JOURNAL OF BACTERIOLOGY, July 1980, p. 285-292
0021-9193/80/07-0285/08$02.00/0

Vol. 143, No. 1

Suppressors of a UGG Missense Mutation in *Escherichia coli*

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As part of our investigation of tRNA structure-function relationships, we isolated and preliminarily characterized translational suppressors of the tryptophan codon UGG in a *trpA* missense mutant of *Escherichia coli*. The parent strain also contained two other mutant alleles relevant to the suppressor search; these were *supD*, which codes for a serine-inserting amber suppressor tRNA, and *glyV55*, the gene for a GGA/G-reading mutationally altered glycine tRNA. On the basis of map location, reversed-phase (RPC-5) column chromatography of glycyl-tRNA, and codon response, several classes have been distinguished so far. The number of suppressors in each class, their codon responses, and their apparent genic identities, respectively, are as follows: class 1—4 suppressors, UGG, *supD*; class 2—12 suppressors, UGG, *glyU*; class 3—9 suppressors, UGA and UGG, *glyT*; class 4—2 suppressors, UGG, *glyT*; class 5—7 suppressors, UGG, *glyV55*. Besides these, one suppressor retains *supD* activity, but so far its map location has not been distinguished from that of *supD*. Another suppressor clearly does not map near *supD* or any of the glycine tRNA genes mentioned. These last two suppressors may represent novel missense suppressors such as misacylated tRNA's or mutationally altered aminoacyl-tRNA synthetases, tRNA modification enzymes, or ribosomes. Finally, three other suppressors were obtained from a strain containing *glyT56*, the gene for an AGA/G-reading form of *glyT* tRNA. All three occurred at the expense of *glyT56* activity and exhibited the transductional linkage to *argH* that is characteristic of *glyT*.

As part of our mutational approach to the study of the role of tRNA structure in determining the coding specificity (codon response) of a given tRNA, two of the immediate goals of this laboratory have been (i) to convert existing translational suppressors, particularly glycine tRNA mutants, to new coding specificities and (ii) to obtain novel translational suppressors (for example, mutationally altered ribosomes, tRNA modification enzymes, aminoacyl-tRNA synthetases, polypeptide release factors, etc.). To do this, we have been using suppressible "mutant codons" that correspond to position 211 of the alpha chain of tryptophan synthetase (EC 4.2.1.20) of *Escherichia coli* (15). Of the seven amino acids that yield a nonfunctional alpha chain when they occur at position 211, suppressors have not been found for the codons corresponding to three: tryptophan (UGG), tyrosine (UAU or UAC), and glutamine (CAG). The wild-type amino acid at position 211 is glycine, and seven other amino acids (alanine, serine, threonine, isoleucine, leucine, valine, and asparagine) at that position yield a functional enzyme (15). Consequently, the tryptophan synthetase alpha-chain mutants provide an opportunity to obtain new kinds of suppressors, as well as new coding specificities of glycine tRNA's. This paper reports the use of the mutant *trpA*(UGG211) for this dual purpose and describes the isolation and preliminary characterization of the new UGG suppressors.

(Preliminary reports of portions of this work were presented at the fall meeting of the Texas Branch of The American Society for Microbiology, 1 December 1978, Houston, Tex., and at the Tenth Annual Gulf Coast Molecular Biology Conference, 19-21 January 1979, Corpus Christi, Tex.)

MATERIALS AND METHODS

All bacterial strains used were derivatives of *E. coli* K-12. The principal strains are described in Table 1. *glyV55* (originally *ins*394) codes for a GGA/G-reading mutationally altered form of *glyV* tRNA (2, 3). *glyT56* is a *glyT*-derived suppressor of AGA (3, 7, 16) and AGG (5). Δ(*tonB-trp*) designates a deletion extending from *tonB* to at least *trpB* (strain MDA 1031 contains a longer deletion that extends into *trpE*). In addition to being tryptophan auxotrophs, such strains cannot grow on indole, unless a functional *trpB* is carried on an episome. In most of the strains used in this study, the *trp* genes which contain a specific *trpA* mutation are contained in the *cysB*... *trp*... *tonB* region of the chromosome carried by the Fredericq episome (4), which covers a chromosomal *tonB-trp* deletion. Construction of *trpA* mutant derivatives of the Fredericq

episome has been described previously (12). References to these derivatives contain only the specific *trpA* mutation present. Hence, F'*trpA*(UGG211) designates the entire Fredericq episome with the normal alleles of all genes between *cysB* and *tonB* except for the *trpA* mutation, in which the codon UGG occurs in place of the GGA, corresponding to position 211 of the wild-type tryptophan synthetase alpha chain.

The ability of strains to suppress mutations in phage T4 was examined by using the spot test method, in which droplets of preparations of each phage mutant are deposited at intervals along a streak of bacterial cells on tryptone agar plates. In every test the controls included were wild-type phage T4, a suppressor-free bacterial strain, and strains containing suppressors of each of the phage mutants. The UGA phage mutant, which was obtained from C. Yanofsky, was derived from an ochre mutant in the presence of a tryptophan-inserting UGA suppressor.

Mutagenesis with ethyl methane sulfonate was performed by the method of Yanofsky et al. (18). The media and genetic procedures that we used are described elsewhere (1, 14) or below.

The details of tRNA preparation and characterization by reversed-phase (RPC-5) column chromatography were described previously (12). For comparisons of the glycyl-tRNA profiles of individual suppressors with an appropriate control strain, we did the following. Each *lysA*- or *metB*-linked suppressor was removed from the mutagenized or selective background and introduced into another strain nonselectively by transductional linkage with the appropriate marker, namely *lysA* or *metB*. At the same time, we saved a *lysA*$^+$ or *metB*$^+$ transductant that remained suppressor-free to serve as a control strain. Consequently, in each case the control strain was as isogenic as possible. In the case of the *purA*-linked suppressors, however, the columns were run with tRNA from the original suppressor strains against tRNA of the parent strain (MDA 1011).

RESULTS

Suppressor isolation. The parent of most of the suppressors referred to in this paper was MDA 1011 (Table 1). The relevant markers are indicated in Table 2. *supD*, which is approximately 8% cotransducible with *his*, is a serine-inserting amber suppressor. The genome of MDA 1011 contains an amber mutation in *argE*, but this strain is phenotypically Arg$^+$ due to suppression by *supD*. Because of the presence of *supD*, this strain also supports the growth of several phage T4 amber mutants. *glyU*$^+$, the normal gene for the GGG-reading $tRNA_1^{Gly}$ (3), is linked to *lysA*. *glyT*$^+$, which is cotransducible with *argH* and *metB*, codes for $tRNA_2^{Gly}$, which responds to both GGA and GGG (3). *glyV55* is linked to *purA* and codes for a GGA/G-reading, mutationally altered form of *glyV* tRNA (which normally reads GGU and GGC). The mutant tRNA represents a guanine to uridine change in the "wobble position" of the anticodon.

The expectations concerning possible UGG suppressors were as follows. Suppressors derived from *supD* should not suppress UGA and UAG mutants of phage T4 and should cause the strain to become Arg$^-$. All other suppressors should

TABLE 1. *Bacterial strains*

Strain	Genotype
MDA 892	*glyV55 argH* Δ(*tonB-trp*)/F'*trpA*(UGG211)
MDA 1011	*glyV55 argE*(Am) *supD* Δ(*tonB-trp*)/F'*trpA*(UGG211)
MDA 1031	*his* Δ(*tonB-trpE*)*15*/F'*trpA*(UGG211)
MDA 1102	*ilvD130 argH lysA* Δ(*tonB-trp*)/F'*trpA*(UGG211)
MDA 1103	*glyV55 metB* Δ(*tonB-trp*)/F'*trpA*(UGG211)
MDA 1136	*purA46 thr-1 leu-1 thi-1 arg-43 proA35 str-126 tonA25 tsx-71* T2^r T3^r Δ(*tonB-trp*)/F'*trpA*(UGG211)
MDA 1151	*glyV55 metB glyT56* Δ(*tonB-trp*)/F'*trpA*(UGG211)

TABLE 2. *Possible tRNA sources of UGG suppressors*[a]

Gene	Map location (min)	Gene product	Codon response	Linked markers
glyU	61	$tRNA_1^{Gly}$	GGG	*lysA* (55)[c]
glyT	88	$tRNA_2^{Gly}$	GGA, GGG	*metB* (25) *argH* (65)
glyV55	94	Mutant form of $tRNA_3^{Gly}$	GGA, GGG	*purA* (68)
supD	42	$tRNA_{su1}^{Ser}$ [b]	UAG	*his* (8)

[a] Except for footnote *b*, the information on this table has been published previously (2, 3, 5–7, 11–13a, 17).

[b] The *supD* product has been identified as a serine-accepting tRNA derived from a minor serine tRNA by a single base change in the middle of the anticodon (Steege, personal communication).

[c] Numbers in parentheses are approximate percent co-transducibilities.

allow the strain to remain Arg$^+$ and continue to support the growth of T4 amber mutants due to the presence of *supD*. They should differ, however, in their ability to suppress a T4 UGA mutant. Specifically, suppressors derived from *glyT* and *glyV55* should respond to UGA in addition to UGG. The retention of third-position wobble by mutant glycine tRNA's altered in their response to the first or second nucleotides of a codon has been observed before with suppressors of AGA, GAA, and AAG (5, 12; unpublished data). Finally, *glyU*-derived suppressors should be UGG specific.

Consequently, spontaneously occurring or ethyl methane sulfonate-induced Trp$^+$ derivatives of the parent strain were selected on glucose minimal medium containing arginine. The colonies were examined by replica plating for the Arg phenotype. To distinguish revertants from suppressed mutants, the Fredericq episome (see above) for each survivor was transferred by mating to a suppressor-free *tonB-trp* deletion strain, and the Trp phenotypes of the exconjugants were determined. At the same time, the episomes were transferred to a *supD*-containing *tonB-trp* deletion strain to determine whether any Trp$^+$ colonies were due to conversion of *trpA*(UGG211) to *trpA*(UAG211) and suppression by the *supD* already present in the strain. All UGG suppressor strains were then tested for response to UGA and UAG by examining their ability to support the growth of one UGA and four UAG mutants of phage T4.

In this fashion, many UGG suppressors were identified. Representatives of each class predicted on the basis of the criteria described above were purified for further study. They are listed in Table 3 with some of their properties.

Mapping. After each suppressor strain was purified by single-colony isolation, phage P1 was grown on each and used in transductions with strains MDA 1031, MDA 1102, MDA 1103, and MDA 1136 (Table 1) to test for linkage to *his*, *lysA*, *metB*, and *purA*, respectively (Table 3). The four suppressors that also became Arg$^-$ and were unable to support the growth of the four T4 UAG mutants were linked to *his*. This result supports the tentative conclusion that they were derived from *supD*. All of the rest of the suppressors retained the *supD* activities, and one showed no linkage to any of the four markers. Another suppressor (SP-12) remained Arg$^+$ and retained *supD* activity, but it was approximately 8% cotransducible with *his*. We have not yet been able to separate this suppressor genetically from *supD*.

One surprising feature of the class of suppressors displaying the *metB* linkage that is characteristic of *glyT* is that it has two members that do not suppress the T4 UGA mutant. This is contrary to the simple prediction stated above for the *glyT*-derived UGG suppressors. Similarly, although all of the suppressors that are cotransducible with *purA* display linkages characteristic of *glyV55* (12), none appears to respond to UGA. Finally, the *lysA*-linked suppressors seem to display a range of co-transducibilities. Initially, this result suggested the possibility that not all members of this class were derived from *glyU*. The unlikelihood of that, however, is addressed in the Discussion.

RPC-5 column chromatography. The glycyl-tRNA profile, after RPC-5 chromatography, of strains containing *glyV55* was characterized previously (12). The tRNA of the parent strain in this study (MDA 1011) displayed a profile that was the same as that of the control strain in Fig. 1; Fig. 1 also indicates the glycine tRNA genes that code for each peak of glycine-accepting tRNA. For RPC-5 chromatography, *metB*-linked suppressors were introduced by transduction into MDA 1103, which contains *glyV55*, whereas *lysA*-linked suppressors were introduced into MDA 1102, which does not contain *glyV55* (Fig. 2, solid line).

Figure 1 shows the profiles of one *metB*-linked suppressor (EMS-9) and its control strain. It is clear that EMS-9 affects *glyT* tRNA, as evidenced by the loss of the first two peaks of the profile (Fig. 1). The loss of an entire peak of aminoacyl-tRNA has been observed before with several missense suppressors (3, 7, 10a, 12, 13, 17) and may be due to reduced aminoacylation of the mature tRNA, reduced transcription of the mutant gene, or improper precursor maturation. The nature of the small peak of mutant tRNA just in front of the major *glyT* peak of the control is not known at this time. The profiles of three other *metB*-linked suppressors (SP-9, SP-11, and SP-13) turned out to be the same as the profile of EMS-9 (Fig. 1). In Fig. 2, the control strain was a Lys$^+$ transductant of MDA 1102. Figure 2 also shows the profile of a *lysA*-linked suppressor, SP-15. It is clear that this suppressor affects *glyU* tRNA. The profiles of three other *lysA*-linked suppressors (EMS-10, EMS-15, and SP-2) were affected in the same way. Figure 3 shows the profile of the glycyl-tRNA of the parent strain chromatographed with that of SP-1, a *purA*-linked UGG suppressor. The *glyV55* peak was entirely missing from the mutant. The same altered profile was obtained with the six other *purA*-linked suppressors (SP-3, SP-4, SP-5, SP-6, SP-7, and SP-14).

UGG suppressors obtained in a *glyT56*-containing strain. The *glyT* suppressors de-

TABLE 3. *UGG suppressors*

Designation of suppressor mutation[a]	Arg phenotype[b]	Growth of T4 amber mutants[c]	Suppressible codon(s)[d]	Linked marker	RPC-5 glycyl-tRNA profile[e]
EMS-1	−	−	UGG	*his* (9)[f]	
EMS-2	−	−	UGG	*his* (8)	
EMS-3	−	−	UGG	*his* (6)	
EMS-4	−	−	UGG	*his* (5)	
EMS-5	+	+	UGA, UGG	*metB* (28)	
EMS-6	+	+	UGA, UGG	*metB* (32)	
EMS-7	+	+	UGA, UGG	*metB* (33)	
EMS-8	+	+	UGA, UGG	*metB* (24)	
EMS-9	+	+	UGA, UGG	*metB* (28)	*glyT*
EMS-10	+	+	UGG	*lysA* (55)	*glyU*
EMS-11	+	+	UGG	*lysA* (55)	
EMS-12	+	+	UGG	—[g]	
EMS-13	+	+	UGG	*lysA* (71)	
EMS-14	+	+	UGG	*lysA* (56)	
EMS-15	+	+	UGG	*lysA* (65)	*glyU*
EMS-16	+	+	UGG	*lysA* (69)	
SP-1	+	+	UGG	*purA* (70)	*glyV55*
SP-2	+	+	UGG	*lysA* (40)	*glyU*
SP-3	+	+	UGG	*purA* (69)	*glyV55*
SP-4	+	+	UGG	*purA* (67)	*glyV55*
SP-5	+	+	UGG	*purA* (75)	*glyV55*
SP-6	+	+	UGG	*purA* (64)	*glyV55*
SP-7	+	+	UGG	*purA* (71)	*glyV55*
SP-8	+	+	UGG	*lysA* (74)	
SP-9	+	+	UGG	*metB* (20)	*glyT*
SP-10	+	+	UGG	*lysA* (49)	
SP-11	+	+	UGA, UGG	*metB* (20)	*glyT*
SP-12	+	+	UGG	*his* (9)	
SP-13	+	+	UGG	*metB* (25)	*glyT*
SP-14	+	+	UGG	*purA* (60)	*glyV55*
SP-15	+	+	UGG	*lysA* (67)	*glyU*
SP-16	+	+	UGG	*lysA* (63)	
SP-17	+	+	UGG	*lysA* (41)	
SP-18	+	+	UGA, UGG	*metB* (26)	
SP-19	+	+	UGA, UGG	*metB* (28)	
SP-20	+	+	UGA, UGG	*metB* (28)	

[a] EMS indicates mutations obtained after treatment with ethyl methane sulfonate, and SP indicates mutations that occurred spontaneously.

[b] The parent strain has an amber *argE* mutation but is phenotypically Arg^+ due to the presence of *supD*.

[c] The parent strain supports the growth of the four T4 amber mutants tested, due to the presence of *supD*.

[d] The UGG response was shown by suppression of the *E. coli* mutation *trpA*(UGG211), and the UGA response was shown by suppression of a phage T4 UGA mutant.

[e] Gene designation for the affected peak.

[f] Numbers in parentheses are approximate percent co-transducibilities.

[g] —, Not linked to *his*, *lysA*, *metB*, or *purA*.

scribed above were derived from the wild-type *glyT*. To determine whether different kinds of UGG suppressors could be derived from a mutant form of *glyT*, we constructed MDA 1151 (Table 1), which contains the AGA/G-reading suppressor *glyT56* (3, 7, 16). Strain MDA 1152 (not shown in Table 1) has the same genotype as MDA 1151 and should be essentially the same. However, some of the genetic markers in it were not obtained from exactly the same sources. Both Trp^- strains were plated onto glucose minimal medium containing methionine to select for Trp^+ survivors. One suppressor was found among 60 Trp^+ derivatives of MDA 1151, and 2 were found among 79 derivatives of MDA 1152. The three suppressors (designated SP-27, SP-28, and SP-29, respectively) were then tested for ability to support the growth of T4B1, a *glyT56*-suppressible missense mutant of phage T4 (5). By this criterion, all three lost *glyT56* activity and as such were candidates for being *glyT56*-derived UGG suppressors.

To determine the map location of the new suppressors, all three suppressor strains, which

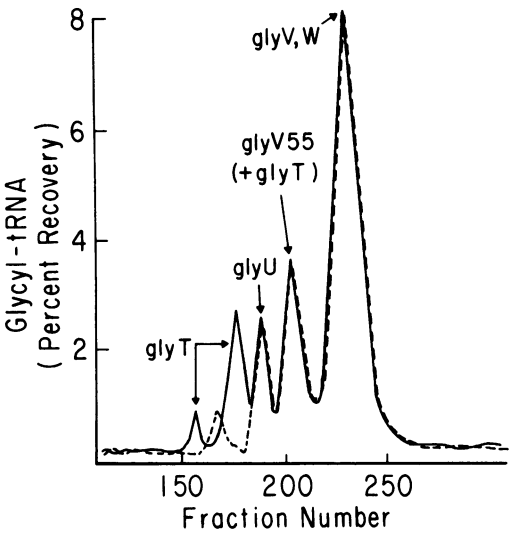

FIG. 1. *Glycyl-tRNA profiles obtained after RPC-5 column chromatography. Particular glycine tRNA genes associated with each peak are also indicated. The solid line represents the profile of the control strain (see text), and the dashed line represents the profile of the metB-linked UGG suppressor designated EMS-9 (Table 3). Percent recovery was determined as follows: counts per minute of each fraction divided by total counts per minute eluted from the column, multiplied by 100. In practice, the total counts per minute was the sum of the counts per minute from every second fraction.*

were Met$^-$ due to *metB*, were used as recipients in transductions with phage P1 grown on a wild-type (*metB*$^+$ *glyT*$^+$) strain. Selection was made for Met$^+$ colonies on glucose minimal medium containing tryptophan. The Met$^+$ transductants were then tested for inability to grow in the absence of tryptophan (that is, for recombinational loss of the UGG suppressor). The result was that all three suppressors were cotransducible with *metB* and exhibited the linkage to *metB* that is characteristic of *glyT* (approximately 25%). To determine suppressor linkage to *argH*, phage P1 was grown on *metB*$^+$ derivatives of each suppressor, and each phage preparation was used to transduce strain MDA 892 to Arg$^+$. Again, all three suppressors were approximately 65% cotransducible with *argH*, as is *glyT*.

To determine whether SP-27, SP-28, and SP-29 were dominant or recessive as suppressors, their ability to suppress *trpA*(UGG211) was tested in the presence of an F-prime factor carrying the wild-type genes of the *argH-glyT* region. In this way, all three were shown to be dominant.

The tRNA from each suppressor-containing strain was chromatographed on an RPC-5 column with the tRNA from an isogenic *glyT56*-containing strain. The profile obtained for each suppressor was exactly the same as the profile of the control strain, which was the same as the one obtained with EMS-9 (Fig. 1).

DISCUSSION

In this paper we have described the isolation and preliminary characterization of several new classes of codon-specific missense suppressors.

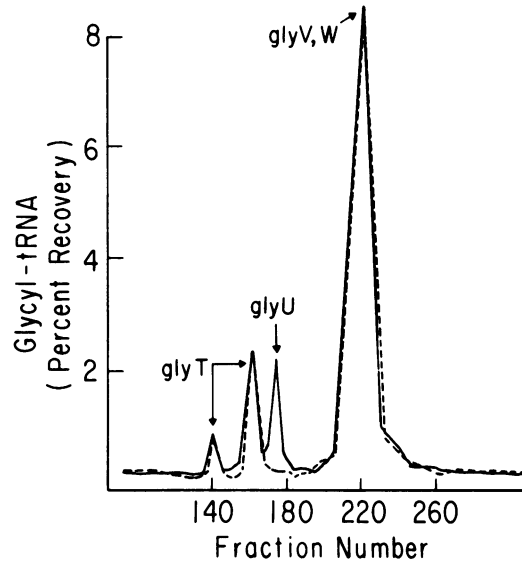

FIG. 2. *RPC-5 glycyl-tRNA profiles of the lysA-linked UGG suppressor SP-15 (dashed line) and its control strain (solid line).*

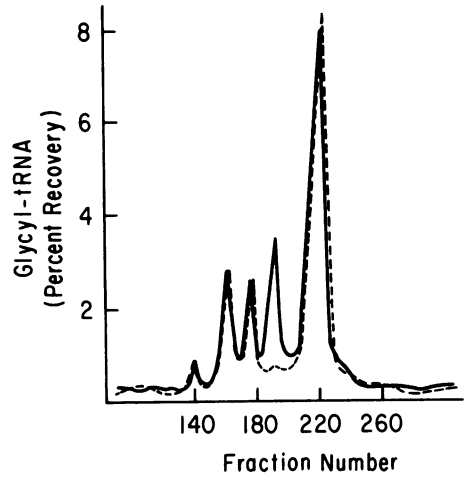

FIG. 3. *RPC-5 glycyl-tRNA profiles of the parent strain MDA 1011 (solid line) and the purA-linked UGG suppressor SP-1 (dashed line).*

All 39 were selected as suppressors of the *E. coli* auxotrophic mutation *trpA*(UGG211). Although some clearly respond to the nonsense codon UGA in addition to UGG, the others may be UGG specific, as judged by their failure to support the growth of a phage T4 UGA mutant. We present genetic evidence that indicates that 4 are derived from *supD*, 12 are derived from *glyU*, 11 are derived from wild-type *glyT*, 3 are derived from *glyT56*, and 7 are derived from *glyV55*. RPC-5 column chromatography of glycyl-tRNA from several suppressor strains demonstrated that four suppressor mutations affect *glyU* tRNA (GGG specific), four affect *glyT* tRNA (responds to GGA and GGG), and seven affect *glyV55* tRNA (a GGA/G-reading mutationally altered form of the GGU/C-reading *glyV* tRNA). The least characterized suppressors include one that does not map at or near *supD*, *glyU*, *glyT*, or *glyV55* and one that maps at *supD* but retains *supD* activity.

Our apparent success in converting *supD* to suppressors of UAA (11), UGG (see above), and UAU or UAC (unpublished data) is consistent with the recent demonstration that the UAG-suppressing *supD* product is indeed a serine-accepting tRNA derived from a minor serine tRNA by a single base change in the middle of the anticodon (D. Steege, personal communication). Whether the conversions that we have obtained are due to predictable anticodon changes remains to be seen. The inability of *supD*-derived UGG suppressors to respond to UGA was expected since *supD* does not suppress UAA. As is discussed below, however, such simple predictions may not be very reliable.

The number of ethyl methane sulfonate-induced suppressors examined was almost as high as the number of spontaneously occurring ones (16 versus 20). Nevertheless, certain classes of suppressors were found in one group only (Table 3). In particular, all four *supD*-derived suppressors were induced by ethyl methane sulfonate. Also, the two apparently UGG-specific suppressors derived from *glyT*, as well as the seven suppressors derived from *glyV55*, occurred spontaneously. The reason for this is not apparent at this time. The other types were rather evenly distributed; these included those derived from *glyU*, *glyT* suppressors that responded to UGA in addition to UGG, and those whose identities are not yet known.

The *lysA*-linked suppressors appear to display a range of co-transducibility with *lysA*. However, since in most cases fewer than 100 Lys^+ transductants were examined, the differences might only reflect the variation sometimes observed with P1 transduction. Furthermore, the RPC-5 profiles of representatives of "high" and "low" linkages turned out to be the same (that is, the *glyU* peak was "missing").

The *glyT*-derived UGG suppressors have several interesting properties. Although those that respond to UGA fit the simplest expectation concerning third-position wobble, there are two that either do not respond to adenine in the third position at all or have had that ability restricted severely. However, we cannot yet rule out the possibility that these two either are acylated with amino acids that are not acceptable at the site of that T4 mutation or fail to respond to the UGA codon when it occurs in the particular mRNA content of that T4 mutation. Nevertheless, this result indicates clearly that at least two different nucleotide changes have given rise to these suppressors. Consequently, a simple anticodon change, predicted on the basis of hydrogen-bonding properties, cannot explain both types. In Fig. 1, the small peak of mutant tRNA that runs just in front of the major *glyT* peak of the control could be the suppressor tRNA. The low glycine acceptance of this species (relative to the acceptance of the major *glyT* peak) could be due to reduced aminoacylation of the mature tRNA, reduced transcription of the mutant gene, or improper precursor maturation. However, if that small peak is the suppressor tRNA, it is interesting that the UGG-specific *glyT* suppressors (SP-9 and SP-13) show the same profile as the wobblers (EMS-9 and SP-11). Recently however, we examined by RPC-5 chromatography *glyT*-derived AGA/G and GAA/G suppressors and found that they display the same small peak in the same position (10a). Consequently, that small peak, rather than being the suppressor tRNA, may represent a portion of glycine-accepting tRNA that is evident only as a result of the mutational alteration of *glyT* tRNA.

Since *glyT* codes for the only GGA-reading glycine tRNA in normal *E. coli* cells (3, 11a) and since the new *glyT*-derived UGG suppressors were isolated in a strain containing mutant GGA-reading *glyV55* tRNA, we wanted to determine whether the viability of these suppressor-containing strains depended on the presence of *glyV55*. This was done as described elsewhere (11a). The result was that, in order for a haploid strain to survive with these *glyT* suppressors in their genomes, *glyV55* must be present. The haplolethality of these suppressors, however, is not an unexpected consequence of conversion of the only normal GGA-reading glycine tRNA to a new coding specificity. Nevertheless, there are haploid strains that do not contain *glyV55* but do survive when $glyT^+$ is replaced by several *glyT*-derived suppressors, including the UGA/G-reading SP-11 (11a). This result indicates that SP-11 and perhaps the other UGA/G-reading

glyT-suppressors have retained the ability to respond to GGA, at least in certain circumstances, while having gained the new coding specificity. This implies that certain genetic backgrounds can allow a somewhat unorthodox first-position wobble. All of these observations are consistent with the growing body of data that lend support to the notion that tRNA conformational changes are involved in the process of codon recognition (8–10, 11a).

The *glyV55*-derived suppressors should also prove to be very interesting mutants. It is not at all apparent why any of the seven should appear to be UGG specific and why, in contrast to the *glyT* suppressors, none seems to respond to UGA. However, one thing to be kept in mind is that, although *glyV55* tRNA is a GGA/G reader, as is *glyT* tRNA, its nucleotide sequence is approximately 25% nonhomologous with that of *glyT*. Experiments are in progress to investigate further the possible UGG specificity of *glyV55*- and *glyT*-derived suppressors.

An unexpected *his*-linked suppressor is SP-12. It is approximately 8% cotransducible with *his* and retains *supD* activity. Since we have not yet been able to separate it genetically from *supD*, it may represent an unusual mutant form of *supD* that can still respond to UAG while having gained the new coding specificity (UGG but not UGA). Such wobble with regard to the middle position of a codon was indicated previously in the case of mutant glycine tRNA's (11a, 12). It could be, however, that a *supD* duplication was involved in the suppressor selection, with one copy becoming changed to the new suppressor form. Another possibility is that the suppressor mutation occurred in a very closely linked (to *supD*) gene that codes for another tRNA or for a tRNA-related molecule.

SP-27, SP-28, and SP-29 appear to be derived from *glyT56*. It will be interesting to see whether UGA/G suppressors derived from a single-step *glyT* mutant (*glyT56*) are different from such suppressors derived from wild-type *glyT* (EMS-9, SP-11, etc.).

Some speculation can be raised concerning the molecular nature of the *his*-linked SP-12 and of EMS-12, which does not map at *supD*, *glyU*, *glyT*, or *glyV55*. Since both serine and leucine at position 211 of the tryptophan synthetase alpha chain yield a functional protein (15), a coding specificity change in either of those tRNA's could result in suppression of *trpA*(UGG211). (The behavior of cysteine at position 211 is not known yet.) The suppressors could also involve misacylated tryptophan tRNA or other tRNA's in which the mutation simultaneously alters the specificities for both codon response and amino acid acceptance. Another possibility would be specific mutant tRNA modification enzymes, perhaps affecting (although not necessarily) one of the glycine tRNA's or *supD* tRNA. Finally, it is possible that mutations in genes for rRNA or ribosomal proteins can affect the codon-dependent interactions of tRNA's and mRNA.

In summary, we have isolated a large variety of UGG suppressors that promise to be of great interest for questions of translational fidelity and tRNA structure-function relationships.

ACKNOWLEDGMENTS

For discussions and technical assistance with different portions of this work, we are most grateful to Frances Pagel, Kathryn LaBoon, Patrick Condreay, and Thomas Glaser. We are also indebted to Thomas Glaser, Charlotte Matney, and Norman Prather for developing a computer program for performing the calculations and profile print-outs of RPC-5 column runs. For assistance in preparation of the manuscript we thank Janet Naquin and Walter Pagel.

Portions of this work were supported by grant NP-167 from the American Cancer Society and by Public Health Service grant GM21499 from the National Institute of General Medical Sciences. E.J.M. is the recipient of Public Health Service research career development award GM 00047 from the National Institute of General Medical Sciences.

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