*MISSENSE SUPPRESSION OF THE TRYPTOPHAN SYNTHETASE A-PROTEIN MUTANT A78**

By Naba K. Gupta and H. Gobind Khorana

INSTITUTE FOR ENZYME RESEARCH OF THE UNIVERSITY OF WISCONSIN, MADISON

Communicated June 23, 1966

Certain suppressor mutations are known which lead to the formation of a functional enzyme in a mutant strain which normally produces an inactive protein.[1] This type of suppression, missense suppression, has been shown to result in the replacement of one amino acid by another in a small fraction of the protein molecules.[2] Thus both the inactive mutant protein and an active enzyme resembling the wild-type protein are produced by the suppressed mutant. For example, mutant A78 of *E. coli* produces an inactive tryptophan synthetase A protein which has a particular glycine residue replaced by cysteine.[3] A suppressed mutant, strain A78-su78, has been isolated in which A-protein activity is restored to the extent of about 2 per cent that of the wild-type A protein.[4] It has been suggested that missense suppression may result from the alteration of one of the cellular components involved in translation, such that the "incorrect" amino acid is occasionally incorporated in response to a given sense codon. Specifically, in the case of strain A78-su78, missense suppression is believed to involve the insertion of glycine in response to a cysteine codon (UGU or UGC).

Synthetic ribopolynucleotides containing two nucleotides in alternating sequence have been shown to direct the *in vitro* formation of specific copolypeptides containing two amino acids in alternating sequence.[5, 6] Thus, for example, poly UG[7] directs the synthesis of a valine-cysteine copolypeptide in a cell-free amino acid incorporating system from *E. coli* B.[6] If suppression resulted in the occasional misreading of one of the codons in these ribopolynucleotides such that an incorrect amino acid were incorporated, it should be demonstrable in the cell-free amino acid-incorporating system, and the nature of the altered component of the translation apparatus could thus be specified. At the suggestion and encouragement of Dr. Charles Yanofsky, we have investigated the nature of the change in the suppressed strain A78-su78. The results, which are reported herein, show that poly UG does, in fact, direct the synthesis of a valine-glycine copolypeptide when the amino acid-incorporating system from the B strain of *E. coli* is supplemented specifically by tRNA from the suppressed mutant A78-su78.

A similar result has been obtained by Carbon, Berg, and Yanofsky[8] with a different suppressed mutant, A36-su36. These authors found that poly AG,[7] which normally directs the synthesis of a glutamic acid-arginine copolypeptide,[6] directs the incorporation of C^{14}-glycine in the presence of glutamic acid when tRNA from the suppressed mutant is added to the amino acid-incorporating system from the nonsuppressed strain. Previously, tRNA has been shown to be responsible for suppression of an amber codon in the genetic material specifying a bacteriophage coat protein.[9, 10]

Materials and Methods.—Poly d(TG:CA)[7] was prepared[11] and generously supplied by Dr. R. D. Wells. Uniformly labeled C^{14}-glycine (66 μc/μmole) and C^{14}-valine (185 μc/μmole) were commercial products. Unlabeled ribonucleoside triphosphates were purchased from Pabst Labora-

tories. Valylglycine and related di- and tripeptides were obtained from Mann Research Laboratories.

Cultures and culture procedures: Mutant A78 was produced by ultraviolet irradiation of the Ymel stock of the K-12 strain of *E. coli*.[3] Suppressed mutant A78-su78 was isolated as a slow-growing prototroph arising from A78. Genetic tests established that prototrophy was due to suppression. Mutants and suppressed mutants were grown on minimal medium[12] and harvested in late log phase. Cell pastes of mutant A-78 and of suppressed mutant A78-su78 were generously supplied by Dr. Yanofsky.

Preparation of ribosomes and the supernatant fraction from E. coli B: *E. coli* B ribosomes and dialyzed 100,000 *g* supernatant fractions were prepared by a modification of the procedure described by Nishimura *et al.*[13] To the *S*30 supernatant solution was slowly added solid ammonium sulfate (0.14 gm/ml) as described by Wood and Berg.[14] During the addition of solid ammonium sulfate the solution was stirred and the pH of the solution was adjusted to 7.0 by the dropwise addition of 1 *M* ammonium hydroxide. After stirring for 10 min the suspension was centrifuged at 20,000 *g* for 20 min. The supernatant fraction was centrifuged at 100,000 *g* for 2 hr. The ribosomal pellet thus obtained was suspended gently in 2 *M* KCl, 0.03 *M* magnesium acetate, 0.006 *M* β-mercaptoethanol and 0.01 *M* Tris-HCl pH 7.8 overnight at 4°C. The suspension was then gently dispersed and the solution centrifuged at 10,000 *g* for 10 min to remove any insoluble material. The ribosomes were then sedimented at 100,000 *g* for 2 hr and the pellet was suspended in the standard buffer and the suspension stored frozen in liquid nitrogen.

The 100,000 *g* supernatant fraction obtained above after 2 hr centrifugation was again centrifuged at 100,000 *g* for 6 hr. Approximately $^2/_3$ of the supernatant fraction was carefully pipetted off and dialyzed against standard buffer for 20 hr at 2°C with three changes of the buffer. The dialyzed solution was stored in liquid nitrogen.

Preparation of RNA polymerase: The RNA polymerase preparation was fraction 4 in the procedure of Chamberlin and Berg.[15] The preparation incorporated 2750 mμmoles of C^{14} CTP/hr/mg, using calf thymus DNA as the template under the assay conditions of Chamberlin and Berg.[15]

Preparation of tRNA: *E. coli* B tRNA, prepared according to Zubay's method,[16] was kindly supplied by Dr. D. S. Jones. A36-su36 tRNA was a gift from Dr. P. Berg; A78 tRNA and A78-su78 tRNA were prepared by a modification of Zubay's method. The preparation was carried through to the second ethanol precipitation step as described by Zubay.[16] The precipitate was dissolved in 0.3 *M* sodium acetate and *iso*propyl alcohol fractionation was carried out as described. The tRNA fraction was dissolved in 0.1 *M* Tris HCl, pH 9.0, and the solution was incubated at 37° for 1 hr. The solution was further purified by chromatography on a DEAE-cellulose column in 0.02 *M* Tris HCl, pH 7.4.

Paper chromatography: Descending technique with Whatman 3 MM paper was used. The solvent systems used were solvent I, *n*-butanol–water–acetic acid (4:5:1); solvent II, ethanol–water (77:23). The R_f values for glycine and the peptides in solvent I were gly, 0.19; gly-gly, 0.18; val-gly, 0.37; gly-val, 0.40; val-gly-gly, 0.28; gly-gly-val, 0.41.

Assay of polypeptide synthesis: The two-step reaction procedure described by Jones *et al.*[6] was used for cell-free polypeptide synthesis. The components of the reaction mixture in step 1 were the same as described before. The reaction mixture in step 2 contained per ml 42 μmoles Tris-HCl (pH 7.8), 7 μmoles of magnesium acetate, 1.25 μmoles of magnesium chloride, 12 μmoles of β-mercaptoethanol, 0.3 μmole of manganese chloride, 27 μmoles of potassium chloride, 0.2 μmole of GTP, 1.6 μmoles of ATP, 4 μmoles of phosphoenolpyruvate, 6 μg of phosphoenolpyruvate kinase, 22.5 OD_{260} units of ribosomes, 20–30 OD_{260} units of tRNA, 2.5 μc of C^{14} amino acids as specified, 125 mμmoles of C^{12} amino acids where indicated, and poly d(TG:AC), poly UG, excess nucleoside triphosphates, and RNA polymerase as introduced from the first stage of the reaction. The reaction mixture was incubated at 0°C for 10 min and then 320 μg of 100,000 *g* supernatant fraction was added. Incubation was carried out at 37°C and aliquots (50 μl) of the reaction mixture were removed and assayed for C^{14}-incorporation as described by Jones *et al.*[6]

Large-scale preparation of C^{14}-labeled copolypeptide: The first-stage reaction mixture contained in 0.36 ml: 165 mμmoles each of UTP and GTP, 100 mμmoles of poly d(TG:AC), and 0.75 mg of RNA polymerase in addition to the other components as described above. The incubation was carried out at 37° for 1 hr. After the first-stage reaction, 2.5 μc of C^{14}-glycine and 25 OD of either A78 tRNA or A78-su78 tRNA were added in addition to other components described

above, in a total volume of 1.25 ml. After incubation for $3^1/_2$ hr at 37°, the reaction mixtures were chilled in ice and 12 μl of 10 N sodium hydroxide was added. The solution was allowed to stand at room temperature for 1 hr. The total protein fraction was then precipitated by the addition of 60 μl of 100% trichloroacetic acid. The precipitate was washed 3 times with cold 5% trichloroacetic acid, twice with ethyl alcohol–ether (1:1) and twice with ether.

Characterization of polypeptidic product: Acidic hydrolysis and Edman degradation of C^{14}-valylglycine dipeptide: The C^{14}-labeled product obtained in the large-scale preparation was dissolved in 0.5 ml of 10 N HCl, and the solution was incubated at 37 or 50°C. Aliquots were spotted on Whatman no. 3 paper along with appropriate markers. After development in solvent I, the chromatograms were cut into $^1/_2$-in. pieces and the strips counted in a liquid scintillation spectrometer. The radioactive spot at the position corresponding to the valylglycine dipeptide was eluted from the paper and the eluate (700 cpm) evaporated to dryness. The residue was dissolved in 0.1 ml and to it was added 0.2 ml of a 5% solution in pyridine[17] of phenylisothiocyanate, and the resulting solution was kept overnight at room temperature. After addition of 0.2 ml of water, excess phenylisothiocyanate was removed by four extractions with 3 ml of benzene, and a portion (0.1 ml) of the aqueous solution was applied to a paper chromatogram. The remainder of the aqueous solution was evaporated and 0.2 ml of acetic acid saturated with anhydrous hydrogen chloride was added. After 1 hr at room temperature, the acid was removed *in vacuo* and 50 mμ-moles of cold glycine in 1 ml of water was added to the residue. The solution was evaporated to dryness and the residue was dissolved in 0.1 ml water and applied to paper chromatograms alongside a glycine marker. The chromatogram was developed in solvent I.

Results.—Comparison of tRNA from E. coli strains A78 and A78-su78 for poly UG-directed synthesis of valine-cysteine copolypeptide: The results shown in Figure 1 provide, in part, a confirmation of the previous finding of Jones, Nishimura, and Khorana[6] in that the incorporation of C^{14}-valine is dependent on the presence of poly UG and cysteine. The incorporation of C^{14}-valine is seen to be dependent on the addition of tRNA to the amino acid-incorporating system used in the present experiments. The results in Figure 1 also show that equivalent amounts of tRNA's prepared from strains A78 and A78-su78 are indistinguishable in their capacity to stimulate the incorporation of C^{14}-valine.

Comparison of different tRNA preparations for C^{14}-glycine incorporation: Poly UG specifically directs the incorporation of valine and cysteine only.[6] When C^{14}-glycine was tested in the presence of the remaining 19 cold amino acids, no

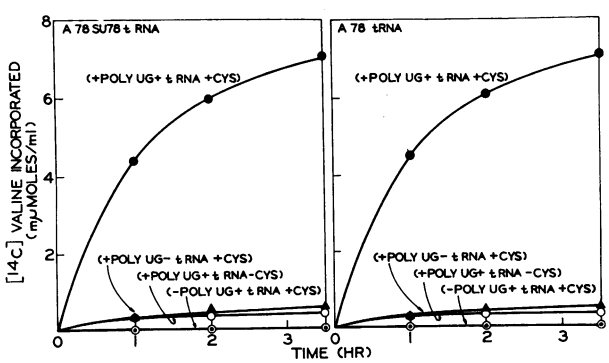

FIG. 1.—Characteristics of the incorporation of C^{14}-valine in the presence of A78-su78 tRNA and A78 tRNA. The reaction mixture at the first stage contained, per ml, 0.62 mg of RNA polymerase, 20 mμmoles of poly d(TG:AC), 0.33 μmole of UTP, and 0.33 μmole of GTP. Incubation was at 37° for 1 hr. These reaction mixtures were taken to the second stage as described in *Materials and Methods*. In the second-stage reaction 13.5 mμmoles of C^{14}-valine (185 μc/μmole), 125 mμmoles cold cysteine, 10 OD tRNA were added where indicated.

incorporation was previously detected. In the present experiments, C^{14}-glycine incorporation was tested in the presence of tRNA preparations from different sources. The results in Figure 2 show that a clear stimulation of C^{14}-glycine incorporation was given by A78-su78 tRNA. This incorporation was dependent on the presence of cold valine and poly UG. Some glycine incorporation was also observed in the absence of cold valine. As discussed below, the nature of this incorporation is not understood. The results summarized in Table 1 show that of the various tRNA preparations tested, only A78-su78 tRNA and A36-su36-su78 tRNA were active in valine-glycine copolypeptide synthesis. The *E. coli* strain A36-su36-su78 has previously been shown to contain two suppressor genes, one specific for mutant A36 and the other specific for mutant A78.[18] Carbon *et al.*[8] have shown that the tRNA preparation from this strain is also active in glutamic acid-glycine copolypeptide synthesis under the direction of poly AG.

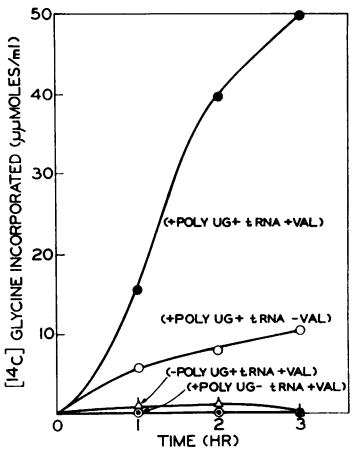

FIG. 2.—Characteristics of the incorporation of C^{14}-glycine in the presence of A78-su78 tRNA preparations. The reaction mixtures at the first stage were the same as described in Fig. 1. In the second-stage reaction 37.5 mμmoles C^{14}-glycine, 125 mμmoles cold valine, 20 OD A78-su78 tRNA were added.

Characterization of the C^{14}-glycine-valine copolypetide: (a) *Acidic hydrolysis:* Acid-insoluble polypeptides containing C^{14}-glycine were prepared as described under *Materials and Methods.* The tRNA used was either from A78-su78 or from A78, and both the incubations were in the presence and absence of cold valine. The radioactive polypeptides obtained were incubated in 10 *N* HCl at 37° and the products were separated andanalyzed for radioactivity as described above. Figure 3 shows only the results obtained after acidic hydrolysis for 15 days. The C^{14}-products obtained using tRNA from A78-su78 but no valine (Fig. 3*A*) or using tRNA from A78 with and without valine (Fig. 3*C* and *D*) all released the total of radioactivity in the form of a peak corresponding to glycine. The appearance of this peak was rapid, the maximum being reached within 1 day at 37°. The poly-

TABLE 1

COMPARISON OF DIFFERENT PREPARATIONS FOR POLY UG-STIMULATED C^{14}-GLYCINE INCORPORATION IN THE PRESENCE OF COLD VALINE

tRNA added	μμMoles C^{14}-glycine incorporated/ml incubation mixture
A78-su78	41.4
A36-su36-su78	18.2
A78	2.0
E. coli B	0
A36-su36	0

The reaction conditions were the same as described in Fig. 2. Equivalent amounts of different tRNA preparations were added at the second stage of the reaction in addition to the other components. C^{14}-glycine incorporation in the absence of poly UG was subtracted as background. Incubation time was 2 hr.

TABLE 2

EFFECT OF CYSTEINE ON POLY UG-STIMULATED C^{14}-GLYCINE INCORPORATION IN THE PRESENCE OF COLD VALINE

tRNA used	Additions	μμMoles C^{14}-glycine incorporated/ml
A78-su78	None	56
A78-su78	Cysteine	43.8
A78	None	5.8
A78	Cysteine	43.5

The reaction conditions were the same as described in Fig. 2. Where indicated, cold cysteine (125 mμmoles/ml) was added. Incubation time was 3½ hr at 37°.

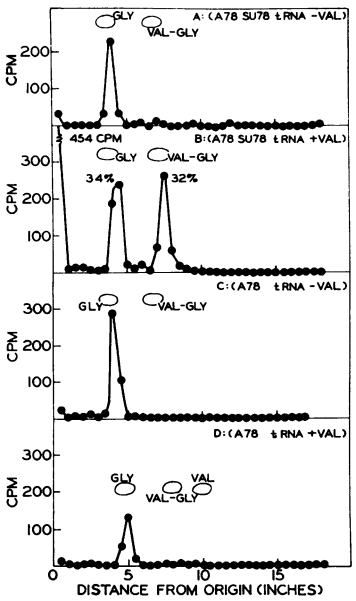

FIG. 3.—Characterization of C^{14}-glycine peptides formed in the presence of A78-su78 tRNA and A78 tRNA. The polypeptide was dissolved in 10 *N* HCl and incubated at 37° for 15 days. An aliquot was then spotted on Whatman no. 3 paper and developed in solvent I. The chromatograms were cut in $^1/_2$-in.-wide strips and counted for radioactivity in the scintillation counter.

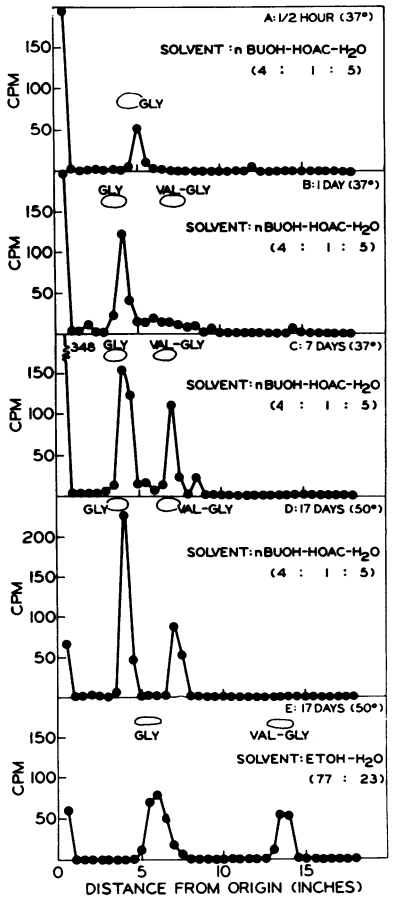

FIG. 4.—Kinetics of acidic hydrolysis of C^{14}-glycine peptide formed in the presence of A78-su78 tRNA and valine. The polypeptide was kept in 10 *N* HCl and portions were removed and chromatographed in two solvent systems as indicated. Time and temperature of incubation conditions are shown in the figure.

peptidic products obtained in the presence of tRNA from A78-su78 and valine gave the glycine peak, but, in addition, this incubation mixture was *unique* in that the major portion of the radioactivity appeared in the form of a product which slowly hydrolyzed to form a dipeptide with the R_f of markers valylglycine and glycylvaline. Kinetics of acidic hydrolysis of C^{14}-glycine-containing products obtained in the presence of tRNA from A78-su78 and cold valine are further shown in Figure 4. The patterns in Figure 4*D* and *E* are those of another sample similarly prepared but incubated at 50° for 17 days. The use of two different solvent systems (Fig. 4*D* and *E*) further demonstrates the formation of a product different from glycine and identical in R_f value with the marker dipeptide, valylglycine. It should be added that under the prolonged incubation periods used, the radioactivity in the

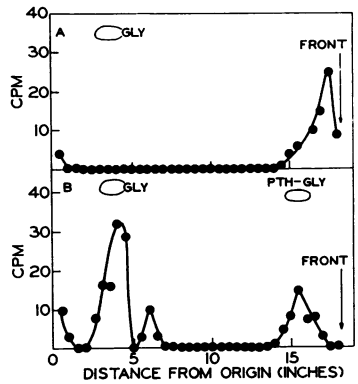

FIG. 5.—Characterization of C^{14}-valyl-glycine dipeptide by Edman degradation. (*A*) Product of reaction of valyl-glycine dipeptide with phenyl*iso*thiocyanate. (*B*) Products released after thiohydantoin step in Edman degradation. Paper chromatography was in solvent I.

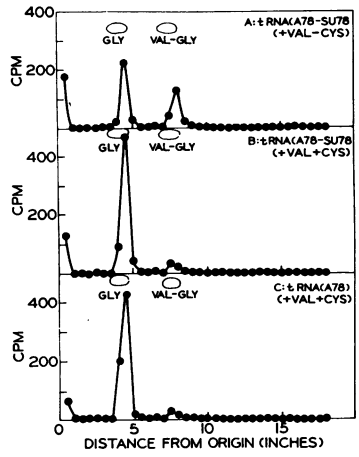

FIG. 6.—Effect of cysteine on the nature of C^{14}-glycine peptide formation in the presence of A78 tRNA and A78-su78 tRNA. The polypeptide formed in the presence or absence of cysteine was dissolved in 10 *N* HCl and incubated at 50° for 5 days. An aliquot was then spotted on Whatman no. 3 paper and developed in solvent I.

C^{14}-glycine peak continued to increase steadily. Under these conditions an authentic sample of valylglycine was found to undergo considerable hydrolysis, and it is therefore likely that the radioactive material at the origin was first converted to valine-glycine dipeptide and/or oligopeptides, which, in turn, hydrolyzed to free amino acids.

(b) *Edman degradation:* The above radioactive product with the R_f of valyl-glycine was reacted with phenylisothiocyanate and the resulting product was chromatographed in solvent I. The pattern of radioactivity (Fig. 5*A*) showed the expected formation of a compound with an R_f much higher than that of the starting material. This derivative was treated with acid and the product was chromatographed in the same solvent. The results are presented in Figure 5*B*. Approximately 65 per cent of the total radioactivity appeared in the form of free glycine, indicating that at least 65 per cent of the original dipeptide consisted of valyl-C^{14}-glycine.[19] The formation of a second radioactive product traveling near the front was also noted. This presumably was phenylthiohydantoin of C^{14}-glycine and could have originated from C^{14}-glycylvaline.

Inhibition of valine-glycine copolypeptide synthesis by cysteine: Preparations of tRNA from A78-su78 support the synthesis of a valyl-cysteine copolypeptide (in the presence of valine and cysteine) and of a valyl-glycine copolypeptide (in the presence of valine and glycine). It was of interest to examine the effect of cysteine on C^{14}-glycine incorporation in the presence of valine. The results of isotope incorporation experiments (Table 2) using A78-su78 sRNA showed about 20 per cent inhibition of C^{14}-glycine incorporation in the presence of cold cysteine. With A78 tRNA, a severalfold increase of C^{14}-glycine incorporation on addition of cysteine

was actually observed. Because of the above-described incorporation of glycine into material other than a valine-glycine copolypeptide, the C^{14}-labeled products (experiments of Table 2) were further analyzed by acidic hydrolysis. The results shown in Figure 6 indicate that the increased incorporation of C^{14}-glycine occurred in a form that rapidly released free glycine. Cysteine markedly inhibited C^{14}-glycine incorporation into the valine-glycine copolypeptide in the presence of A78-su78 tRNA.

Discussion.—The studies in this paper clearly show that the suppressed mutant, A78-su78, but not its parental strain, A78, contains an altered tRNA which brings about the incorporation of glycine in response to a codon for cysteine. Thus when poly UG, which contains alternating cysteine (UGU) and valine (GUG) codons, is used as the messenger in the *in vitro* amino acid-incorporating system, glycine is incorporated in the presence of valine and A78-su78 tRNA. By characterization of the polypeptidic product formed, the above incorporation has been demonstrated to occur in positions adjacent to valine. This substitution of glycine in place of cysteine accounts for the formation of a functional A protein by the suppressed mutant, since the change responsible for the inactivity of the A protein in A78 mutant is from glycine → cysteine. It is also clear from the *in vitro* data obtained in this study that in the presence of poly UG, tRNA preparations from the suppressed mutant incorporate glycine poorly in the presence of valine, compared with cysteine incorporation in the presence of valine. This observation is consistent with *in vivo* data which suggest that only a small fraction of the A-protein molecules produced by the suppressed mutant have glycine at the critical position.

The data obtained in this study show that the addition of cold cysteine inhibited the formation of the valine-glycine copolypeptide. While in these experiments unfractionated tRNA was used, further experiments have been carried out using fractionated tRNA species. Countercurrent distribution of A78-su78 tRNA yields an enriched (at least tenfold) suppressor tRNA preparation, which is well resolved from the bulk of glycine-tRNA and cysteine-tRNA.[20] Addition of cold cysteine and cysteine-tRNA again inhibited the suppressor tRNA activity as measured by poly UG-directed incorporation of C^{14}-glycine in the presence of cold valine and valine-tRNA. From these results, it may tentatively be concluded that the altered A78-su78 tRNA competes with normal cysteine-donating tRNA and that, therefore, the presence of an excess of cysteine competitively inhibits the incorporation of glycine.

In their study of the *in vitro* suppression of the A36 change (glycine→arginine), Carbon *et al.* observed that the addition of arginine had no effect on glutamic acid-glycine copolypeptide synthesis under the direction of poly AG. This result is in contrast with the inhibition herein described of valine-glycine copolypeptide synthesis upon the addition of cysteine. Further studies are clearly necessary to explain the different results obtained in the two systems.

While the involvement of tRNA in missense suppression in A78-su78 and A36-su36[8] is firmly established, the origin of the altered tRNA in the suppressed mutants remains unknown. The most likely possibilities for A78-su78 tRNA are that it is a glycine tRNA with a change in its anticodon, or it is an altered cysteine tRNA which now accepts glycine in place of cysteine. Whatever the nature of the change, it would appear that a very minor species of tRNA is involved since the counter-

current distribution studies referred to above have shown that the suppressor tRNA constitutes a very small fraction of the total glycine acceptor activity present in strain A78-su78.

Summary.—Poly UG, which contains uridylate and guanylate nucleotides in alternating sequence, has previously been shown to direct the synthesis of a valine-cysteine copolypeptide in the cell-free amino acid-incorporating system prepared from *E. coli* B. The same messenger is now shown to direct the synthesis of a copolypeptide of valine and glycine in the presence of valine and glycine when the standard incorporating system from *E. coli* B is supplemented by transfer RNA from the missense suppressed strain A78-su78. Missense suppression in this strain of *E. coli* is thus shown to operate at the level of transfer RNA.

* This is paper LVIII in the series "Studies on Polynucleotides." Paper LVII is by Söll, D., D. S. Jones, E. Ohtsuka, R. D. Faulkner, R. Lohrmann, H. Hayatsu, H. G. Khorana, J. D. Cherayil, A. Hampel, and R. M. Bock, *J. Mol. Biol.*, in press.

[1] Yanofsky, C., D. Helinski, and B. Maling, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 26 (1961), p. 11.

[2] Brody, S., and C. Yanofsky, these PROCEEDINGS, **50**, 9 (1963).

[3] Guest, J. R., and C. Yanofsky, *J. Biol. Chem.*, **240**, 679 (1965).

[4] Guest, J. R., and C. Yanofsky, *J. Mol. Biol.*, **12**, 793 (1965).

[5] Nishimura, S., D. S. Jones, and H. G. Khorana, *J. Mol. Biol.*, **13**, 302 (1965).

[6] Jones, D. S., S. Nishimura, and H. G. Khorana, *J. Mol. Biol.*, **16**, 454 (1966).

[7] Poly UG and poly AG are the abbreviations for ribopolynucleotides containing, respectively, uridylate and guanylate units, and adenylate and guanylate units in alternating sequence. Poly d(TG:CA) is the abbreviation for double-stranded DNA-like polymer, one strand of which contains alternating thymidylate and deoxyguanylate units and the complementary strand of which contains alternating deoxycytidylate and deoxyadenylate units.

[8] Carbon, J., P. Berg, and C. Yanofsky, these PROCEEDINGS, **56**, 764 (1966).

[9] Capecchi, M. R., and G. N. Gussin, *Science*, **149**, 417 (1965).

[10] Engelhardt, D. L., R. E. Webster, R. C. Wilhelm, and N. D. Zinder, these PROCEEDINGS, **54**, 1791 (1965).

[11] Wells, R. D., E. Ohtsuka, and H. G. Khorana, *J. Mol. Biol.*, **14**, 221 (1965).

[12] Vogel, H., and D. M. Bonner, *J. Biol. Chem.*, **218**, 97 (1956).

[13] Nishimura, S., D. S. Jones, E. Ohtsuka, H. Hayatsu, T. M. Jacob, and H. G. Khorana, *J. Mol. Biol.*, **13**, 283 (1965).

[14] Wood, W. B., and P. Berg, these PROCEEDINGS, **48**, 94 (1962).

[15] Chamberlin, M., and P. Berg, these PROCEEDINGS, **48**, 81 (1962).

[16] Zubay, G., *J. Mol. Biol.*, **4**, 317 (1962).

[17] Gray, W. R., and B. S. Hartley, *Biochem. J.*, **89**, 379 (1963).

[18] Yanofsky, C., unpublished observations.

[19] This result is in accord with the theoretical expectation that a copolypeptide containing alternating valine and glycine residues would undergo preferential acidic hydrolysis at glycine sites rather than at the sterically hindered valine sites. Thus valylglycine would be the main expected dipeptidic product.

[20] Gupta, N. K., U. L. RajBhandary, and H. G. Khorana, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31 (1966), in press.