Recognition of tRNA\textsuperscript{Gln} by \textit{Helicobacter pylori} GluRS2—a tRNA\textsuperscript{Gln}-specific glutamyl-tRNA synthetase

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Received July 6, 2009; Revised and Accepted August 27, 2009

ABSTRACT

Accurate aminoacylation of tRNAs by the aminoacyl-tRNA synthetases (aaRSs) plays a critical role in protein translation. However, some of the aaRSs are missing in many microorganisms. \textit{Helicobacter pylori} does not have a glutaminyl-tRNA synthetase (GlnRS) but has two divergent glutamyl-tRNA synthetases: GluRS1 and GluRS2. Like a canonical GluRS, GluRS1 aminoacylates tRNA\textsuperscript{Glu1} and tRNA\textsuperscript{Glu2}. In contrast, GluRS2 only misacylates tRNA\textsuperscript{Gln} to form Glu-tRNA\textsuperscript{Gln}. It is not clear how GluRS2 achieves specific recognition of tRNA\textsuperscript{Gln} while rejecting the two \textit{H. pylori} tRNA\textsuperscript{Glu} isoacceptors. Here, we show that GluRS2 recognizes major identity elements clustered in the tRNA\textsuperscript{Gln} acceptor stem. Mutations in the tRNA anticodon or at the discriminator base had little to no impact on enzyme specificity and activity.

INTRODUCTION

In protein translation, each aminoacyl-tRNA synthetase (aaRS) recognizes and connects its cognate tRNA to its cognate amino acid (aa), forming a specific aminoacyl-tRNA (or isoacceptor set). These aminoacyl-tRNAs are then brought into the ribosome by elongation factor (EF-Tu) where they are used in protein translation. Intuitively, a complete set of 20 aaRSs is required with one enzyme matching each of the cognate 20 amino acids to the appropriate tRNA(s) (1). However, many microorganisms lack a full set of aaRSs. For example, \textit{Helicobacter pylori} has two tRNA\textsuperscript{Glu} isoacceptors; Figure 1). GluRS2 is consequently responsible for the bio-synthesis of Glu-tRNA\textsuperscript{Gln}. Interestingly, although GluRS1 and GluRS2 are closely related, GluRS2 does not make Glu-tRNA\textsuperscript{Glu} (13,14).

Instead of a canonical ND-GluRS, \textit{H. pylori} and a small subset of other bacteria utilize two paralogous GluRSs—GluRS1 and GluRS2. \textit{Helicobacter pylori} GluRS1 is discriminatory and only generates Glu-tRNA\textsuperscript{Glu1} and Glu-tRNA\textsuperscript{Glu2}. (\textit{H. pylori} has two tRNA\textsuperscript{Glu} isoacceptors; Figure 1). GluRS2 is consequently responsible for the bio-synthesis of Glu-tRNA\textsuperscript{Gln}. Interestingly, although GluRS1 and GluRS2 are closely related, GluRS2 does not make Glu-tRNA\textsuperscript{Glu} (13,14).

The close evolutionary relationship between GluRS1 and GluRS2 and the unusual non-cognate tRNA\textsuperscript{Gln} specificity of GluRS2 led to the proposal that GluRS2 could represent an abortive or ongoing attempt by bacteria to evolve a bacterial GlnRS (14). (All known glutamyl- and aspartyl-tRNA synthetases (ND-GluRS and ND-AspRS) misacylate tRNA\textsuperscript{Gln} and tRNA\textsuperscript{Asn}, respectively, to generate Glu-tRNA\textsuperscript{Gln} and Asp-tRNA\textsuperscript{Asn} (Reactions 1 and 3, respectively, misacylated tRNAs are shown in bold) (1,12). These enzymes are non-discriminatory because they still recognize and aminoacylate their cognate tRNAs to generate Glu-tRNA\textsuperscript{Glu} and Asp-tRNA\textsuperscript{Asp} (Reactions 2 and 4, respectively). Next, a glutamine-dependent amidotransferase (AdT) identifies and repairs Glu-tRNA\textsuperscript{Gln} and Asp-tRNA\textsuperscript{Asn} to generate Glu-tRNA\textsuperscript{Gln} and Asn-tRNA\textsuperscript{Asn}, respectively (AdT rxn not shown) (4,6,9).

(1) ND-GluRS: Glu + ATP + tRNA\textsuperscript{Gln} $\rightarrow$ Glu-tRNA\textsuperscript{Gln} + AMP + PPi
(2) ND-GluRS: Glu + ATP + tRNA\textsuperscript{Glu} $\rightarrow$ Glu-tRNA\textsuperscript{Glu} + AMP + PPi
(3) ND-AspRS: Asp + ATP + tRNA\textsuperscript{Asp} $\rightarrow$ Asp-tRNA\textsuperscript{Asp} + AMP + PPi
(4) ND-AspRS: Asp + ATP + tRNA\textsuperscript{Asn} $\rightarrow$ Asp-tRNA\textsuperscript{Asn} + AMP + PPi

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GlnRSs originated in eukarya (15–18) and the factors that have prevented the emergence and/or utilization of GlnRS in most bacteria are not well understood.) It has also been proposed that the divergence of GluRS1 and GluRS2 occurred to accommodate changes in the length of the tRNA<sup>Glut</sup> and tRNA<sup>Glu2</sup> D-stems (4 versus 3 base pairs, respectively) (13).

We are interested in understanding how GluRS2 diverged from GluRS1 to gain unique specificity for tRNA<sup>Gln</sup> while rejecting the two tRNA<sup>Glu</sup> isoacceptors tRNA<sup>Glu1</sup> and tRNA<sup>Glu2</sup>. We have previously shown that a single point mutation in the anticodon-binding domain of GluRS2 converts this enzyme into one that only aminoacylates tRNA<sup>Glu1</sup> instead of tRNA<sup>Gln</sup>, demonstrating recognition of the tRNA anticodon by GluRS2. Unexpectedly, this G417T mutation did not induce aminoacylation activity towards tRNA<sup>Glu2</sup>, despite the fact that this tRNA has the same UUC anticodon (19). In order to identify the mechanisms used by GluRS2 to select tRNA<sup>Gln</sup> and reject tRNA<sup>Glu2</sup> here we have introduced varying degrees of tRNA<sup>Glu2</sup> character into tRNA<sup>Gln</sup>. Analysis of these tRNAs demonstrates that the anticodon loop and the discriminator base are not identity elements for GluRS2 aminoacylation of tRNA<sup>Gln</sup>. Instead, the major identity elements are localized in the acceptor stem of tRNA<sup>Gln</sup>. These results are put into an evolutionary context.

**MATERIALS AND METHODS**

**Materials**

Oligonucleotides were purchased from Invitrogen and used without further purification. The pCR 2.1 TOPO plasmid was also from Invitrogen. Pfu polymerase was purchased from Stratagene. Taq polymerase was from New England Biolabs. Radiolabeled glutamate (L-[3,4-<sup>3</sup>H]-glutamic acid) was purchased from Perkin Elmer. All buffers were filtered through a 0.22 μm filter prior to use. When appropriate, solutions were autoclaved. Unless otherwise stated, reagents were used without further purification. All gene constructs were verified by DNA sequencing of the entire gene insert.

**Cloning of Hp tRNA variants**

For each tRNA chimera, two partially overlapping primers were designed to reconstitute the entire tRNA gene with appended BamHI and EcoRI restriction sites onto the 5′- and 3′-ends of the gene, respectively (see Supplementary Table S1). Each primer pair was used in a template-independent polymerase chain reaction (PCR) with Pfu polymerase. Each PCR product was inserted into the pCR2.1 TOPO vector after incubation with Taq polymerase. The correct insert was verified by DNA sequencing and then sub-cloned into the BamHI and EcoRI sites of the pES300 vector to enable isopropyl β-D-1-thiogalactopyranoside (IPTG)-induced in vivo overtranscription of the cloned tRNA gene (14).

Mutations were introduced by QuickChange<sup>®</sup> mutagenesis according to the directions provided by Stratagene. Primer sequences are provided in Supplementary Table S1.

**Overtranscription and purification of Hp tRNAs and tRNA chimeras**

Each tRNA and chimera was overexpressed in the *Escherichia coli* strain MV1184 at 37°C in Luria Broth (500 ml LB) supplemented with ampicillin (100 μg/ml) and glucose (0.5% w/v). When the A600 nm was between 0.4 and 0.6, IPTG (to a final concentration of 1 mM) was added to induce production of the tRNA. Cells were pelleted by centrifugation 4–5 h after induction and stored at −80°C for future use. Each overproduced tRNA was purified by Nucleobond affinity chromatography (Clontech) as previously described (14). This procedure generates a mixture of *E. coli* tRNAs that is enriched with the encoded *H. pylori* tRNA of interest. In vitro tRNA production was conducted (instead of in vitro transcription) to allow for the introduction of a 2-thiouridine at position 34 of all three wild-type tRNAs and all mutations. This modification is essential for aminoacylation by GluRS (20). Thus, any tRNAs that lack this modification will have negligible activity in the experiments described below.

The concentration of each tRNA was determined using GluRS1 or GluRS2, depending on the substrate specificity...
of the tRNA, in standard aminoacylation assays (see below). Expression of tRNA variants that were not robust substrates for either GluRS1 or GluRS2 was verified by urea gel and northern blot (see next section). Final tRNA concentrations were highly variable and ranged from being too low to accurately quantify (<100 pmol/A260 nm) to ~700 pmol/A260 nm (see Supplementary Table S3).

Northern blots of acceptor mutations and D stem/loop tRNAs

Northern blots were performed for tRNAs that could not be robustly quantified by aminoacylation. Total tRNA concentration was measured by A260 nm value; either 0.5 or 0.05 A260 nm aliquots were used for analysis of each tRNA. Each tRNA was diluted to 40 μl in 100 mM NaOAc, pH 5.0, 8 M urea, 0.05% (w/v) xylene cyanol; the sample was boiled for 5 min. Each sample (20 μl) was immediately loaded onto a 12% urea gel. Electrophoresis was performed for 75 min at 150 V. The tRNAs were transferred from the gel to an Immobilon-NY+ membrane (Millipore) using a Semi-Dry Blotting Unit (Fisher Biotech); transfer was conducted with a 32P-labeled oligonucleotide (TLH-14C: 5' TCAGATTGCCAGGACCAA 3') selected to be specific for all mutant tRNAs (15). The membrane was washed four times with 20 ml of the following buffer: 450 mM NaCl, 90 mM Tris–HCl, pH 8.0, 6 mM Na2EDTA, 0.1% SDS w/v, before exposure to a storage phosphor screen (Molecular Dynamics). Bound radioactivity was visualized using a Typhoon 9210 (Amersham Biosciences).

Aminoacylation assays with GluRS1 and GluRS2

*Helicobacter pylori* GluRS1 and GluRS2 were purified to homogeneity as previously described (14). Aminoacylation reactions were conducted in 40 mM HEPES-OH, pH 7.5, 4 mM ATP, 8 mM MgCl2, 200 μM unlabelled Glu and 50 μCi ^3^H-Glu at 37°C. For assays aimed at measuring the expression level of different tRNAs, the experiments were performed for 90 min with 1 μM GluRS1 or GluRS2. For initial rate assays, 0.1 μM GluRS1 or GluRS2 was used with 10 μM enriched tRNA (concentration was estimated from A260 nm readings) and time points were taken at shorter intervals. The unit definition of GluRS1 is defined as the amount of enzyme that aminoacylates 0.1 pmol tRNA^Glu1^ per second; a unit of GluRS2 aminoacylates tRNA^Gln^ at a rate of 0.1 pmol per second (14). All assays were conducted in triplicate and the reported error measurements reflect standard deviation.

**RESULTS**

The tRNA acceptor stem is important for the tRNA^{Gln} specificity of GluRS2

There are two tRNA^{Glu} isoacceptors in *H. pylori*—tRNA^{Glu1} and tRNA^{Glu2}—and one tRNA^{Gln} (2) (Figure 1; the sequences of *E. coli* tRNA^{Glu} and tRNA^{Gln} are given for comparison, with known identity elements marked by circles and squares) (21–23). While tRNA^{Glu1} and tRNA^{Gln} share 78% sequence identity, *H. pylori* GluRS2 apparently uses different mechanisms to reject these two tRNAs. It has been shown that a single mutation in the GluRS2 anticodon-binding domain can switch this enzyme’s tRNA substrate specificity from tRNA^{Gln} to tRNA^{Glu1}. However, this mutated GluRS2 failed to aminoacylate tRNA^{Glu2} (19).

A series of four tRNA chimeras were designed according to different domains of the tRNA. These tRNA^{Glu2} chimeras each contain ~75% tRNA^{Gln} and ~25% tRNA^{Glu2} character (Figures 2 and 3; Supplementary Table S2). Chimera 2 was further modified to contain the tRNA^{Glu2} variable loop in order to maintain stable tertiary structure (21). All chimeras, including chimera 3, retain the tRNA^{Gln} anticodon. Each tRNA was overtranscribed *in vivo* and purified by ion exchange chromatography, as previously described (14). Levels of overexpression were quantified by aminoacylation assays using excess GluRS1 and GluRS2, and results from the assay that produced the highest aminoacylation levels were used. (Note: The calculated expression levels for each tRNA are included in Supplementary Table S3.)

Each chimeric tRNA was assayed with GluRS1 and with GluRS2; for comparison, identical assays were conducted with the three wild-type *H. pylori* tRNAs. The results of these initial rate assays are shown in Figure 2 (for wild-type tRNAs) and in Figure 3 (for the chimeric
tRNAs). (Note: the primary sequences of these chimeras and all other tRNA constructs are included in the Supplementary Table S2). Only Chimera 1, wherein the acceptor stem of tRNAGlu2 was transplanted into tRNAGln, has tRNA Glu2-like activity. Unlike the parent tRNAGln, Chimera 1 is a strong substrate for GluRS1 but not for GluRS2. In contrast, Chimeras 2, 3 and 4 all retain tRNA Gln-like activity as they are predominantly aminoacylated by GluRS2 but not by GluRS1. These results argue that the key identity elements for GluRS2 recognition of tRNA Gln are localized only within the acceptor stem of tRNA Gln. In fact, the aminoacylation profiles of Chimeras 3 and 4 are virtually indistinguishable from that of tRNA Gln. (Note: For tRNA Gln, the low observed rate of GluRS1-catalyzed aminoacylation is the result of aminoacylation of contaminating E. coli tRNAs (14); thus, it is likely that the GluRS1 data for chimeras 2–4 is misleadingly high. Given the negligible impact that these chimeras had on GluRS2 activity, the role of contaminating tRNAs was not investigated further).

Interestingly, Chimera 2 is actually a better substrate for GluRS2 than for tRNAGln. This result is seemingly in contradiction with the proposed role of the D-stem length in the emergence of GluRS2 (13); see below for further discussion and analysis.

Based on these results and the known identity elements for other tRNA Gln aminoacylation systems (21–23), we chose to further dissect the acceptor stem, D-stem/loop, and anticodon stem/loop to more precisely define the role(s) of these regions and to confirm the unexpected results that neither the D-stem/loop nor the anticodon stem/loop are strong sources of identity for GluRS2 recognition of tRNA Gln.

The role of the D-stem/loop
Transfer tRNA Glu isoacceptors typically have an augmented D-stem, containing four base pairs instead of the three base pairs seen in tRNA Gln. In E. coli tRNA Gln, this larger D-stem contains major identity elements that are recognized by the discriminating E. coli GluRS (Figure 1) (21). Moreover, it has also been proposed that the size of the tRNA Gln D-stem is an important feature for the divergence in tRNA specificity between GluRS1 and GluRS2; this hypothesis was partially based on the observation that Acidithiobacillus ferrooxidans GluRS1 aminoacylates one of its tRNA Gln isoacceptors and this tRNA Gln has a four base pair D-stem (13). Because of the apparent contradiction between these previous observations and our data showing that Chimera 2, which contains an engineered four base pair D-stem, is still tRNA Gln-like in activity, we evaluated two additional D-stem/loop constructs. In the first construct, the D-loop of tRNA Glu2 was transplanted into tRNA Gln; in the second, the tRNA Glu2 D-stem was introduced into tRNA Gln. Neither of these tRNAs were robust substrates for either GluRS1 or GluRS2, a result that is strikingly different from both tRNAGln and Chimera 3 (Figure 4A). Interestingly, when the concentration of GluRS1 or GluRS2 and the length of the assay is increased (the conditions we use to quantify tRNA expression levels), both of these tRNAs can be aminoacylated by either GluRS1 or GluRS2 (see Supplementary Table S3). Consequently, neither the D-stem nor the D-loop contain major identity elements for GluRS1 or GluRS2. However, because both the D-stem and the D-loop tRNAs have diminished activity...
towards GluRS2, the possibility that this region contains minor identity elements cannot be ruled out.

**Mutagenesis of the anticodon loop**

Next, we focused our attention on the anticodon stem/loop of tRNAGln. In *Thermus thermophilus* D-GluRS, a single mutation in the anticodon-binding domain switched this enzyme to an ND-GluRS with dual specificity for tRNA^Glu^ and tRNA^Gln^ (24). And in *H. pylori*, a G417T mutation in the GluRS2 anticodon-binding domain was sufficient to introduce tRNA^Glu^ but not tRNA^Glu2^ aminoacylation activity into GluRS2 (19). It has also been shown that both the anticodon of *E. coli* tRNA^Glu^ (UUC) and *E. coli* tRNA^Gln^ (UUG) are important for *E. coli* GluRS and *E. coli* GlnRS recognition, respectively (21–23). Also, post-transcriptional modification of position 34 in the anticodon, to generate 5-methylaminomethyl 2-thiouridine (mnm5s2U34) enhances both aminoacylation activity and specificity (20). This modification is expected to be present in all three *H. pylori* tRNA isoacceptors (2) and in our different tRNAs (14). Because U34 is present in all three tRNAs, it was not evaluated. The *H. pylori* tRNA^Glu^, tRNA^Gln2^, and tRNA^Gln^ anticodon loops vary at four positions (Figure 1). Among these four nucleotides, N36, N37, and N38 have been shown to be important identity elements for both *E. coli* GluRS and GlnRS (21–23). We individually evaluated each of these positions by mutating the nucleotide in tRNA^Gln^ into that of tRNA^Glu2^ (Figure 5). Consistent with the wild-type behavior of Chimera 3, mutagenesis at each of these positions had no effect on the substrate behavior of tRNA^Gln^ . These results show that the anticodon loop is not important for GluRS2 recognition, in contrast to patterns seen with other GluRSs and with many other tRNA/aaRS pairs (12,19,24,26,27).

**Mutagenesis of the tRNA^Gln^ acceptor stem and discriminator base**

Finally, we turned our attention to the acceptor stem of tRNA^Gln^, the region that holds the most promise based on the original survey of tRNA chimeras. We first examined the discriminator base (N73), another position that is often used by aaRSs to achieve tRNA specificity (27,28). Unexpectedly, given the common importance of this position, the identity of the discriminator base is not important for GluRS2 recognition of tRNA^Gln^ . This high level of tRNA production and the lack of activity with GluRS2 clearly demonstrate that the G1:C72 base pair of tRNA^Glu2^ is a major antideterminant for GluRS2. The fifth base-pair mutation (G5:C68 inverted to C5:G68) was overtranscribed at moderate levels, confirming that this position is also important as an antideterminant in tRNA^Glu2^, preventing GluRS2 recognition. The second,
fourth and seventh base pairs were not robustly overexpressed (Figure 6C and Supplementary Figure S1). Each of these tRNAs was assayed at the maximum possible concentration. Consequently, the poor aminoacylation activities with each of these mutations likely indicate that each of these positions is an important antideterminant that prevents tRNA\textsubscript{Glu2} from being aminoacylated by GluRS2, however the possibility that tRNA expression levels were simply too low to observe aminoacylation activity cannot be ruled out.

Interestingly, while many of the acceptor stem positions in tRNA\textsubscript{Glu2} are clearly important for rejection by GluRS2, no single base-pair mutation led to recognition by GluRS1. This observation is in sharp contrast to Chimera 1, which is a robust substrate for GluRS1. Clearly, some or all of these positions are tRNA\textsubscript{Glu2} determinants for GluRS1, but they are only strong enough to induce recognition when combined. Perhaps, tRNA\textsubscript{Gln} also contains an antideterminant for GluRS1 distal to the acceptor stem.

**DISCUSSION**

GluRS2 uses specialized mechanisms to recognize tRNA\textsubscript{Gln}

These studies demonstrate that GluRS2 achieves its unique tRNA\textsubscript{Gln} specificity, rejecting tRNA\textsubscript{Glu2}, solely by distinguishing between differences in the acceptor stems of these two tRNAs. It is surprising that neither the anticodon nor the discriminator base is important, as these positions are critical for tRNA\textsubscript{Glu} and tRNA\textsubscript{Gln} aminoacylation in other systems (21–23,27). In *E. coli* tRNA\textsubscript{Glu}, identity elements are spread throughout the tRNA scaffold, with major determinants located in the augmented D-stem (Figure 1) (21). The identity elements of *E. coli* tRNA\textsubscript{Gln} are mainly located in the two distal ends of the tRNA, at the discriminator base, the second and third base pairs in the acceptor stem, and the anticodon loop (Figure 1) (22,23). Furthermore, in *T. thermophilus* D-GluRS the size and identity of the amino acid that interacts with the third nucleotide of the anticodon plays an important role in discrimination between tRNA\textsubscript{Glu} and tRNA\textsubscript{Gln} (24); this region is important for GluRS2 rejection of tRNA\textsubscript{Glu1} as well (19). In contrast, here we show that GluRS2 rejects tRNA\textsubscript{Glu2} by predominantly looking at only one region of the tRNA—the acceptor stem.

Although this work focused on the rejection of tRNA\textsubscript{Glu2}, the results also provide some insight into how GluRS2 rejects tRNA\textsubscript{Glu1}. As we have previously reported, a G417T mutant GluRS2 aminoacylates tRNA\textsubscript{Glu1} but not tRNA\textsubscript{Gln} or tRNA\textsubscript{Glu2} (19). In light of the present work, this result is surprising because tRNA\textsubscript{Glu1} contains the same G1:C72 base pair as tRNA\textsubscript{Glu2}, a strong antideterminant for GluRS2 (Figure 6A and B). Thus, it appears that the G417T mutation unmasks a role for the tRNA\textsubscript{Glu1} anticodon that is sufficient to overcome the potency of the G1:C72 acceptor stem antideterminant. The combination of these results suggest that tRNA\textsubscript{Glu1}, unlike tRNA\textsubscript{Glu2}, contains
determinants for GluRS2 that are located in both the acceptor stem and the anticodon. This observation is unexpected since tRNA\textsubscript{Glu\textsuperscript{1}} and tRNA\textsubscript{Glu\textsuperscript{2}} contain the same UUC anticodon, and it suggests subtle differences in the shapes of the two tRNAs. Mutagenesis experiments within the tRNA\textsubscript{Glu\textsuperscript{2}} framework are needed in order to truly understand the differences in how these tRNAs are rejected by GluRS2.

Our results indicate that the first acceptor stem base pair (U1:A72) is critically important for GluRS2s tRNA specificity—namely, the accurate recognition of tRNA\textsubscript{Gln}\textsuperscript{Glu} and the rejection of tRNA\textsubscript{Glu\textsuperscript{1}} and tRNA\textsubscript{Glu\textsuperscript{2}}, which both contain a G1:C72 base pair. The importance of this position is conserved throughout indirect aminoacylation. Like GluRS2, ADt, the amidotransferase that converts Glu-tRNA\textsubscript{Gln} into Gln-tRNA\textsubscript{Gln}, relies on the U1:A72 base pair for recognition of Glu-tRNA\textsubscript{Gln} (29,30); the archaeal type ADt (Methanothermobacter thermotrophicus GatDE) also relies on this position for recognition of tRNA\textsubscript{Gln} (in this case, it is an A1:U72 base pair which is recognized by GatDE) (25). The archaeal GatCAB does not use the first base pair as a strong identity element (31).

Evolutionary Implications

Substantial evidence has accumulated to suggest that progenitor tRNAs were smaller than their modern counterparts and consisted of either a single acceptor-stem microhelix or a minihelix comprised of the acceptor stem and TPC-stem/loop (32–34). These smaller RNAs were putatively aminoacylated by ancestral aaRSs, comprised solely of catalytic domains (34,35). Acquisition of divergent anticodon-binding domains was a likely key step in the separation of GluRS and GlnRS (34,36). GluRS2 deviates from this picture, however, because it contains a GluRS-like anticodon-binding domain (13,14). Instead, this enzyme has capitalized on primordial mechanisms of tRNA recognition, in effect rendering the anticodon-binding domain useless, at least with respect to distinguishing between tRNA\textsubscript{Gln} and tRNA\textsubscript{Glu}\textsuperscript{2}.

A very recent report also demonstrated that the truncated catalytic domain of \textit{E. coli} D-GluRS is capable of discriminating against tRNA\textsubscript{Gln}, in favor of tRNA\textsubscript{Glu}, even in the presence of a GlnRS anticodon-binding domain (37). While this D-GluRS truncation was large enough to include recognition of known D-stem/loop identity determinants, this report further supports the evolutionary hypothesis that ancestral identity elements were recognized solely by the catalytic domain of GluRSs, as we see predominately here for GluRS2.

The data presented herein also show that the strongest determinants for \textit{H. pylori} GluRS1 are localized within the acceptor stem of tRNA\textsubscript{Glu}. Chimera 1 was the only chimera to show robust aminoacylation activity with GluRS1. However, individual base pair mutations in the acceptor stem of tRNA\textsubscript{Glu} were insufficient to induce GluRS1 recognition. Thus, tRNA\textsubscript{Glu\textsuperscript{2}} does not contain a single potent determinant for GluRS1, rather aminoacylation activity is induced by recognition of a set of identity elements apparently distributed throughout the acceptor stem. Thus, like GluRS2, GluRS1 uses ancestral mechanisms to recognize its tRNA substrates and to reject tRNA\textsubscript{Gln}.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

The authors thank Liangjun Zhou and Shirin Fatma for critical reading of the article and the two anonymous reviewers for their thoughtful and thorough comments on the article.

FUNDING

National Institutes of Health (GM071480); Wayne State University. Funding for open access charge: National Institutes of Health.

Conflict of interest statement. None declared.

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