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Three Different Missense Suppressor Mutations Affecting the $tRNA_{GGG}^{Gly}$ Species of *Escherichia coli*

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The *Escherichia coli* suppressor mutation, *supT*, has been shown to cause a C → U substitution in the middle position of the $tRNA_{GGG}^{Gly}$ anticodon. This is the same tRNA species that is altered by the $glyUsu_{AGA}$ mutation studied previously. This finding indicates that the *supT* mutant tRNA reads the glutamic acid codon, GAG. The *supT* suppressor has also been converted to a new suppressor, called $glyUsu_{GAA}$, which will suppress the GAA mutation, *trpA46*. The in vivo suppression efficiencies of each of these three missense suppressors has been measured and are as follows: $glyUsu_{AGA}$, 3.6%; *supT*, 1.6%; and $glyUsu_{GAA}$, 0.4%. Mistranslation by these mutant glycine tRNA species has no adverse affects on cell growth since cultures possessing the suppressors grow as fast as cells without. The *supT* tRNA species can be observed as a peak in the profile of glycyl-tRNA fractionated on a RPC-5 chromatographic column, indicating that the mutant tRNA can be aminoacylated with reasonable efficiency. This finding contrasts with previous findings concerning the $glyUsu_{AGA}$ mutant tRNA which is not significantly aminoacylated under the same conditions.

Escherichia coli has three distinct species of glycine transfer ribonucleic acid (tRNA) which can be distinguished by their chromatographic mobilities and codon responses (6, 14). Studies of an AGA specific missense suppressor mutation, $glyUsu_{AGA}$, showed that the $tRNA_{GGG}^{Gly}$ species was coded by a single structural gene, *glyU*$^+$, which is closely linked to the *lysA* locus. When these observations were made, we noted that another suppressor mutation, *supT*, had been shown by Eggertsson and Adelberg (9) to have a map position very similar to that found for the $glyUsu_{AGA}$ mutation. This suggested that the *supT* and *glyU* loci might be identical and that *supT* might also affect $tRNA_{GGG}^{Gly}$. In this work we report that the *supT* mutation does affect the $tRNA_{GGG}^{Gly}$ species, causing a C → U transition in the anticodon. The anticodon of the *supT* mutant tRNA is CUC, indicating that it should read the glutamic acid codon, GAG. We also report the conversion of the *supT* suppressor to a suppressor specific for the other glutamic acid codon, GAA. This third missense suppressor derived from $tRNA_{GGG}^{Gly}$ is called $glyUsu_{GAA}$. Including the frameshift suppressor isolated by Riddle and Roth (17) from a *Salmonella typhimurium* culture, this brings to four the number of different suppressors derived from this one species of tRNA.

MATERIALS AND METHODS

Bacteria. *E. coli* K-12 strains used are described in Table 1. Standard genetic symbols and abbreviations have been used (8, 21). Strains AB2077 and AB2551 have been described previously (9), as have been CH354 and CH465 (6). Strain CH548 was prepared by mating CH354 with the Hfr-12 strain AB2077, selecting for Met$^+$, Pur$^+$ recombinants, and scoring for the other markers. The *lysA* marker was introduced into CH550 and CH571 by using trimethroprim selection (20) to isolate a *thyA* mutant of CH548 and then using P1 transduction to co-transduce *thyA*$^+$ *lysA* into this derivative. The final step in the preparation of CH550 involved the introduction of the $glyUsu_{AGA}$ mutation using a P1 lysate of CH465. The *supT* mutation in CH591 was isolated in this laboratory by selecting an Ilv$^+$ revertant of CH548 after nitrosoguanidine mutagenesis; the suppressor was then transduced into CH571 to prepare CH591. The *supT* mutation so isolated is indistinguishable from *supT10* by suppressor specificity, genetic linkage, or growth rates of suppressed mutants. CH726 was derived from CH571 by first selecting a T1 phage-resistant deletion mutant (10) in which the deletion included the *trp* operon; then *trpA46* was introduced by using a P1 lysate of a *trpA46* strain supplied by Charles Yanofsky, selecting for ability to grow on indole without tryptophan supplementation. The $glyS_H$ and $glyS_L$ alleles of the glycyl-tRNA synthetase gene were introduced into appropriate strains as described previously (14). Strains CH757 and CH760 are single lysogens for defective lambda transducing phages. Isolation of

TABLE 1. *Genotypes of E. coli strains*

Strain designation	Genotype
AB2077	*ilvD130 argH1 purC1 xyl-4* Hfr-12
AB2551	*ilvD132 thi-1 leu-6 lac-24 supT10* Hfr-13
CH354	*trpA36 glyS*$_H$ *metB*
CH465	*trpA36 glyUsu*$_{AGA}$ *glyS*$_H$ *argH thi*
CH548	*trpA36 glyS*$_H$ *xyl-4 ilvD130 argH1*
CH550	*trpA36 lysA glyUsu*$_{AGA}$ *glyS*$_H$ *xyl-4 ilvD130 argH1*
CH571	*trpA36 lysA glyS*$_H$ *xyl-4 ilvD130 argH1*
CH591	*trpA36 lysA supT glyS*$_H$ *xyl-4 ilvD130 argH1*
CH726	*trpA46 lysA supT glyS*$_H$ *xyl-4 ilvD130 argH1*
CH727	*trpA461 lysA supT glyS*$_H$ *xyl-4 ilvD130 argH1*
CH729	*trpA46 lysA glyUsu*$_{GAA}$ *glyS*$_H$ *xyl-4 ilvD130 argH1*
CH735	*trpA36 lysA glyUsu*$_{AGA}$ *glyS*$_H$ *xyl-4 ilvD130 argH1*
CH757	*trpA36 lysA glyS*$_H$ *xyl-4 ilvD130 argH1 su*$_{III}^-$ (λ*cI857 S*$^-$ *d lysA*$^+$ *glyU*$^+$)
CH760	*trpA461 lysA*$^-$ *supT glyS*$_H$ *xyl-4 ilvD130 argH1 su*$_{III}^-$ (λ*cI857 S*$^-$ *d lysA*$^+$ *supT*)

these defective lambda transducing phages utilized the lambda lysogen KS72 (18) which has the lambda prophage integrated near lysA. The *glyUsu*$_{AGA}$ suppressor was introduced into KS72, and isolation of λ*dlysA glyUsu*$_{AGA}$ transducing phages was according to procedures classically used for λ*dg* isolation. Other *glyU* alleles were then placed on the phage by recombination. These procedures will be described fully in a later publication. The *supT* alleles present in CH760 are identical to that in CH591.

Mating and mutagenesis procedures. Transduction with phage P1*kc* and Hfr mating were carried out as described previously (13). Agar plates used in genetic procedures contained 1.5% agar, minimal-citrate medium (22), 0.5% glucose, and appropriate supplementation of amino acids and other factors as necessary. When introduction of a suppressor allele was being selected directly, efficient recovery of recombinants or mutants was aided by supplementation of a trace amount (0.1 μg/ml) of the limiting amino acid. When *ilvD130 supT* cultures were grown without isoleucine and valine supplementation, leucine (50 μg/ml) was always added to enhance the growth rate. Ultraviolet mutagenesis was carried out by spreading 10^8 cells on a selective plate and then irradiating the plate for 10 s under a 15-W General Electric germicidal lamp placed 46 cm from the plate. 2-Aminopurine mutagenesis was accomplished by spreading 10^8 cells on a selective plate, followed by the addition of a drop of 2-aminopurine solution (40 mg/ml) to a sterile 12.7-mm filter disk placed on the plate; revertants appeared in a halo around the disk.

Procedures for tRNA isolation and sequencing. The low-phosphate medium used for growing cultures for tRNA preparations has been described previously (12). When induction of the lysogenic strains was not desired, growth was at 34 C throughout. To induce lysogenic cultures thermally, the cultures were initially grown at 34 C until the optical density measured at 590 nm reached 1.2; at this point an equal volume of medium preheated to 50 C was added, and the culture was shaken at 42 C for 15 min. The temperature was then reduced to 39 C, and the cells were harvested 150 min after thermal induction. When [^{32}P]phosphate labeling was desired, up to 10 mCi of carrier-free [^{32}P]phosphate was added to 200 ml of culture 60 min after thermal induction. Procedures for tRNA isolation and RPC-5 reversed-phase chromatography have been described previously (12). ^{32}P-labeled *supT* tRNA was purified in two ways. The first involved RPC-5 column chromatography of glycyl-tRNA, followed by rechromatography of the pooled material from the first column after phenoxyacetylation; this procedure was identical to the one described previously for the purification of the wild-type tRNA$_{GGG}^{Gly}$ (12). The second method utilized the two-dimensional acrylamide gel system described by Ikemura and Dahlberg (15) as the first step in the purification. In this case, tRNA was isolated from CH760 cells which had been labeled with [^{32}P]phosphate between 60 and 150 min after thermal induction. Upon fractionation by this two-dimensional acrylamide gel procedure, *supT* tRNA could be identified as a relatively discrete spot on the autoradiogram. The appropriate area was eluted from the gel and chromatographed on a RPC-5 column (0.9 by 45 cm) with a 400-ml 0.5 to 0.6 M NaCl gradient in 0.01 M $MgCl_2$-0.01 M sodium acetate (pH 4.5)-0.005 M 2-mercaptoethanol for elution. The *supT* tRNA was eluted as a sharp peak which was more than 90% isotopically pure as judged by the absence of contaminating spots on the fingerprints. Most of the procedures for sequencing are from the protocols of Barrell (2), and all have been described before (12). U_2-ribonuclease (U_2-RNase) from *Ustilago*, which is specific for the 3'-phosphodiester linkage of A residues of T_1 RNase fragments, was a gift of H. Okazaki of the Sankyo Co. U_2 RNase digestion of spot tx (see Results) was with 10 μliters of 0.2 U/ml in 0.05 M sodium acetate-0.002 M ethylenediaminetetraacetate (pH 4.5) containing 0.1 mg of bovine serum albumin per ml for 4 h at 37 C. This prolonged incubation was necessary to obtain complete cleavage between the A-residues of the C-U-U-C-U-C-A-A-G- oligonucleotide (2). However, under these conditions the C-U-U-C-U-C-A- product was often cleaved further to produce C-U-U-C-U-C-, and occasionally this secondary product was obtained predominantly.

RESULTS

Genetic relationship of the glyUsu$_{AGA}$ and supT mutations. The similar linkage relationships of *glyUsu*$_{AGA}$ (14) and *supT* (9) to the *lysA* locus suggested that both mutations might affect the same locus and could possibly repre-

sent independent isolations of the same suppressor. The *glyUsu*$_{AGA}$ and *supT* mutations are not independent isolations of the same mutation, however, since the specificities of the suppression are different, as shown by the following observations. The *glyUsu*$_{AGA}$ mutation was isolated according to its ability to suppress the *trpA36* mutation (14) which has the AGA codon at the mutant site (24). The *supT* mutation was isolated according to its specificity for the *ilvD130* mutation. Therefore if *supT* and *glyUsu*$_{AGA}$ are identical mutations, both *trpA36 ilvD130 supT* and *trpA36 ilvD130 glyUsu*$_{AGA}$ strains should be phenotypically Trp$^+$, Ilv$^+$. However, when such strains were constructed, the *trpA36 ilvD130 glyUsu*$_{AGA}$ strain was Trp$^+$, Ilv$^-$, whereas the *trpA36 ilvD130 supT* strain was Trp$^-$, Ilv$^+$, indicating that *glyUsu*$_{AGA}$ does not suppress *ilvD130* and *supT* does not suppress *trpA36*.

Recombinational experiments (Table 2) showed that, although these two suppressors are not identical, they are very closely linked and quite possibly allelic. In these experiments, the generalized transducing phage P1 was used to transfer the *lysA* region from a *lysA*$^+$ *su*$_x$ donor to a *trpA36 ilvD130 lysA su*$_y$ recipient, selecting for Lys$^+$ transductants and scoring for the suppressor mutation present in the recombinant. As indicated in Table 2, almost all recombinants possessed either the donor or recipient suppressor mutation. None of the recombinants carried both suppressors, and only 1 of a total of 339 tested had neither. It is concluded that there is less than 1% recombination between *supT* and *glyUsu*$_{AGA}$. Since 1% recombination occurs between mutations separated by roughly 160 base pairs (23), it is concluded that *supT* and *glyUsu*$_{AGA}$ are closely linked and possibly allelic.

TABLE 2. *Recombination between glyUsu and supT*[a]

Donor	Recipient	Recombination			
		glyUsu[b]	*supT*	Both	Neither
lysA$^+$ *glyUsu*	*lysA*$^-$ *supT*	156	72	0	1
lysA$^+$ *supT*	*lysA*$^-$ *glyUsu*	28	82	0	0

[a] In the first mating, strain CH465 was donor and CH591 was recipient; in the second experiment, AB2551 was donor and CH550 was recipient. Complete genotypes are listed in Table 1. P1 transduction was performed as indicated in Materials and Methods. Lys$^+$ transductants were selected on agar plates supplemented with glucose, arginine, tryptophan, isoleucine, valine, and leucine. The presence of the *glyUsu* and *supT* alleles was determined by testing the Trp and Ilv phenotypes, respectively.

[b] Suppressor allele of Lys$^+$ transductant.

Effect of the supT mutation on tRNA$_{GGG}^{Gly}$. We have shown previously that the *glyUsu*$_{AGA}$ mutation leads to an alteration of tRNA$_{GGG}^{Gly}$ in both codon recognition (6) and interaction with the glycyl-tRNA synthetase (5). If *supT* is allelic to *glyUsu*$_{AGA}$, the tRNA$_{GGG}^{Gly}$ species should also be altered in *supT* cultures. Such an alteration can be observed in tRNA preparations fractionated on a RPC-5 reverse-phase chromatographic column. For these experiments and the primary sequence determination experiments to be described below, we employed strains which are lysogenic for a defective lambda transducing phage carrying the *lysA glyU* region of the chromosome. In the experiment described in Fig. 1, [^{14}C]glycyl-tRNA from strain CH757 (*glyU*$^+$/λ*dglyU*$^+$) was mixed with [^{3}H]glycyl-tRNA from strain CH760 (*supT*/λ*dsupT*), and the mixture was fractionated on a RPC-5 column. Previous work (12) has shown that the [^{14}C]glycyl-tRNA in fractions 85 to 91 is tRNA$_{GGG}^{Gly}$. From the results of Fig. 1, it is seen that this peak of tRNA$_{GGG}^{Gly}$ is missing in the ^{3}H-labeled preparation from the *supT* culture. The [^{14}C]glycyl-tRNA material in the peak centering in fraction 78 and that in the peak centering in fraction 97 is tRNA$_{GGA/G}^{Gly}$ (ref. 14 and personal communication from John Carbon). There is only one tRNA$_{GGA/G}^{Gly}$ struc-

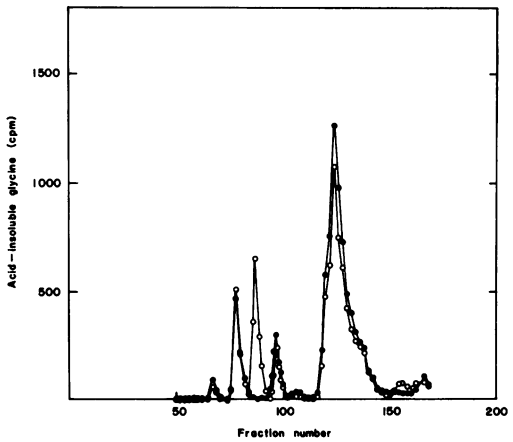

FIG. 1. *Comparison of glycyl-tRNA profiles of supT and wild-type cultures. The tRNA preparations were aminoacylated with either [^{3}H]- or [^{14}C]glycine, mixed, and applied to the RPC-5 reversed-phase column. Elution was with a 400-ml linear gradient of 0.5 to 0.6 M NaCl in 0.01 M sodium acetate (pH 4.5)-0.01 M $MgCl_2$-0.005 M 2-mercaptoethanol. CH757 (glyU$^+$) tRNA aminoacylated with [^{14}C]glycine (○); CH760 (supT) tRNA aminoacylated with [^{3}H]glycine (●).*

tural gene (14) which produces the material in both of these peaks. Fraction 78 $tRNA_{GGA/G}^{Gly}$ possibly differs from fraction 97 $tRNA_{GGA/G}^{Gly}$ by a modification. The relevant point for this study is that the [^{3}H]glycyl-tRNA corresponding to the [^{14}C]glycyl-tRNA in fraction 97 is slightly skewed to the left in Fig. 1. The experiment to be described in Fig. 2 suggests that this lack of coincidence of the [^{3}H]- and [^{14}C]glycyl-tRNA in fractions 94 to 100 is due to the presence of *supT* specific tRNA in the ^{3}H-labeled preparation.

Induction of a prophage which carries a tRNA structural gene should result in a large amplification of that tRNA species (12, 19). Accordingly, strain CH760 was heat induced as described in Materials and Methods, and the tRNA was isolated, aminoacylated with [^{3}H]glycine, and chromatographed on the RPC-5 column. [^{14}C]glycyl-tRNA from CH760 which had not been heat induced served as a control. The results of this experiment (Fig. 2) show that this procedure results in a large increase in glycine acceptor in the region (fractions 110 to 114) just behind the position normally occupied by wild-type $tRNA_{GGG}^{Gly}$ and just ahead of the position occupied by the slower component of $tRNA_{GGA/G}^{Gly}$. From these results, we conclude that like the *glyUsu*$_{AGA}$ mutation, the *supT* mutation causes an alteration in the $tRNA_{GGG}^{Gly}$ species. However, the results of Fig. 2 show that the effect of the *supT* mutation on $tRNA_{GGG}^{Gly}$ is quite different from the effect of the *glyUsu*$_{AGA}$ mutation. The *glyUsu*$_{AGA}$ mutant tRNA has a greatly reduced affinity for the glycyl-tRNA synthetase and cannot be aminoacylated sufficiently for it to appear as an observable peak in an experiment such as that shown in Fig. 2 (5). The *supT* mutation does not have such an extreme effect on the acceptor activity of the mutant tRNA since the *supT* tRNA can be observed as a substantial peak in Fig. 2.

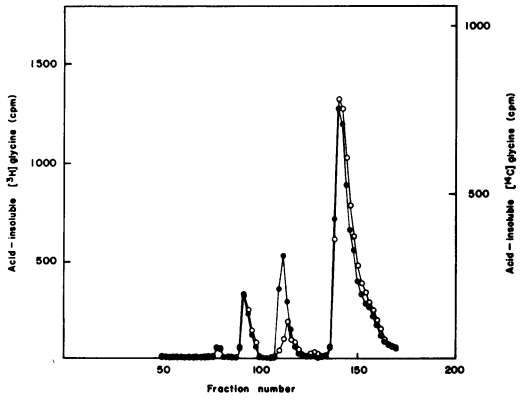

FIG. 2. *Effect on the glycyl-tRNA profile of thermal induction of a supT transducing phage lysogen. Procedures were as indicated in Fig. 1. Transfer RNA isolated from an uninduced culture of CH760 and aminoacylated with* [^{14}C]*glycine* (○); *tRNA isolated from a thermally induced culture of CH760 and aminoacylated with* [^{3}H]*glycine* (●).

Alteration of the $tRNA_{GGG}^{Gly}$ primary sequence by supT. The complete primary sequence of the *E. coli* $tRNA_{GGG}^{Gly}$ species has been determined recently (12). Given that the *supT* mutation alters this tRNA species, it was of obvious interest to determine the alteration in the primary sequence caused by the *supT* mutation. [^{32}P]phosphate-labeled *supT* tRNA was isolated from a thermally induced culture of CH760 as indicated in Materials and Methods. Portions of the purified *supT* tRNA were then digested with pancreatic RNase A and with T_1 RNase, and the digests fractionated by two-dimensional electrophoresis. The pancreatic RNase fingerprint of the *supT* tRNA proved to be identical to the fingerprint of wild-type $tRNA_{GGG}^{Gly}$. Identity of all of the pancreatic RNase fragments was confirmed by base compositional analysis after NaOH digestion and by digestion with T_1 RNase.

The T_1 RNase fingerprint of the *supT* tRNA, however, showed a significant change from the wild-type species (Fig. 3). No material was found at the position normally occupied by fragment t11 from the wild-type molecule, and the position occupied by wild-type fragment t12 had approximately double the normal intensity. This material is designated tx in Fig. 3. All other T_1 RNase fragments appeared in the normal positions and were identical to the wild-type as judged by base composition and characterization by pancreatic RNase digestion. In addition, the modified bases in t8 (D-A-G-) and t10 (T-Ψ-C-G-) were present. The change in fragment t11 was particularly interesting in that fragment t11 contains the anticodon of the wild-type tRNA and has the sequence C-U-U-C-C-C-A-A-G-, where the C-C-C sequence is the anticodon. Fragment t12 has the sequence C-U-C-U-A-U-A-C-G-. Digestion with pancreatic RNase of the spot tx produced molar yields of A-A-G-, A-U-, A-C- and G- in addition to U- and C- mononucleotides. The presence of two different G-containing fragments in this digest shows that spot tx is definitely a mixture of two different T_1 RNase fragments. The A-U-, A-C-, and G- are normally found after digestion of the wild-type t12 fragment, whereas A-A-G- is found in digests of wild-type t11. This result suggested that the *supT* mutation alters frag-

ment t11 such that it has a mobility identical to t12 in the fingerprinting procedure. It might be noted here that the base composition of wild-type t11 is C-$_4$, U-$_2$, A-$_2$, G-, whereas that of t12 is C-$_3$, U-$_3$, A$_2$-, G-. Therefore, a C → U transition in fragment t11 would give it a base composition identical to t12, and such a change would cause these fragments to have identical electrophoretic mobilities.

Further characterization depended on separation of the apparently mutated t11 fragment from t12, a difficult task if these nonanucleotides have identical base compositions. The approach used was to digest the spot tx with U_2 RNase which is specific for the 3'-phosphodiester linkage of A residues of T_1 RNase fragments. Fractionation of the U_2-RNase digest by electrophoresis in 7% formic acid on diethylaminoethyl-cellulose produced two large fragments, y and z. Occasionally, fragment z was cleaved secondarily under the conditions used for U_2 RNase digestion (see Materials and Methods). These fragments were characterized by polynucleotide phosphorylase digestion (12), and by derivatization with the carbodiimide reagent followed by pancreatic RNase digestion (2) (Table 3). U_2 RNase fragment y was shown to be C-U-C-U-A-, and is the product expected after U_2 RNase digestion of wild-type fragment t12. Pancreatic RNase digestion of derivatized fragment z produced A-, C-, U-C-, and U-U-C- (Table 3). Polynucleotide phosphorylase digestion of this fragment showed that C-U-U is the trinucleoside diphosphate at the 5'-terminus of this large U_2 RNase digestion product of tx. From this information, we conclude that the sequence of this oligonucleotide is C-U-U-C-U-C-A-, and that spot tx contains both normal t12 and a new fragment C-U-U-C-U-C-A-A-G-. This new fragment differs from wild-type t11 by a single C → U transition at the position representing the middle base of the anticodon. The complete sequence of the wild-type $tRNA_{GGG}^{Gly}$ and the mutant *supT* tRNA is shown in Fig. 4. The *supT* tRNA has CUC as its anticodon, predicting that *supT* tRNA is specific for the glutamic acid codon, GAG.

The finding that *supT* tRNA differs from the wild type by a single C → U transition is consistent with our observation that hydroxylamine, a mutagen known to cause GC → AT transitions (11) is very effective in generating *supT* mutations.

Conversion of supT to a GAA suppressor. Yanofsky and co-workers (23, 24) deduced that the *trpA46* mutation involves a *gly* (GGA) → *glu* (GAA) transition at position 211 in the *trpA* gene. Normally *trpA46* cultures are only observed to revert to Trp$^+$ by a mutation at the *trpA* locus (24). In view of the above result that the *supT* mutant tRNA has a CUC anticodon and by inference reads the GAG codon, a *trpA46 supT* double mutant should have two additional possibilities for reversion to Trp$^+$ besides simple structural gene reversions. Both of these involve genetic suppression and are illustrated in Fig. 5. First of all, the *trpA46* mutant codon GAA could be changed to GAG; in this case, the *supT* suppressor tRNA would occasionally insert glycine at this codon, producing some active tryptophan A protein. Secondly, the anticodon of the *supT* tRNA could be changed to UUC, creating a new suppressor species that would read the *trpA46* codon GAA as glycine, and also producing some active tryptophan A protein.

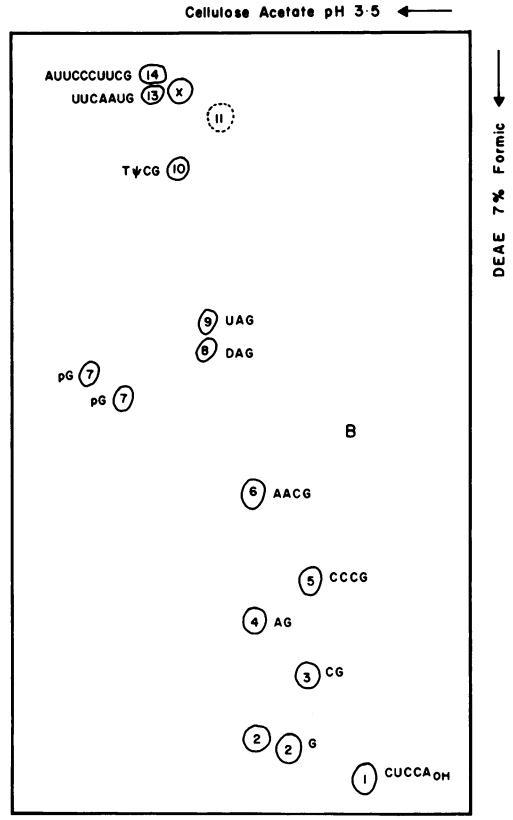

FIG. 3. *Tracing of a fingerprint of a T_1 RNase digest of ^{32}P-labeled supT tRNA. Spots t1–t10, t13, and t14 are in positions identical to those found for wild-type $tRNA_{GGG}^{Gly}$ (12). The area occupied by t11 in digests of wild-type tRNA is indicated by the broken line. The position indicated by x is that normally occupied by t12, but is of approximately twice the normal intensity in the digest of the supT tRNA. B indicates the position of the blue dye marker xylene cyanole FF.*

TABLE 3. *Characterization of U_2 RNase digestion products of spot tx*

U_2 RNase product	Treatment	Product	Electrophoretic mobility[a]	Alkaline hydrolysis[b]			Snake venom phosphodiesterase hydrolysis[b]	
				C-	A-	U-	-C	-U
y	None	C-U-C-U-A-	0.58[c]	2.0	1	1.9		
y	RNase A digestion of carbodiimide derivative	U-C-	-0.55[d]	1		0.9		
		U-A-	-0.35[d]		1	1.0		
y	Polynucleotide phosphorylase	C-U-C	1.4[e]	1		0.8	1	1.1
		C-U	1.8[e]	+				
z	None	C-U-U-C-U-C-A-	0.21[c]					
z	RNase A digestion of carbodiimide derivative	U-U-C-	-0.84[d]	1		1.7		
		U-C-	-0.55[d]	1		1.0		
z	Polynucleotide phosphorylase	C-U-U	1.4[e]	1		0.9		+
		C-U	1.8[e]	+				+

[a] Electrophoretic mobilities are in relation to mobility of the blue dye marker xylene cyanol FF.

[b] After hydrolysis the products were separated by electrophoresis on paper at pH 3.5, and determined quantitatively by liquid scintillation counting. Results are expressed as relative molar yields of nucleotide.

[c] Electrophoresis was on diethylaminoethyl-cellulose in 7% formic acid.

[d] Electrophoresis was on 3MM paper at pH 3.5.

[e] Electrophoresis was on diethylaminoethyl-cellulose at pH 3.5.

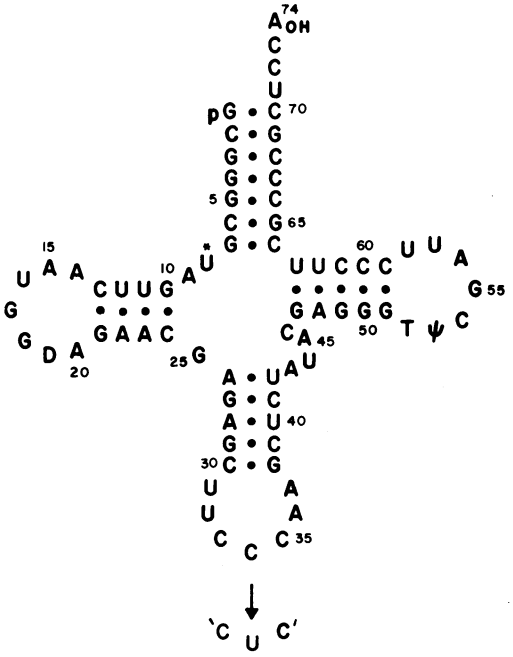

FIG. 4. *Cloverleaf model of $tRNA_{GGG}^{Gly}$ indicating the C → U substitution caused by the supT mutation in the middle of the anticodon.*

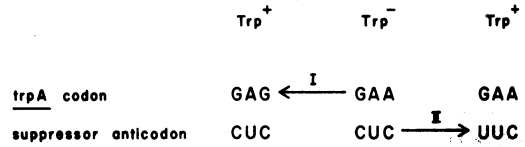

FIG. 5. *Reversion of a trpA46 supT culture to Trp+. Mode I depicts the change of the trpA46 mutant codon, GAA, to GAG such that it can be read as glycine by the supT suppressor tRNA. Mode II depicts the change of the supT tRNA anticodon from CUC to UUC such that it will read the trpA46 mutant codon GAA, as glycine. Neither of these modes of trpA46 reversion is available to a trpA46 culture which does not possess the supT mutation.*

With this rationale in mind, a *trpA46 lysA supT ilvD130* strain, CH726, was constructed (Materials and Methods). CH726 is phenotypically Trp⁻. This strain was mutagenized by various mutagens, and Trp⁺ revertants were selected and purified. Whether or not a *glyU*-derived suppressor was necessary for expression of the Trp⁺ phenotype of these revertants could be tested by using a P1 lysate of a *lysA*⁺ *glyU*⁺ strain to transduce the revertants to Lys⁺ and scoring for loss of the Trp⁺ phenotype among the transductants. If a *lysA* linked suppressor was necessary for the revertant to be Trp⁺, a majority of these Lys⁺ transductants would become simultaneously Trp⁻.

When ultraviolet irradiation was used for mutagenesis, the majority of revertants were extremely slowly growing without tryptophan supplementation, but grew normally with tryptophan supplementation. Four of five examples of these slowly growing mutants were shown to have a *lysA*-linked suppressor necessary for the Trp⁺ phenotype, since 60 to 80% of the Lys⁺ transductants become Trp⁻ when transduced

with a *lysA*$^+$ *glyU*$^+$ donor. Restoration of *supT* to these Trp$^-$ derivatives by additional P1 transduction did not result in restoration of the Trp$^+$ phenotype. Therefore, it is concluded that these UV-induced Trp$^+$ revertants contain a new GAA-specific suppressor. Additional experiments showed that this new suppressor was specific for the *trpA46* allele and would not suppress *trpA36* which has an AGA codon at the mutant site. One of these new suppressed revertants, CH729, was retained as the primary source of this new suppressor, designated *glyUsu*$_{GAA}$.

Spontaneous or 2-aminopurine mutagenesis of the *trpA46 supT* strain, CH726, resulted in many slowly growing Trp$^+$ revertants which could also be shown to depend on suppression for the Trp$^+$ phenotype. In this case, however, the Trp$^+$ phenotype could be restored by the reintroduction of the *supT* suppressor to the Lys$^+$, Trp$^-$ transductants obtained after infection with a P1 lysate of a *lysA*$^+$ *glyU*$^+$ donor. In these cases, it is concluded that a change at the *trpA* locus had produced a *trpA* allele which is suppressible by the *supT* mutation. These most likely occurred by a change at the *trpA46* mutant codon such that it was changed from GAA to GAG. One of these spontaneous revertants, CH727, was retained as the primary source of this new *trpA* allele which we have designated as *trpA461*.

Suppression efficiencies of the glyU-derived suppressors. We now have three different missense suppressors derived from tRNA$_{GGG}^{Gly}$. We also have available three *trpA* mutations each suppressible by a different one of these suppressors; these are *trpA36* (AGA), *trpA46* (GAA), and *trpA461* (GAG). The *trpA36* and *trpA46* mutations are known to affect the same glycine residue in the tryptophan A protein (23) and, from the mode of its origin, *trpA461* almost certainly affects this glycine residue also. With these mutants available, it is possible to examine suppression efficiencies of each of these suppressors. The experiments are based on the observation by Brody and Yanofsky (4) that, whereas only active tryptophan A protein can participate in the conversion of indole glycerol phosphate to indole (reaction 1), all tryptophan A protein (A-CRM) is active in the conversion of indole to tryptophan (reaction 2). Therefore, the quotient of activity in reaction 1 to activity in reaction 2 gives a measure of the suppression efficiency. The measurement of the suppression efficiencies for the three suppressors is given in Table 4. These results show that *glyUsu*$_{AGA}$ is more efficient than *supT* which is in turn more efficient than *glyUsu*$_{GAA}$. This finding is consistent with the observation that when no tryptophan is supplied a *glyUsu*$_{AGA}$-suppressed culture grows faster than one suppressed by *supT* which in turn grows faster than one suppressed by *glyUsu*$_{GAA}$.

TABLE 4. *Suppression efficiencies of glyU suppressors*[a]

Genotype	A (U/ml)	A-CRM (U/ml)	Efficiency of suppression (%)
trpA36 glyS$_H$ *glyUsu*$_{AGA}$ ...	2.8	170	3.6
trpA36 glyS$_L$ *glyUsu*$_{AGA}$ ...	2.4	438	1.2
trpA461 glyS$_H$ *supT*	1.6	187	1.6
trpA461 glyS$_L$ *supT*	1.5	643	0.4
trpA46 glyS$_H$ *glyUsu*$_{GAA}$ ...	2.0	823	0.4
trpA$^+$	12.6	23.2	(100)

[a] Enzyme assays and the calculation of the efficiencies of suppression are as described by Brody and Yanofsky (4).

Two different alleles for the glycyl-tRNA synthetase have been observed to occur in various *E. coli* K-12 cultures. One allele, *glyS*$_H$, produces a product more active than the other allele, *glyS*$_L$ (3, 16). Previously, it was observed that the suppression efficiency of the *glyUsu*$_{AGA}$ mutation depended on which allele was present (14). The results in Table 4 show that the efficiency of *supT* also depends on the *glyS* allele, *supT glyS*$_H$ cultures having a higher suppression efficiency than *supT glyS*$_L$ cultures. Dependence of the suppression efficiency on the *glyS* allele indicates that the glycyl-tRNA synthetase is responsible for the aminoacylation of the suppressor tRNA in vivo. When a P1 lysate of a *glyUsu*$_{GAA}$ culture was used to infect a *trpA46 glyS*$_L$ culture, no Trp$^+$ transductants were obtained, whereas the same P1 lysate could transduce *glyUsu*$_{GAA}$ to a *trpA46 glyS*$_H$ culture. The simplest explanation for this result is that *glyUsu*$_{GAA}$ suppression is also dependent on the *glyS* allele and that when the *glyS*$_L$ allele is present suppression is so weak as to not produce an observable Trp$^+$ phenotype.

None of the tRNA$_{GGG}^{Gly}$ derived suppressors has a detrimental effect on the cell, at least as far as growth rates are concerned. Doubling times were measured for wild-type and mutant strains growing in synthetic medium supplemented with glucose, lysine, isoleucine, valine, leucine, arginine, and tryptophan, and were found to be *glyU*$^+$ (50 min), *glyUsu*$_{AGA}$ (52 min), *supT* (50 min), and *glyUsu*$_{GAA}$ (53 min).

DISCUSSION

The preceding has shown that the $tRNA_{GGG}^{Gly}$ species is affected by three different missense suppressor mutations. The *glyUsu*$_{AGA}$ mutant tRNA reads AGA as glycine; evidence for this is derived from in vitro experiments utilizing a synthetic poly A-G message (14), as well as from the specificity of the *glyUsu*$_{AGA}$ suppressor for a known AGA mutant (*trpA36*) (24). The demonstration in this report that the *supT* mutation changes the anticodon of $tRNA_{GGG}^{Gly}$ from CCC to CUC predicts that the *supT* mutant tRNA should read the GAG glutamic acid codon as glycine. This supposition is supported by the observation that the *trpA46* allele (GAA) can be readily converted to a *supT* suppressible allele after spontaneous or 2-aminopurine mutagenesis. Evidence for the codon specificity of the third suppressor mutation, *glyUsu*$_{GAA}$, is derived from its specificity for a known GAA mutation, *trpA46* (24). At the present time, it seems probable that the conversion of *supT* to *glyUsu*$_{GAA}$ involves a second change in the anticodon of $tRNA_{GGG}^{Gly}$, so that the *glyUsu*$_{GAA}$ anticodon is UUC. We hope to test this hypothesis by isolating and sequencing the *glyUsu*$_{GAA}$ mutant tRNA.

According to the rules of wobble (7), a GAA suppressor should also suppress GAG mutants, given that the amino acid inserted (in this case glycine) is acceptable. Particularly, we would expect *glyUsu*$_{GAA}$ to suppress the mutations *ilvD130* and *trpA461*. We have constructed a *trpA461 ilvD130 glyUsu*$_{GAA}$ strain. This culture will not produce observable colonies in 6 days if isoleucine and valine supplementation is omitted from the plates and produces only tiny colonies in 6 days if tryptophan supplementation is omitted. When tryptophan, isoleucine, and valine are all supplied, large colonies are produced in 2 days. From these rather qualitative observations, it appears that *glyUsu*$_{GAA}$ has a very slight response to the GAG codon, but substantially less than for the GAA codon, since *trpA46 glyUsu*$_{GAA}$ cultures grow substantially faster than the *trpA461 glyUsu*$_{GAA}$ cultures on tryptophan-deficient plates. We conclude that *glyUsu*$_{GAA}$ can read GAG by wobble, but that the resulting suppression is extremely weak (see below.)

All three of the *glyU*-derived suppressor mutations alter the chromatographic profile of glycyl-tRNA. As reported previously (14), the *glyUsu*$_{AGA}$ mutation leads to a disappearance of the $tRNA_{GGG}^{Gly}$ species without the reappearance of a mutant species elsewhere in the profile. The reason that the *glyUsu*$_{AGA}$ mutant tRNA is not seen anywhere in the profile is due to the fact that it is aminoacylated at a much reduced rate by the glycyl-tRNA synthetase (5). On the other hand, the *supT* mutant tRNA does appear in the chromatographic profiles (fractions 110 to 114 of Fig. 2), indicating that the mutant tRNA can be aminoacylated under the conditions used (a 10-fold excess of the glycyl-tRNA synthetase). The amount of *supT* tRNA apparent in the profiles is, however, less than the amount of wild-type $tRNA_{GGG}^{Gly}$ produced in a wild-type strain. For example, the strains CH757 and CH760 contain two copies of *glyU*$^+$ and *supT* genes, respectively. Yet as seen in Fig. 1, a large peak of $tRNA_{GGG}^{Gly}$ is present in the CH757 profile, but there is only enough *supT* tRNA in the CH760 profile to produce a slight skewing of one of the $tRNA_{GGA/G}^{Gly}$ peaks. The basis for this quantitative difference is unknown. When glycyl-tRNA from a *glyUsu*$_{GAA}$ strain is subjected to RPC-5 column chromatography (unpublished data), no material is obseved at the position normally occupied by $tRNA_{GGG}^{Gly}$, nor are any new peaks of glycyl-tRNA observed. There are several possible explanations for this observation. First the *glyUsu*$_{GAA}$ tRNA species may coincide with one of the other peaks of glycyl-tRNA and be obscured by it. A second possibility is that the presence of the mutation may interfere with the biosynthesis of the mutant tRNA. A third possibility is that, like the *glyUsu*$_{AGA}$ mutant tRNA, the *glyUsu*$_{GAA}$ mutant tRNA may be inefficiently aminoacylated under the conditions used and, therefore, not appear in the profiles of glycyl-tRNA. Clearly, further experimentation is necessary to clarify the effect of the *glyUsu*$_{GAA}$ mutation.

The observation that *glyUsu*$_{AGA}$ suppression is more efficient that *supT* suppression (Table 4) is somewhat surprising since the degree of aminoacylation of *supT* tRNA by glycyl-tRNA synthetase in vitro is substantially higher than that observed for *glyUsu*$_{AGA}$ tRNA. However, suppression efficiencies of missense suppressors should depend on several factors other than the efficiency of aminoacylation of the suppressor tRNA. Among these factors are the amount of suppressor tRNA made, the efficiency with which the suppressor tRNA associates with the messenger RNA-ribosome complex, and the amount of normal competing aminoacyl-tRNA present which reads the suppressed codon. Anderson (1) has suggested that there is a very limited amount of $tRNA_{AGA}^{Arg}$ in *E. coli* and that the supply of arginyl-$tRNA_{AGA}^{Arg}$ might control the rate of protein synthesis in cistrons containing the AGA codon. According to Anderson's argument, when arginine must be inserted

at maximal rate, one of the other five arginine codons would be used. If this is true, the limitation on the supply of $tRNA_{AGA}^{Arg}$ might help explain the relatively high efficiency of $glyUsu_{AGA}$ suppression. It might be pointed out that another AGA suppressor, $glyTsu_{AGA}$, is able to achieve 30% suppression of the *trpA36* mutation (14). On the other hand, the relatively inefficient suppression of *trpA461* by *supT*, despite the observable acceptor activity of *supT* tRNA, might be related to the fact that GAG is one of only two glutamic acid codons. Since there are only two glutamic acid codons, one might expect the cell to translate GAG with high efficiency, and this normal capacity would tend to lower the *supT* suppression efficiency.

Mutation of the *glyU* tRNA species has no apparent detrimental effect on the cell, since all three of the suppressor mutants studied here grow as fast as the $glyU^+$ parent when the medium is supplemented to the point that suppression is not required for growth. This finding indicates that neither the loss of the normal GGG translating capacity of $tRNA_{GGG}^{Gly}$ nor the mistranslation of other mRNA molecules caused by the suppressors is detrimental. Presumably the $tRNA_{GGA/G}^{Gly}$ species remaining is able to translate both GGA and GGG with sufficient efficiency. The nonessentiality of $tRNA_{GGG}^{Gly}$ contrasts with the effects of $tRNA_{GGA/G}^{Gly}$—loss of $tRNA_{GGA/G}^{Gly}$ by mutation deprives the cell of GGA translating capacity, resulting in severe, detrimental effects (14).

Future experiments have as their aim the determination of the actual change caused in the $tRNA_{GGG}^{Gly}$ species by the $glyUsu_{AGA}$ and $glyUsu_{GAA}$ mutations and the effect of each of the three suppressor mutations on the tRNA biosynthesis, aminoacylation, and binding to messenger RNA-ribosome complexes.

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LITERATURE CITED

1. Anderson, W. F. 1969. The effect of tRNA concentration on the rate of protein synthesis. Proc. Nat. Acad. Sci. U.S.A. **62**:566-573.
2. Barrell, B. G. 1971. Fractionation and sequence analysis of radioactive nucleotides, p. 751-779. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research, vol. 2. Harper and Row, New York.
3. Bock, A., and F. C. Neidhardt. 1966. Location of the structural gene for glycyl ribonucleic acid synthetase by means of a strain of *Escherichia coli* possessing an unusual enzyme. Z. Vererbungsl. **98**:187-192.
4. Brody, S., and C. Yanofsky. 1963. Suppressor gene alteration of protein primary structure. Proc. Nat. Acad. Sci. U.S.A. **50**:9-16.
5. Carbon, J., C. Squires, and C. W. Hill. 1969. Genetically altered $tRNA^{Gly}$ subspecies in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **34**:505-512.
6. Carbon, J., C. Squires, and C. W. Hill. 1970. Glycine transfer RNA of *Escherichia coli*. II. Impaired GGA-recognition in strains containing a genetically altered transfer RNA; reversal by a secondary suppressor mutation. J. Mol. Biol. **52**:571-584.
7. Crick, F. H. C. 1966. Codon-anticodon pairing: the wobble hypothesis. J. Mol. Biol. **19**:548-555.
8. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61-76.
9. Eggertsson, G., and E. A. Adelberg. 1965. Map positions and specificities of suppressor mutations in *Escherichia coli* K-12. Genetics **52**:319-340.
10. Franklin, N. C., W. F. Dove, and C. Yanofsky. 1965. The linear insertion of a prophage into the chromosome of *E. coli* shown by deletion mapping. Biochem. Biophys. Res. Commun. **18**:910-923.
11. Freese, E., E. Bautz, and E. B. Freese. 1961. The chemical and mutagenic specificity of hydroxylamine. Proc. Nat. Acad. Sci. U.S.A. **47**:845-855.
12. Hill, C. W., G. Combriato, W. Steinhart, D. L. Riddle, and J. Carbon. 1973. The nucleotide sequence of the GGG-specific glycine transfer RNA of *Escherichia coli* and of *Salmonella typhimurium*. J. Biol. Chem. **248**:4252-4262.
13. Hill, C. W., J. Foulds, L. Soll, and P. Berg. 1969. Instability of a missense suppressor resulting from a duplication of genetic material. J. Mol. Biol. **39**:563-581.
14. Hill, C. W., C. Squires, and J. Carbon. 1970. Glycine transfer RNA of *Escherichia coli*. I. Structural genes for two glycine tRNA species. J. Mol. Biol. **52**:557-569.
15. Ikemura, T., and J. E. Dahlberg. 1973. Small ribonucleic acids of *Escherichia coli*. I. Characterization by polyacrylamide gel electrophoresis and fingerprint analysis. J. Biol. Chem. **248**:5024-5032.
16. Ostrem, D. L., and P. Berg. 1970. Glycyl-tRNA synthetase: an oligomeric protein containing dissimilar subunits. Proc. Nat. Acad. Sci. U.S.A. **67**:1967-1974.
17. Riddle, D. L., and J. R. Roth. 1972. Frameshift suppressors. III. Effects of suppressor mutations on transfer RNA. J. Mol. Biol. **66**:495-506.
18. Shimada, K., R. A. Weisberg, and M. E. Gottesman. 1972. Prophage lambda of unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. **63**:483-503.
19. Squires, C., B. Konrad, J. Kirschbaum, and J. Carbon. 1973. Three adjacent transfer RNA genes in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **70**:438-441.
20. Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554-555.
21. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.
22. Vogel, H. J., and D. M. Bonner. 1956. Acetyl-ornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
23. Yanofsky, C., and V. Horn. 1972. Tryptophan synthetase α chain positions affected by mutations near the ends of the genetic map of *trpA* of *Escherichia coli*. J. Biol. Chem. **247**:4494-4498.
24. Yanofsky, C., J. Ito, and V. Horn. 1966. Amino acid replacements and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31**:151-162.