

Rational design and directed evolution of a bacterial-type glutamyl-tRNA synthetase precursor

Li-Tao Guo¹, Sunna Helgadóttir¹, Dieter Söll^{1,2,*} and Jiqiang Ling^{1,*}¹Department of Molecular Biophysics and Biochemistry and ²Department of Chemistry, Yale University, New Haven, CT 06520-8114, USA

Received March 1, 2012; Revised April 24, 2012; Accepted May 7, 2012

ABSTRACT

Protein biosynthesis requires aminoacyl-transfer RNA (tRNA) synthetases to provide aminoacyl-tRNA substrates for the ribosome. Most bacteria and all archaea lack a glutamyl-tRNA synthetase (GlnRS); instead, Gln-tRNA^{Gln} is produced via an indirect pathway: a glutamyl-tRNA synthetase (GluRS) first attaches glutamate (Glu) to tRNA^{Gln}, and an amidotransferase converts Glu-tRNA^{Gln} to Gln-tRNA^{Gln}. The human pathogen *Helicobacter pylori* encodes two GluRS enzymes, with GluRS2 specifically aminoacylating Glu onto tRNA^{Gln}. It was proposed that GluRS2 is evolving into a bacterial-type GlnRS. Herein, we have combined rational design and directed evolution approaches to test this hypothesis. We show that, in contrast to wild-type (WT) GlnRS2, an engineered enzyme variant (M110) with seven amino acid changes is able to rescue growth of the temperature-sensitive *Escherichia coli glnS* strain UT172 at its non-permissive temperature. *In vitro* kinetic analyses reveal that WT GluRS2 selectively acylates Glu over Gln, whereas M110 acylates Gln 4-fold more efficiently than Glu. In addition, M110 hydrolyzes adenosine triphosphate 2.5-fold faster in the presence of Glu than Gln, suggesting that an editing activity has evolved in this variant to discriminate against Glu. These data imply that GluRS2 is a few steps away from evolving into a GlnRS and provides a paradigm for studying aminoacyl-tRNA synthetase evolution using directed engineering approaches.

INTRODUCTION

Translation of the genetic information from DNA to protein is a central process in all three domains of life (bacteria, archaea and eukaryotes). Protein synthesis on the

ribosome requires aminoacyl-transfer RNA (aa-tRNA) substrates, which are formed by aminoacyl-tRNA synthetases (aaRSs) and delivered to the ribosome by elongation factors (1–3). Except for selenocysteine, each of the 22 proteinogenic amino acids has a cognate aaRS that ligates it to the corresponding tRNAs, although several aa-tRNAs use indirect synthesis pathways in defined organisms (4). For example, a number of methanogenic archaea lack a cysteinyl-tRNA synthetase and produce Cys-tRNA^{Cys} via a two-step pathway (5). Many bacteria and archaea do not encode an asparaginyl-tRNA synthetase; in such organisms, synthesis of Asn-tRNA^{Asn} begins with misacylation of aspartate (Asp) to tRNA^{Asn} by a non-discriminating (ND) aspartyl-tRNA synthetase, followed by conversion of Asp to Asn by an amidotransferase (AdT) (4). Similarly, most bacteria and all archaea lack a glutamyl-tRNA synthetase (GlnRS) and use a Glu-AdT to produce Gln-tRNA^{Gln} from Glu-tRNA^{Gln}, which is synthesized by a glutamyl-tRNA synthetase (GluRS) (4,6) (Figure 1). In contrast, eukaryotes have evolved a GlnRS to directly synthesize Gln-tRNA^{Gln} (1).

In most bacteria and archaea, glutamate (Glu) is attached to both tRNA^{Glu} and tRNA^{Gln} by a ND-GluRS. Certain bacteria (e.g. *Helicobacter pylori*) encode two types of GluRSs: GluRS1 that ligates Glu to tRNA^{Glu} and GluRS2 that specifically attaches Glu to tRNA^{Gln} (7,8) (Figure 1). Phylogenetic and structural studies suggested that modern GluRSs and GlnRSs evolved from a common ancestral ND-GluRS (9). GlnRS evolved from ND-GluRS after the split between archaea and eukaryotes, and the gene encoding eukaryotic GlnRS was later transferred horizontally to certain bacterial species (e.g. *Escherichia coli*) (10–12). In this work, we have combined rational design and directed evolution approaches to obtain a *H. pylori* GluRS2 variant that is capable of rescuing a *glnS* temperature-sensitive *E. coli* strain (13,14), and aminoacylating Gln to tRNA^{Gln} *in vitro*. We have thus identified an important precursor that could further evolve into a bacterial-type GlnRS.

*To whom correspondence should be addressed. Tel: +1 203 432 6205; Fax: +1 203 432 6202; Email: jiqiang.ling@yale.edu
Correspondence may also be addressed to Dieter Söll. Tel: +1 203 432 6200; Fax: +1 203 432 6202; Email: dieter.soll@yale.edu

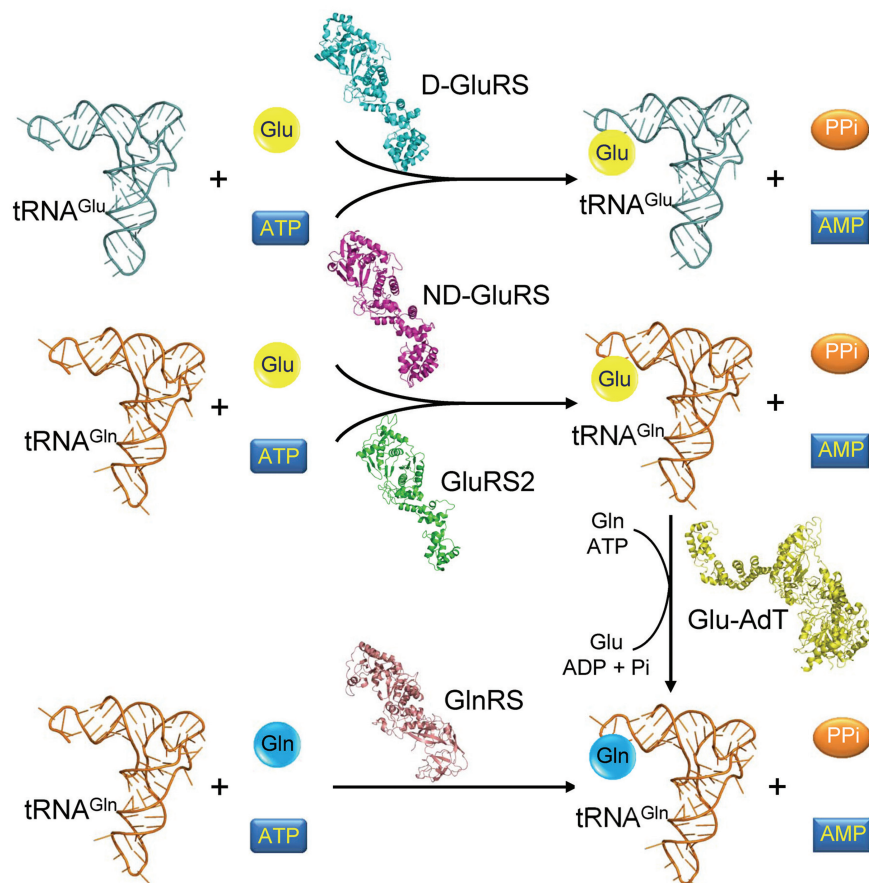


Figure 1. Pathways for Glu-tRNA and Gln-tRNA synthesis. Discriminating-GluRS (D-GluRS) selectively attaches Glu to tRNA^{Glu}; ND-GluRS attaches Glu to both tRNA^{Glu} and tRNA^{Gln} and GluRS2 specifically attaches Glu to tRNA^{Gln}. The resulting Glu-tRNA^{Gln} is converted to Gln-tRNA^{Gln} by Glu-AdT. Alternatively, Gln-tRNA^{Gln} is directly synthesized by GlnRS. The structures are taken from PDB 1Q7Q, 3AL0 and 3AII.

MATERIALS AND METHODS

Strains, plasmids and protein purification

Escherichia coli UT172 *glnS* temperature-sensitive strain was used for selection of the *H. pylori* GluRS2 mutation library carried on pERS2 vector, which was derived from pUC18. *Escherichia coli* total tRNA used in this study was purchased from Roche. Wild-type (WT) and mutant *H. pylori* GluRS2 were cloned into pET20b vector for overexpression in the BL21(DE3) *E. coli* strain. Expression of WT GluRS2 was induced in Luria-Bertani (LB) broth with 1 mM isopropyl β -D-1-thiogalactopyranoside for 1 h at 37°C when A_{600} reached 1.2. M110 GluRS2 formed inclusion bodies when overexpressed at 37°C. Its expression was thus induced at 30°C with 0.1 mM isopropyl β -D-1-thiogalactopyranoside for 5 h at $A_{600} \sim 1.2$. The proteins were purified with Ni-NTA affinity resin (Qiagen) according to the standard procedures and dialyzed against a storage buffer containing 50 mM Hepes (pH 7.5), 100 mM NaCl, 10 mM 2-mercaptoethanol, 3 mM MgCl₂ and 50% glycerol.

Construction and selection of *H. pylori* GluRS2 mutation library

To construct a pERS2 plasmid, the full-length gene encoding GluRS2 was inserted into pUC18 plasmid

between EcoRI and PstI sites. An additional G nucleotide was included after the EcoRI site to allow GluRS2 controlled by Plac promoter.

Seven residues (A5, S7, E39, C178, I190, R192 and H196) located in the amino acid binding pocket of *H. pylori* GluRS2 were chosen based on the crystal structure of *Thermus thermophilus* GluRS:tRNA^{Glu} (2CV0) to generate the mutation library. I190 and R192 were rationally mutated to serine and cysteine, respectively. A5, S7, E39, C178 and H196 were randomized. Overlap polymerase chain reaction was performed as described previously (15). 4×10^8 transformants were obtained for the library, which was 12 times larger than calculated library size (3.4×10^7).

The mutation library was selected in *E. coli* UT172 strain, in which mutants charging tRNA^{Gln} with glutamine would survive on LB plate supplemented with 100 μ g/ml ampicillin at 42°C. During the first round of selection, 1 μ g plasmid was transformed into UT172 electrocompetent cells to obtain 1×10^9 transformants. The transformants were recovered in 20 ml Super Optimal broth with Catabolite repression (SOC) at 30°C for 2 h, followed by the addition of 100 μ g/ml ampicillin and 8 h of growth. 0.3 ml saturated culture was diluted into 15 ml SOC medium and grown for 5 h at 30°C. Next, 1 ml culture was spread on 20 \times 20 cm LB plate supplemented

with 100 µg/ml ampicillin and the plate was incubated at 42°C for 24 h. Colonies growing on the plate were scraped off and extracted for plasmids. The plasmids from the first round of selection were subjected to a second round of selection with the same procedure.

Enzymatic assays

Adenosine triphosphate (ATP)–pyrophosphate (PPi) exchange reaction was performed in the buffer containing 100 mM Hepes–KOH, pH 7.2, 30 mM KCl, 12 mM MgCl₂, 2 mM dithiothreitol (DTT), 2 mM KF, 5 mg/ml total *E. coli* tRNA, 5 mM ATP, 5 mM PPi, 0.1 µCi [γ -³²P]ATP, 9 µM enzymes and 0.24–10 mM Glu and Gln. The 1 µl reaction mixture was spotted onto thin-layer chromatography (TLC) polyethylenimine (PEI) cellulose F plates (Merck). Then, the TLC plates were developed in 1 M KH₂PO₄ and 1 M urea to separate ATP and PPi. Detection and quantification of signals were performed as described before (16).

Aminoacylation assay was essentially performed as described before (8) with slight modifications. The 40 µl reaction mixture contained 100 mM Hepes–KOH, pH 7.2, 30 mM KCl, 12 mM MgCl₂, 2 mM DTT, 5 mg/ml total *E. coli* tRNA, 5 mM ATP, 40 µM [¹⁴C]Glu or [³H]Gln and 2.3 µM enzymes; 8 µl aliquots were taken out at each time point for scintillation counting.

ATP consumption was performed in the presence of 100 mM Hepes–KOH (pH 7.2), 30 mM KCl, 12 mM MgCl₂, 2 mM DTT, 5 mg/ml total *E. coli* tRNA, 0.1 µCi [α -³²P]ATP, 1 mM cold ATP, 10 mM Glu or Gln and 9 µM GluRS2. At each time spot, a 2-µl aliquot was quenched with 2 µl acetic acid. The 1 µl resulting mixture was spotted on PEI cellulose plates and separated in 0.1 M ammonium acetate and 5% acetic acid. The Adenosine monophosphate (AMP)/ATP ratios were quantified with phosphorimaging. No aminoacyl adenylate spot was observed during the reaction time course.

RESULTS

Design and evolution of a GluRS2 variant that rescues a *glnS* temperature-sensitive strain

Previous studies have shown that unlike ND-GluRS that aminoacylates Glu to both tRNA^{Glu} and tRNA^{Gln}, *H. pylori* GluRS2 (*HpGluRS2*) specifically charges Glu to tRNA^{Gln}, indicating that GluRS2 may be on the way to evolve into a bacterial-type GlnRS that is distinct from the eukaryotic-type GlnRS found in modern organisms (7,8). Phylogenetic analyses have shown that GluRS2 is closely related to bacterial GluRSs but is more distant from GlnRSs present in eukaryotes and certain bacteria (9). Our sequence alignment revealed multiple active site residues that have distinct patterns in GluRS and GlnRS proteins (Figure 2). For example, position 190 (*H. pylori* GluRS2 numbering) is either an isoleucine or valine in bacterial GluRSs but is changed to a conserved serine in GlnRSs, and R192 of *HpGluRS2* corresponds to a cysteine in GlnRSs. The crystal structure of *T. thermophilus* GluRS shows that these two residues interact with the side chain of the substrate Glu in

the presence of tRNA (17) (Figure 3). The structures of *E. coli* GlnRS also reveal that S227 and C229 (corresponding to I190 and R192 in *HpGluRS2*, respectively) are located close to the side chain of Gln at the active site (18,19).

To evolve *HpGluRS2* into a GlnRS, we rationally introduced mutations I190S and R192C. We further randomized five active site residues that are important for amino acid binding based on the structure of *T. thermophilus* GluRS (17) (Figure 3). The plasmid library expressing randomized *HpGluRS2* was transformed into *E. coli* strain UT172, which harbored a temperature-sensitive *glnS* gene (13,14). This *E. coli* strain was not able to grow at 42°C due to a defect in Gln-tRNA^{Gln} synthesis, and as expected, WT *HpGluRS2* did not complement growth at 42°C (Figure 4). Selection of the transformed library at 42°C led to one GluRS2 variant (named M110) that rescued the growth of UT172 (Figure 4). Growth analysis revealed that UT172 transformed with M110 exhibited a doubling time of 94 min at 42°C, compared with 32 min for the strain transformed with *E. coli* GlnRS (Figure 4B). Sequencing results showed that M110 contained mutations A5L, S7R, E39R, C178L, I190S, R192C and H196Q. The *in vivo* complementation assay suggested that M110 GluRS2 likely supplied the *E. coli* cells with sufficient Gln-tRNA^{Gln} to support growth. Alternatively, M110 GluRS2 might possess a higher Glu-tRNA^{Gln} synthesis activity than the WT at 42°C, and Glu misincorporation at Gln codons rescued the growth of UT172. Such possibilities were further investigated using biochemical assays *in vitro*.

M110 GluRS2 has evolved a GlnRS activity

To probe the amino acid specificity of the evolved enzyme, we purified the WT and M110 GluRS2 variants and performed *in vitro* pyrophosphate exchange experiments, which measured the activation of amino acids with ATP. To our surprise, WT GluRS2 exhibited similar activation efficiencies (determined by the k_{cat}/K_m value) for Glu and Gln (Table 1). Both the k_{cat} and K_m values for Gln were ~2-fold lower than Glu. Conversely, the M110 variant showed 2.3-fold preference for Gln over Glu. The Gln activation efficiency was 9-fold higher for M110 than WT due to increased k_{cat} and decreased K_m values.

Next, we measured aminoacylation activities of WT and M110 GluRS2. Despite similar amino acid activation efficiencies, WT GluRS2 only attaches Glu, but not Gln, to tRNA^{Gln} at both 37 and 42°C (Figure 5). M110 showed 4-fold increased aminoacylation efficiency for Gln than Glu, confirming that this variant had evolved a GlnRS activity. M110 still maintained the Glu charging activity, although it was reduced by 30% compared with the WT GluRS2 at 37°C. The WT and M110 variants showed almost identical Glu charging efficiency at 42°C. Given that only M110 but not the WT GluRS2 rescued the *glnS* temperature-sensitive strain, our aminoacylation data suggested that instead of Glu-tRNA^{Gln} production, the Gln-tRNA^{Gln} synthesis activity that had evolved in M110 was responsible for rescuing the growth phenotype of UT172.

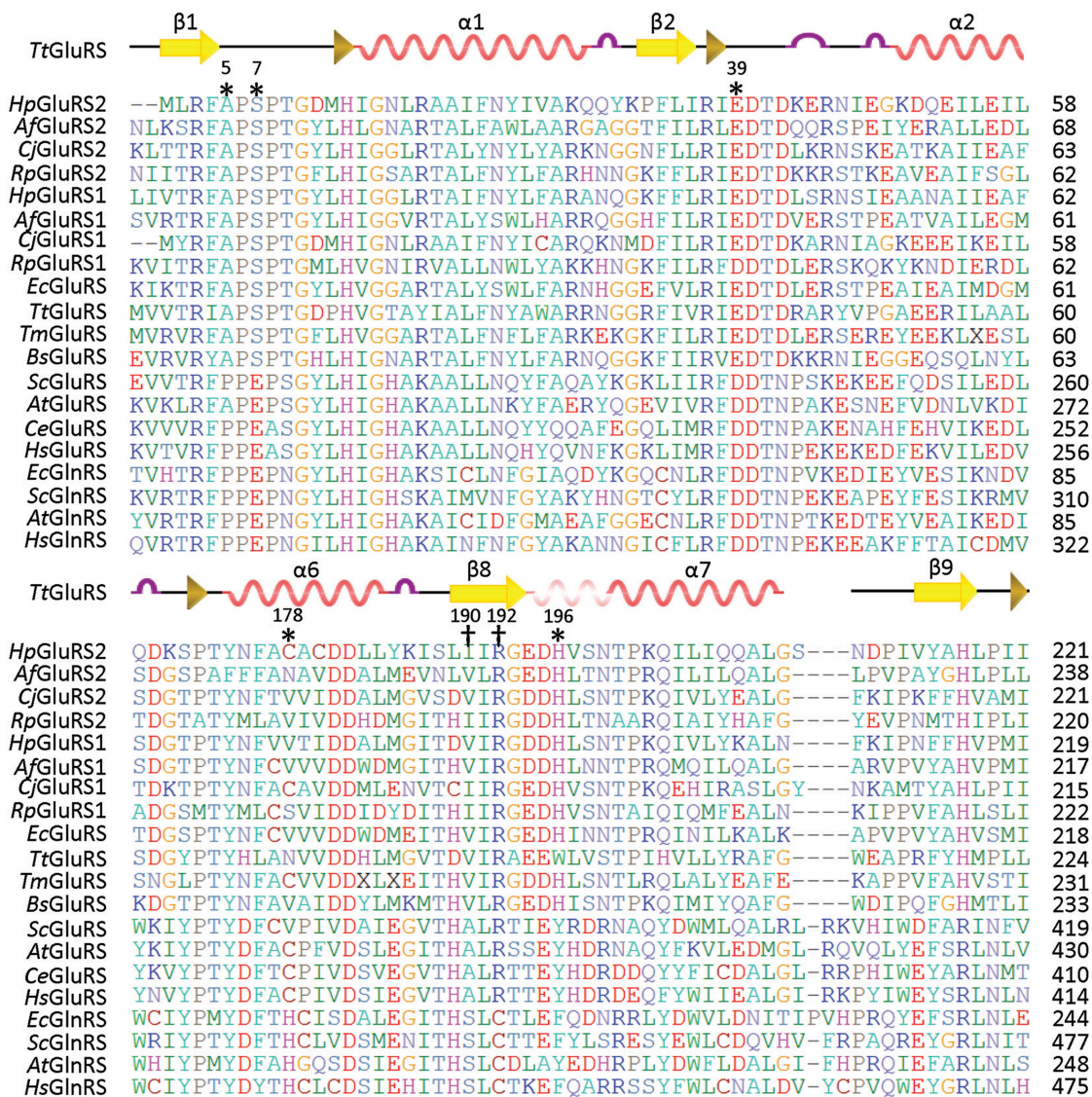


Figure 2. Sequence alignment of the GluRS/GlnRS family proteins. The residues that are rationally engineered in this work are indicated by daggers and the randomized residues are asterisked. *Hp*, *Helicobacter pylori*; *Af*, *Acidithiobacillus ferrooxidans*; *Cj*, *Campylobacter jejuni*; *Rp*, *Rickettsia prowazekii*; *Ec*, *Escherichia coli*; *Tt*, *Thermus thermophilus*; *Tm*, *Thermotoga maritima*; *Bs*, *Bacillus subtilis*; *Sc*, *Saccharomyces cerevisiae*; *At*, *Arabidopsis thaliana*; *Ce*, *Caenorhabditis elegans* and *Hs*, *Homo sapiens*.

M110 GluRS2 uses an editing mechanism to reduce aminoacylation of Glu to tRNA^{Gln}

The discrepancies between amino acid activation and aminoacylation results led us to investigate whether a quality control mechanism might help GluRS2 variants to discriminate between Glu and Gln. It has been previously shown that *E. coli* GlnRS hydrolyzes glutaminyl adenylate (Gln-AMP) in the presence of tRNA (20), mimicking a pre-transfer editing activity discovered in other aaRSs (21–24). We measured the hydrolysis of [α -³²P]ATP by WT and M110 GluRS2 in the presence of tRNA and Glu or Gln (Figure 6). WT GluRS2 hydrolyzed ATP about 3-fold more slowly in the presence of Gln than Glu, suggesting that the WT enzyme does not use an editing activity to prevent Gln from being attached to tRNA^{Gln}. Rather, the activated Gln-AMP is likely

positioned in a non-productive fashion at the active site of WT GluRS2, restricting the transfer of Gln to tRNA. The M110 variant showed 3-fold higher ATP hydrolysis rate than the WT in the presence of Glu, and the end point of hydrolyzed ATP was 15-fold higher than the available pool of tRNA^{Gln} in the reaction (Figure 6). This suggests that M110 uses an editing mechanism to selectively hydrolyze activated Glu-AMP. Collectively, our results indicate that M110 uses both kinetic discrimination and editing mechanisms to preferentially aminoacylate Gln over Glu.

DISCUSSION

Evolution of the GluRS/GlnRS family enzymes

It has been widely accepted that the indirect pathway of Gln-tRNA^{Gln} synthesis predates the direct pathway and

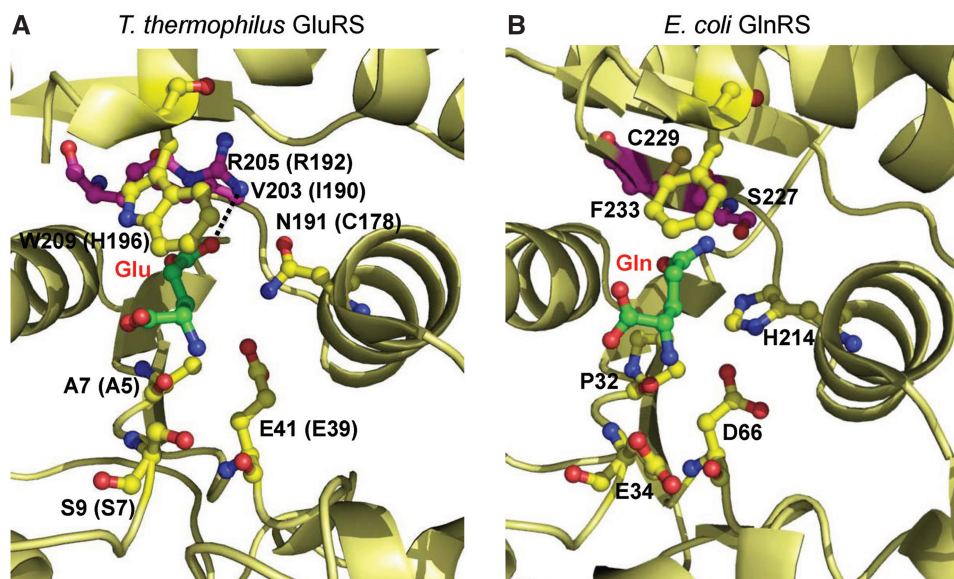


Figure 3. Active sites of GluRS and GlnRS. The crystal structures of (A) *T. thermophilus* GluRS (2CV0) and (B) *E. coli* GlnRS (1O0B) are shown. In parentheses are corresponding residues in *H. pylori* GluRS2. The rationally mutated residues are shown in purple and randomized residues are shown in blue.

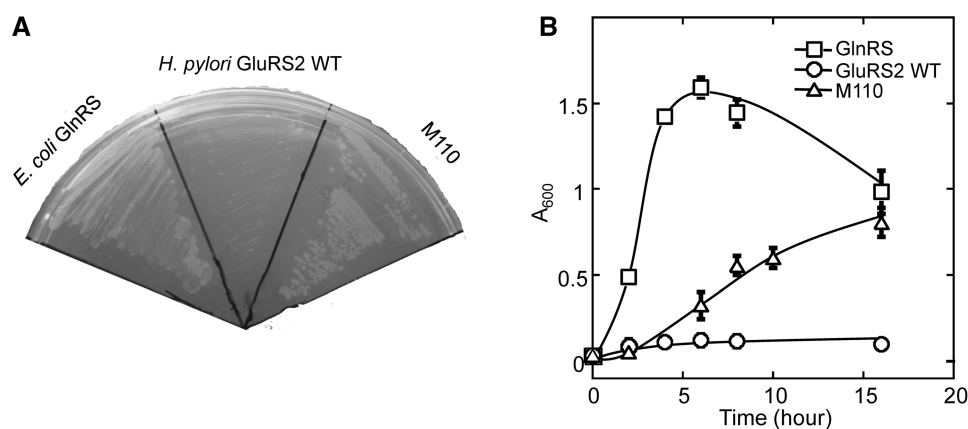


Figure 4. Complementation of the *glnS* temperature-sensitive *E. coli* strain UT172. (A) GlnRS and M110 GluRS2, but not WT GluRS2, are able to rescue the growth of strain UT172 at 42°C on LB plates. (B) UT172 expressing M110 shows a slower growth rate compared with the strain expressing *E. coli* GlnRS at 42°C in LB broth. The results are the average of at least four repeats with standard deviations indicated.

Table 1. Pyrophosphate exchange activities of WT and M110 GluRS2 at 37°C

	Glu			Gln			Selectivity (Gln/Glu)
	k_{cat} ($\times 10^{-3}$ s $^{-1}$)	K_m (mM)	k_{cat}/K_m ($\times 10^{-3}$ mM $^{-1}$ s $^{-1}$)	k_{cat} ($\times 10^{-3}$ s $^{-1}$)	K_m (mM)	k_{cat}/K_m ($\times 10^{-3}$ mM $^{-1}$ s $^{-1}$)	
WT	18 ± 0.1	0.44 ± 0.07	41 ± 7	8.9 ± 0.5	0.17 ± 0.04	49 ± 11	1.2
M110	18 ± 0.4	0.09 ± 0.02	190 ± 40	26 ± 2	0.06 ± 0.01	430 ± 110	2.3

that GlnRS was absent in the last universal common ancestor (LUCA) (4,10,12). The GluRS in LUCA was a ND enzyme and charged Glu to both tRNA^{Glu} and tRNA^{Gln} (Figure 1). Glu-tRNA^{Gln} was then converted

to Gln-tRNA^{Gln} by a Glu-AdT (GatCAB in chloroplasts and mitochondria of bacteria; GatFAB in mitochondria of yeast and GatDE in archaea) (4,25–28). GlnRS evolved in early eukaryotes from a duplicated ND-GluRS to gain

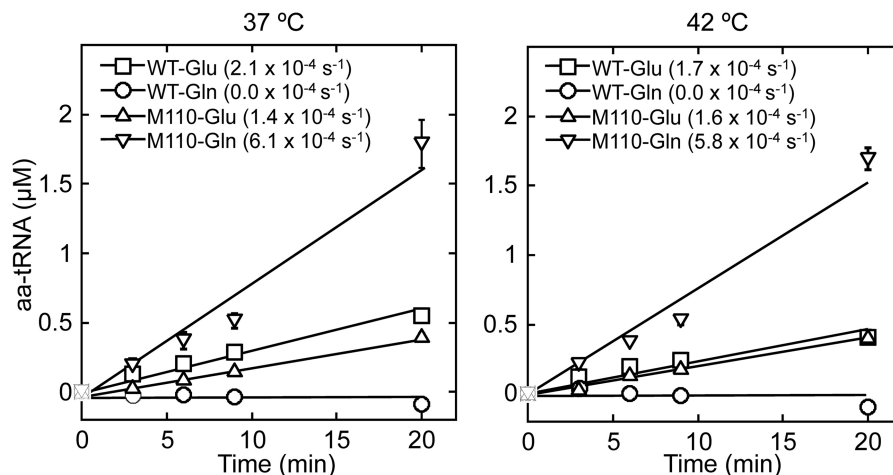


Figure 5. Aminoacylation by GluRS2 variants. WT GluRS2 (2.3 μM) charges Glu (40 μM) but not Gln (40 μM) to tRNA^{Gln} (5 mg/ml total *E. coli* tRNA containing 3 μM tRNA^{Gln} as determined by plateau charging), whereas M110 (2.3 μM) preferentially aminoacylates Gln over Glu at both 37 and 42°C.

specificity for Gln and tRNA^{Gln} and was later transferred to certain bacteria (9,29). A group of proteobacteria, including *H. pylori*, acquired a second GluRS (GluRS2) through horizontal gene transfer. GluRS2 specifically recognizes tRNA^{Gln}, leading to the hypothesis that it has been evolving to become a bacterial-type GlnRS (7,8). We show here that WT *H. pylori* GluRS2 already possesses the power to activate Gln, yet the resulting Gln-AMP may not be correctly positioned for the transfer of the amino acid moiety to tRNA. Our engineered GluRS2 variant (M110) has gained Gln charging activity and thus represents an important precursor towards the evolution of a bacterial-type GlnRS. The aminoacylation activities of our GluRS2 variants are low compared with previously published results (30), presumably because to be consistent with our *in vivo* tests, we have used total *E. coli* tRNA instead of purified *H. pylori* tRNA^{Gln}. The heterologous tRNA used and the competition from non-cognate tRNAs could decrease the aminoacylation efficiency of GluRS2.

Structural insights into substrate recognition by WT and M110 GluRS2

WT GluRS2 specifically attaches Glu (but not Gln) to tRNA, although it also activates Gln during the pyrophosphate exchange assay (Figure 5 and Table 1). In contrast, the M110 variant acylates Gln 4-fold more efficiently than Glu (Figure 5). We propose that WT GluRS2 binds activated Gln-AMP in a mode not suitable for aminoacylation. Structural studies of *T. thermophilus* GluRS reveal that R205 (equivalent of R192 in *H. pylori* GluRS2 and C229 in *E. coli* GlnRS) directly interacts with the side-chain oxygen of Glu (Figure 3). This residue might serve as a negative determinant for proper positioning of Gln-AMP during the amino acid transfer step. The evolved M110 variant contains a R192C mutation, which could be critical for glutamylation. In line with this notion, a C229R

mutation has been shown to significantly improve the K_m of *E. coli* GlnRS for Glu (31). It is interesting that the M110 variant maintains the same glutamylation activity as in the WT, presumably due to the cumulative effects of other mutations present in M110.

Quality control mechanisms in natural and engineered aaRSs

Selection of the correct amino acid is a big challenge for many aaRSs due to the structural and chemical similarities between amino acids. To maintain translational fidelity, such aaRSs use editing mechanisms to hydrolyze incorrect aminoacyl adenylates (pre-transfer editing) or aa-tRNAs (post-transfer editing) (32,33), whereas post-transfer editing requires a separate editing domain or a free-standing protein (34–36) and pre-transfer editing mainly occurs at the active site (21,37,38). It is intriguing that our engineered M110 GluRS2 appears to have evolved an editing mechanism to discriminate against Glu in favor of Gln (Figure 6). GluRS2 lacks a post-transfer editing domain, prompting us to hypothesize that M110 uses a pre-transfer editing strategy to hydrolyze activated Glu-AMP, presumably at the active site resembling *E. coli* GlnRS (20). It is worth noting that a recent study shows that WT GluRS2 modestly increases the hydrolysis of Glu-tRNA^{Gln} by an unknown mechanism (30). The fidelity mechanism for GluRS2 thus remains to be addressed in future studies. Should editing occur at the active site before amino acid transfer, a water molecule is likely required for hydrolysis of Glu-AMP and Gln-AMP. We have recently shown that yeast mitochondrial threonyl-tRNA synthetase edits seryl adenylate faster than threonyl adenylate due to the minor movement of a potential catalytic water molecule (Ling *et al.*, unpublished work). A similar mechanism could explain the preferential hydrolysis of Glu-AMP over Gln-AMP by M110, which needs to be clarified by future structural studies.

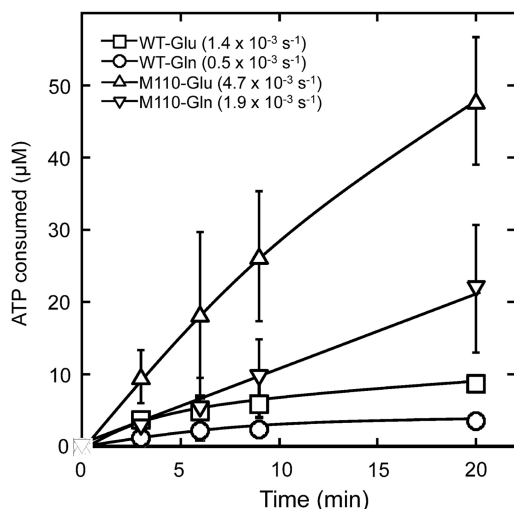


Figure 6. ATP hydrolysis by GluRS2 variants. M110 (9 µM) hydrolyzes ATP 2.5-fold faster in the presence of Glu (10 mM) than Gln (10 mM) at 37°C, suggesting that this mutant discriminates against Glu using an editing mechanism.

Engineering aaRSs towards expanded substrate recognition

Several aaRSs have been successfully engineered to co-translationally incorporate a variety of unnatural amino acids into proteins in bacteria and eukaryotes (15,39–46). Both rational design and directed evolution methods have been used for unnatural amino acid incorporation, yet only a few studies have used such methods to understand the evolution of natural aaRSs. A rationally engineered *E. coli* GlnRS has obtained a misacylation activity to produce Glu-tRNA^{Gln} (31), and transplanting the GlnRS acceptor stem loop to an archaeal ND-GluRS makes it specifically recognize tRNA^{Gln} (29). In this work, we have combined rational design and directed evolution approaches to understand the evolutionary potential from GluRS2 to GlnRS. Such a strategy could be used as a model to investigate how primordial aaRSs have acquired new functions to become the enzymes present in modern organisms.

ACKNOWLEDGEMENTS

We are grateful to Kelly Sheppard (Skidmore College), Patrick O'Donoghue and Ilka Heinemann (Yale University) for insightful discussion and comments.

FUNDING

Funding for open access charge: National Institute of General Medical Sciences [GM022854 to D.S.].

Conflict of interest statement. None declared.

REFERENCES

- Ibba,M. and Söll,D. (2000) Aminoacyl-tRNA synthesis. *Annu. Rev. Biochem.*, **69**, 617–650.
- Steitz,T.A. (2008) A structural understanding of the dynamic ribosome machine. *Nat. Rev. Mol. Cell Biol.*, **9**, 242–253.
- Dale,T. and Uhlenbeck,O.C. (2005) Amino acid specificity in translation. *Trends Biochem. Sci.*, **30**, 659–665.
- Sheppard,K., Yuan,J., Hohn,M.J., Jester,B., Devine,K.M. and Söll,D. (2008) From one amino acid to another: tRNA-dependent amino acid biosynthesis. *Nucleic Acids Res.*, **36**, 1813–1825.
- Sauerwald,A., Zhu,W., Major,T.A., Roy,H., Palioura,S., Jahn,D., Whitman,W.B., Yates,J.R. 3rd, Ibba,M. and Söll,D. (2005) RNA-dependent cysteine biosynthesis in archaea. *Science*, **307**, 1969–1972.
- Nureki,O., Vassylyev,D.G., Katayanagi,K., Shimizu,T., Sekine,S., Kigawa,T., Miyazawa,T., Yokoyama,S. and Morikawa,K. (1995) Architectures of class-defining and specific domains of glutamyl-tRNA synthetase. *Science*, **267**, 1958–1965.
- Skouloubris,S., Ribas de Pouplana,L., De Reuse,H. and Hendrickson,T.L. (2003) A noncognate aminoacyl-tRNA synthetase that may resolve a missing link in protein evolution. *Proc. Natl Acad. Sci. USA*, **100**, 11297–11302.
- Salazar,J.C., Ahel,I., Orellana,O., Tumbula-Hansen,D., Krieger,R., Daniels,L. and Söll,D. (2003) Coevolution of an aminoacyl-tRNA synthetase with its tRNA substrates. *Proc. Natl Acad. Sci. USA*, **100**, 13863–13868.
- Nureki,O., O'Donoghue,P., Watanabe,N., Ohmori,A., Oshikane,H., Arais,Y., Sheppard,K., Söll,D. and Ishitani,R. (2010) Structure of an archaeal non-discriminating glutamyl-tRNA synthetase: a missing link in the evolution of Gln-tRNA^{Gln} formation. *Nucleic Acids Res.*, **38**, 7286–7297.
- Lamour,V., Quevillon,S., Diriong,S., N'Guyen,V.C., Lipinski,M. and Mirande,M. (1994) Evolution of the Glx-tRNA synthetase family: the glutaminyl enzyme as a case of horizontal gene transfer. *Proc. Natl Acad. Sci. USA*, **91**, 8670–8674.
- Woese,C.R., Olsen,G.J., Ibba,M. and Söll,D. (2000) Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process. *Microbiol. Mol. Biol. Rev.*, **64**, 202–236.
- O'Donoghue,P. and Luthey-Schulten,Z. (2003) On the evolution of structure in aminoacyl-tRNA synthetases. *Microbiol. Mol. Biol. Rev.*, **67**, 550–573.
- Englich-Peters,S., Conley,J., Plumbridge,J., Leptak,C., Söll,D. and Rogers,M.J. (1991) Mutant enzymes and tRNAs as probes of the glutaminyl-tRNA synthetase: tRNA^{Gln} interaction. *Biochimie*, **73**, 1501–1508.
- Weygand-Durasevic,I., Schwob,E. and Söll,D. (1993) Acceptor end binding domain interactions ensure correct aminoacylation of transfer RNA. *Proc. Natl Acad. Sci. USA*, **90**, 2010–2014.
- Park,H.S., Hohn,M.J., Umehara,T., Guo,L.T., Osborne,E.M., Benner,J., Noren,C.J., Rinehart,J. and Söll,D. (2011) Expanding the genetic code of *Escherichia coli* with phosphoserine. *Science*, **333**, 1151–1154.
- Guo,L.T., Chen,X.L., Zhao,B.T., Shi,Y., Li,W., Xue,H. and Jin,Y.X. (2007) Human tryptophanyl-tRNA synthetase is switched to a tRNA-dependent mode for tryptophan activation by mutations at V85 and I311. *Nucleic Acids Res.*, **35**, 5934–5943.
- Sekine,S., Shichiri,M., Bernier,S., Chenevert,R., Lapointe,J. and Yokoyama,S. (2006) Structural bases of transfer RNA-dependent amino acid recognition and activation by glutamyl-tRNA synthetase. *Structure*, **14**, 1791–1799.
- Rath,V.L., Silvian,L.F., Beijer,B., Sproat,B.S. and Steitz,T.A. (1998) How glutaminyl-tRNA synthetase selects glutamine. *Structure*, **6**, 439–449.
- Bullock,T.L., Uter,N., Nissan,T.A. and Perona,J.J. (2003) Amino acid discrimination by a class I aminoacyl-tRNA synthetase specified by negative determinants. *J. Mol. Biol.*, **328**, 395–408.
- Gruic-Sovulj,I., Uter,N., Bullock,T. and Perona,J.J. (2005) tRNA-dependent aminoacyl-adenylate hydrolysis by a nonediting class I aminoacyl-tRNA synthetase. *J. Biol. Chem.*, **280**, 23978–23986.
- Boniecki,M.T., Vu,M.T., Betha,A.K. and Martinis,S.A. (2008) CP1-dependent partitioning of pretransfer and posttransfer editing in leucyl-tRNA synthetase. *Proc. Natl Acad. Sci. USA*, **105**, 19223–19228.
- Hendrickson,T.L., Nomanbhoy,T.K., Crécy-Lagard,V., Fukai,S., Nureki,O., Yokoyama,S. and Schimmel,P. (2002) Mutational

- separation of two pathways for editing by a class I tRNA synthetase. *Mol. Cell*, **9**, 353–362.
23. Splan, K.E., Ignatov, M.E. and Musier-Forsyth, K. (2008) Transfer RNA modulates the editing mechanism used by class II prolyl-tRNA synthetase. *J. Biol. Chem.*, **283**, 7128–7134.
 24. Minajigi, A. and Francklyn, C.S. (2010) Aminoacyl transfer rate dictates choice of editing pathway in threonyl-tRNA synthetase. *J. Biol. Chem.*, **285**, 23810–23817.
 25. Pujol, C., Bailly, M., Kern, D., Marechal-Drouard, L., Becker, H. and Duchene, A.M. (2008) Dual-targeted tRNA-dependent amidotransferase ensures both mitochondrial and chloroplastic Gln-tRNA^{Gln} synthesis in plants. *Proc. Natl Acad. Sci. USA*, **105**, 6481–6485.
 26. Frechin, M., Senger, B., Braye, M., Kern, D., Martin, R.P. and Becker, H.D. (2009) Yeast mitochondrial Gln-tRNA^{Gln} is generated by a GatFAB-mediated transamidation pathway involving Arc1p-controlled subcellular sorting of cytosolic GluRS. *Genes Dev.*, **23**, 1119–1130.
 27. Nagao, A., Suzuki, T., Katoh, T. and Sakaguchi, Y. (2009) Biogenesis of glutamyl-tRNA^{Gln} in human mitochondria. *Proc. Natl Acad. Sci. USA*, **106**, 16209–16214.
 28. Tumbula, D.L., Becker, H.D., Chang, W.Z. and Söll, D. (2000) Domain-specific recruitment of amide amino acids for protein synthesis. *Nature*, **407**, 106–110.
 29. O'Donoghue, P., Sheppard, K., Nureki, O. and Söll, D. (2011) Rational design of an evolutionary precursor of glutamyl-tRNA synthetase. *Proc. Natl Acad. Sci. USA*, **108**, 20485–20490.
 30. Huot, J.L., Fischer, F., Corbeil, J., Madore, E., Lorber, B., Diss, G., Hendrickson, T.L., Kern, D. and Lapointe, J. (2011) Gln-tRNA^{Gln} synthesis in a dynamic transamidosome from *Helicobacter pylori*, where GluRS2 hydrolyzes excess Glu-tRNA^{Gln}. *Nucleic Acids Res.*, **39**, 9306–9315.
 31. Bullock, T.L., Rodriguez-Hernandez, A., Corigliano, E.M. and Perona, J.J. (2008) A rationally engineered misacylating aminoacyl-tRNA synthetase. *Proc. Natl Acad. Sci. USA*, **105**, 7428–7433.
 32. Mascarenhas, A.P., An, S., Rosen, A.E., Martinis, S.A. and Musier-Forsyth, K. (2008) Fidelity mechanisms of the aminoacyl-tRNA synthetases. In: RajBhandary, U.L. and Köhrer, C. (eds), *Protein Engineering*. Springer, New York, pp. 153–200.
 33. Ling, J., Reynolds, N. and Ibba, M. (2009) Aminoacyl-tRNA synthesis and translational quality control. *Annu. Rev. Microbiol.*, **63**, 61–78.
 34. Schmidt, E. and Schimmel, P. (1994) Mutational isolation of a sieve for editing in a transfer RNA synthetase. *Science*, **264**, 265–267.
 35. An, S. and Musier-Forsyth, K. (2004) Trans-editing of Cys-tRNA^{Pro} by *Haemophilus influenzae* YbaK protein. *J. Biol. Chem.*, **279**, 42359–42362.
 36. Ahel, I., Korencic, D., Ibba, M. and Söll, D. (2003) Trans-editing of mischarged tRNAs. *Proc. Natl Acad. Sci. USA*, **100**, 15422–15427.
 37. SternJohn, J., Hati, S., Siliciano, P.G. and Musier-Forsyth, K. (2007) Restoring species-specific posttransfer editing activity to a synthetase with a defunct editing domain. *Proc. Natl Acad. Sci. USA*, **104**, 2127–2132.
 38. Zhu, B., Yao, P., Tan, M., Eriani, G. and Wang, E.D. (2009) tRNA-independent pretransfer editing by class I leucyl-tRNA synthetase. *J. Biol. Chem.*, **284**, 3418–3424.
 39. Yoo, T.H. and Tirrell, D.A. (2007) High-throughput screening for methionyl-tRNA synthetases that enable residue-specific incorporation of noncanonical amino acids into recombinant proteins in bacterial cells. *Angew. Chem. Int. Ed. Engl.*, **46**, 5340–5343.
 40. Datta, D., Wang, P., Carrico, I.S., Mayo, S.L. and Tirrell, D.A. (2002) A designed phenylalanyl-tRNA synthetase variant allows efficient in vivo incorporation of aryl ketone functionality into proteins. *J. Am. Chem. Soc.*, **124**, 5652–5653.
 41. Tang, Y. and Tirrell, D.A. (2002) Attenuation of the editing activity of the *Escherichia coli* leucyl-tRNA synthetase allows incorporation of novel amino acids into proteins in vivo. *Biochemistry*, **41**, 10635–10645.
 42. Liu, W., Brock, A., Chen, S. and Schultz, P.G. (2007) Genetic incorporation of unnatural amino acids into proteins in mammalian cells. *Nat. Methods*, **4**, 239–244.
 43. Wan, W., Huang, Y., Wang, Z., Russell, W.K., Pai, P.J., Russell, D.H. and Liu, W.R. (2010) A facile system for genetic incorporation of two different noncanonical amino acids into one protein in *Escherichia coli*. *Angew. Chem. Int. Ed. Engl.*, **49**, 3211–3214.
 44. Chin, J.W., Cropp, T.A., Anderson, J.C., Mukherji, M., Zhang, Z. and Schultz, P.G. (2003) An expanded eukaryotic genetic code. *Science*, **301**, 964–967.
 45. Neumann, H., Peak-Chew, S.Y. and Chin, J.W. (2008) Genetically encoding N(epsilon)-acetyllysine in recombinant proteins. *Nat. Chem. Biol.*, **4**, 232–234.
 46. Liu, C.C. and Schultz, P.G. (2010) Adding new chemistries to the genetic code. *Annu. Rev. Biochem.*, **79**, 413–444.