Effects of mutations at position 36 of $tRNA^{Glu}$ on missense and nonsense suppression in *Escherichia coli*

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Abstract Mutations in the anticodon of $tRNA^{Glu}$ (UUC) were isolated or constructed and characterized for their ability to suppress cognate nonsense or missense mutations in vivo. The C36-to-A36 transversion mutation was isolated as an ochre and an amber suppressor, while the G36 transversion was selected as a CAG missense suppressor. $tRNA^{Glu}$ suppressors of an AAG missense mutation could not be isolated, and a U36 transition mutation introduced into $tRNA^{Glu}$ in vitro conferred no suppressor phenotype. Over-expression of glutamyl-tRNA synthetase did not increase the activity of the U36 mutant $tRNA^{Glu}$, suggesting a defect at the level of translation rather than at the level of synthetase recognition.

Key words: Suppressor tRNA; Translational efficiency; $tRNA^{Glu}$; Anticodon

1. Introduction

Anticodons influence the activity of a tRNA through interactions with various components of the translational apparatus. While the role of anticodon nucleotides in recognition by many aminoacyl-tRNA synthetases has been well documented [1], there is also evidence that the anticodon loop interacts with rRNA [2,3] and adjacent tRNAs [4] in translating ribosomes. The nature of these interactions and their contribution to the accuracy and efficiency of translation are largely unknown.

The efficiency of codon–anticodon recognition also appears to depend on a relationship between anticodon sequence and the sequence of adjacent nucleotides in the anticodon loop, forming the basis of the extended anticodon hypothesis [5]. Introduction of a C-to-A change at position 36 of $tRNA^{Glu}$ by oligonucleotide-directed mutagenesis produced only a weak ochre suppressor [6]. Further experimentation led to the conclusion that the lack of strong suppressor activity was attributable to a defect in codon recognition or tRNA–ribosome interaction, rather than to a defect in aminoacylation [7]. Similar conclusions were reached during analysis of amber suppressor derivatives of $tRNA^{Glu}$ containing a C34/A36 double mutation [8,9].

In this report we describe the isolation and characterization of ochre suppressor and missense suppressor derivatives of $tRNA^{Glu}$. A genetic selection was employed to isolate C to A transversions at position 36 as ochre or amber suppressors, while a C-to-G transversion at the same position was selected as a suppressor of a GAG(glu)-to-CAG(gln) missense mutation in *lacZ*. Both mutants were found to be very weak glutamic acid-inserting suppressors. Attempts to isolate suppressors of a GAG(glu) to AAG(lys) missense mutation at the same position in *lacZ* were unsuccessful. A $tRNA^{Glu}$ allele containing a C-to-U transition at position 36 constructed by oligonucleotide-directed mutagenesis failed to suppress the same *lacZ* AAG missense mutation. The activity of the U36 mutant $tRNA^{Glu}$, measured as β-galactosidase activity, was not increased by over-expression of glutamyl-tRNA synthetase (GluRS). While defects in aminoacylation cannot be excluded, our observations are consistent with previous findings [6,7] and indicate that mutations at position 36 in the anticodon of $tRNA^{Glu}$ fail to produce efficient translational suppressors due primarily to a defect in codon recognition or tRNA–ribosome interaction.

2. Materials and methods

2.1. Bacterial strains and plasmids

All bacterial strains are derivatives of *E. coli* K12 and their genotypes are described in Table 1. EF167 is an F^- Gal^+ Pro^+ *srlC*::Tn*10* derivative of CSH41 [10] bearing the *lacI22* and *lacZ13*(Ochre) mutations from CA167 [11]. EF168 is a spontaneous Lac^+ revertant of EF167; true revertants were distinguished from extragenic suppressors by the ability of P1 grown on this strain to transduce XA21 (*lacZΔM15*) [12] to Lac^+. CSH101, CSH103 and CSH106 carrying an F′ *lac-pro* with amber or missense mutations at codon 461 of *lacZ* were obtained from Cold Spring Harbor Laboratory. CSH142 F′100 was derived from a spontaneous revertant of CSH102 [13] and is isogenic to CSH101, CSH103 and CSH106. The *gltT* gene encoding $tRNA^{Glu}$ was expressed in the context of the *rrnB* operon on the pSC101-derived plasmid pMO10 [14]. Plasmid pLQ7611ΔNruI contains the *gltX* gene encoding glutamyl-tRNA synthetase (GluRS) [15] and was a kind gift of Dr. Lee A. Sylvers.

2.2. Mutagenesis

Cells containing pMO10 were mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine for 10 and 20 min as described [13]. Outgrowth was omitted and mutagenized cells were plated directly onto M9 lactose minimal medium [10] containing 50 μg/ml kanamycin. Oligonucleotide-directed mutagenesis was performed on M13mp19 clones containing the *Hin*dIII–*Sph*I fragment spanning the spacer region of *rrnB* [16].

2.3. Analysis of mutants

Assays for β-galactosidase activity were performed as described [17]. In vivo suppression was detected by streaking overnight cultures onto M9 lactose minimal plates plus kanamycin and incubating at 37°C.

3. Results

Suppressor mutations in the *gltT* gene encoding $tRNA^{Glu}$ were isolated in the context of the *rrnB* operon carried on plasmid pMO10 [14]. The first mutants isolated were suppressors of the *lacZ13*(Ochre) mutation in EF167. EF167 containing pMO10 was mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and plated on minimal lactose medium containing kanamycin. Suppressor-encoding plasmids were extracted from pooled Lac^+ colonies and used to transform EF167 to Lac^+.

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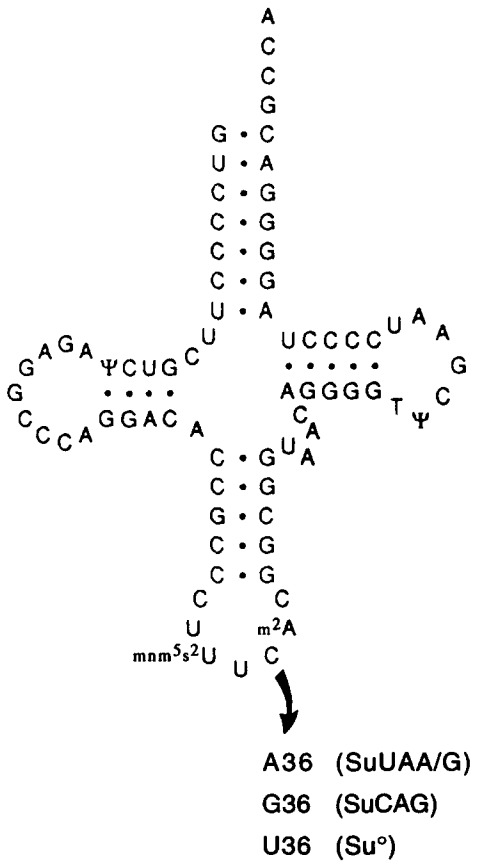

Fig. 1. Secondary structure model of $tRNA^{Glu}$ showing the sites of mutations analyzed in this study and their associated phenotypes. SuUAA/G, suppressor of UAA (ochre) and UAG (amber) mutations; SuCAG, suppressor of CAG missense mutations; Su°, no observed suppressor phenotype.

Individual Lac^+ pMO10 transformants were then screened for co-inheritance of the suppressor phenotype by a second round of transformations of EF167. Suppressor mutations were identified by sequencing the *gltT* gene. All *lacZ13*(Ochre) suppressors were found to bear a C-to-A transversion at position 36 of the anticodon, producing a canonical ochre suppressor anticodon ([18]; see Fig. 1).

This procedure was repeated to select $tRNA^{Glu}$ mutants in a series of strains bearing amber or missense mutations at codon 461 of *lacZ* [13,19]. Because synthesis of functional β-galactosidase requires the insertion of glutamic acid at codon 461, suppression of these mutations requires decoding by a tRNA species charged with glutamic acid. Since each of the mutant codons used in this selection differs from the wild-type GAG(glu) codon only at the first position, suppressors were expected to bear complementary mutations at position 36 in the $tRNA^{Glu}$ anticodon. Three *lacZ* mutations, UAG(amber), CAG(gln) and AAG(lys), were used in selections.

We succeeded in isolating two of the three possible suppressor alleles. The $tRNA^{Glu}$A36(SuUAA/G) (ochre suppressor) allele was re-isolated as a suppressor of *lacZ*(UAG461), and mutants bearing the G36 allele were isolated as suppressors of the GAG(glu) to CAG(gln) missense mutation. The G36 mutation proved to be somewhat toxic and was rapidly lost in the absence of selection. Attempts to isolate the third allele, U36, as a suppressor of the AAG(lys) missense mutation were unsuccessful.

We have previously used this selection procedure to isolate nonsense suppressor mutations in the 16 S and 23 S rRNA genes of *rrnB* (Gregory and Dahlberg, unpublished results). We therefore reconstructed the A36 and G36 alleles by oligonucleotide-directed mutagenesis to confirm the relationship between these tRNA mutations and their suppressor phenotypes and to eliminate possible rRNA suppressor mutations. The $tRNA^{Glu}$ mutants constructed by oligonucleotide-directed mutagenesis produced suppressor phenotypes identical to those of the mutants isolated in vivo. The U36 allele was also constructed in vitro to determine if the inability to isolate this mutant in vivo was due to a lack of suppressor activity.

Suppression was quantitated by measurement of β-galactosidase activity. The results of this analysis are presented in Table 2. The A36 allele produced low level suppression of both the *lacZ13*(Ochre) mutation (3%) and the *lacZ*(UAG461) mutation (3.4%). The G36(SuCAG) allele exhibited 1.4% suppression of *lacZ*(CAG461). Although low, this level of activity is sufficient to facilitate growth on lactose minimal medium. These results stand in contrast to the failure of the U36 mutation to act as a suppressor of the *lacZ*(AAG461) mutation. Very low levels of β-galactosidase activity were detected (less than 1 unit of activity), while absolutely no activity was detectable in the absence of the U36 allele (Table 2). This mutant showed no growth advantage over wild-type on lactose minimal medium, consistent with our failure to isolate it in vivo. No substantial growth defect was observed with the U36 mutant, indicating that this tRNA is probably not very active at other AAG(lys) codons.

While the level of β-galactosidase activity resulting from the U36 allele is insufficient to support growth on lactose minimal medium, it does indicate that this tRNA is at least partially aminoacylated with glutamic acid. We attempted to enhance the suppressor activity of the U36 allele by introducing a plasmid which leads to an approximately 100-fold over-expression of GluRS [15]. CSH106 (*lacZ*(AAG461)) and CSH142F'100 were transformed with combinations of plasmids pMO10 (WT $tRNA^{Glu}$ or $tRNA^{Glu}$U36) and either pBR322 or pLQ7611DNruI (pBR322 containing the *gltX* gene encoding GluRS) and assayed for β-galactosidase activity. As presented in Table 3, no increase in β-galactosidase activity was observed, suggesting that the $tRNA^{Glu}$U36 species is aminoacylated.

Table 1
Bacterial strains used in the selection and analysis of *gltT* mutants

Strain	Genotype	Source
EF167	F⁻ *lacI22 lacZ13* (Ochre) *thi srlC*::TN*10*	This study
EF168	F⁻ *lacI22 lacZ*⁺ *thi srlC*::Tn*10*	This study
CSH142 F'100	*araΔ* (*gpt-lac*)*5*/F' *proA*⁺*B*⁺*lacZ*⁺	M. O'Connor
CSH101	*araΔ* (*gpt-lac*)*5*/F' *proA*⁺*B*⁺*lacZ* (UAG461)	CSH[a]
CSH103	*araΔ* (*gpt-lac*)*5*/F'*proA*⁺*B*⁺*lacZ* (CAG461)	CSH
CSH106	*araΔ* (*gpt-lac*)*5*/F'*proA*⁺*B*⁺*lacZ* (AAG461)	CSH

[a]Cold Spring Harbor Laboratory.

4. Discussion

Efficient suppression by $tRNA^{Glu}$ is highly sensitive to the nucleotide identity of position 36. None of the three possible base changes at this position produced an efficient nonsense (in the case of A36) or missense (in the cases of G36 and U36) suppressor tRNA. While C-to-A or C-to-G transversions produced suppressors which could be isolated by genetic selection, C-to-U transitions could not be isolated. A $tRNA^{Glu}$U36 mutant allele constructed in vitro failed to suppress the *lacZ*(AAG461) mutation, producing less than one unit of β-galactosidase activity.

The lack of suppression by $tRNA^{Glu}$U36 could result either from a translational defect or from a lack of recognition by GluRS. The failure of GluRS over-expression to enhance suppression suggests that this tRNA is fully aminoacylated. The alternative explanation, that the U36 mutant is completely unrecognized by GluRS, is less appealing and is inconsistent with the observed (albeit low level) production of β-galactosidase activity in CSH106. Previous studies with $tRNA^{Glu}$A36(SuUAA/G) [8,9], $tRNA^{Glu}$C34/A36(SuUAG) (amber suppressor) [7], and $tRNA^{Glu}$G37 [20], demonstrated that at least some alterations in the anticodon permit recognition by GluRS. Mischarging by LysRS is also unlikely since $tRNA^{Glu}$ contains several negative determinants for lysine identity [20].

The effects of the G36 and U36 alleles on growth rate correlated with their activity as missense suppressors. We believe the toxicity of the G36 mutation to be the result of insertion of glutamic acid at glutamine codons in other coding sequences. In contrast, the slight reduction in colony size resulting from the U36 mutation suggests that misincorporation at lysine codons by this tRNA does not occur at physiologically significant levels. This is consistent with its absence of suppressor activity.

Construction of effective missense suppressor derivatives of $tRNA^{Glu}$ may require additional mutations situated outside the anticodon. More efficient $tRNA^{Glu}$(SuUAA/G) [7] and $tRNA^{Glu}$(SuUAG) [8,9] derivatives have been constructed in vitro by introduction of mutations in the anticodon loop and stem. Such mutations may be necessary to facilitate efficient tRNA–ribosome interactions or tRNA–tRNA interactions during translation.

One approach to characterizing such interactions is to identify mutations in ribosomal proteins, rRNA, or other tRNAs which relax the C36 requirement. The nearly complete absence of suppressor activity makes $tRNA^{Glu}$U36 an ideal substrate for the selection of such second-site suppressors. We have isolated several mutants which exhibit improved suppression of *lacZ*(AAG461) by $tRNA^{Glu}$U36 by greater than an order of magnitude. The characterization of these mutants may provide some fundamental insights into the nature of interactions occurring with tRNAs during translation.

Table 2
Effect of $tRNA^{Glu}$ mutant alleles on β-galactosidase activities in *lac* mutants

$tRNA^{Glu}$ allele	*lacZ* allele[a]	β-Galactosidase activity (units)[b]	% suppression[c]
C36 (WT)	*lacZ13* (Ochre)	n.d.	n.a.
	lacZ$^+$	1103.6 ± 177.5	n.a.
	lacZ(UAG461)	n.d.	n.a.
	lacZ(CAG461)	0.66 ± 0.05	n.a.
	lacZ(AAG461)	n.d.	n.a.
	lacZ$^+$(F'100)	1557.7 ± 61.1	n.a.
A36(SuUAA/G)	*lacZ13* (Ochre)	30.0 ± 1.6	3.0
	lacZ$^+$	1002.8 ± 72.1	
	lacZ(UAG461)	81.7 ± 3.6	3.4
	lacZ$^+$(F'100)	2384.3 ± 142.3	
G36(SuCAG)	*lacZ*(CAG461)	16.0 ± 2.3	1.4
	lacZ$^+$(F'100)	1126.0 ± 240.4	
U36	*lacZ*(AAG461)	0.72 ± 0.18	0.06
	lacZ$^+$(F'100)	1149.6 ± 201.8	

n.a., not applicable; n.d., no detectable activity observed.
[a] *lacZ* alleles are described in the text and in Table 1.
[b] β-Galactosidase activity is expressed as Miller units ± s.D. [10].
[c] % suppression is calculated as the activity in the *lacZ* mutant divided by the activity in the corresponding isogenic *lacZ*$^+$ strain multiplied by 100.

Table 3
Effect of over-expression of GluRS on suppressor activity of $tRNA^{Glu}$U36

$tRNA^{Glu}$, GluRS plasmid[a]	β-Galactosidase activity (units)[b]		% suppression[c]
	lacZ(AAG461)	*lacZ*$^+$	
WT, pBR322	0.013 ± 0.003	1760.8 ± 148.1	–
WT, GluRS	0.003 ± 0.002	1386.4 ± 168.5	–
U36, pBR322	0.310 ± 0.008	1187.1 ± 48.0	0.026
U36, GluRS	0.215 ± 0.024	775.1 ± 48.6	0.028

[a] Wild-type or U36 $tRNA^{Glu}$ alleles were combined either with pBR322 or with pLQ7611ΔNruI, which over-expresses GluRS by approximately 100 fold [15].
[b] β-Galactosidase activity is expressed as Miller units [10].
[c] % suppression is calculated as the activity in CSH106 (*lacZ*(AAG461)) divided by the activity in CSH142F'100 (*lacZ*$^+$) multiplied by 100.

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