tRNA Recognition by Glutamyl-tRNA Reductase*

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During the first step of porphyrin biosynthesis in Archaea, most bacteria, and in chloroplasts glutamyl-tRNA reductase (GluTR) catalyzes the NADPH-dependent reduction of glutamyl-tRNA to glutamate-1-semialdehyde. Elements in tRNA that are important for utilization by *Escherichia coli* GluTR were determined by kinetic analysis of 51 variant transcripts of *E. coli* tRNA*Glu* (53). Base U8, the U13*G22**A46 base triple, the tertiary Watson-Crick base pair 19*56, and the lack of residue 47 are required for GluTR recognition. All of these bases contribute to the formation of the unique tertiary core of *E. coli* tRNA*Glu*. Two tRNA*Glu* molecules lacking the entire anticodon stem/loop but retaining the tertiary core structure remained substrates for GluTR, while further decreasing tRNA size toward a minihelix abolished GluTR activity. RNA footprinting experiments revealed the physical interaction of GluTR with the tertiary core of tRNA*Glu*. *E. coli* GluTR showed clear selectivity against mischarged Glu-tRNA*Glu*. We concluded that the unique tertiary core structure of *E. coli* tRNA*Glu* was sufficient for *E. coli* GluTR to distinguish specifically its glutamyl-tRNA substrate.

Glutamyl-tRNA synthetase (GluRS) esterifies the cognate tRNA*Glu* with glutamate to form glutamyl-tRNA (Glu-tRNA*Glu*), an aminoacyl-tRNA that possesses a dual function in the metabolism of most organisms. Apart from its well studied role in protein biosynthesis (1), Glu-tRNA*Glu* is the initial precursor for the synthesis of tetrapyrroles, e.g. chlorophyll, heme, and vitamin B_{12} (2, 3). In plants, Archaea, and most bacteria the common precursor of all tetrapyrroles, 5-aminolevulinic acid, is formed in a two-step reaction from the five-carbon chain of glutamate. Glutamyl-tRNA reductase (GluTR) catalyzes the NADPH-dependent reduction of glutamate to glutamate-1-semialdehyde (GSA), which is subsequently converted to 5-aminolevulinic acid by glutamate-1-semialdehyde-2,1-amino-mutase (GSA-AM) (4, 5). To maintain the fidelity of protein biosynthesis and, at the same time, an adequate metabolite flux for 5-aminolevulinic acid formation, accurate recognition of various cellular tRNAs by their cognate aminoacyl-tRNA synthetases and of Glu-tRNA by GluTR must be secured. The characteristic nucleotides in tRNAs (identity elements) needed for recognition by their cognate aminoacyl-tRNA synthetases have been characterized for many systems (6). For the *Esche- richia coli* GluRS-tRNA*Glu* system, 5-methylaminomethyl-2-thiouridine in position 34, U35, C36, A37 in the anticodon loop, G1*C72, U2*A71 in the acceptor stem, the U11*A24 base pair, the U13*G22**A46 base triple, and the lack of residue 47 (Δ47) determine the charging identity (7, 8, 33). The crystal structure of the GluRS-tRNA*Glu* complex (9, 10) provided further insight to tRNA*Glu* recognition by GluRS. In contrast, there is little information on the recognition of Glu-tRNA*Glu* by GluTR. Specific sequence information is required, as 5-aminolevulinic acid synthesis in barley chloroplasts demands chloroplast tRNA*Glu* and not cytoplasmic tRNA*Glu* (11). Specificity of GluTR for tRNA*Glu* was also shown for the enzyme from *Chlorella vulgaris* (31), *Chlorobium vibrioforme* (32), *Chlamydomonas reinhardtii* (13), and *Synechocystis 6803* (12). Based on a sequence comparison of tRNA*Glu* species that are substrates for various GluTRs with tRNA species which are not utilized, putative identity elements were postulated (14). A point mutation (C56–U56) in *Euglena gracilis* chloroplast tRNA*Glu* was reported to uncouple protein and chlorophyll biosynthesis. This mutant tRNA was still aminoacylated by chloroplast GluRS and utilized in protein biosynthesis but was not a substrate for GluTR and therefore did not support tetrpyrrole biosynthesis (15). The solution of the long sought-after crystal structure of GluTR (30) and the biochemical characterization of *E. coli* GluTR (16, 17) generated much interest in the details of tRNA recognition by this protein. The role of the glutamate part of the substrate was elucidated by site-directed mutagenesis of the *E. coli* GluTR substrate binding pocket and the co-crystallization of glutamycin, a glutamate analogue, with the *Methanopyrus kandleri* enzyme (29). Glutamycin was found tightly coordinated in the highly conserved catalytic pocket, which also includes the cysteine residue (Cys-50) known to form an intermediate thioester bond between the enzyme and glutamate (16). In contrast, much less is known about the contribution of the tRNA portion of the substrate to recognition and catalysis. Here we present an analysis of tRNA identity in GluTR recognition.

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* The abbreviations used are: GluRS, glutamyl-tRNA synthetase; CD, circular dichroism; GSA, glutamat-1-semialdehyde; GSA-AM, glutamate-1-semialdehyde-2,1-amino-mutase; GluTR, glutamyl-tRNA reductase; HPLC, high performance liquid chromatography; T7 RNAP, T7 RNA polymerase; N*N, secondary base pair; N**N, tertiary base pair.

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EXPERIMENTAL PROCEDURES

Production of Recombinant E. coli GluTR, GluRS, GSA-AM, and T7 RNAAP—E. coli GluTR was produced as a six-histidine N-terminal fusion protein, renatured from inclusion bodies and purified as described (17). Recombinant E. coli GluRS and GSA-AM were purified to apparent homogeneity according to published procedures (18, 20). Bacteriophage T7 RNAAP was purified from an overproducing E. coli strain harboring the plasmid pAR1219 as outlined previously (19).

Construction of Expression Vectors for tRNA<sup>Ala</sup> Variants—The majority of expression vectors for the production of E. coli tRNA<sup>Ala</sup> variants were described before (7). The plasmid pKR320 (20) contains the E. coli tRNA<sup>Ala</sup> gene cloned downstream of a T7 RNA polymerase promoter. The tRNA<sup>Ala</sup> variants encoding tRNAs harboring the point mutations C56U, G19A, C56U/G19A, U8C, C56G, and G23C were constructed in the plasmid pKR320 using the QuikChange<sup>TM</sup> site-directed mutagenesis kit (Stratagene, Heidelberg, Germany). To obtain the minimalista tRNA constructs an expression vector encoding a tRNA<sup>Ala</sup> transcript that lacks the entire anticodon stem/loop (pKR320ΔA/C) was generated by deletion of 22 nucleotides using the oligonucleotide 5'-CTAGAGGCCCAACAGGTTGTCAGAATCC-3' (ΔA/C3-'). The following oligonucleotides were used to insert the linker region in plasmid pKR320ΔA/C underlined, 5'-CTAGAGGCCCAACAGGTTGTCAGAATCC' (ΔA/C1) and 5'-CCAACAGGTTGTCAGAATCC (ΔA/C2). The minimalista tRNA was constructed by the deletion of 19 nucleotides of plasmid pKR320ΔA/C using the oligonucleotide 5'-CACTATAGTCCCTAGGGGTTGCACGCCCAGGACAATTAACAGGGGTTCGAATCC-3'. All introduced mutations were verified by complete DNA sequence determination.

Preparation and Purification of tRNA Gene Transcripts—E. coli tRNA<sup>Ala</sup> (UCA) variants and minimalista tRNA molecules were synthesized by in vitro T7 RNAAP run-off transcription (21). The DNA fragments carrying the E. coli tRNA<sup>Ala</sup> gene variants including the T7 RNAAP promoter were amplified using PCR. To generate the appropriate 3'-CCA-end of the tRNA<sup>Ala</sup> transcripts, the various PCR-amplified template DNAs carrying the tRNA<sup>Ala</sup> genes were digested with NspI and using the oligonucleotide 5'-CTAGAGGCCCAACAGGTTGTCAGAATCC-3' (ΔA/C). The various PCR-amplified tRNA<sup>Ala</sup> templates were subsequently purified by MonoQ anion exchange chromatography as described (23).

Aminoacylation of tRNA<sup>Ala</sup>—The tRNA<sup>Ala</sup> substrate [1<sup>4</sup>C]Glu-tRNA<sup>Ala</sup> was prepared in 100 or 500 μl in 0.5 μl isolated E. coli tRNA<sup>Ala</sup> reaction mixtures containing 30 mm Na-HEPES, pH 7.5, 15 mm MgCl<sub>2</sub>, 25 mm KCl, 4 mm dithiothreitol, 4 mm spermidine, 200 μg/ml bovine serum albumin, 2.0–0.5 mm each nucleoside triphosphate (depending on the template), the PCR-amplified template DNA (∼0.5 μm), and 100 μg/ml (1 mm) T7 RNAAP. The E. coli tRNA<sup>Ala</sup> (UGU) transcript was prepared according to published procedures (22). The RNA transcripts were subsequently purified by MonoQ anion exchange chromatography as described (23).

Circular Dichroism (CD) and UV Absorption Measurements—CD and UV absorption spectra were recorded on a Jasco J-810 spectropolarimeter equipped with Neslab RET-110 temperature control unit. Each tRNA sample (∼0.3 mg/ml) was initially stabilized at 37 °C for 15 min. Melting experiments were performed at 1 °C increments using a temperature range of 30 °C in the presence of either 10 mM EDTA or 10 mM MgCl<sub>2</sub>.

RESULTS AND DISCUSSION

Because both GluTR and GluRS recognize the same tRNA<sup>Ala</sup>, we wanted to compare which nucleotides are crucial for recognition. The earlier studies on GluRS identity not only presented a basis for comparison but also provided the large set of in vitro synthesized Glu-tRNA<sup>Ala</sup> variants required to identify residues in E. coli tRNA<sup>Ala</sup> that are important for E. coli GluTR activity.

Kinetic Analysis of tRNA<sup>Ala</sup> Variants—The recognition of Glu-tRNA<sup>Ala</sup> by GluTR was examined by the kinetic analysis of unmodified wild type tRNA<sup>Ala</sup> (Fig. 1) and 51 tRNA<sup>Ala</sup> variants that were prepared in vitro run-off transcription. All trans- scripts were aminoaacylated with [1<sup>4</sup>C]glutamate using E. coli GluRS and tested as GluTR substrates in the depletion assay by monitoring the loss of charged tRNA<sup>Ala</sup> (24). The formation of the reaction product GSA was verified by HPLC analysis. It was shown previously that E. coli GluTR utilizes fully modified E. coli Glu-tRNA<sup>Ala</sup> and unmodified Glu-tRNA<sup>Ala</sup> transcripts at comparable catalytic rates (16). The specific activity for this process was measured as 0.47 μmol min<sup>−1</sup> mg<sup>−1</sup> (k<sub>cat</sub>) and was set to 100%. All other values obtained in this study were related to this value. The majority of charged tRNA<sup>Ala</sup> transcripts harboring single, double, or triple mutations were utilized by E. coli GluTR without any significant reduction of the catalytic activity (Table I). Interestingly, this set also includes tRNA<sup>Ala</sup> transcripts carrying the mutations of bases that are known to be identity elements in tRNA<sup>Glu</sup> for recognition by E. coli GluRS (7, 8).

GluTR Does Not Recognize the Acceptor Arm of tRNA and the Anticodon Stem/Loop—Each single base pair of the anticodon stem and all bases of the anticodon loop of tRNA<sup>Ala</sup> were exchanged without affecting GluTR activity. This contrasts with observations made with GluRS, which is known to recognize the residues U34, U35, C36, and A37 of the anticodon loop. In agreement with this, the loss of the 5-methylaminomethyl-2-thiol (mnm5s2) modification of the base U34 in the tRNA<sup>Ala</sup> transcript, which significantly contributes to tRNA<sup>Ala</sup> identity for GluRS (33), does not affect GluTR activity (16). Surprisingly, no tRNA<sup>Glu</sup> identity elements for GluTR were found in the acceptor stem. The discriminator base G73 and all base pairs of the acceptor stem (G1*C72, U2*A71, C3*G70, C4*G69, C5*G68, C6*G67, U7*A66) were mutated without affecting
tRNAGlu variants harboring mutations in the tertiary core reduced GluTR activity are summarized in Table II. A set of tRNAGlu variants designed to facilitate folding of the tertiary structure of the tRNA are indicated by dotted lines. The distinct domains of the tRNA are indicated by base triplets (taken from Ref. 7), are indicated by pairs, and base triplets (see Fig. 1).

**TABLE I**

Investigated Glu-tRNAGlu variants with no influence on GluTR activity

| tRNAs | Base exchange |
|-------|---------------|
| tRNAGlu transcript | G73 → A73 |
| Acceptor stem | C72 → A72 |
| U2*A71 → C2*G71 |
| C3*G70 → U3*A70 |
| C3*G70 → A3*U70 |
| C4*G69 → U4*A69 |
| C5*G68 → U5*A68 |
| C6*G67 → U6*A67 |
| C7*G66 |
| T-stem | A49*U65 → G49*C65 |
| G50*C64 → A50*U64 |
| G51*C63 → A51*U63 |
| G52*C62 → A52*U62 |
| D-stem/loop | A26**T44 → G26**C44 |
| C29*G10 → A45 → U25*A10**G45 |
| C25*G10 → A45 → C25*G10**G45 |
| U11*A24 → C11*G24 |
| C12*G23**C9 → U12*A23**A9 |
| C12*G23**C9 → C12*G23**A9 |
| U13*G22 → G19*U22 |
| A16 → G16 |
| C20 → U20 |
| C20a → U20a |
| Anticodon stem | C27*G43 → U27*A43 |
| C28*G42 → U28*A42 |
| C29*C41 → A29*U41 |
| C30*G40 → U30*A40 |
| C31*G39 → U31*A39 |
| Anticodon loop | C32 → U32 |
| U33 → C33 |
| U34 → A34 |
| U34 → C34 |
| U35 → A35 |
| C36 → U36 |
| C36 → G36 |
| A37 → G37 |
| C38 → A38 |

GluTR activity. In contrast, GluRS was shown to recognize G1*C72 and U2*A71 (7).

The Unique Tertiary Core Structure of tRNAGlu Is Recognized by GluTR—The tRNAGlu variants that led to significantly reduced GluTR activity are summarized in Table II. A set of tRNAGlu variants harboring mutations in the tertiary core (D-stem/loop, variable loop, and T-stem/loop) was synthesized by T7 RNAP transcription. The U13*G22**A46 base triple is formed via a tertiary base pairing between the phylogenetically conserved pyrimidine 13-purine 22 motif (U13*G22 in tRNA-Glu) in the D-stem and base 46 (A46) in the variable loop (36) (see Fig. 2C). A tRNAGlu transcript carrying a mutation of U13–C13, which allows the formation of the Watson-Crick base pair C13*G22, was still a reasonable substrate for GluTR but led to significantly reduced GluRS activity (7). However, for both GluRS and GluTR mutations of the bases G22 and A46 (to A22, G46, U46) almost abolished substrate utilization. Furthermore, the tertiary core of tRNAGlu is characterized by the lack of residue 47 in the four nucleotide short variable loop. The insertion of U into position 47 decreased the catalytic activity of GluTR as well as of GluRS. As the base triple 13*22**46 was shown to play an important role in maintaining the overall folding of the tRNA (34), it seems likely that GluTR recognition depends on the unique core structure of tRNAGlu. In addition, the lack of residue 47 is thought to stabilize the mentioned base triple via stable positioning of base A46 (35). Furthermore, the strictly conserved tertiary base pair U8*A14, which forms a reverse Hoogsteen pairing configuration, was disrupted in a tRNAGlu variant, which harbored a mutation of the base U8–C8. The transcript, although charged by GluRS, was only a weak substrate for GluTR (Table II). The finding that the tertiary core structure of tRNAGlu is recognized by GluTR was supported by in vivo data from *E. gracilis*. It was shown that a point mutation of tRNAGlu led to a chlorophyll-deficient phenotype, with a tRNAGlu variant still participating in chloroplast protein biosynthesis but no longer able to be utilized by GluTR (15). The *E. gracilis* tRNAGlu harbored the single mutation of C56–U56. As this base forms a tertiary base pairing (A19**U56) in the variable loop, C, structure and sequence of the investigated minihelix comprising the T-stem/loop and the acceptor stem.

![Fig. 1. Two-dimensional representation of the tertiary structure of the tRNA molecules employed in this study.](Image)
Conformational Stability of the tRNA Transcripts—Because of the central role of the tRNA\textsubscript{Glu} backbone in GluTR recognition, the mutated tRNA\textsubscript{Glu} transcripts used in this study were analyzed for their structural integrity. The stabilities of wild type tRNA\textsubscript{Glu} transcripts and the variants affecting tertiary base interactions were investigated by thermal unfolding experiments. The changing spectroscopic features of various tRNA\textsubscript{Glu} transcripts were investigated by UV absorbance and CD spectroscopy and compared. The increasing UV absorbance during the thermal tRNA unfolding process was monitored between 250 and 270 nm. This region of the tRNA spectrum showed the highest degree of absorbance and hyperchromicity (data not shown). However, the UV absorbance spectra of the various tRNA\textsubscript{Glu} molecules changed only slightly during the thermal unfolding process. In contrast, clear changes of the corresponding CD spectra between 80 and 320 nm were observed. The CD spectrum of folded wild type tRNA\textsubscript{Glu} at 27 °C showed a minimum centered at 210 nm and a maximum centered at 270 nm. During the melting process of this tRNA\textsubscript{Glu} molecule the absorbance at 210 nm increased with the rising temperature, whereas the absorbance at 270 nm maximum decreased in amplitude and shifted its maximum to 277 nm (Fig. 3A). Fig. 3B shows a differential representation of the melting curves based on changes in the ellipticity at the single wavelength at 210 nm for the wild type tRNA\textsubscript{Glu} transcript. In the same figure the corresponding data for the tRNA\textsubscript{Glu} variant transcript harboring the base exchange C56–U56 is shown. Only minimal differences (<1 °C) of the melting point between

### Table II

| tRNA\textsubscript{Glu} transcript | Base exchange | Affected structural element | Relative GluTR activity |
|-----------------------------------|---------------|-----------------------------|-------------------------|
| T-loop/D-loop                     | C56 → U56    | G19**C56                    | 4 ± 1                   |
| G19 → A19                         | G19**C56      | 2 ± 1                       |
| G19**C56 → A19**U56               |               | 85 ± 5                      |
| D-stem                            | U8 → C8      | U8**A14                     | 30 ± 3                  |
| U13 → C13                         | U13**G22**A46| 90 ± 5                      |
| G22 → A22                         | U13**G22**A46| 10 ± 2                      |
| A46 → G46                         | U13**G22**A46| 5 ± 1                       |
| A46 → U46                         | U13**G22**A46| 3 ± 1                       |
| U13**G22**A46 → C13**A22**G46     | U13**G22**A46| 2 ± 1                       |
| Δ47 → U47                         |               | 2 ± 1                       |
| Anticodon stem/loop               | ΔAC1\textsuperscript{b} | 90 ± 5                      |
|                                   | ΔAC2\textsuperscript{b} | 85 ± 4                      |
| Minihelix                         | Minihelix\textsuperscript{b} | Tertiary core              | 0                       |
| E. coli tRNA\textsubscript{Glu}  |               |                             | 0                       |

\textsuperscript{a} GluTR activity was measured by the substrate depletion assay as described in detail under "Experimental Procedures." Product formation was verified by HPLC analysis. The specific activity (0.47 μmol min\textsuperscript{-1} mg\textsuperscript{-1}) of GluTR with wild type tRNA\textsubscript{Glu} represents 100%.

\textsuperscript{b} The truncated tRNA mutants are described under "Experimental Procedures."
wild type tRNA\textsubscript{Glu} transcript and the U56 variants were noticed, indicating an almost identical stability of these transcripts. Similar to identical results were obtained during the analysis of the tertiary core mutants G56, G56, A19, and A19*U56. In agreement with these observations, the melting temperature for wild type tRNA\textsubscript{Glu} transcript and all investi-
gated variants was shifted from 33 °C in the presence of 10 mM EDTA to 56 °C in the presence of 10 mM MgCl₂. In conclusion, the performed melting experiments indicate no altered conformational stability for any investigated variants. We conclude that mutations in the tertiary base pair C56*G19 cause only minor alterations in structure and stability. Nevertheless, this part of the tRNA(Glu) molecule represents the central recognition element for GluTR.

Minimization of the RNA Substrate—To further narrow down the influence of the tRNA tertiary core on overall tRNA(Glu) recognition by GluTR, minimalista tRNA(Glu) substrates were designed (Fig. 1). For the truncated construct ΔAC1 the anti-codon stem/loop region was deleted and replaced by a linker region of the two nucleotides AU. For the molecule ΔAC2 the linker consists of four nucleotides (AAUA). These linker regions were designed to allow the necessary base interactions of the tertiary core. Aminoacylation of these truncated transcripts by GluRS to wild type level was achieved by increasing the RNA and GluRS concentration up to 3 and 10 μM, respectively. Both constructs ΔAC1 and ΔAC2 served as efficient substrates in the standard GluTR depletion assay (Table II). We concluded from these results that the whole anticodon stem/loop region is not needed for the recognition of Glu-tRNAGlu by GluTR. To examine the effect of further reduction of the tRNA substrate, a minihelix comprising the acceptor stem, the T-stem, and T-loop was constructed. This minihelix is missing the tertiary core of tRNA(Glu). The minihelix transcript was charged with glutamate in an aminoacylation reaction containing 16 μM GluRS for 1.5 h. This glutamyl-minihelix was not a substrate for GluTR in the standard GluTR depletion assay (Table II). In conclusion, these experiments provided further evidence for the role of the tertiary core of tRNA(Glu) in GluTR recognition.

RNAse Footprinting Reveals Glu-tRNAGlu-GluTR Interactions at the Tertiary Core—RNAse footprinting was employed to investigate GluTR-tRNAGlu interactions. Initial experiments were hampered by substrate destruction caused by GluTR. In the absence of NADPH GluTR possesses an esterase activity that hydrolyzes the Glu-tRNAGlu substrate into tRNA(Glu) and glutamate. To stabilize the GluTR-bound substrate GluTR esterase activity was inhibited by the NADPH analogue β-nicotinamide-mononucleotide (reduced form) as described before (24). We used RNases V1, T1, and nuclease S1 to digest Glu-tRNAGlu alone and in complex with GluTR to investigate changes in the protection pattern. During the precharging reaction nonspecific cleavage of the transcript at certain weak points was inevitable and might be induced by GluRS (28). These positions were omitted from further analysis. Surprisingly, the addition of up to 100 nM GluTR reproducibly led to increased RNAse V1 digestion at the T-stem, D-stem, and parts of the anticodon stem. These findings might indicate conformational changes of the tRNA structure induced by docking to GluTR or alternatively the occurrence of GluTR RNase V1 protein-protein interactions. Thus, changes in the protection pattern of tRNA(Glu) were only observed by RNAse T1 and nuclease S1 treatment. Protection of the base G10 in the D-stem and the bases G53, U54, and U55 in the T-loop from RNAse digestion by GluTR was observed (Fig. A4). The protection of these positions was verified by digestion of phosphorothioate containing tRNA transcripts (data not shown). Therefore, the direct interaction of GluTR with the tertiary core of tRNA(Glu) was demonstrated. In the current model of Glu-tRNAGlu-GluTR interactions (29), based on the position of glutamycin, which mimics the 3' end of Glu-tRNAGlu in the active site pocket, the tRNA(Glu) is bound from the inside of the L-shape of the molecule, similar to its binding to GluRS. It is in precisely this region of the tRNA that the protected bases are localized (Fig. 4). The Glu-tRNAGlu-GluTR model is based on the M. kandleri GluTR structure, which reveals significant catalytic and structural similarities to the E. coli enzyme (16) so that an analogue tRNA orientation is proposed.

GluTR Shows tRNA(Glu) Specificity—Finally, we investigated whether the unique tertiary core of tRNA(Glu) provides enough information to discriminate against the closely related tRNA(Gln) when mischarged with glutamate. The tRNA(Gln) transcript of E. coli was mischarged with glutamate using 31 μM GlhR and 80 μM glutamate and tested for GluTR acceptance in standard depletion assays. However, [14C]-Glu-tRNAGln was not a substrate for GluTR. Because many organisms carrying GluTR form glutamyl-tRNA via naturally mischarged Glu-tRNA(Gln) (20) this observation is of biological significance.

General Discussion—GluTR is an enzyme that uses aminoacyl-tRNA as substrate. Specific recognition of Glu-tRNA by E. coli GluTR is brought about by the unique tertiary core of tRNA(Glu) created by the U13*G22*A64 base triple, Δ47, and the tertiary base pair G19*G56. Neither the anticodon nor acceptor stem are major identity elements, as they are for most aminoacyl-tRNA synthetases. The accuracy of this process is further sustained by the tight coordination of the glutamate part of the substrate in the active site pocket of GluTR.

Do other enzymes that utilize aminoacyl-tRNA as a substrate recognize tRNA in a similar fashion? For instance, E. coli methionyl-tRNA(Sec) formyltransferase, the enzyme required for formylating the initiator tRNA, requires a cluster of determinants in the acceptor stem and the A11*U24 base pair in the D-stem (37, 38). Furthermore, similar to GluTR, increased RNase V1 digestion was observed in the anticodon stem, although this region is located distal to the tRNA-protein contacts observed in the co-crystal structure (39). This observation was explained by conformational changes of the tRNA structure induced by Met-tRNA formyltransferase binding (40). In addition, enhanced RNase V1 cleavage in the anticodon stem was also described for the EF-Tu-Phe-tRNA(Phe)-GTP and IF2-Met-tRNA complexes suggesting changes in anticodon stem conformation even though these proteins interact with the distal acceptor stem (41, 42). It will be interesting to explore conformational changes in Glu-tRNA(Glu) structure possibly induced by GluTR binding. Finally, selenocysteinyl-tRNA(Sec) is an aminoacyl-tRNA substrate specifically recognized by selenocysteine synthase and the SELB translation factor. The unusually long eight-base-pair acceptor stem of E. coli tRNA(Sec) was found to be the major recognition determinant for SELB (43). The mechanisms of further enzymes utilizing an aminoacyl-tRNA substrate, e.g. Glu-tRNA(Gln) amidotransferase, which transamidates misacylated Glu-tRNA(Glu), are still under investigation. These results suggest that enzymes utilizing aminoacyl-tRNA as substrate interact directly with the aminoacylated acceptor stem and the D-stem, whereas the anticodon domain serves as a major recognition element of aminoacyl tRNA synthetases.

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