JOURNAL OF BACTERIOLOGY, June 1978, p. 801-807
0021-9193/78/0134-0801$02.00/0
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Vol. 134, No. 3
Printed in U.S.A.

Variations Among *glyV*-Derived Glycine tRNA Suppressors of Glutamic Acid Codons

EMANUEL J. MURGOLA,* NORMAN E. PRATHER, AND KATHLEEN H. HADLEY

Department of Biology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Received for publication 16 December 1977

Glutamic acid codon suppressors in 18 isogenic strains of *Escherichia coli* have been further characterized as to map location, dominance, growth rates in various media, suppression of the GAG codon, and tRNA profiles after reversed-phase column chromatography. In general the evidence supports the conclusion that all of these suppressors are due to mutations in *glyV55*, the gene for a GGA/G-reading mutant form of *glyV* tRNA, and that they represent several different classes that may correspond to at least as many different nucleotide changes. Furthermore, 17 ot the 18 suppressors can coexist in a haploid genome with a *glyT* suppressor that is devoid of GGA-reading ability. This result indicates the retention by those *glyV* suppressors of some ability to respond to GGA as well as the acquisition of the ability to read GAA, and suggests the possibility of "wobble" in the middle position of the anticodons of those tRNA's.

In a previous paper (15) we described the isolation and preliminary characterization of 18 suppressors of mutant *trpA46* (Gly[GGA] → Glu[GAA] at position 211 of the tryptophan synthetase [TS; EC 4.2.1.20] alpha chain) of *Escherichia coli*. All 18 were shown to be 40% linked by transduction to *tsm-1*, a temperature-sensitive mutation that is 40% cotransducible with *glyV* (16), a gene for the $tRNA^{Gly}$ that reads GGU/C. One suppressor was shown, by benzoylated diethylaminoethyl-cellulose (BDC) column chromatography, to affect the tRNA peak specified by *glyV55*, the gene for $tRNA^{Gly}_{ins}$ (4, 24), a GGA/G-reading, mutationally altered form of *glyV* tRNA. The results indicated that all 18 might be due to alterations of *glyV55* tRNA that convert it from a GGA/G reader to a GAA/G reader. In this paper we (i) describe the more precise map location of *glyV* and the similar linkage of all 18 suppressors to that region, (ii) demonstrate, by reversed-phase (RPC-5) column chromatography, that all 18 mutations affect the *glyV55* tRNA peak, and (iii) present evidence for variation among the suppressors, reflecting presumably different nucleotide changes.

(A preliminary report of a portion of the work was presented at the Sixth Annual Gulf Coast Molecular Biology Conference, 24-25 January 1975, Corpus Christi, Tex.)

MATERIALS AND METHODS

Bacterial strains and procedures. The parent strain, designated MV1119, was described previously (15), as well as the isolation of the suppressor strains. The relevant genetic markers are *glyV55* (originally ins_{394}), the gene for a GGA/G-reading, mutationally altered form of *glyV* tRNA (4, 24), and *trpA46*, a missense mutant of *trpA* in which the glycine codon GGA corresponding to position 211 of the TS alpha chain is replaced by the glutamic acid codon GAA. Of the 18 suppressors (Su46) described in this study, 14 were obtained after treatment with hydroxylamine and 4 were obtained after exposure to UV light (see Table 1). Other strains are described in the next paragraph or at first mention in Results. All of the studies reported in this paper were done with isogenic strains obtained by phage P1-mediated transduction of each suppressor back into the parent strain or into other appropriate recipients.

To determine the dominance or recessiveness of each suppressor mutation, an F-prime (F117) carrying a wild-type *glyV* region and harbored in strain KLF17/KL132 (= strain no. 4255 from the *E. coli* Genetic Stock Center) was introduced by conjugation into all 18 suppressor strains. Each resulting partial diploid was tested for maintenance of suppressor activity (suppression of *trpA46*) and for presence of F117 (ability to transfer the wild-type allele of *purA* to a $PurA^-$ recipient in conjugation).

Estimations of relative growth rates were obtained as follows: 20 ml of a stationary-phase culture (glucose minimal medium) of each suppressor was added to 200 ml of the appropriate medium. Cultures were incubated at 37°C with aeration on a gyratory shaker. Periodically, 5-ml samples were taken and the absorbancy was determined at 550 nm.

Introduction of specific *trpA* mutations into the Fredericq episome (8) was accomplished as follows. Using a phage P1*vir* lysate made on a Cys^+ strain containing any one of the *trpA* mutations, the strain *pro cysB* Δ(*tonB-trp*)/F′*cysB* trp^+ (obtained from C.

TABLE 1. *Summary of some properties of 18 suppressors of trpA46*

Suppressor	Mutagen used	Growth class[a]	Profile class[b]	Suppression of *ilvD130*	Coexistence with *glyT56*
Su46−3	UV		L	Yes	Yes
−4	UV	A	L	Yes	Yes
−5	UV	A	L	Yes	Yes
−9	UV	A	L	Yes	Yes
−17	HA[c]		L	Yes	Yes
−18	HA	B	L	Yes	Yes
−20	HA		H	Yes	Yes
−21	HA		H	Yes	Yes
−23	HA		H	Yes	Yes
−24	HA	C	L	Yes	Yes
−26	HA	D	L	Yes	Yes
−27	HA	C	H	No	No
−28	HA	E	L	Yes	Yes
−29	HA	F	L	Yes	Yes
−31	HA	G	H	Yes	Yes
−33	HA	A	H	Yes	Yes
−34	HA	B	H	Yes	Yes
−35	HA	G	L	Yes	Yes

[a] See Table 2.
[b] H (high) and L (low) refer to the relative amounts of glycine-accepting tRNA remaining in the *glyV55* region of the profile.
[c] HA, Hydroxylamine.

Yanofsky) was transduced to Cys$^+$ on glucose minimal medium plus tryptophan (and proline). The Cys$^+$ transductants were then screened for Trp$^-$, i.e., for those in which the recombination had occurred in the episome. Presence of the particular *trpA* mutation on the episome was verified by transfer of the episome to a suppressor-free *trp* deletion strain and also to a *trp* deletion strain containing an appropriate translational suppressor (missense or nonsense). In this paper, references to these various derivatives of the Fredericq episome will contain only the specific *trpA* mutation. Hence, the description F'*trpA*(GAA211) stands for the entire Fredericq episome, with the normal allele of *cysB* and the entire *trp* operon (consisting of all normal *trp* genes except for the particular *trpA* mutation).

The media and other genetic procedures we used are described elsewhere (1, 16) or in Results.

tRNA preparation and characterization. Cells were grown in 2 liters of glucose minimal medium (plus any necessary supplements) and harvested in mid (to late) log phase (absorbancy at 550 nm = 0.5). tRNA was extracted according to the method of Carbon and Curry (3). Preliminary characterizaton of tRNA's was done by using RPC-5 reversed-phase chromatography (19) of aminoacyl-tRNA according to a modification of the procedure of Hill et al. (11). For aminoacylation of the tRNA, a 0.25-ml reaction mixture contained 0.5 mg of unfractionated tRNA, 15 mM $MgCl_2$, 1 mM ATP, 10 mM KCl, 100 mM potassium cacodylate (pH 7.0), 12 mM 2-mercaptoethanol, 0.05 mg of bovine serum albumin, 0.1 mM [^{3}H]glycine (500 mCi/mmol) or [^{14}C]glycine (50 mCi/mmol), and 4 U of glycyl-tRNA synthetase, obtained from step IV according to the procedure of Ostrem and Berg (18). After incubation of the mixture for 10 min at 37°C, the pH was adjusted to 4.5 with 1 M sodium acetate, pH 4.5. The mixture was centrifuged at 20,000 × *g* for 15 min, and the precipitate was discarded.

The buffers used for reversed-phase chromatography contained, in addition to the appropriate NaCl concentration, 10 mM sodium acetate, pH 4.5, 10 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. A jacketed, 0.6- by 25-cm column packed with RPC-5 material was equilibrated with buffer containing 0.2 M NaCl. For dual-label experiments, 0.15 ml of each reaction mixture was added to 0.25 ml of buffer containing 0.2 M NaCl and applied to the column, using a sample injection valve equipped with a 0.5-ml sample loop. The column was washed with 10 ml of 0.2 M NaCl buffer to remove acid-soluble radioactivity (unreacted glycine) and then eluted with a 150-ml, 0.4 to 0.6 M linear gradient. Finally, the column was washed with 15 ml of 2.0 M NaCl buffer. Throughout the sample loading and column run, the temperature was maintained at 32°C and the flow rate was held constant at 1 ml/min (approximately 200 lb/inch^2). Fractions of 0.5 ml were collected directly into liquid scintillation minivials. For counting, 4.5 ml of Fisher Scintiverse was added to every other fraction.

RESULTS

Map location. Preliminary mapping of the 18 suppressors (15) located them in the region of *glyV*, a gene for the tRNA^{Gly} that reads GGU/C, by demonstrating transductional linkage to *tsm-1*, a temperature-sensitive mutation that is 40% cotransducible with *glyV* (16), but whose precise location is not known. Recently we noticed (unpublished observations) that isolates of certain *glyV* mutants exhibited high mutation frequencies for one marker or another and that in some cases the increased mutability seemed to cotransduce with *glyV*. Consequently, we examined the *E. coli* map for mutator gene loci in the general region in which *glyV* had been roughly mapped by conjugation (24). After noticing the *purA*-linked *mutL* as a possible *glyV*-linked mutator, we obtained a *purA* strain from the Coli Genetic Stock Center (CGSC strain no. 4436 contains *purA46* and has also been referred to as PC0273). After a *tonB-trp* deletion of that strain was obtained by selecting for resistance to ϕ80*vir* and colicins V and B (6), *trp* regions containing the *trpA58* and *trpA46* mutations were independently introduced by transduction. *trpA58* is a missense mutant of *trpA* resulting from a Gly(GGU/C) to Asp(GAU/C) change at position 234 of the TS alpha chain (9, 25). *glyV50* (= SuGAU/C, or Su58) designates a *trpA58* suppressor, originally isolated in the laboratory of Charles Yanofsky. It maps in the *glyV* region (7) and affects 30% of the GGU/C-reading tRNA^{Gly} peak on BDC columns (24). Using a phage P1*vir* lysate made on a Pur$^+$ strain containing *glyV50*, we transduced the *purA trpA58*

strain to Pur^+ and then tested the transductants for Trp^+ (= Su^+). Of 1,231 Pur^+ transductants examined, 840 became Trp^+ (= Su^+), indicating a cotransduction frequency of *glyV50* with *purA* of approximately 68%. Similar transductions were done with lysates on each Su46 (all are Pur^+), using as recipient the *trpA46* derivative of the *purA* strain. Approximately 200 transductants were analyzed in each case, and each suppressor showed roughly 68% cotransduction with *purA*. These results suggest a location of *glyV* at approximately min 93.5 on the recalibrated linkage map of *E. coli* (2).

Knowing the precise map location of the suppressors allowed examination of the dominance or recessiveness of each one (see Materials and Methods). Each mutation was found to be dominant, expressing the suppressor activity in the presence of an F-prime carrying the wild-type *glyV* region.

Specificity of GAA and GAG. We showed previously (15) that each of the 18 suppressors could suppress *trpA*(GAG211) in addition to *trpA*(GAA211). Having on hand several *trpA* mutants with different codons corresponding (mainly) to position 211 of the TS alpha chain, we wanted to determine whether the suppressors differed with regard to their expected inability to suppress codons other than GAA and GAG. Consequently, *tonB-trpAB* deletions were obtained in each of several suppressor strains (Su46-9, Su46-27, Su46-28, and Su46-34; see Table 2), and each *trpA* mutation was introduced into each deletion strain either by P1 transduction or by mating with strains carrying a given *trpA* mutation on the Fredericq episome. The mutant codons represented were AGA and GAU (25), UAG (15), UAA (14), AAG, UAU, and CAG (17). The result was that none of the 18 suppressors displayed suppression of any of these codons, and it strengthens the conclusion that they are specific for GAA and GAG.

RPC-5 column chromatography. tRNA extracted from the parent strain, MV1119, aminoacylated in vitro with ^3H- or ^{14}C-labeled glycine and chromatographed on an RPC-5 column presents the profile shown in Fig. 1. The first two peaks are the products of a single gene, *glyT*, the gene for the normal GGA/G-reading glycine tRNA (21, 23; unpublished observations in our laboratory). The third peak represents $tRNA^{Gly}_{GGG}$, the *glyU* gene product (10). Since the fourth peak is too large to be simply the third peak of *glyT* tRNA (21, 23), we surmised initially that it contained mainly *glyV55* tRNA. *glyV55* can be crossed out of the genome by introduction of Su58 into the strain, resulting in the loss of the *glyV55* peak from the BDC column profile (24). To prepare isogenic strains with and without *glyV55*, we made a *trpA58* derivative of MV1119 (= *glyV55 trpA58*) and then introduced Su58 by transduction (= *glyV50 trpA58*). Cochromatography of the glycyl-tRNA from these two strains on RPC-5 gave results identical to those shown in Fig. 2 (but the solid line would represent *glyV55 trpA58* and the dashed line *glyV50 trpA58*). The fourth peak of the parental profile was clearly affected and almost entirely lost. The remaining tRNA in the region presum-

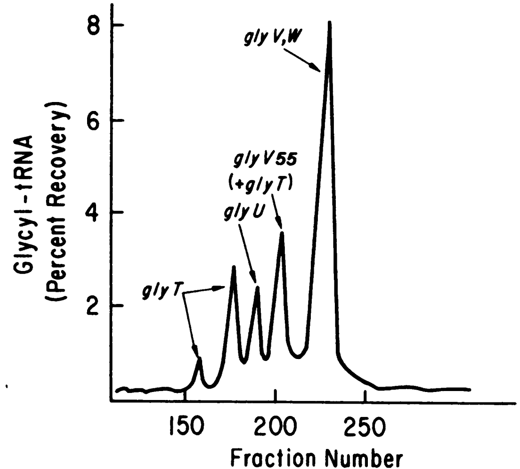

FIG. 1. *Glycyl-tRNA profile of parental MV1119 tRNA obtained after RPC-5 column chromatography. Particular glycine tRNA genes associated with each peak are also indicated. Percent recovery represents the cpm of each fraction divided by the total cpm eluted from the column, multiplied by 100. In practice, the "total cpm" is the sum of cpm from every second fraction.*

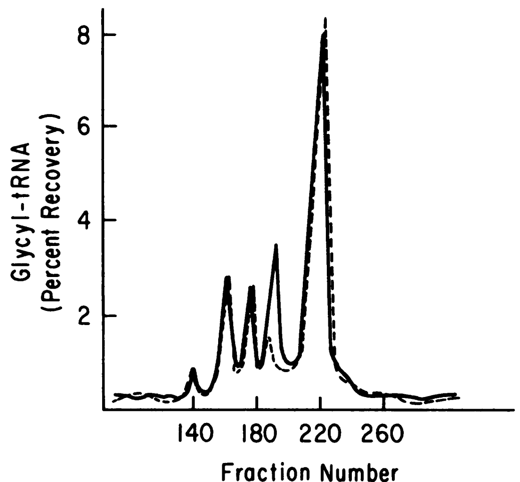

FIG. 2. *Chromatography, on RPC-5, of glycyl-tRNA from MV1119 (solid line) with glycyl-tRNA from Su46-34 (dashed line).*

ably represents the third peak of *glyT* tRNA (21, 23). The last and largest peak of the parental glycyl-tRNA profile represents tRNA specified by *glyW* and presumably two other copies of the *glyV* gene (7).

The tRNA from MV1119 was chromatographed on RPC-5 with the tRNA from each suppressor strain after in vitro aminoacylation with ^{3}H- or ^{14}C-labeled glycine. For most suppressors the labels were reversed, and some were examined several times, including also the extraction of their tRNA's from cultures grown at different times. The glycyl-tRNA profile for Su46-34 chromatographed with MV1119 tRNA is shown in Fig. 2. The result for each of the 18 suppressors was essentially the same; that is, the *glyV55* peak was clearly affected and almost entirely lost. A small but reproducible difference exists among the suppressors with regard to how much glycine-accepting tRNA is left in the region of the *glyV55* peak. According to this criterion, the suppressors fall into two classes: those that display a profile exactly like that of Su46-34 (designated "H" in Table 1) and those showing less glycine-accepting tRNA in the region of the *glyV55* peak (designated "L" in Table 1). The reproducibility of the difference has been shown by repeated runs not only of suppressors of each class against the parental tRNA but also of representatives of one class against those of the other. The nature of the glycyl-tRNA remaining in that region is not known at this time.

Growth rates in liquid media. The growth of each of 13 isogenic suppressor strains in four different media, relative to the control strain, is shown in Table 2. As a first approximation, the glucose minimal medium/acid casein hydrolysate (min/ACH) ratios indicate the existence of three classes. Some of those suppressor strains that show essentially the same min/ACH ratio, however, can be distinguished by examination of the values for minimal medium and for ACH. The results suggest that the suppressors fall into about seven different classes.

Suppression of *ilvD130*. Besides GAA, all 18 suppressors had been shown to suppress the other glutamic acid codon GAG when it occurred in *trpA* at a position corresponding to amino acid 211 of the TS alpha chain (15). It has been deduced by Hill et al. (10) that GAG is the mutant codon in *ilvD130*, a mutant form of the *D* gene of the *ilv* operon. It was of interest to see whether the 18 suppressors of *trpA*(GAG211) were similar in their ability to suppress the GAG codon when it occurred in a gene other than *trpA*. The *ilvD130* mutation was introduced into MV1119 by transduction in two steps. First, a P1 lysate was made on a strain containing a *metE* mutation and also a mutation (isolated by M. B. Coukell) that is 100% cotransducible with certain *ilv* mutations and confers valine resistance. The lysate was used to transduce MV1119 to valine resistance on medium containing methionine. Approximately 10% of the valine-resistant transductants were also Met$^-$. Using the *metE* marker, the *ilvD130* was introduced. After all 18 Su46's were put into the resulting strain (*ilvD130 glyV55 trpA46*) by transduction, selecting for Trp$^+$ on glucose minimal plus isoleucine and valine, each Su46 derivative was examined for suppression of *ilvD130*. The results were that 17 of the 18 suppressors did exhibit

TABLE 2. *Relative growth of suppressor strains in liquid media*[a]

Su46 strain no.	min[b,c]	Trp min[b,c]	ACH[b,c]	Trp ACH[b,c]	Min/ACH[c]	Growth class
4	1.44	1.06	1.45	1.20	0.99	A
5	1.44	1.12	1.41	1.20	1.02	A
9	1.42	1.07	1.48	1.12	0.96	A
18	1.28	1.10	1.61	1.25	0.80	B
24	1.56	1.10	1.57	1.17	0.99	C
26	1.72	ND[d]	2.07	ND[d]	0.83	D
27	1.50	1.10	1.59	1.25	0.94	C
28	1.22	0.99	1.77	1.15	0.69	E
29	1.00	1.09	1.30	1.15	0.77	F
31	1.31	1.12	1.30	1.20	1.01	G
33	1.50	1.03	1.49	1.15	1.01	A
34	1.28	1.09	1.54	1.20	0.83	B
35	1.33	1.02	1.30	1.05	1.02	G

[a] Media used were: glucose minimal (min), min plus tryptophan (Trp min), min plus acid casein hydrolysate (ACH), and ACH plus Trp (Trp ACH).

[b] Each number is a ratio of absorbancy (at 550 nm) doubling times to each suppressor value divided by the control value. The control strain is a "full revertant" (see ref. 25) of MV1119. Control doubling times (in minutes) were 108 (average of 108, 108, and 108), 96 (average of 93 and 99), 83 (average of 80, 84, and 85), and 65 (average of 64 and 66) for min, Trp min, ACH, and Trp ACH, respectively.

[c] For the control strain, these ratios are 1.00 by definition.

[d] ND, Not determined.

suppression of *ilvD130*; the one that did not was Su46-27.

Conversion of GAA/G suppressors to nonsense suppressors. As part of our continuing studies on missense and nonsense suppression (also see next section), it was considered important to try to convert glutamic acid codon suppressors to amber (UAG) or ochre (UAA) suppressors. The two suppressors chosen, Su46-34 and Su46-27, appear to represent different mutations as indicated by growth rates, suppression of *ilvD130*, and coexistence with *glyT56* (described in the next section). Each suppressor was introduced by transduction into the strain *argE glyV55 Δ(tonB-trp)/F′trpA46*. The *argE*, obtained from Jeffrey Miller, is an amber mutation that requires only a low level of suppression for growth in the absence of arginine. Serial dilutions, in L broth, of each Su46 (−34 and −27) derivative were grown overnight. The highest dilution that grew was washed twice in minimal salts and plated on glucose minimal medium plus tryptophan, selecting for Arg^+ survivors. The Arg^+ colonies were examined by replica plating for any that had become simultaneously Trp^-.

Several Arg^+ Trp^- colonies were obtained with both Su46 strains. Each Trp^-, when tested with phage T4 nonsense mutants, was shown to possess a new ochre suppressor. To provide evidence that each new ochre suppressor was derived from Su46-34 or Su46-27, phage P1 was grown on the new mutants. Each lysate was used in transductions with the following recipient strains: (i) *purA trpA*(UAA211), (ii) *purA trpA46*, and (iii) *argE*(Am) *glyV*(Su46) Δ(*tonB-trp*)/F′*trpA46*. The *trpA*(UAA211) in strain (i) was derived mutationally from *trpA46* in the presence of an ochre suppressor (14). The transduction results were the same for each ochre suppressor donor, namely: with strain (i), about 68% of the Pur^+ transductants were Trp^+; with strain (ii), all of the Pur^+ transductants were Trp^-; with strain (iii), all of the Arg^+ transductants were Trp^-. These results support the conclusion that the new ochre suppressors were derived from the GAA suppressors.

tRNA was extracted from Su46-34, from Su46-27, and from one ochre suppressor derived from each Su46 (designated SuUAA/G-34 and SuUAA/G-27). After aminoacylation with radioactively labeled glycine, each suppressor tRNA was chromatographed on an RPC-5 column with its parental tRNA. Whereas the SuUAA/G-34 profile was the same as that of its parent, the SuUAA/G-27 profile displayed a difference (Fig. 3). The same result was obtained with reversed labels and also when the two ochre suppressors were co-chromatographed. The RPC-5 profile

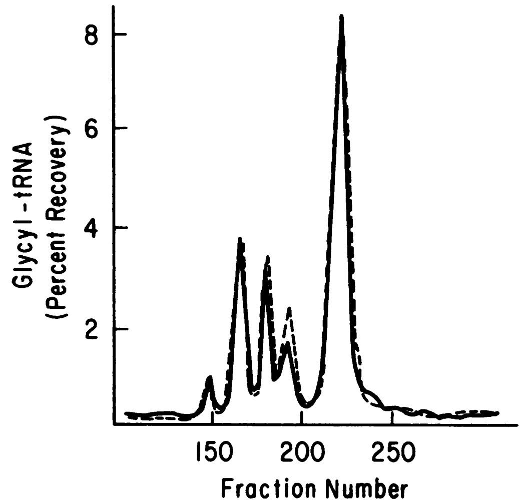

FIG. 3. *Chromatography, on RPC-5, of glycyl-tRNA from Su46-27 (solid line) with glycyl-tRNA from SuUAA/G-27 (dashed line).*

difference between these two ochre suppressors provides further support for the conclusion that Su46-34 and Su46-27 are due to different mutational changes.

Coexistence with *glyT56*. Since our results indicated that all 18 suppressors are derived from *glyV55* tRNA, which reads GGA (and also GGG), it was of interest to ask whether these suppressors retained any ability to respond to GGA as well as having gained the ability to respond to GAA. A genetic approach to the question involved constructing the strain we designate as CH527A46, since it is derived in two steps from CH527 (4). The relevant genetic markers are *glyT56, glyV55,* and *trpA46*. *glyT56*, which is derived from *glyT* tRNA (22, 23) and responds to AGA, is a haplolethal suppressor of *trpA36* (Gly[GGA] → Arg[AGA]). It has been shown by others (4) and by us (unpublished data) that this suppressor is deficient in its ability to read GGA, and that its presence in a haploid genome is lethal unless it is accompanied by *glyV55* tRNA.

Phage P1 lysates made on each Su46 were used to attempt to transduce CH527A46 to Trp^+, that is, to introduce each Su46 into the *glyT56*-containing strain. The transduction was plated on glucose minimal medium and also on glucose minimal plus ACH. In such a transduction, Trp^+ transductants that occur should be due to introduction of an Su46 into the recipient, and it is expected that the *glyV55* will be crossed out simultaneously. Since the viability of the *glyT56*-containing recipient depends on the presence of *glyV55* tRNA, no transductants were expected unless a particular Su46 retained some

ability to respond to GGA. The outcome was that Trp$^+$ transductants were obtained, on minimal medium, with 17 of the 18 suppressors (all but Su46-27). All of the colonies retained *glyT56*, as judged by suppression of a phage T4 AGA mutant (20), and the transductant frequencies were not abnormal (although the colonies took 4 to 5 days to come up). This result, repeated several times for many of the suppressors, suggests that all but Su46-27 retain some ability to respond to GGA. That the GGA response is limited, however, is indicated by the failure of those 17 suppressors to yield transductants on ACH. Furthermore, the colonies obtained on minimal medium were shown to be rich medium sensitive (see reference 4 for discussion of a similar and related situation).

Chromatography of tRNA from a strain containing both *glyT56* and Su46-34, on BDC columns as described previously (15), showed no evidence of a *glyV55* peak. This result rules out the possibility of a duplication having occurred, allowing the introduction of Su46-34 and the retention of *glyV55*. It also rules out a *glyV55*-type mutation occurring in another *glyV* gene.

To provide further evidence that the ability of the 17 suppressors to coexist with *glyT56* has nothing to do with the transduction event, the attempt was made to construct double suppressor strains nonselectively. The strains *argE* (*glyT*$^+$) *glyV*(Su46-34) *trpA46* and *argE* (*glyT*$^+$) *glyV*(Su46-27) *trpA46* were transduced to Arg$^+$ with a lysate grown on an *argE*$^+$ *glyT56* strain (*argE* and *glyT* are approximately 50% cotransducible). When the transductants were tested for suppression of the T4 AGA mutant, the presence of *glyT56* was verified in some Arg$^+$ derivatives of the Su46-34 strain (but not in any of the Su46-27 Arg$^+$ colonies).

In the previous section, we described an ochre suppressor that was derived, presumably by a single mutation, from Su46-34. This ochre suppressor could not be introduced by transduction into the strain *argE*(Am) *glyT56 glyV55* Δ*(tonB-trp)*/F′*trpA*(UAA211), selecting for Arg$^+$ (that is, suppression of the amber *argE*). By using the same P1 lysate, however, the ochre suppressor could be introduced into an isogenic strain that was *glyT*$^+$ instead of *glyT56*. As a further control, the transduction was also performed by using a P1 lysate made on a strain containing *supD83*, an ochre suppressor derived from a serine-inserting amber suppressor (14). This ochre suppressor was able to be introduced into the *glyT56* recipient as well as into the *glyT*$^+$ recipient. These results support the conclusion that the residual GGA response of the Su46-34 strain is indeed a property of the suppressor tRNA itself and that this was lost (as would be expected) when the Su46 was converted to SuUAA/G.

DISCUSSION

This paper presents the results of a systematic continuation of the characterization of 18 suppressors of glutamic acid codons. We have located *glyV* more precisely on the *E. coli* genetic map and found it to be approximately 68% cotransducible with *purA*. All 18 suppressors were shown to be similarly linked to *purA*. Chromatographic analysis (RPC-5) of the glycyl-tRNA from each suppressor strain clearly demonstrated an alteration of the *glyV55* peak. Furthermore, each suppressor was shown to be dominant in a strain diploid for the *glyV* region. These results support the conclusion that all 18 suppressors are due to mutations in *glyV55*, which is itself derived mutationally from *glyV*.

Isogenic strains containing each suppressor have been found to exhibit differences according to growth rates in different media, suppression of the GAG codon, tRNA profiles after RPC-5 column chromatography, and coexistence with *glyT56*. These studies indicate that the suppressors represent several different classes, which may correspond to at least as many different nucleotide changes. Assuming that each suppressor is due to a single mutational event, it is likely that most of the changes (perhaps all but one) have occurred outside the anticodon of *glyV55* tRNA. If so, then nucleotide sequence analysis of the mutant tRNA's should be very enlightening regarding the involvement of tRNA tertiary structure in the determination of coding specificity.

We have shown that 17 of the 18 suppressors are able to coexist in a haploid genome with a *glyT* suppressor that is devoid of GGA-reading ability. This result indicates the retention by those GAA suppressors of some ability to respond to GGA. We presented evidence that our ability to obtain the double suppressor strains is not due to new events occurring during the transduction, and that the GGA response is indeed a property of the suppressor tRNA. Recently, we have been able to induce, with both hydroxylamine and ethyl methane sulfonate, *glyV*-derived GAA suppressors in a strain containing *glyT56* (E. J. Murgola et al., manuscript in preparation).

The ability of the 17 *glyV* suppressors to respond to GAA and GGA suggests the possibility of "wobble" in the middle position of the anticodons of those tRNA's. The anticodon of *glyV55* tRNA is UCC. We would like to suggest the possibility that Su46-27, which cannot coexist with *glyT56*, is due to the expected C → U change in the middle position of the anticodon

and that the other 17, which appear to be able to wobble, reading A or G in the middle position of the codon, are due to nucleotide changes outside the anticodon. There is a well-studied mutant tRNA in which a change outside the anticodon affects the coding specificity: the *trpT*-derived UGA suppressor reported by Hirsh (12). That case is particularly relevant to the GAA suppressors since the change outside the anticodon allows an anticodon C to pair with A or G in the codon. An important difference, however, is that in such a proposal for the present cases, the unorthodox wobble would be occurring at a position in which it was hypothesized that no wobble would occur (5). And yet, it may be that pairings thought of as "illicit" in wild-type cells are indeed possible if an appropriate alteration occurs in tRNA (or ribosome) structure (13).

ACKNOWLEDGMENTS

For excellent technical assistance, we are indebted to Maggie Jackson and Betsy Mims. For gifts of bacterial strains, we are grateful to Charles Yanofsky, Jeffrey Miller, and Barbara Bachmann.

Portions of this work were supported by grants to E.J.M. from the American Cancer Society (NP-167) and Public Health Service (GM 21499, from the National Institute of General Medical Sciences) and aided by Public Health Service institutional grant RR5511 from the Division of Research Resources. K.H.H. was the recipient of a Rosalie B. Hite Predoctoral Fellowship. 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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