Anticodon shift in tRNA: A novel mechanism in missense and nonsense suppression†

(UGG-specific glycine tRNA suppressor/eight-nucleotide anticodon loops/hypermodified nucleosides/frameshift mutations)

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ABSTRACT In a previous publication, an unusual UGG-reading missense suppressor caused by insertion of an extra adenylate residue in the anticodon loop of an *Escherichia coli* glycine tRNA was described. In this study, we provide *in vivo* evidence that the additional nucleotide causes an “anticodon shift” by one nucleotide in the 3′ direction and that the “new” anticodon can explain the unanticipated coding properties of the suppressor. We converted the UGG suppressor with ethyl methanesulfonate, a base-substitution mutagen, to suppressors that read codons related to UGG by a single base change. Sequence analysis of each mutant tRNA revealed that its mutational alteration was an anticipated base change in one of the three nucleotides of the “new” anticodon. Although the new suppressors read codons beginning with A or U, the mutant tRNAs lack the customary hypermodified nucleosides on the 3′ side of the anticodon. As determined on the basis of their *in vivo* coding specificities, the new mutant tRNAs do not continue to utilize the original anticodon triplet for decoding. Furthermore, the failure of the UGG suppressor to correct frameshift mutations throughout each of three genes of the *trp* operon suggests that the addition of a nucleotide to the anticodon loop of a tRNA does not necessarily result in out-of-frame decoding by the tRNA. Therefore, a “frameshift” mutation in a tRNA has principally changed the triplet codon recognition properties of the molecule.

In a previous publication, we reported two independent but identical cases of missense suppression caused by insertion of an extra nucleotide in the anticodon loop of a glycine tRNA (1). The glycine tRNA specified by *glyT* in *Escherichia coli* reads the glycine codons GGA and GGG. The nucleotide sequence of its anticodon loop (nucleotides 32–38) is 5′-C-U-U*-C-C-A-A-3′ (anticodon underlined), in which U* represents a uridine with an unidentified modification. In the suppressor tRNA, which was isolated as a UGG suppressor and was shown not to suppress UGA, the anticodon loop sequence is 5′-C-U-U-C-C-A-A-A-3′ (U-34 is unmodified). To explain the codon recognition properties of the UGG suppressor, we suggested that the additional nucleotide in the loop causes an “anticodon shift” by one nucleotide in the 3′ direction and that the “new” anticodon allows the tRNA to read UGG but not UGA. To test this proposal, we converted the *glyT*-derived UGG suppressor with a base-substitution mutagen to suppressors that read codons related to UGG by a single base change. The new suppressor strains were further characterized for codon recognition properties *in vivo*, and their *glyT* tRNAs were isolated for nucleotide sequence analysis. The results provide evidence of an anticodon shift in the mutant tRNAs.

MATERIALS AND METHODS

All bacterial strains used in this study were derived from *E. coli* K-12. The *trp* genes, with specific suppressible missense and nonsense mutations in *trpA*, were contained in the *cysB trp tonB* region of the chromosome carried by the Fredericq episome, a conjugative plasmid (2). Each episome was maintained in a strain with a deletion in the *tonB-trp* region. All the new suppressors described in this report were obtained from the *glyT*-derived, UGG-specific suppressor originally designated SP-13 (3, 4). Both SP-13 and an independently isolated suppressor, SP-9, have an extra adenine nucleotide on the 3′ side of the original anticodon, resulting in an anticodon loop composed of eight nucleotides instead of the usual seven (1). For convenience, then, we will refer to SP-13 phenotypically as SuUGG-8 and genotypically as *glyT*(SuUGG-8). Suppressors derived from SP-13 will be designated “Su” followed by the codon that they read. Previous publications describe growth media and routine genetic procedures (4–6), the procedure for mutagenesis with ethyl methanesulfonate (7), tRNA purification, and nucleotide sequence analysis by the direct-readout method of Gupta and Randerath (1, 8, 9).

RESULTS

Selections for Suppressor Derivatives of *glyT*(SuUGG-8). In general, mutant derivatives of *glyT*(SuUGG-8) were selected for their ability to suppress specific, well-characterized auxotrophic mutations in *trpA* (5, 6, 10, 11). In addition to selecting for single-step mutants that are suppressors of AGC, UAG, UGU, and UGA, we planned to convert SuUAG to a suppressor of GAG. We reasoned that if SuUGG-8 uses the “new” anticodon 5′-C-C-A-3′, then the suppressor’s mutant derivatives should result from single-base changes of nucleotides C-35, C-36, or A-37 (Fig. 1). To construct the strains for each selection, a particular *trpA* mutation carried on the Fredericq episome was introduced by conjugation into a strain containing both *glyT*(SuUGG-8) and a *tonB-trp* deletion. A culture of each strain was treated with the base-substitution mutagen ethyl methanesulfonate. To obtain mutagen-induced, tryptophan-independent colonies, dilutions that were calculated not to yield spontaneous revertants were plated on glucose minimal medium without tryptophan supplementation. The tryptophan-independent colonies were screened for suppressed mutants (as opposed to revertants), for suppressors linked in transduction to the *glyT* region, and for loss of the UGG-suppressing activity (Table 1). Mutants meeting these criteria were tested further

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FIG. 1. Comparison of the anticodon loop region of wild-type *E. coli glyT* tRNA with that of *glyT*(SuUGG-8) tRNA. U*, a uridine with an unidentified modification; Sp, the suppressor mutant arose spontaneously; solid arc, the original anticodon; broken arc, the proposed new anticodon triplet.

for codon recognition properties. (No ethyl methanesulfonate-induced, *glyT*-linked UGA suppressors were obtained.) As we anticipated, we found that SuAGG does not read AGA and that SuUAG does not read UAA (Table 1). In addition, SuUGU recognizes neither UGA nor UGG. Finally, the GAG suppressor derived secondarily from the new SuUAG does not read GAA. Except in the case of UGU, these coding properties were tested with pairs of *trpA* mutants; each member of a pair contained the mutant codon at either position 211 or position 234 of the *trpA* polypeptide. The other coding properties that were tested are indicated in Table 1 and are discussed below.

Nucleotide Sequence Analysis of Mutant tRNAs. Mutational changes in tRNA primary structure were determined by directly comparing the entire sequence of SuUGG-8 tRNA with the mutant tRNAs derived from it. Alterations were observed only in the anticodon loop region. The rest of the molecule in each mutant tRNA was unchanged and is not shown. Fig. 2 presents the nucleotide sequence of the anticodon loop region of four *glyT*-derived, ethyl methanesulfonate-induced suppressor tRNAs. Each mutant tRNA was obtained from *glyT*(SuUGG-8) in either one (Fig. 2 *a-c*) or two (Fig. 2*d*) selection steps. The sequences can be read directly from 5' to 3' (left to right) according to the characteristic migratory behavior of specific nucleoside diphosphates (9). The anticodon of wild-type *glyT* tRNA is composed of nucleotides 34, 35, and 36 (Fig. 1). The anticodon shift proposed to explain the coding properties of *glyT*(SuUGG-8) would result in an anticodon composed of nucleotides 35, 36, and 37. SuAGG (Fig. 2*a*) resulted from the substitution of U for A at position 37, SuUAG (Fig. 2*c*) from a C to U change at position 36, and SuUGU (Fig. 2*b*) from the replacement of C by G at position 35. An unidentified modified uridine appears in SuUGU tRNA at position 34. From its mobility, we know that it is not the same unidentified modified U-34 found

FIG. 2. Analysis of the 5' terminus (9) of fragments from polyethyleneimine-cellulose prints of gel ladders (not shown) obtained by hydrolysis, 5',³²P labeling with polynucleotide kinase, and gel electrophoresis of four mutant *glyT* tRNAs. After RNase T₂ treatment *in situ*, the released 5'-terminal nucleoside diphosphates were contact-transferred to a polyethyleneimine-cellulose sheet for chromatographic analysis in either 0.55 M ammonium sulfate or 1.75 M ammonium formate (pH 3.5). Each tRNA was examined in both chromatographic systems. The sequences are shown from 5' to 3' (left to right). (a) SuAGG tRNA, chromatographed in sulfate; (b) SuUGU tRNA, in formate; (c) SuUAG tRNA, in formate; (d) SuGAG tRNA, in formate. The broken circle in *b* indicates a spot that corresponds to position 34 and that shows up more clearly on longer exposure.

in wild-type *glyT* tRNA (Fig. 1). SuGAG, obtained by ethyl methanesulfonate treatment of SuUAG (Fig. 2c), is shown in Fig. 2d. It is derived from SuUAG by an A-37 to C-37 change, and it differs from SuUGG-8 at two positions, U-36 and C-37. All of these sequence alterations are diagrammed in Fig. 3.

Do the New Suppressors Also Use the "Old" Anticodon? To study this question *in vivo*, we constructed appropriate strains to determine whether any of four of the suppressors (SuUGG-8, SuAGG-8, SuUAG-8, or SuGAG-8) have coding properties that are predictable from the triplet composed of nucleotides 34, 35, and 36 (Fig. 3). With SuUGG-8 and SuAGG-8, GGA-reading ability was evaluated by testing the ability of the suppressor *glyT* alleles to replace the wild-type *glyT* in a haploid strain (12). Other coding specificities were examined according to the ability of each suppressor to correct specific auxotrophic

Table 1. Suppression of mutant codons by *glyT*(SuUGG-8) and by four of its ethyl methanesulfonate-induced derivatives*

Suppressor	Mutant codons													
	<i>E. coli</i> (<i>trpA</i>) [†]										Phage T4			
	UGG	AGG	AGA	UAG	UAA	GAG	GAA	UGU	UGA	GGA	AGA	UGA	UAA	UAG
SuUGG-8	+	—	—	—	0	—	—	—	—	— [‡]	—	—	—	—
SuAGG-8	—	+	—	—	0	—	0	0	0	—	—	0	0	0
SuUAG-8	—	—	—	+	—	—	—	0	0	0	—	0	—	—
SuGAG-8	—	—	—	—	0	+	—	0	—	0	—	0	0	0
SuUGU-8	—	0	0	0	0	0	0	+	—	0	0	—	0	0

* +, Suppresses; -, does not suppress; 0, not determined.

[†] Most codons were tested at both position 211 and position 234 of *trpA*.

‡ Result previously reported (12).

FIG. 3. Comparison of the anticodon loop region of *E. coli glyT* (SuUGG-8) tRNA with that of several suppressor tRNAs derived from it. U* represents an unidentified modified uridine at position 34 in SuUGU-8 tRNA. This modified uridine differs, however, from the unidentified modified uridine found at position 34 in wild-type *glyT* tRNA (Fig. 1).

mutations at positions 211 and 234 of *trpA*. SuUGU-8, however, could not be tested in this way because no appropriate *trpA* mutant was available. The results of these tests are shown in Table 1. SuUGG-8 and SuAGG-8 do not read GGA; SuUAG does not read AGA or AGG; and SuGAG does not read AGA, AGG, UGA, and UAG.

Attempts to Suppress Frameshift Mutations. Because the anticodon loop of SuUGG-8 contains an extra nucleotide, this suppressor may conceivably be able to suppress some frameshift mutations, particularly +1 types. Such a situation has been described for the frameshift suppressor *sufD* in *Salmonella* (13). Eighteen frameshift mutations, distributed throughout the *trpE*, *trpD*, and *trpC* genes, were kindly provided by C. Yanofsky. One mutant, *trpE9777*, has an additional adenylate residue located in the region coding for amino acids 4 and 5 of the *trpE* polypeptide (14). Each *trp* frameshift mutation was transduced into a strain containing both *glyT*(SuUGG-8) and a *tonB-trpAE* deletion; the deletion was replaced by the corresponding mutant *trp* region. Parallel transductions were done with an isogenic *glyT*⁺ strain. In all cases, transductants were selected for their ability to grow on indole (*trpB*⁺). After they were purified, the transductant colonies were tested for their ability to grow on minimal medium without tryptophan supplementation (that is, for their ability to suppress the frameshift mutation). The result was that none of the frameshift mutations was suppressed by SuUGG-8.

DISCUSSION

We have provided evidence that the insertion of an extra adenylate residue in the anticodon loop of *glyT* tRNA changes the coding specificity of the tRNA (from GGA to UGG) through an anticodon shift. Suppressor tRNAs derived from the UGG suppressor with a base-substitution mutagen were found to come about by predictable base changes in the appropriate nucleotides of the "new" anticodon. The extra nucleotide in the anticodon loop apparently causes a structural alteration in the loop

region that shifts the effective anticodon one nucleotide in the 3' direction, from 5'-U-C-C-3' to 5'-C-C-A-3'. Our results do not answer the question: Does the mutant tRNA actively "present" the new anticodon for mRNA translation (perhaps through new constraints on base stacking); or does the ribosome, by interacting differently with the mutant tRNA, "see" a different anticodon (perhaps by "counting" nucleotides from the 3' end of the anticodon loop)? Nevertheless, conformational aspects of the tRNA, of the ribosome, or of their interaction are clearly involved in the specificity of codon recognition.

An alternative explanation of the coding specificity of the UGG suppressor is based on the possibility that the stacking of two or three adenines in the loop leads to "bulging" (15, 16) of C-36 or perhaps of C-35. Such bulge stacking might cause the nucleotides U-34, C-35, and A-37 to be used as the anticodon. Our data, however, make this hypothesis implausible. The decisive factor was the finding that SuUGU arose from a change not of U-34 to G, but of C-35 to G (Figs. 2 and 3).

In *E. coli*, all wild-type tRNAs that read codons beginning with U have methyl-2-thio-6-isopentenyladenosine (ms²t⁶A) next to the anticodon on the 3' side. We noted in our earlier study that the *glyT* UGG suppressor has the same anticodon-containing sequence, 5'-U-C-C-A-A-3' (anticodon underlined), as wild-type *E. coli* tryptophan tRNA (1). But the latter has ms²t⁶A next to the anticodon, whereas the mutant glycine tRNA does not. In this study, we observed that the UAG- and UGU-suppressing tRNAs derived from SuUGG-8 do not contain this or any other modified base on the 3' side of the anticodon (Figs. 2 and 3). Furthermore, although *E. coli* tRNAs that read codons beginning with A have N⁶-threonylcarbamoyl-adenosine (t⁶A), or a similarly modified A, on the 3' side of the anticodon, the new AGG suppressor we isolated has no such hypermodification (Figs. 2 and 3). Therefore these two hypermodifications are not necessary for reading such codons. These results also indicate that the enzymes responsible for these modifications must recognize more than a short oligonucleotide sequence. This must also be true of the unidentified modification of U-34 in SuUGU-8 tRNA.

We have provided *in vivo* evidence that the UGG suppressor and its derivatives do not utilize effectively (if at all) the "old" anticodon for decoding. It is not known what conditions are necessary for observing such an "anticodon shuttle," if it can occur. But it is interesting to inquire whether mutational alteration of the ribosome or of any other component of the translational apparatus or environment might allow a tRNA to use each of two triplets as an anticodon. Under such conditions, anticodon shuttle might be exhibited not only by mutant tRNAs but also by normal tRNAs, perhaps in some undermodified state.

The UGG suppressor was examined for its ability to suppress a variety of frameshift mutations throughout each of three genes of the *E. coli trp* operon: *trpE*, *trpD*, and *trpC*. The list of mutations includes *trpE9777*, which is known to result from a single nucleotide insertion (14). The failure of SuUGG-8 to correct any of the 18 tested frameshift mutations may indicate that addition of a nucleotide next to the anticodon of a tRNA does not necessarily result in a four-nucleotide anticodon. Even in the case of the *Salmonella* frameshift suppressor *sufD*, it can be argued that the suppressor tRNA, which has an extra C in the anticodon (the wild-type anticodon is 5'-C-C-C-3'), does not function by the pairing of a four-nucleotide anticodon with four nucleotides in the mRNA (see, for example, ref. 17). Regardless, despite the extra nucleotide in the anticodon loop, the suppressor tRNAs described in this report read primarily, and perhaps only, three-nucleotide codons. We conclude that the reading frame for mRNA translation is not determined strictly by the number of nucleotides in the anticodon region; it may also in-

volve conformation-dependent interactions between the tRNA and the ribosome (17).

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