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$tRNA_2^{Gln}$ Su^+2 Mutants That Increase Amber Suppression

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We selected mutants of λpSu^+2 which had an increased ability to suppress an *Escherichia coli trp B9601* amber mutation on translationally stringent *rpsL594* streptomycin-resistant ribosomes. $tRNA_2^{Gln}$ Su^{+2} molecules produced from eight independent mutants were purified, and their ribonucleic acid sequences were determined. Two types of mutations were mapped to the $tRNA_2^{Gln}$ Su^+2(*glnV*) gene by this method. Both altered the pseudouridine at position 37 of the tRNA anticodon loop. Seven of the isolates were transitions (pseudouridine to cytosine), and one was a transversion (pseudouridine to adenine). These mutations resulted in Su^+ transfer ribonucleic acid molecules that exhibited higher transmission coefficients than their parent Su^+2 transfer ribonucleic acids. As judged by their suppressor spectra on T4 amber mutants, which were almost identical to that of Su^+2, the two mutant Su^+ transfer ribonucleic acids inserted glutamine at amber sites.

The molecular nature of tRNA-ribosome interactions is poorly understood (2). In particular, very little is known about the contributions of tRNA bases outside anticodons to cognate tRNA selection and binding by ribosomes. We have taken a genetic approach to answering this question by isolating mutations that increase translation of amber codons by suppressor tRNA's.

rpsL strains of *Escherichia coli* are resistant to the antibiotic streptomycin. This is due to a mutation in the 30S ribosomal protein S12, the product of the *rpsL* gene (15). Streptomycin-resistant ribosomes also reduce the efficiency of tRNA-mediated suppression of nonsense codons (4, 21). However, the addition of streptomycin to a growth medium increases the level of nonsense suppression by 2 to 50%, depending on the *rpsL* allele and the suppressor tRNA involved (21). Suppression of amber codons by Su^+2 is particularly affected in *rpsL* strains (21). The efficiency of suppression in such strains is reduced almost 10-fold compared with an isogenic, streptomycin-sensitive strain (L. Soll, Ph.D. thesis, Stanford University, Stanford, Calif., 1971). Suppression by Su^+2 of the *trpB9601* amber mutation in an *rpsL594* strain is so low that the strain becomes a tryptophan auxotroph (Soll, Ph.D. thesis). Growth of this strain is restored by adding either tryptophan or streptomycin; the latter acts by relieving the *rpsL* restriction. We have used the *rpsL594* restriction of suppression to select mutants of $tRNA_2^{Gln}$ Su^+2(*glnV*) that can efficiently suppress the *trpB9601* mutation in *rpsL594* strains in the absence of streptomycin.

In this paper we describe the isolation and characterization of two mutants; on the basis of sequence analysis, the mutations in these strains were shown to alter the base sequence of the $tRNA_2^{Gln}$ Su^+2 transcribed from phage DNA. Direct measurements of the transmission coefficients in strains lysogenic for these mutants showed that the two new tRNA's had increased efficiencies of amber suppression in both *rpsL*$^+$ and *rpsL594* genetic backgrounds. We also performed suppression spot tests with a number of T4 amber mutants. The results of these experiments suggested that the specificities of aminoacylation for the two mutant Su^+2 tRNA's were not altered from the specificity of the $tRNA_2^{Gln}$ Su^+2 progenitor.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains which we used are listed in Table 1. All were F^- W3110 strains except LS333, which is F^-; λ lysogens were tested to insure that each derivative was a single lysogen (16).

Bacteriophages. λ cI^+ pSu^+2 was obtained from H. Ozeki and has been described previously (9). λ cI^+ pSu^+2 mutants are phages with spontaneous mutations in the *glnV* Su^+2 gene carried by λ *c*I pSu^+2 (see below).

Reagents. Carrier-free [^{32}P]phosphoric acid was purchased from New England Nuclear Corp. Cellulose (MN300) and polyethyleneimine cellulose (MN2100) were obtained from Brinkmann Instruments. Acrylamide was a product of Eastman Kodak. RNase T_1 and RNase T_2 were products of Sankyo, and the pancreatic RNase was purchased from Worthington Biochemicals Corp. Streptomycin was obtained from

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TABLE 1. *E. coli strains used*

Strain	Genotype	Source, derivation and/or reference
LS1	*trpR trpA9605*(Am)	C. Yanofsky
LS22	*rpsL594*	M. Meselson
LS102	*trpR trpB9601*(Am)	C. Yanofsky
LS178	*trpA109 trpE3*	C. Yanofsky
LS181	*trpR trpA9605*(Am) *rpsL594*	LS1 + P1 (LS22), select for Str^r
LS200	*trpR trpB9605*(Am) *rpsL594*	LS102 + P1 (LS22), select for Str^r
LS268	*trpR trpA9605*(Am) *his-29*(Am) *ilv*	20
LS270	*trpR trpA9605*(Am) *his-29*(Am) *ilv thy*	Trimethoprim selection (13) from LS268
LS333	*leu met proC try lysA purE ara xyl lacZ* Azi^r Str^r T1^r T5^r T6^r	X478; C. Hill
LS340	*trpR trpA9605*(Am) *his-29*(Am) *metE*	LS268 + P1 (LS333), select for Ilva$^+$, score for Met$^-$
LS340(λSu$^+$2)	LS340(λ *cI$^+$ glnV44*[a])	This work
LS340(λSu$^+$2-88)	LS340(λ *cI$^+$ glnV44 glnV88*[b])	This work
LS340(λSu$^+$2-89)	LS340(λ *cI$^+$ glnV44 glnV89*)	This work
LS415	*trpR trpE10220 trpB9601*(Am) *his-29*(Am) *tna nvrA glaK* Str^r (λpapa)	This laboratory
LS1166(λ)		S159(λpapa); E. Lund
LS1364	*trpR trpA9605*(Am) *his-29*(Am) *metE rpsL594*	LS340 + P1 (LS181), select for Str^r
LS1364(λSu$^+$2)	LS1364(λ *cI$^+$ glnV44*	This work
LS1364(λSu$^+$-88)	LS1364(λ *cI$^+$ glnV44 glnV88*)	This work
LS1364(λSu$^+$-89)	LS1364(λ *cI$^+$ glnV44 glnV89*)	This work
LB50	*trpR trpA9605*(Am) *his-29*(Am) *ilv thi* Su$^+$1	L. Breeden; derived from LS268
LB51	*trpR trpA9605*(Am) *his-29*(Am) *ilv thi* Su$^+$2	L. Breeden; derived from LS268
LB52	*trpR trpA9605*(Am) *his-29*(Am) *ilv thi* Su$^+$3	L. Breeden; derived from LS268

[a] Alternate designation, *supE*.

[b] Mutations *glnV88* and *glnV89* were previously referred to by Yarus et al. (25) as E-1 and E-2, respectively.

Sigma Chemical Co. Yeast tRNA was a gift from M. Yarus.

Media. M63 minimal medium (13) was supplemented as required with amino acids (50 μg/ml) and with sugars (0.2%). Minimal medium plates were prepared with agar (final concentration, 1.5% Difco Laboratories). YT medium (13) was the rich medium used for growing bacterial cultures and preparing λ phage stocks. YT medium plates contained 1.2% agar. When required, streptomycin was added to both solid and liquid media to a final concentration of 200 μg/ml.

Preparation of phage-encoded tRNA. ^{32}P-labeled tRNA was prepared for sequence analysis as described by Lund et al. (11). Strain LS1166(λ) was grown in 20 ml of morpholinepropanesulfonic acid (14) containing 0.2 mM phosphate and 0.4% maltose to a concentration of 3×10^8 cells per ml, centrifuged, and suspended in morpholinepropanesulfonic acid containing no phosphate and 0.4% maltose. Bacteria were irradiated for 5 min with a General Electric germicidal lamp (model G15T8; 15 W) at a height of 30 cm; then a 1 M $MgSO_4$ solution was added to the cells (final concentration, 25 mM). After 5 min on ice, 0.2 ml of bacteria was infected with 0.1 ml of phage at a multiplicity of 15 phage per cell. Adsorption was allowed to continue for 15 min at 37°C. The infected bacteria were then diluted 10-fold into prewarmed morpholinepropanesulfonic acid and incubated at 37°C for 10 min. At this point [^{32}P]phosphoric acid was added to a concentration of 0.7 to 1.0 mCi/ml, and the cultures were incubated for another 30 min at 37°C. Carrier yeast RNA (20 to 40 μg/ml) was added, and the cells were extracted twice with 1 volume of redistilled phenol saturated with 0.1 M Tris (pH 7.4)–3.5 mM phosphate. Labeled nucleic acids were then precipitated twice by adding 0.5 M NaCl and 2 volumes of 95% ethanol. Small RNAs were then separated by two-dimensional acrylamide gel electrophoresis as described by Ikemura and Dahlberg (7), except that 0.1% *N,N,N',N'*-tetramethylethylenediamine was substituted for 0.4% 3-dimethylaminopropionitrile. Electrophoresis in the first dimension was at 140 V for 2.0 h, and electrophoresis in the second dimension was at 220 V for 20 h. The dimensions of the gel plates were 14 by 16 cm. tRNA spots were located by autoradiography, cut out, and eluted in a solution containing 0.5 M NaCl, 0.5% phenol, and 20 μg of yeast tRNA per ml. The eluted tRNA was precipitated by adding 0.1 volume of 5 M NaCl and 2 volumes of 95% ethanol. The identity of the Gln_2 Su$^+$2 tRNA species was confirmed by RNase T_1 fingerprinting (9, 22).

Sequence analysis. RNase T_1 fingerprinting was performed as described by Griffin (6). After the nucleotides were transferred onto polyethyleneimine cellulose plates, the plates were carefully washed in 20% ethanol and dried. The bottom 1.5 cm of each plate was sprayed with distilled water and chromatographed for 3 to 4 cm with 1 M pyridinium formate and then to the top of the plate with 2.2 M pyridinium formate. Subsequent nuclease digestions and analyses of oligonucleotides were by the methods of Barrell (1). Mononucleotides from RNase T_2 digestions were sometimes analyzed by chromatography on polyethyleneimine cellulose thin-layer chromatography plates (plastic backed; Brinkman Instruments), using 0.3 M lithium formate (pH 3) as the developing buffer (K. Danna, personal communication).

Enzyme assays. Cultures for tryptophan synthe-

tase A and B assays were inoculated with 5×10^8 cells and grown with vigorous shaking at 37°C for 12 to 15 h in 500 ml of M63 minimal medium supplemented with amino acids (50 μg/ml) and glucose (0.2%). Cells were grown to log phase and harvested by centrifugation. The cells were washed once with 0.15% NaCl, sedimented, and suspended in 2 ml of 0.1 M Tris-hydrochloride buffer (pH 7.8). The cells were then disrupted by sonic oscillation (Sonic 300; Artek) in the presence of glass beads (Sigma catalog no. G-3753). Debris was removed by centrifugation at 18,000 × *g* for 10 min.

Tryptophan synthetases A and B were assayed as described by Smith and Yanofsky (18).

Test for suppression of phage T4 amber mutants. Strains lysogenic for λ *cI*$^+$ pSu$^+$2, λ *cI*$^+$ pSu$^+$2-88, and λ *cI*$^+$ pSu$^+$2-89 were tested for the ability to suppress various T4 amber mutants as follows. Bacterial cultures were grown to log phase in YT medium, mixed with H-top agar (13), and then spread onto YT medium plates. About 10^4 phage were spotted in 2 to 4 μl, and the plates were incubated overnight at 30 and 42°C before reading.

The gene 32 amber mutants and the T4$^+$ phage stocks were supplied by Peter Gauss and Larry Gold. The remaining T4 amber stocks were obtained from Casimir Ryzewski and Bill Wood.

RESULTS

Isolation of spontaneous λpSu$^+$2 suppressor efficiency mutants. Infection of strain LS102 (*trpR trpB9601*) with λpSu$^+$2 resulted in the efficient formation of Trp$^+$ transductants (Table 2). The same transduction experiment performed with strain LS200 (*trpR trpB9601 rpsL594*) yielded no Trp$^+$ transductants unless 200 μg of streptomycin per ml was present on the selective plates. Spontaneous mutants of λpSu$^+$2 capable of giving rise to Trp$^+$ transductants of strain LS200 in the absence of streptomycin were found at a rate of 10^{-8} phage per PFU. Many of these Trp$^+$ colonies were purified, and UV-induced lysates were prepared from them. The lysates were then tested for their Trp$^+$ transducing activity on strain LS200 (Table 2). Eight phage isolates exhibiting the mutant transducing phenotype were retained for further study.

Sequence analysis of the Su$^+$ tRNA produced upon infection with λpSu$^+$2 and λpSu$^+$2 mutants. Because of the nature of the mutant selection, we hypothesized that the mutation(s) responsible for the new suppressor phenotype of λpSu$^+$2 affected either the synthesis or the nucleotide sequence of $tRNA_2^{Gln}$ Su$^+$2. To test this hypothesis, we examined the ^{32}P-labeled tRNA's produced in bacteria infected at a multiplicity of 15 phage per cell with λpSu$^+$2 and the λpSu$^+$2 mutants. The bacterial cells were irradiated heavily with UV light before infection to reduce the synthesis of tRNA's encoded by the host chromosome. The labeled tRNA's produced during the infection were extracted from the bacterial cells and prepared for gel electrophoresis as described above. As Fig. 1 shows, two predominant tRNA species were produced by λpSu$^+$2 and λpSu$^+$2-88. All eight of the mutants examined had tRNA patterns qualitatively identical to those shown in Fig. 1. In addition, all of the mutants seemed to synthesize the same amount of both tRNA species per infected cell.

Each sample of tRNA was eluted from the gel with about 90% recovery. RNase T_1 fingerprints of the eluted tRNA species demonstrated that the fast-migrating tRNA spot was $tRNA_2^{Gln}$ Su$^+$2 (Fig. 2a). This assignment agrees with the results of Inokuchi et al. (9), who also showed that the slow-moving tRNA species was a methionine-accepting tRNA.

The RNase T_1 fingerprints of tRNA Su$^+$2 produced by λpSu$^+$2 and two mutants (λpSu$^+$2-88 and λpSu$^+$2-89) are shown in Fig. 2. (The

TABLE 2. *Phenotypic basis of the mutant selection*[a]

Strain	Genotype	Transducing phage	Streptomycin (200 μg/ml)	Growth on plates	
				Without tryptophan	With tryptophan
LS102	*trpR trpB9601*		Absent	0	+
LS102	*trpR trpB9601*	λpSu$^+$2	Absent	+	+
LS200	*trpR trpB9601 rpsL594*		Absent	0	+
LS200	*trpR trpB9601 rpsL594*	λpSu$^+$2	Absent	0	+
LS200	*trpR trpB9601 rpsL594*	λpSu$^+$2	Present	+	+
LS200	*trpR trpB9601 rpsL594*	λpSu$^+$2 mutants	Absent	+	+
LS200	*trpR trpB9601 rpsL594*	λpSu$^+$2 mutants	Present	+	+

[a] A total of 10^6 PFU of the appropriate phage was adsorbed to 4×10^6 to 6×10^6 bacterial cells for 15 min at 37°C. The bacterium-phage mixture was then mixed with minimal F-top agar (13), spread onto minimal glucose plates, and incubated for 2 days at 37°C. +, Appearance of bacterial colonies; 0, no bacterial colonies were observed after several days of incubation.

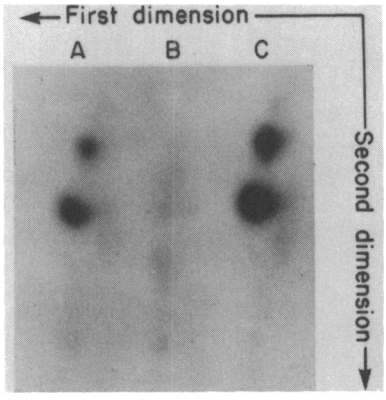

FIG. 1. *Autoradiograph showing the ^{32}P-labeled tRNA's produced by λpSu$^+$2-infected cells (lane A) and λpSu$^+$2-88-infected cells (lane C). Each sample was electrophoresed through a 10% polyacrylamide gel (first dimension), turned 90°, and then electrophoresed in a second dimension (see text). Lane B contained an uninfected control culture and shows the background of cellular tRNA's produced after UV irradiation.*

other six λpSu$^+$2 mutants isolated were identical to λpSu$^+$2-88 and are not discussed further.) tRNA Su$^+$2 from λpSu$^+$2 gave a fingerprint pattern consistent with a UAG-reading suppressor mutation of $tRNA_2^{Gln}$ (9). Each RNase T_1 oligonucleotide spot gave the expected products when redigested with pancreatic RNase and electrophoresed on DEAE paper at pH 3.5 (data not shown) (22).

In Fig. 2b the RNase T_1 fingerprint of tRNA Su$^+$2-88 is shown. Only the RNase T_1 oligonucleotide representative of the anticodon stem and loop was altered in mobility compared with the fingerprint pattern of tRNA Su$^+$2 (Fig. 2a, spot I, and Fig. 2b, spot II). The pancreatic RNase digestion products of all of the RNase T_1 oligonucleotides except spots I and II were identical for all tRNA's (data not shown). Figure 3, lanes a and b, show the relevant pancreatic RNase digestion products of the RNase T_1 anticodon oligonucleotides from Su$^+$2 (Fig. 2a, spot I) and Su$^+$2-88 (Fig. 2b, Spot II). The Su$^+$2 RNase T_1 oligonucleotide produced the pancreatic digestion products expected from an AUmUCUAA*ΨΨCCG oligonucleotide (ψ, pseudouridine) (22). However, pancreatic digestion of the Su$^+$2-88 RNase T_1 anticodon oligonucleotide yielded an AAC oligonucleotide not found in the Su$^+$2 digestion products. Because of these results, the sequence of the mutant Su$^+$2-88 amber suppressor RNase T_1 fragment must have been AUmUCUAA*CΨCCG. Therefore, the mutational event giving rise to the Su$^+$2-88 tRNA allele was a change from pseudouridine to cytosine in position 37 of $tRNA_2^{Gln}$ (Fig. 4).

Figure 2c shows the RNase T_1 fingerprint of tRNA Su$^+$2-89. The mobility of the anticodon oligonucleotide (spot III) was not noticeably different from that of the Su$^+$2 anticodon (Fig. 2a, spot I). However, pancreatic RNase digestion of the Su$^+$2-89 anticodon fragment revealed an AAAU oligonucleotide product (Fig. 3, spot III) not found in pancreatic RNase digestions of either the Su$^+$2-88 or the Su$^+$2 RNase T_1-generated anticodon fragments (Fig. 3, lanes a through c). We concluded that the Su$^+$2-89 mutation was also at position 37 of $tRNA_2^{Gln}$ and changed the pseudouridine at this position to an adenine (Fig. 4).

Efficiency of suppression of the $tRNA_2^{Gln}$ Su$^+$2 mutants. Su$^+$2 is an inefficient suppressor of amber mutants in streptomycin-resistant bacteria (4, 21). The Su$^+$2-88 and Su$^+$2-89 mutants were selected for their ability to overcome this *rpsL* effect. Therefore, to begin understanding the mode of action of these mutations, we measured the transmission coefficients in strains lysogenic for λpSu$^+$2-88 and λpSu$^+$2-89.

The transmission coefficient is a measure of the capacity of a suppressor tRNA to prevent termination of protein synthesis at nonsense codons through the insertion of an amino acid. We measured the transmission coefficients for Su$^+$2 and the mutant Su$^+$2 suppressors in strain LS340 and its isogenic *rpsL594* derivative, strain LS1364. Both of these strains contain a *trpA9605* amber mutation and are constitutive for expression of the *trp* operon (*trpR*$^-$). The *trp* operon is composed of five cistrons, including the *trpA* gene, which are transcribed into a single mRNA from the same operator-promoter region (23). Enzyme production from these genes is coordinate under most conditions, resulting in a constant ratio of *trp* enzyme activities. The *trpB* gene is adjacent to the most promoter distal of the *trp* genes, *trpA*. Therefore, the ratio of *trpA* enzyme activity to *trpB* enzyme activity is determined by the number of ribosomes that translate through the mRNA's of the two genes. When a *trpA9605* amber mutation is present, this ratio of *trpA* activity to *trpB* activity is very small. However, when amber suppressing tRNA's are present in the cells, the ratio of *trpA* activity to *trpB* activity is larger, and this ratio is indicative of the number of ribosomes that continue translation through the *trpA9605* codon.

The enzyme activity of a suppressed protein could be affected by changes in the amino acid inserted by the suppressor tRNA. This was not a complication in our measurements of suppres-

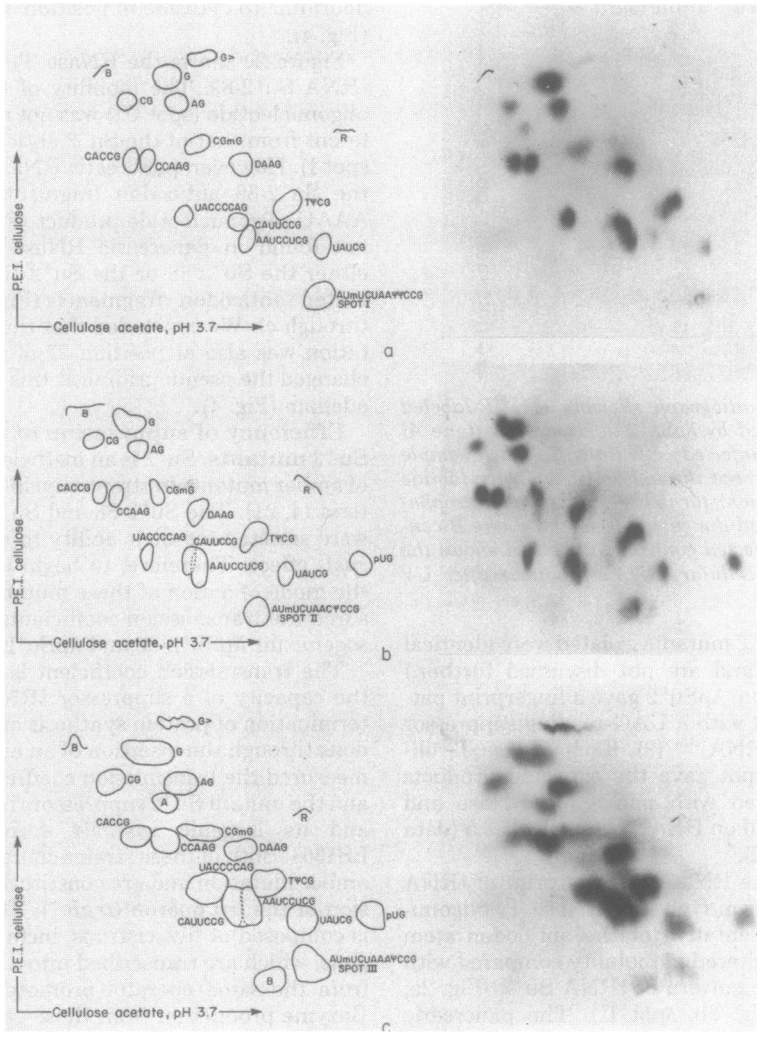

FIG. 2. *RNase T_1 fingerprints of Su⁺2 tRNA (a), Su⁺2-88 tRNA (b) and Su⁺2-89 tRNA (c). In (c), spot A was due to an impurity that appeared only rarely; spot B was a small fraction of oligonucleotide TΨCG, which remained at the origin. The marker dyes B and R have been described previously by Barrell (1). Hyphens were omitted from the figure for clarity. G> indicates cyclic guanine ribonucleotides. P.E.I., Polyethyleneimine.*

sor activity for two reasons. First, Maling and Yanofsky (12) have shown that the *trpA* proteins produced by a large number of *trpA* missense mutants all have similar enzyme activities. Therefore, the amino acid inserted in response to the *trpA9605* codon probably has little or no effect on *trpA* enzyme activity. And second, the results presented below suggest that the Su⁺2 mutant tRNA's inserted the same amino acid (glutamine) as the Su⁺2 tRNA. Thus, even if the enzyme activity of the suppressed *trpA* protein were altered compared with the wild-type protein activity, all of the comparisons made here would still be valid.

The *trpA* and *trpB* enzyme activities were measured in sonic extracts of bacteria lysogenic for λpSu⁺2, λpSu⁺2-88, and λpSu⁺2-89 (12). The ratios of suppressed *trpA* enzyme activity to *trpB* enzyme activity were then normalized to the same ratio measured in isogenic *trpA*⁺ cells.

Table 3 shows the transmission coefficients measured in lysogenic strain LS340 and LS1364 cells. The two mutant suppressor tRNA's dramatically increased the fraction of *trpA* protein that was completed in *rpsL*⁺ cells. Both Su⁺2-88 and Su⁺2-89 exhibited threefold increases in

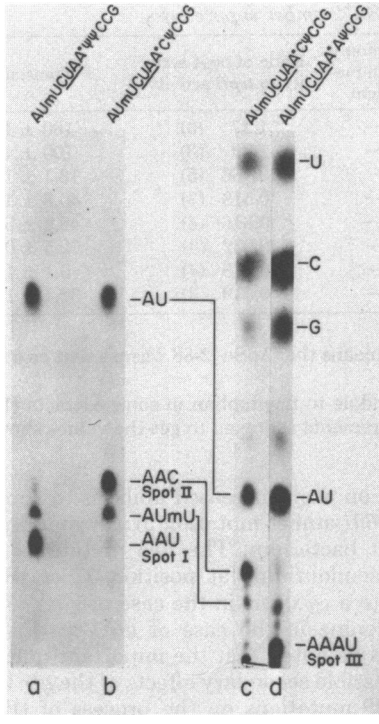

FIG. 3. *DEAE-cellulose paper electropherogram (pH 3.5) of oligonucleotides derived from pancreatic RNase digestions of spots I through III in the RNase T_1 fingerprints of Fig. 2. Lanes a and b contained spots I (Su⁺2) and II (Su⁺2-88) of Fig. 2 and were electrophoresed for 8 h at 1,000 V to obtain better resolution of AAU (spot I) and AAC (spot II) from the AUmU oligonucleotide. The molar yields of guanine, cytosine, and uridine from these digestions were identical (data not shown). Lanes c and d contained spots II (Su⁺2-88) and III (Su⁺2-89) of Fig. 2 and were electrophoresed for 4 h at 1,000 V. The cathode was at the top. The identities of the digestion products in the gels were determined by their electrophoretic mobilities (1) and by elution, digestion with RNase T_2, and chromatography on polyethyeleimine cellulose plates (see text). Sequences from this analysis and from the published sequences of $tRNA_2^{Gln}$ (9, 22) are written above each lane. In some molecules, uridine in AUmU was not modified, so that AU resulted. The anticodon loop pseudouridine residues (designated Ψ in the sequences written above the lanes) were not present in these oligonucleotides, resulting in uridine residues at these positions. The modified adenine derivative A* is also incomplete. All of these undermodifications are probably due to the irradiation of host cells before phage infection (Dahlberg, personal communication).*

suppressor activity compared with the activity measured for Su⁺2. The two mutant suppressor tRNA's were still more active than Su⁺2 in *rpsL594* cells (strain LS1364), but a distinction in behavior between the two mutations could be discerned. Su⁺2-88 was very sensitive to the change in ribosomes, decreasing in efficiency by 75%; in contrast, Su⁺2-89 decreased in efficiency by only 25%. The two mutants clearly responded very differently to streptomycin-resistant ribosomes.

Amino acids inserted by Su⁺2-88 and Su⁺2-89. Table 4 shows the suppressor patterns generated by spotting T4 amber mutants on various Su⁺ bacterial strains. Su⁺1 (inserts serine), Su⁺2 (inserts glutamine), and Su⁺3 (inserts tyrosine) bacteria contain chromosomal suppressor alleles. The other Su⁺ strains tested were the same series of λpSu⁺2, λpSu⁺2-88, and λpSu⁺2-89 lysogens used for the measurement of the transmission coefficients (Table 3). The T4 amber mutants were selected for their sensitivities to the amino acid inserted during suppression (C. Ryzewski, personal communication). Table 4 shows that the Su⁺ control bacterial hosts allowed growth of different T4 phage amber mutants. The patterns of suppression were made more distinctive by performing the tests at 30 and 42°C. Phage proteins resulting from suppression are sometimes temperature sensitive for function because of the amino acid inserted at the amber codon. Thus, any changes in the amino acid inserted by a mutant suppressor tRNA should be observable when the growth of T4 amber mutants is tested on bacterial hosts containing the mutant suppressor tRNA (5, 8, 17, 19).

The suppression patterns shown in Table 4 demonstrate that, although a few differences did

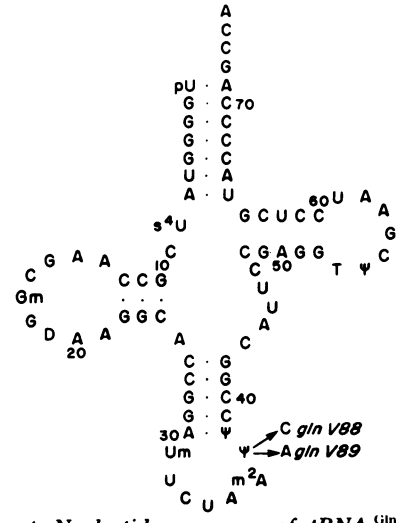

FIG. 4. *Nucleotide sequence of $tRNA_2^{Gln}$ Su⁺2. Su⁺2 is shown with the single base changes caused by the glnV88 and glnV89 mutations at position 37. Ψ, Pseudouridine.*

TABLE 3. *Transmission coefficients of Su⁺2 amber suppressors*

Strain[a]		Suppressor[b]	Streptomycin in medium	Ratio of *trpA* activity to *trpB* activity[c]	% Transmission
trp allele	*rpsL* allele				
trp⁺	*rpsL*⁺	Su⁻	–	1.27 (3)	100 ± 1.9[d]
trp⁺	*rpsL594*	Su⁻	–	1.29 (3)	100 ± 1.8
trpA9605	*rpsL*⁺	Su⁺2	–	0.166 (5)	13.1 ± 1.9
trpA9605	*rpsL*⁺	Su⁺2-88	–	0.518 (3)	40.8 ± 1.3
trpA9605	*rpsL*⁺	Su⁺2-89	–	0.616 (4)	48.6 ± 5.4
trpA9605	*rpsL594*	Su⁺2	–	0.032 (4)	2.5 ± 0.5
trpA9605	*rpsL594*	Su⁺2-88	–	0.118 (4)	9.1 ± 1.2
trpA9605	*rpsL594*	Su⁺2-89	–	0.419 (3)	32.4 ± 1.3

[a] All strains were derivatives of strains LS340 and LS1364.

[b] Su⁺2 means that λpSu⁺2 was present as a lysogen, Su⁺2-88 means that λpSu⁺2-88 was present as a lysogen, and Su⁺2-89 means that λpSu⁺2-89 was present as a lysogen.

[c] *trpA* and *trpB* activities were measured by conversion of indole to tryptophan in sonic extracts (18). The numbers in parentheses are the numbers of independent measurements averaged to get the values shown.

[d] Mean ± standard error of the mean.

exist between λpSu⁺2 and the mutants (λpSu⁺2-88 and λpSu⁺2-89), the patterns were very similar. There were only 3 differences in the 15 comparisons made between λpSu⁺2 and λpSu⁺2-88 in an *rpsL*⁺ background. In each case a T4 mutant that was temperature sensitive when suppressed by Su⁺2 became temperature insensitive when suppressed by Su⁺2-88. When the Su⁺2-88 tRNA was tested in *rpsL594* cells, the suppression pattern changed dramatically; under these conditions Su⁺2-88 suppressed fewer T4 mutants than Su⁺2 in *rpsL*⁺ bacteria. There were 2 differences in the 15 comparisons made between Su⁺2 (*rpsL*⁺) and Su⁺2-88 (*rpsL594*) suppression of the T4 amber mutants. Both of these differences (amber B251 and amber NG430) could be explained by the reduced transmission coefficient of Su⁺2-88 upon transfer from *rpsL*⁺ cells (41%) to *rpsL594* cells (9%). This conclusion was reinforced by an examination of the changes in the T4 suppression patterns elicited by Su⁺2 in *rpsL*⁺ and *rpsL594* bacteria. Four of the six T4 mutants that responded differently to Su⁺2 depending upon the *rpsL* allele present were the same T4 mutants that were suppressed differently by Su⁺2-88 and Su⁺2. The suppression patterns displayed by λpSu⁺2-89 in *rpsL*⁺ and *rpsL594* strains differed in 5 of 15 and 3 of 15 comparisons with λpSu⁺2(*rpsL*⁺), respectively. Again, all of the variations in the T4 amber suppression patterns among Su⁺2, Su⁺2-88, and Su⁺2-89 could be correlated with the *rpsL* allelic background and the resulting changes in transmission coefficients for these tRNA's (Tables 3 and 4). We conclude that all of our data are consistent with the hypothesis that both Su⁺2-88 and Su⁺2-89 tRNA's insert glutamine in response to amber codons.

DISCUSSION

We isolated two mutants of $tRNA_2^{Gln}$ Su⁺2 based on their increased abilities to suppress a *trpB9601* amber mutation in a streptomycin-resistant bacterium. The two mutations change the pseudouridine at position 37 of $tRNA_2^{Gln}$ Su⁺2 to a cytosine in the case of *glnV88* and to an adenine in the case of *glnV89* (Fig. 4). It should be noted that the important question of the possible secondary effects of the *glnV88* and *glnV89* mutations on the process of $tRNA_2^{Gln}$ base modification was not resolved by our tRNA sequence data. tRNA molecules transcribed in UV-irradiated *E. coli* are very poorly modified (J. Dahlberg, personal communication). Therefore, the resolution of this question must await the purification of these mutant tRNA's from cells grown under physiological conditions.

There are several ways in which the mutations in the anticodon region of $tRNA_2^{Gln}$ Su⁺2 might affect the *rpsL* restriction of suppression. The three most probable explanations are as follows. (i) The *glnV88* and *glnV89* mutations increase the affinity or reactivity of the Su⁺2 tRNA with glutaminyl-tRNA synthetase. This could elevate the cellular pool of charged Su⁺ tRNA's available for protein synthesis, resulting in increased suppression. (ii) The mutations increase the ability of the mutant tRNA's to interact with the ribosomes, thereby increasing the fraction of ribosomal transits of an amber codon that yield insertion of an amino acid, rather than termination (the transmission coefficient). And (iii) the mutations alter the specificity of aminoacylation, thereby causing the $tRNA_2^{Gln}$ Su⁺2 mutants to be charged with another amino acid. This class of mutants could relieve the *rpsL*-imposed tryptophan auxotrophy of *trpB9601* by causing insertion of a new amino acid in response to the amber codon. The tryptophan synthetase B protein synthesized under such conditions could be more active enzymatically, thus allowing the biosynthesis of enough tryptophan for growth.

TABLE 4. *Growth of T4 amber mutants on various suppressor strains*

Strains and conditions[a]	Growth of the following T4 mutants:																													
	NG522 (5)[b]		B256 (5)		NG495 (5)		NG572 (5)		B251 (6)		NG145 (6)		NG466 (6)		NG430 (10)		NG197 (18)		H18 (32)		SR51 (32)		HL618 (32)		K6 (34)		NG187 (37)		$T4^+$ (wild type)	
	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C	30°C	42°C
Su^-	0[c]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+
Su^+1	+	0	+	0	+	±	+	+	+	+	+	±	+	+	+	+	0	0	+	+	+	0	+	+	0	0	+	0	+	+
Su^+2	+	0	±	0	+	0	+	0	±	0	±	0	±	0	±	0	0	0	+	+	0	0	+	+	0	0	0	0	+	+
Su^+3	+	+	±	+	+	+	+	+	+	+	±	±	+	+	+	+	+	+	+	+	+	+	±	+	0	0	+	+	+	+
λSu^+2	+	0	±	0	+	0	+	0	+	+	±	0	+	+	±	0	+	+	+	+	0	0	+	+	0	0	0	0	+	+
λSu^+2-88	+	0	±	0	+	+	+	+	+	+	±	±	+	+	+	+	+	+	+	+	0	0	+	+	0	0	0	0	+	+
λSu^+2-89	+	0	±	0	+	+	+	+	+	+	±	±	+	+	+	+	+	+	+	+	±	0	+	+	0	0	±	0	+	+
rpsL594 λSu^+2	±	0	0	0	±	±	±	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	+	+
rpsL594 λSu^+2-88	+	0	±	0	+	+	+	+	+	+	±	0	+	+	+	+	0	0	±	±	0	0	0	0	0	0	0	0	+	+
rpsL594 λSu^+2-89	+	0	±	0	+	+	+	+	+	+	±	±	+	+	+	+	0	0	+	+	0	0	0	0	0	0	0	0	+	+

[a] Spot tests were performed as described in the text. The Su^- strain was LS340. Su^+1, Su^+2, and Su^+3 were strains LB50, LB51, and LB62, respectively. All other strains were lysogenic derivatives of LS340 and LS1364.

[b] The numbers in parentheses refer to the T4 genes affected by the amber mutation.

[c] +, Complete clearing; ±, partial clearing; 0, no clearing.

To distinguish between the latter two possibilities, we measured directly the transmission coefficients for Su^+2, Su^+2-88, and Su^+2-89 in *trpA9605* strains containing an *rpsL*$^+$ or *rpsL594* allele (Table 3). Since the *trpA* protein is not sensitive to the amino acid that is inserted at the *trpA9605* amber codon, the increased transmission coefficients of Su^+2-88 and Su^+2-89 tRNA's compared with Su^+ tRNA must be interpreted as being due to increased suppressor function. Furthermore, the increase in Su^+2 tRNA function brought about by these mutations is not limited to the *rpsL* allele present in the cell or to the *trpA9605* amber mutation. As mentioned above, the tRNA mutants were isolated by selecting for increased suppression of a *trpB9601* amber mutation. In addition, the differences in the suppression patterns of Su^+2-88 and Su^+2-89 compared with Su^+2 for many different T4 amber mutations suggest that the increase in the suppression function of these Su^+2 mutations is general (Table 4).

The suppression spectra of the Su^+2 and mutant suppressor tRNA's on T4 amber mutants imply that the *glnV88* and *glnV89* mutations do not also affect the amino acids inserted by these mutant tRNA's in response to amber codons (Table 4). However, this conclusion does not rule out the possibility that these mutant Su^+2 tRNA's might affect the specificity of aminoacylation. For example, Su^+7 tRNA (*supU*) (20) is acylated equally with tryptophan and glutamine (10, 19, 24), yet it usually inserts glutamine during protein synthesis (3, 19). All of the differences in T4 amber suppression among Su^+2, Su^+2-88, and Su^+2-89 can be accounted for by the differences in their transmission coefficients, rather than by the amino acids inserted during suppression. This conclusion is not unexpected because the Su^+2-88 and Su^+2-89 tRNA's retain the proper sequence features in their CCA stems and the central uridines of their anticodons, which appear to be essential for glutaminyl-tRNA synthetase action (24).

The Su^+2, Su^+2-88, and Su^+2-89 sequences differ in only one position, which is at the top of the anticodon loop (Fig. 4). The finding that this position might be important for ribosomal function is also consistent with the recent results of Yarus et al. (25). These workers transplanted the DNA encoding the anticodon loop and the stem of Su^+2-88 into the *trpT* gene that encodes $tRNA^{Trp}$. In doing so, they created a new functional suppressor tRNA, Su^+271. The Su^+271 tRNA sequence differs in four positions from the sequence of Su^+7 $tRNA^{Trp}$, and all four are at the top of the anticodon loop. The transmission coefficients measured for Su^+7 and Su^+271 tRNA's, like those obtained for Su^+2-88 and Su^+2-89 tRNA's, also suggest that this region of

the anticodon loop affects chain propagation on ribosomes. We hope that biochemical studies of these mutant Su^+2 tRNA's will generate more insight into the functional role of the bases in the anticodon loop with regard to ribosomal binding and the other steps in the ribosomal cycle of tRNA.

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