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Genetics

Functional communication in the recognition of tRNA by *Escherichia coli* glutaminyl-tRNA synthetase

(opal suppressor/tRNA specificity/aminoacyl-tRNA synthetase/genetic selection/mutation)

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ABSTRACT **Wild-type *Escherichia coli* glutaminyl-tRNA synthetase (GlnRS; EC 6.1.1.18) poorly aminoacylates opal suppressors (GLN) derived from $tRNA^{Gln}$. Mutations in *glnS* (the gene encoding GlnRS) that compensate for impaired aminoacylation were isolated by genetic selection. Two *glnS* mutants were obtained by using opal suppressors differing in the nucleotides composing the base pair at 3·70: glnS113 with an Asp-235 → Asn change selected with GLNA3U70 (GLN carrying G3 → A and C70 → U changes), and glnS114 with a Gln-318 → Arg change selected with GLNU70 (GLN carrying a C70 → U change). The Asp-235 → Asn change was identified previously by genetic selection. Additional mutants were isolated by site-directed mutagenesis followed by genetic selection; the mutant enzymes have single amino acid changes (Lys-317 → Arg and Gln-318 → Lys). A number of mutants with no phenotype also were obtained randomly. *In vitro* aminoacylation of a $tRNA^{Gln}$ transcript by GlnRS enzymes with Lys-317 → Arg, Gln-318 → Lys, or Gln-318 → Arg changes shows that the enzyme's kinetic parameters are not greatly affected by the mutations. However, aminoacylation of a $tRNA^{Gln}$ transcript with an opal (UCA) anticodon shows that the specificity constants (k_{cat}/K_m) for the mutant enzymes were 5–10 times above that of the wild-type GlnRS. Interactions between Lys-317 and Gln-318 with the inside of the L-shaped tRNA and with the side chain of Gln-234 provide a connection between the acceptor end-binding and anticodon-binding domains of GlnRS. The GlnRS mutants isolated suggest that perturbation of the interactions with the inside of the tRNA L shape results in relaxed anticodon recognition.**

Accurate recognition of tRNA by aminoacyl-tRNA synthetases is a vital step ensuring the fidelity of gene expression. Genetic studies implicating the anticodon and acceptor stem of tRNA as important sites for recognition by *Escherichia coli* glutaminyl-tRNA synthetase (GlnRS; EC 6.1.1.18) have been confirmed by x-ray crystallographic analysis of the GlnRS–$tRNA^{Gln}$ complex (1, 2) and by kinetic data *in vitro* (3). Mutants of GlnRS with altered tRNA recognition have been obtained by genetic selection. Suppression of a glutamine-specific amber mutation in *lacZ*[*lacZ*(*UAG1000*)] allowed selection for mutants of GlnRS able to mischarge the *tyrT*(*UAG*) amber suppressor (4–6). The mutants isolated were found to affect tRNA acceptor stem recognition (7). Implications from these studies were confirmed by a more extensive mutational analysis of defined regions of the acceptor binding domain of GlnRS (8) with genetic selection using an amber suppressor derived from $tRNA^{Ser}$ (9).

The availability of the opal suppressor (GLN) derived from $tRNA_2^{Gln}$ (10) prompted a study of the amino acids inserted by the opal suppressor (11). We determined by amino acid sequencing of a reporter protein that GLN inserts predominantly tryptophan (88%) as well as glutamine (12%). Imposition of either C70 → U or G3 → A, C70 → U mutations on GLN resulted in opal suppressors (designated GLNU70 and GLNA3U70, respectively) that failed to suppress an opal mutation at position 15 in *trpA*, the gene for the α subunit of tryptophan synthetase (12). Insertion of glutamine but not tryptophan at position 15 in the α subunit derived from suppression of *trpA*(UGA15) leads to active *trpA* gene product. The lack of suppression by GLNU70 and GLNA3U70 was attributed to reduced aminoacylation by GlnRS (11).

This study describes the use of *trpA*(*UGA15*) suppression in a genetic selection to isolate *glnS* mutants that compensate for the impaired aminoacylation of GLNU70 and GLNA3U70. The location of these mutations and implications for the accurate recognition of tRNA by GlnRS are defined.

MATERIALS AND METHODS

Strains. *E. coli* strains with *trpA* mutations (12) were KL2576 [*metB glyV55* Δ(*tonBtrpAB17*)/F′*trpA*(*UGA15*)] and KL2725 [*trpT*(*UGA/G*) *glyV55* Δ(*tonBtrpAE15*)/F′-*trpA*(*UGA15*)]. The *lacZ*(*UAG1000*) strains have been described (4, 13): BT32 [relevant genotype *lacZ*(*UAG1000*) *tyrT*(*UAG*)], BT13 [isogenic to BT32 except for *serU*(*UAG*)], and BT235 [*lacZ*(*UAG1000*) su^0]. Strains DH5α and a *recA* derivative of JM101 were used for cloning, and CJ236 was used for *in vitro* mutagenesis. Strain X3R2 carries a chromosomal deletion of the *glnS* gene and was transformed with *glnS* mutants on pBR322 and cured of the prophage carrying $glnS^+$ (5).

Phages and Plasmids. Phage λgti is a cI_{857} and *nin5* *Eco*RI-insertion vector with an intact *att* region. The 2-kb *Dra* I fragment containing the *glnS* gene (14, 15) was recloned in λgti from pMN20 by digestion with *Eco*RI, ligated, and packaged by standard methods. Phage λgtiglnS carries the *glnS* gene in the leftward orientation relative to the λ arms. Opal suppressors GLNU70 or GLNA3U70 were cloned on plasmid pGFIB (11, 16) and recloned in the *Eco*RV site of pACYC184 as a *Pvu* II fragment. M13mp19 and pBR322 were used for recloning and sequencing of the *glnS* mutants. Clones of the synthetic amber suppressors Ala2 and GluA derived from $tRNA^{Ala}$ and $tRNA^{Glu}$, respectively, were gifts of J. Miller (17). The 2-kb *Eco*RI *glnS*-containing fragment from pMN20 (15) was ligated in pUC2119 (pUCglnS) for preparation of U-containing single-stranded DNA (ssDNA)

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Abbreviations: GlnRS, glutaminyl-tRNA synthetase; GLN, opal suppressor derived from $tRNA_2^{Gln}$; ssDNA, single-stranded DNA; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MetRS, methionyl-tRNA synthetase.

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as a template for *in vitro* mutagenesis (18). Induction of ssDNA was with M13K07 (19).

Mutagenesis and Selection of *glnS* Mutants. Phage λgtiglnS was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) as described (11). The mutagenized lysate was mixed with 0.1 ml of cells of an overnight culture of strain KL2576 transformed with GLNU70 or GLNA3U70 and resuspended in 10 mM $MgCl_2$. After preadsorption, the mixture was plated on minimal medium lacking tryptophan. After 2–3 days of incubation at 32°C, colonies were reisolated, and phage stocks from individual isolates were prepared by temperature induction. Isolates were retested for the ability to confer a Trp^+ phenotype on KL2576 transformed with plasmids carrying the appropriate opal suppressor. Single-copy lysogens were made of strain BT32, and β-galactosidase activity was assayed (5, 20).

***In Vitro* Mutagenesis of the 316–318 Region of GlnRS.** Oligonucleotides (20- or 21-mers) with an equal mixture of A/C/G/T at the codons corresponding to Thr-316, Lys-317, and Gln-318 of GlnRS were used to prime second-strand synthesis of U-containing ssDNA from pUCglnS (19). An aliquot was transformed in KL2576 transformed with the appropriate opal suppressor and was plated on minimal medium containing ampicillin and chloramphenicol. Plasmid DNA isolated from colonies after 2–4 days of incubation at 32°C was retested for the phenotype and sequenced. A portion of the mutagenic mixture was also transformed in strain DH5α, and plasmid DNA was sequenced from colonies picked at random; mutants identified by sequencing were retransformed in KL2576 transformed with the opal suppressor, and the phenotype was tested on minimal plates.

Sequencing of the *glnS* Mutants. Where appropriate, ssDNA and double-stranded DNA were used as templates for sequencing by the dideoxy chain-termination method. The 2-kb *glnS*-containing *Eco*RI fragment from each mutant isolate of λgtiglnS was recloned for sequencing in M13 or pBR322. Oligonucleotides ≈300 nucleotides apart were used for sequencing and screening by sequencing isolates for the sites of the *glnS113* and *glnS114* mutations and isolates from *in vitro* mutagenesis.

Enzyme Purification and *in Vitro* Aminoacylation. Strain X3R2 transformed with a pBR clone of $glnS^+$ was used as the source of $GlnRS^+$ enzyme. Mutant GlnRS proteins, named to show the substituted position followed by the replacement amino acid, were purified from transformants of strain X3R2 cured of the prophage carrying $glnS^+$ (see above) by chromatography on DEAE-cellulose followed by phosphocellulose (21, 22). The specific activity of the enzymes was 1026 nmol of Gln-$tRNA^{Gln}$ formed per min per ng of $GlnRS^+$; 857, per ng of GlnRS317Arg; 731, per ng of GlnRS318Lys; and 162, per ng of GlnRS318Arg. Transcription *in vitro* with T7 RNA polymerase of a *Bst*NI digest of plasmid DNA of the cloned $tRNA^{Gln}G1$ and $tRNA^{Gln}G1U34C35A36$ genes was as described (11, 23), with purification by electrophoresis on denaturing polyacrylamide gels followed by elution. Aminoacylation assays (50 μl) contained 50 mM potassium Hepes (pH 7.2), 16 mM $MgCl_2$, 2 mM ATP, 395 μM glutamine, and 5 μM [^{3}H]glutamine (specific activity, 47–55 mCi/mmol; 1 Ci = 37 GBq). For determination of kinetic parameters for $tRNA^{Gln}G1$ transcript, a range of 0.1–10 μM tRNA with 2 nM enzyme was used, and for $tRNA^{Gln}G1U34C35A36$ transcript, a range of 5–50 μM tRNA with 20 nM mutant enzyme and a range of 20–200 μM tRNA with 20 nM wild-type enzyme were used. Aliquots were taken at time points, washed, and counted (21). Kinetic parameters were calculated from Eadie–Hofstee plots.

RESULTS

The procedure for mutant isolation with the opal suppressors derived from $tRNA^{Gln}$ is illustrated in Fig. 1. Isolates of λgtiglnS (shortened henceforth to λglnS) from random mutagenesis with MNNG that conferred a Trp^+ phenotype as lysogens were tested and characterized according to the opal suppressor used; λglnS114 was isolated on GLNU70, and λglnS113 was isolated on GLNA3U70. Although not an exhaustive screen, the two classes of mutants showed a distinct spectrum of suppression when retested for Trp^+ phenotype in strain KL2576 transformed with the appropriate opal suppressor. The λglnS114 mutant suppressed with both GLNU70 and GLNA3U70 opal suppressors, while λglnS113 suppressed only with GLNA3U70 (Table 1).

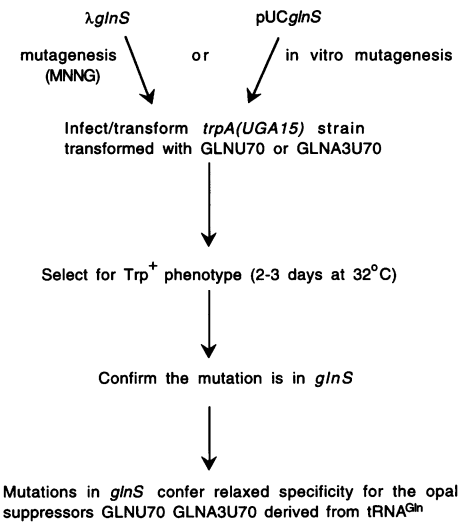

FIG. 1. Genetic selection for *E. coli glnS* mutants by using opal suppressors GLN derived from $tRNA_2^{Gln}$.

Mutants of λglnS had been isolated previously by the ability to mischarge *tyrT(UAG)*, conferring a Lac^+ phenotype in strain BT32 from suppression of the *lacZ(UAG1000)* mutation (4, 6). The λglnS114 and λglnS113 mutants obtained in this study were also tested for mischarging of *tyrT(UAG)*. The λglnS113 mutant complemented BT32 (Table 1) and therefore was thought to be similar to mutants isolated previously. This was confirmed by DNA sequencing to identify the mutation responsible for both a Trp^+ phenotype in strain KL2576 transformed with GLNA3U70 and a Lac^+ phenotype (corresponding to 66.7 units of β-galactosidase activity) in strain BT32. However, the λglnS114 mutant showed marginal growth in BT32 with low β-galactosidase activity (8.4 units; Table 1), indicating a different mutation.

Apart from the *tyrT*(UAG) suppression, the λglnS mutants isolated by this selection (Fig. 1) did not misacylate a number of other suppressors tested. Strain BT13, which contains *serU(UAG)*, was Lac^- with the λglnS mutants present as a lysogen. Strain BT235, transformed with the amber suppressor GluA-A73 [derived from $tRNA^{Gln}$ with additional mutations at A38 to increase suppressor efficiency (17) and G73 → A to confer glutamate-specificity (24)], was Lac^- with the λglnS mutants present as lysogens. This was also the case for

Table 1. Suppression by *glnS* mutants

Strain [relevant marker(s)]	Suppressor tRNA	Phage isolate		
		λglnS113	λglnS114	λglnS
KL2576 [*trpA(UGA15)*]	GLNA3U70	+	+	−
KL2576 [*trpA(UGA15)*]	GLNU70	−	+	−
BT32 [*lacZ(UAG1000)*]	tyrT(UAG)	+ (66.7)	± (8.4)	− (1.2)

Suppression was assayed by growth of lysogens at 30°C on minimal plates due to suppression of *trpA(UGA15)* in KL2576 and suppression of *lacZ(UAG1000)* in BT32. +, Trp^+ phenotype in strain KL2576 and Lac^+ in BT32; ±, weak growth; −, no growth. Numbers in parentheses indicate units of β-galactosidase activity determined as described (20).

the amber suppressor Ala2 derived from $tRNA^{Ala}$ in strain BT235 (Table 1). The opal suppressor derived from $tRNA^{Trp}$ by mutation G24 → A in the D-stem [*trpT(UGA)*] (25) also was not mischarged with glutamine by the mutants of λglnS in strain KL2725. Therefore these mutants of GlnRS are not generally relaxed in tRNA specificity.

Changes in the λglnS Mutants Isolated by Random Mutagenesis and Selection. The *glnS* gene from a number of independent isolates of λglnS114 and λglnS113 was recloned in plasmid pBR322, compatible with pACYC plasmids containing the opal suppressors GLNU70 and GLNA3U70. A double transformant of KL2576 with the appropriate opal suppressor and *glnS* mutant then had a Trp^+ phenotype, confirming the mutation(s) is on the 2-kb *glnS*-containing fragment. Sequencing showed that a single nucleotide change was responsible for the phenotype. The λglnS113 mutant has a G → A change corresponding to Asp-235 → Asn in GlnRS, and the λglnS114 mutant has an A → G change corresponding to a Gln-318 → Arg change in GlnRS (Fig. 2). The mutations are transitions, correlating with the known preference of MNNG (20). A number of isolates from the selection were screened by DNA sequencing to confirm that the mutations corresponding to amino acids at positions 235 and 318 were present in λglnS113 and λglnS114, respectively. No other mutations were found in the *glnS* gene.

Other *glnS* Mutants Isolated by *in Vitro* Mutagenesis. The codons corresponding to amino acids Thr-316, Lys-317, and Gln-318 were mutated with degenerate oligonucleotides. Mutants were identified by either randomly selecting colonies on rich media or selecting for a Trp^+ phenotype on minimal media. Strikingly, only GlnRS317Arg and GlnRS318Lys mutants were identified from a Trp^+ phenotype with either opal suppressor derivative (Fig. 2). More conspicuously, only the arginine codon AGG was identified from more than 30 independent isolates of the codon 317 mutagenic reaction, and similarly only the lysine codon AAG was identified from a similar number of isolates of the codon 318 mutagenic reaction. Both codons correspond to ones used in low-abundance proteins; the arginine codon AGG is very rarely used in *E. coli* (20) and the presence of tandem arginine codons AGA and AGG has been implicated in frame-shifting due to the low efficiency of read-through (26). It is pertinent to note that the mutation resulting in GlnRS318Arg (isolated as a λ lysogen) is the CGG codon and causes severe inhibition of growth when recloned in pBR322. This mutation could not be recloned into high-copy number plasmids. The rationale for the limited number of substitutions at codons 316–318 is that toxicity/lethality may occur with substitutions that lead to significant changes in tRNA–GlnRS interactions. Substitutions are tolerated at a low level of read-through of the mutated codon and/or to amino acids that do not result in significant structural perturbations. Toxicity has also been observed for mutants of GlnRS (8) and tyrosyl-tRNA synthetase (27). Limited substitutions at amino acids 316–318 could be obtained from random selection on rich media, and

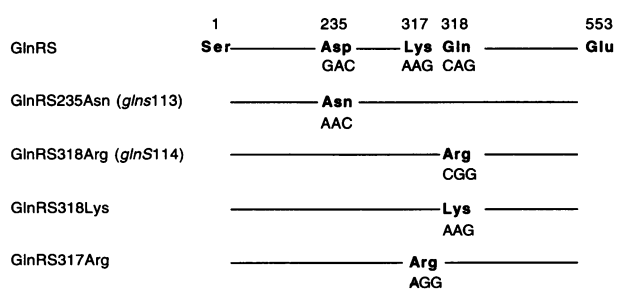

FIG. 2. Location of mutations and corresponding amino acid changes in GlnRS selected *in vivo*. The mutated codon is indicated below the corresponding amino acid.

Table 2. Suppression by mutations of *glnS* at codons corresponding to Thr-316, Lys-317, and Gln-318

GlnRS mutations (codon)	GLN (pACYC derivative) GLNU70	GLNA3U70
Thr-316 → Ala (GCC)	−	−
Thr-316 → Gly (GGC)	−	−
Thr-316 → Arg (CGC)	−	−
Lys-317 → Glu (GAG)	−	−
Lys-317 → Arg (AGG)	++	++
Lys-317 → Thr (ACT)	−	−
Gln-318 → Glu (GAG)	−	−
Gln-318 → Leu (CTG)	−	−
Gln-318 → Lys (AAG)	+	±

The isolation of mutants by *in vitro* mutagenesis was as described in text. ++, Strong growth on minimal plates after 2 days at 32°C; +, weaker growth after 2–3 days; ±, marginal growth after 3 days; −, no growth after 4 days.

subsequent testing showed no phenotype associated with these substitutions (Table 2); this was true for mutants with substitutions at Thr-316 and substitutions at 317 and 318 resulting in Glu-317, Thr-317, Glu-318, and Leu-318. The phenotype is then dependent on changes to Arg-317 and Arg-318, with a weaker phenotype with Lys-318 (Table 2).

***In Vitro* Aminoacylation of $tRNA^{Gln}$ Transcripts.** GlnRS mutants effecting suppression with GLNU70 and GLNA3U70 were purified from a strain with the *glnS* gene interrupted. In this strain the $glnS^+$ gene is supplied by a prophage containing *glnS*. After induction and confirmation that the only functional copy of *glnS* in the cell is the plasmid copy, plasmid DNA was sequenced to confirm the mutation. After purification, the GlnRS318Arg enzyme had a specific activity about 1/10th that of wild-type GlnRS, with the GlnRS317Arg and GlnRS318Lys enzymes having specific activities comparable to that of the wild-type enzyme.

Aminoacylation of a $tRNA^{Gln}$ transcript with the wild-type (CUG) anticodon was tested. Also analyzed was a transcript of $tRNA^{Gln}G1U34C35A36$, as the anticodon corresponds to the anticodon (UCA) of the opal suppressors. Compared with wild-type enzyme, the kinetic parameters for aminoacylation of the transcript with the cognate anticodon are not greatly affected by mutations in the GlnRS enzymes, although k_{cat} of the GlnRS318Arg enzyme was reduced about half compared with that of the wild-type and other mutant enzymes (Table 3) accounting for the lower specific activity of the GlnRS318Arg mutant enzyme. The GlnRS mutants are not enhanced in binding or catalytic rate for the cognate anticodon. However, kinetic parameters for the mutant enzymes with the $tRNA^{Gln}$ transcript with the U34C35A36 anticodon are improved compared with the wild-type enzyme. The GlnRS317Arg and GlnRS318Arg enzymes show a decrease in K_m and increase in k_{cat}, with an overall specificity constant (k_{cat}/K_m) that is almost 10 times that of the wild-type enzyme (Table 3). The GlnRS318Lys enzyme has a smaller effect on aminoacylation of $tRNA^{Gln}$ with the UCA anticodon, primarily an increase in k_{cat}, with an overall increase in k_{cat}/K_m of

Table 3. Aminoacylation of $tRNA^{Gln}$ transcripts by wild-type and mutant GlnRS enzymes

Enzyme	tRNA transcript: $tRNA^{Gln}G1$ K_m, μM	k_{cat}, s^{-1}	k_{cat}/K_m ratio	$tRNA^{Gln}G1U34C35A36$ K_m, μM	k_{cat}, s^{-1}	k_{cat}/K_m ratio
GlnRS (wild-type)	0.10	1.32	1.0	>71.4	0.05	1.0
GlnRS317Arg	0.10	1.05	0.8	29.3	0.20	9.7
GlnRS318Arg	0.13	0.43	0.3	15.6	0.10	9.1
GlnRS318Lys	0.10	1.98	1.5	42.8	0.14	4.7

nearly 5 times that of wild type. GlnRS318Lys also has the weakest *in vivo* phenotype of the mutant enzymes isolated by genetic selection (Table 2). The Trp^+ phenotype with the *glnS* mutants correlates with enhanced aminoacylation of $tRNA^{Gln}$ with the UCA anticodon, with an improvement of 5–10 times in the specificity constant. This illustrates the sensitivity of *in vivo* genetic selection, as the mutants exhibit relatively small differences in kinetic parameters of aminoacylation *in vitro*.

DISCUSSION

Position of the Mutations in GlnRS. A genetic approach has implicated important functional interactions without assumptions from biochemical or structural data. The *glnS113* mutation (Asp-235 → Asn) is the same amino acid change identified in the mutation *glnS7* isolated based on mischarging of *tyrT(UAG)* (4, 6). Therefore, two genetic selections using tRNAs with different anticodons have identified the same change in GlnRS. The nature of the previously described mischarging enzymes GlnRS7 and GlnRS10 (GlnRS235Gly) was rationalized from the structure of the GlnRS–$tRNA^{Gln}$ complex (7). The side chain of Asp-235 makes sequence-specific interactions with base pair G3·C70 of $tRNA^{Gln}$ (ref. 7; Fig. 3*A*), and mutations at position 235 result in relaxed specificity. Therefore, mischarging of *tyrT(UAG)*, which has a U3·A70 base pair, and of GLNA3U70 occurs because of relaxed specificity of *glnS7*/*glnS113* for tRNAs that do not have G3; furthermore, this mutation was not isolated from the selection with GLNU70. This emphasizes the importance of Asp-235 and the G3·C70 base pair (9) as major elements ensuring the specificity of aminoacylation by GlnRS. However, it is remarkable that both amber and opal suppressor tRNAs are aminoacylated by the GlnRS235Asn mutant enzyme.

The *glnS114* mutation (Gln-318 → Arg) and the Lys-317 → Arg, Gln-318 → Lys mutations are changes at amino acid positions not implicated to date as being important for specificity in aminoacylation of $tRNA^{Gln}$ (Fig. 3*A*). However, from the structure of the GlnRS–$tRNA^{Gln}$ complex (1, 2), the side chain of amino acid Gln-318 is in the proximity of the sugar phosphate backbone of $tRNA^{Gln}$ and makes a number of interactions with the inside of the L-shaped $tRNA^{Gln}$ (Fig. 3*B*). Additionally, the side chain of Lys-317 interacts with the side chain of Gln-234 and with the tRNA backbone at the inside of the L-shaped tRNA. Mutations at positions 317 and 318 isolated by genetic selection (Fig. 2) may provide additional interactions with $tRNA^{Gln}$. However, the GlnRS318-Arg mutant enzyme is not relaxed in specificity for other tRNAs. The suppressors tested were *serU(UAG)*, $tRNA^{Glu}$ (GluA73), and *trpT(UGA)* because they are derived from tRNAs known to be mischarged by GlnRS *in vivo* or *in vitro* (9, 17, 24, 28). In the case of $tRNA^{Ala}$ (Ala2), this tRNA has a G3·U70 base pair to mimic GLNU70 used in the selection. Although there was marginal suppression by λglnS114 with *tyrT(UAG)* (Table 1), this mutant retains specificity for $tRNA^{Gln}$-derived suppressors. This explains why this mutation was not identified from the previous selection. Also, *in vitro* aminoacylation assays (Table 3) show that the mutant enzymes are not improved in kinetic parameters for tRNA with the cognate (CUG) anticodon and are elevated in the overall specificity constant for tRNA with the UCA anticodon compared with wild-type enzyme. The effect of these mutations is then to enhance aminoacylation of a tRNA with the UCA anticodon.

Role of the 317, 318 Loop in tRNA Anticodon Recognition. An explanation for suppression by enzymes with mutations at 317 and 318 is propagation of a long-range structural effect to relax or bypass specificity for recognition of the anticodon (ref. 2; Fig. 3*A*). The inside of the L-shaped tRNA molecule contains many conserved nucleotides and is not thought to be

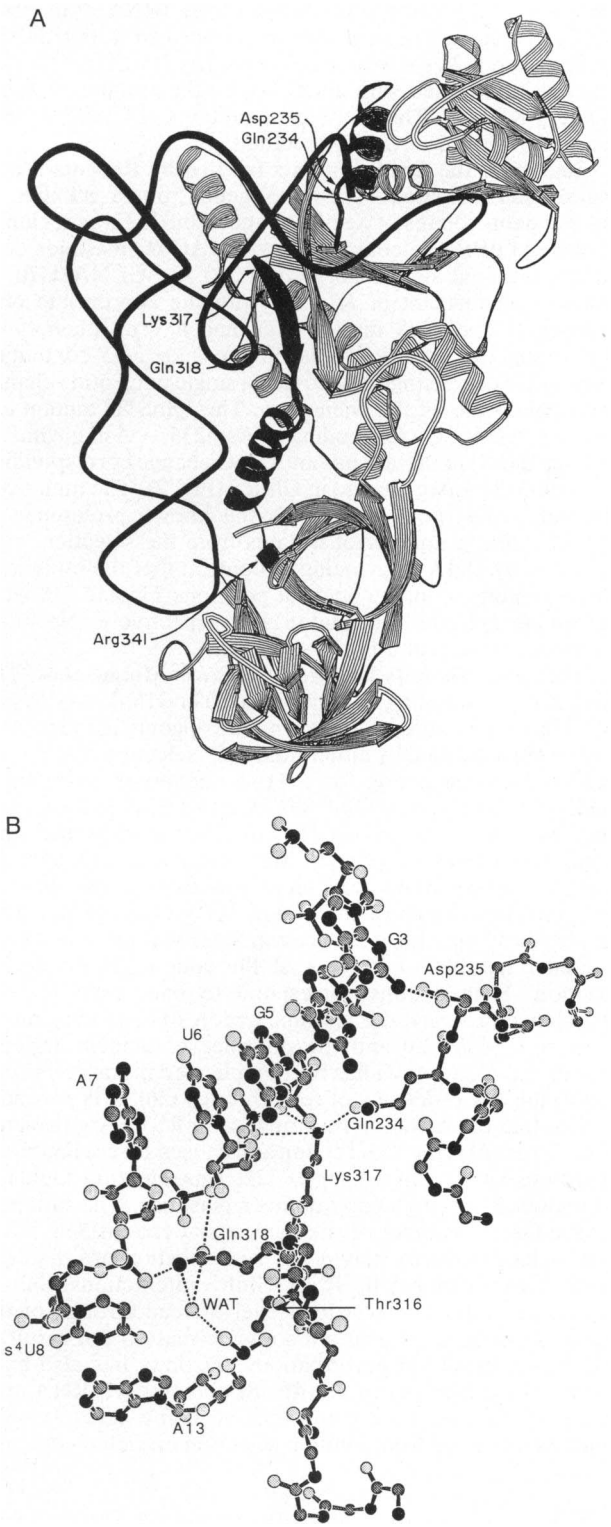

FIG. 3. (*A*) Structure of the GlnRS–$tRNA^{Gln}$ complex with the tRNA drawn as the dark line, and relevant parts of the protein that communicate anticodon recognition to acceptor-stem recognition are shaded. (*B*) Location of the side chains of amino acids Asp-235, Gln-234, Thr-316, Lys-317, and Gln-318 in the GlnRS–$tRNA^{Gln}$ crystal structure (ref. 1; J. Perona, personal communication). The relevant nucleotides of the inside of the L-shape of $tRNA^{Gln}$ are indicated with potential hydrogen-bonding interactions with the side chain of the amino acid and tRNA. The α-carbon backbone of GlnRS is shown from residue 230 to 240 and from 310 to 320, with a coordinated water molecule (WAT) also shown.

a major site of discrimination between tRNAs by aminoacyl-tRNA synthetases. However, interaction with the inside of the L shape has been implicated for many years in tRNA recognition (29); a covalent adduct has been proposed between U8 (a conserved nucleotide usually modified as s^4U in *E. coli* tRNAs) and alanyl-tRNA synthetase (30) and also with lysine residues in methionyl-tRNA synthetase (MetRS; ref. 31). As shown by *in vitro* aminoacylation (Table 3), the Lys-317 → Arg, Gln-318 → Lys, and Gln-318 → Arg mutations increase both binding and catalysis of tRNA with the UCA anticodon.

The Lys-317 and Gln-318 residues are at the start of a β-strand that continues as an α-helix to the binding pocket for U35 of the anticodon (Fig. 3*A*). This motif ends in Arg-341, which is responsible for sequence-specific recognition of U35 (2, 32). The mutations resulting in Arg-317, Lys-318, and Arg-318 may provide a "hinge" and allow the C35 of the opal suppressors to fit in a pocket specific for U35. In addition, Lys-317 interacts with Gln-234 to connect acceptor stem recognition with this loop (Fig. 3*B*). Gln-234 lies near amino acid Thr-230, which is in the proximity of ATP and Leu-228, which contributes to specificity for glutamine (33). Therefore, anticodon recognition may be functionally communicated with the active site through these interactions. Propagation of specificity through the enzyme–tRNA complex via the Lys-317 and Gln-318 interactions is responsible for connecting acceptor stem recognition with aminoacylation of the cognate tRNA. The structure of the loop containing Lys-317 and Gln-318 is conserved between GlnRS and MetRS (Fig. 3*B*). Residues 317–322 of GlnRS align with residues 354–359 of MetRS (34). Mutations in this region of MetRS have an effect on the K_m for ATP and on the rate of ATP–PP_i exchange (35). Although the mutations do not affect aminoacylation of $tRNA^{fMet}$, the structure of MetRS was determined by using a catalytically active monomer (36) and may not represent the complex with tRNA.

The importance of arginine and lysine residues in the altered recognition of tRNA is apparent from this study; other investigations have shown the relevance of arginine in RNA–protein interactions—for example, HIV Tat protein interacting with TAR RNA (37). Are additional positions in GlnRS (and tRNA) responsible for communication of anticodon specificity to the acceptor stem and the active site? Compensating mutations in tRNA may be selected for improved aminoacylation by GlnRS (W. H. McClain, personal communication), and the selection may be extended to target two long β-ribbons that span the anticodon binding pockets and extend to the active site (1, 2). The structure of the GlnRS enzymes mutated in the 317, 318 loop with tRNAs altered in the anticodon may reveal the nature of this proposed long-range conformational change. Therefore, a genetic analysis has provided information on recognition within a complex system that is complementary to the structural studies.

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