $tRNA^{Asp}$ anticodon branch on YadB (Fig. 5) suggests that this branch has physical contact with the protein, a conclusion in agreement with the competition of $tRNA^{Asp}$ deprived of Q for glutamylation of native $tRNA^{Asp}$ with Q34 (Fig. 3B). This *a priori* unpredicted biological function of YadB as a tRNA modifying enzyme recognizing the anticodon stem and loop of tRNA can be generalized to other YadB-containing microorganisms, as supported by sequence comparison of their $tRNA^{Asp}$ and $tRNA^{Glu}$ (Fig. 4). Therefore, YadB proteins can be renamed glutamyl-Q $tRNA^{Asp}$ synthetases (Glu-Q-RSs).

From another perspective, one can question the evolutionary origin of the YadB proteins. Whether an ancestral YadB protein was a precursor of modern prokaryotic GluRSs or whether the YadB family originates from an ancestral GluRS remains an open question. However, their contemporary biological function has likely a recent evolutionary origin, given the reaction that YadB enzymes catalyze. Because the presence of YadB proteins is restricted to prokaryotic organisms, glutamylation of the Q base in $tRNA^{Asp}$, which only occurs in such organisms, is intriguing, since Q base is also found in eukaryotes. However, hypermodified Q residues have been detected in higher eukaryotes, with a mannosyl or galactosyl residue instead of a Glu fixed on the cyclopentenyldiol ring. Note also that the mannosyl and galactosyl moieties, like the ribose and the cyclopentenyldiol ring,

possess two adjacent *cis*-diols that could also be potential amino acid acceptors via catalysis by aaRS-mimicking proteins. These observations raise interesting questions about the structure of the enzymes attaching mannose or galactose to Q bases and about the possible existence of proteins that may esterify these sugars. Like YadB, such proteins could be aaRS-mimicking proteins.

To conclude, let us comment on tRNA structure in the light of the new paradigm and of concepts underlying the origin of tRNA and the genetic code. Here it is shown that a mimic of an aaRS catalytic domain makes no fundamental difference in recognizing a tRNA anticodon branch instead of a tRNA amino acid accepting branch. This property of YadB is in line with the idea that contemporary tRNA originated by fusion of two primordial minihelix or stem–loop structures (3). Likewise, primordial aaRSs were simplified structures restricted to their catalytic core, existing in nature as two versions (the class I and the class II versions), that became more complex in later evolution by the addition of extra domains, the most important being the anticodon-binding domain (1). In the course of such structural tinkering it is likely that primitive aaRSs were able to recognize a variety of RNA architectures with tRNA features. This potential recognition was maintained in some modern proteins as a remnant of their early evolution. YadB (Glu-Q-RS) is such a protein. We anticipate that other aaRS mimics remain to be discovered and believe that such proteins will use their catalytic and RNA recognition potential in participating in metabolic pathways as yet undeciphered.

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