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Participation of Redundant Transfer Ribonucleic Acids from Yeast in Protein Synthesis*

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ABSTRACT: Four glycine tRNAs have been isolated from brewers yeast. They appear to recognize the same code word but are known to be structurally different. In the present experiments, each glycine tRNA has been precharged with radioactive glycine and has been used in the *in vitro* synthesis of protein directed by bacteriophage R17 RNA in an *Escherichia coli* S-30 system that was dependent on added transfer ribonucleic acid (tRNA) for maximal activity. Preliminary experiments established that the yeast glycyl-tRNAs transferred their amino acid into the same proteins that were synthesized when *E. coli* tRNA was used. Large-scale preparations fractionated on sucrose gradients allowed the isolation of the coat protein subunits synthesized *in vitro*.

Thin-layer mapping of tryptic digests of protein synthesized *in vitro* has shown that for the most part, the glycine code words are translated with considerable fidelity by the yeast glycyl-tRNAs. The yeast tRNAs show a characteristic pattern of competition between themselves, and also when compared to *E. coli* $tRNA^{Gly}$ when used in pairs for the synthesis of coat protein. One of the peptides in the R17 coat has more glycine incorporated into it from yeast $tRNA^{Gly}$ than would be expected from the known composition of the peptide. It appears likely that a code word for another amino acid in the R17 RNA is being translated as glycine by the yeast, but not the *E. coli*, tRNAs. These observations have formal similarities to reports of suppression of missense being brought about by tRNA.

Results from several laboratories have shown that the genetic code is highly degenerate (Nirenberg *et al.*, 1965; Söll *et al.*, 1965, 1966). Fractionation of tRNA has demonstrated the existence of more than one acceptor RNA for many amino acids (for review, see Miura, 1967). A number of workers have reported that degenerate tRNAs recognize distinct code words (Weisblum *et al.*, 1962, 1965; von Ehrenstein and Dais, 1963; Bennett *et al.*, 1965; Gonano, 1967).

Recognition of synonym code words by separable amino acid specific RNAs also has been demonstrated (Kellog *et al.*, 1966; Söll *et al.*, 1967; Söll and RajBhandary, 1967). The basis of synonym code word recognition almost certainly residues in the codon–anticodon relationship of m- and tRNAs. It is not known how a single tRNA is able to recognize more than one triplet, although a theoretical basis for the phenomenon has been suggested by Crick (1966).

If many tRNAs show multiple codon recognition it would be anticipated that a large number of species would not be required for recognition of all the sense code words. In fact, evidence from fractionation studies shows that the number of separable tRNA species may be large (Bergquist *et al.*, 1965; Söll *et al.*, 1966; Gillam *et al.*, 1967). The multiplicity of tRNAs specific for a single amino acid appears to arise from two causes. Degenerate species are able to recognize the synonym triplets in the genetic code and differ minimally in the sequence of their anticodon triplets. It is reasonable to assume that no more than 64 degenerate tRNA species could exist.

Acceptor RNA species that respond to the same code word but are structurally different have been termed redundant (Söll *et al.*, 1966; Bergquist, 1966) and, theoretically, their number may be limited only by the pos-

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sible combinations of base sequences that allow recognition of a tRNA by the various enzymes involved with it in protein synthesis.

The role of redundant tRNAs in the cell is unknown. It has been suggested that redundant tRNAs may play a role in genetic suppression (Söll *et al.*, 1966). The occurrence of redundant species of tRNA may be related to the existence of multiple copies of particular tRNA cistrons in the genome of an organism. Presumably, mutations have occurred which have altered the primary sequences of the DNA except that coding for the anticodon or enzyme recognition sites of the tRNAs.

We have reported the isolation of four species of glycine-acceptor RNA and lysine-acceptor RNA from yeast (Bergquist, 1966). At least some of these species were able to recognize the same code words but were demonstrably different in primary structure. The purpose of this communication is to report on the ability of the redundant glycine tRNAs to translate the code words for glycine that occur in a natural mRNA. Our results show that yeast tRNAs may be used in the translation of R17 RNA *in vitro*. Each one of the redundant glycine tRNAs when used by itself recognized all glycine codons in the message for R17 coat protein. They compete with each other and with *Escherichia coli* $tRNA^{Gly}$ when used in combination. Examination of the incorporation of glycine into tryptic peptides from the coat protein synthesized *in vitro* suggests that glycine may be substituted for another, as yet unknown, amino acid when transferred into protein from $tRNA^{Gly}_{(yeast)}$.

Methods

Preparation of tRNA. tRNA was prepared from mid-log phase *E. coli* S26Rle (an su^+ K12 strain obtained from M. Capecchi) by a phenol procedure modified from Capecchi (1966) as follows. (1) The cells were washed twice in 0.01 M Tris acetate–0.01 M magnesium acetate buffer (pH 7.8) (TM buffer); (2) the washed cells were resuspended in TM buffer and extracted with distilled phenol saturated with buffer; (3) the 1 M NaCl extraction of the crude RNA pellet was carried out only once; and (4) the tRNA was purified on DEAE-cellulose and then stripped of esterified amino acids by the method of Sarin and Zamecnik (1964).

Brewers yeast tRNA was prepared and fractionated on partition columns as previously described (Bergquist *et al.*, 1965). The fractions enriched for glycine-acceptor activity were purified further on columns of DEAE-cellulose operated at high temperature (Bergquist, 1966). The four glycine tRNAs used here correspond to those previously described and are numbered 1–4 in order of their elution off the descending temperature gradient column (Bergquist, 1966). tRNA from *E. coli* that lacked a substantial portion of its glycine-acceptor activity was prepared by column chromatography on DEAE-cellulose.

Aminoacylation of tRNA. Crude or purified tRNA was charged with amino acid using a partially purified synthetase either from yeast or from *E. coli* (Bergquist, 1966). The reaction mixture contained per milliliter: 100 μmoles of Tris acetate (pH 7.8), 10 μmoles of ATP,[1] 10 μmoles of phosphoenolpyruvate, 0.5 μmole of CTP, 4 μmoles of magnesium acetate, 50 μmoles of 2-mercaptoethanol, 2 μg of pyruvic kinase, 200 mμmoles of glycine, 200 mμmoles of 19 [^{12}C]amino acids minus glycine, 0.5–1.0 mg of tRNA, and 2.5 A_{280} units of synthetase. The specific activities were 0.5–1.3 $\times$ 10^5 cpm/mμmole for [^{14}C]glycine and 1.0–1.6 $\times$ 10^5 cpm/mμmole for [^{3}H]-glycine. After incubation at 25 (yeast) or 30° (*E. coli*) for 20 min, the enzyme was removed by shaking twice with an equal volume of TM-saturated phenol. Traces of phenol were removed by ether extraction, the solution was adjusted to 0.5 M by addition of 1.0 M sodium acetate (pH 5.0), and then the RNA was precipitated as the cetyltrimethylammonium (CTB) salt (Bergquist, 1965). The CTB–RNA was converted into the sodium salt by three washings with 70% ethanol–0.1 M sodium acetate (pH 5.0) and then washed with 95% ethanol, acetone, and finally with ether. The residual ether was removed in a vacuum desiccator and the RNA was dissolved and stored frozen in 0.02 M sodium acetate (pH 5.0).

These incubation conditions provide the greatest yield of charged tRNA but are suboptimal for the charging of purified tRNAs. The glycine tRNAs accepted about half the amount of amino acid that could be loaded under optimal conditions of amino acid, tRNA, and enzyme concentration.

E. coli Cell-Free Extracts. Cell-free extracts (S-30 fraction) were prepared from *E. coli* S26 according to the method of Capecchi (1966), except that the S-30 was dialyzed against 0.01 M Tris acetate (pH 7.8)–0.01 M magnesium acetate–0.03 M ammonium chloride–0.006 M 2-mercaptoethanol.

In Vitro Protein Synthesis. The *in vitro* amino acid incorporation experiments directed by R17 RNA were carried out as described by Capecchi and Gussin (1965) and Capecchi (1966) except for buffer modifications as described in the text. The S-30 extract was preincubated for 15 min at 37°. Incubation was carried out at the same temperature. Addition of R17 RNA to the preincubated S-30 extract gave a 20–50-fold stimulation of glycine incorporation, and addition of S26Rle tRNA resulted in a further threefold stimulation of incorporation. Equal amounts of tRNA in terms of charged amino acid were added to reaction mixtures in all experiments involving the addition of more than one precharged tRNA. The amount of purified or unpurified but precharged tRNA to be added to reaction mixture where the *in vitro* synthesized coat protein was to be recovered was determined by a small-scale experiment. We adjusted the amount of tRNA to give the maximal incorporation into coat protein in a 30-min incubation period at 37°.

[1] Abbreviations used that are not listed in *Biochemistry* 5, 1445 (1966), are: TCPK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; SSC, 0.15 M NaCl–0.015 M sodium citrate (pH 7.0); TM, 0.01 M Tris acetate–0.01 M magnesium acetate buffer (pH 7.8); CTB, cetyltrimethylammonium salt; MES, 2-*N*-(morpholino)-ethanesulfonic acid; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

R17 RNA and Coat Protein. Bacteriophage R17 was grown in broth and purified according to the procedure of Gesteland and Boedtker (1964). Phage stocks were grown and assayed on *E. coli* Hfr_1. Phage RNA was prepared by adding sodium dodecyl sulfate solution to a final concentration of 0.1% to 5 ml of a 2% phage suspension in 0.15 M NaCl–0.015 M sodium citrate (SSC). The phage solution was shaken for 10 min at 2° with an equal volume of redistilled SSC-saturated phenol. The phases were separated by centrifugation and the aqueous phase was removed carefully and again extracted with an equal volume of phenol. After centrifugation, the RNA was precipitated from the aqueous phase by the addition of 2.5 volumes of ethanol. The RNA was collected by centrifugation, dissolved in a minimal volume of water, reprecipitated with ethanol, centrifuged again, redissolved in water, and freeze dried. In some experiments, where there was evidence of rRNA contamination of the R17 RNA, 5-mg portions were centrifuged on 5–20% sucrose gradients.

Preparation of R17 in Vivo. [^{14}C]Glycine-labeled R17 was prepared according to Capecchi (1966). The host bacteria were grown in glycerol–Casamino Acids medium (Capecchi, 1966), with the Casamino Acids replaced by an amino acid mixture minus glycine. [^{14}C]Glycine (80 μCi) was added per liter 10 min after infection.

Sucrose density gradient centrifugation was carried out with 5- or 25-ml linear gradients of 5–20% sucrose containing 0.08 M KCl–0.01 M Tris acetate–0.01 M magnesium acetate (pH 7.8). Preparative gradients (25 ml) were run on a Spinco Model L at 24,000 rpm for either 10 hr (R17-directed incorporation system) or 14.5 hr (RNA fractionation). Thirty 10-drop fractions were collected and 25 μl was removed from each fraction for optical density and radioactivity assays. The 5-ml gradients were centrifuged at 38,000 rpm in a Spinco Model L for 2.75 hr. Thirty 2-drop fractions were collected. The optical density of fractions was measured with a Zeiss spectrophotometer.

For radioactivity assays, the fractions were treated at pH 12 for 10 min at 30° to discharge the aminoacyl-tRNA. Two drops of 0.3% bovine serum albumin was added and the protein was precipitated with 7% TCA, collected on Millipore filters (type AA), dried, and counted in a Packard scintillation counter (Model 3324 or 3365).

Fingerprinting. Contamination of the *in vitro* synthesized R17 coat protein by the 20S RNA synthetase protein was reduced by RNase treatment of the total protein-synthesizing reaction mixture as described by Capecchi (1966) prior to sucrose gradient centrifugation. Carrier R17 coat protein was added to the pooled glycin-labeled fractions which were precipitated and washed with 7% trichloroacetic acid and then with ether (Capecchi, 1966). Residual ether was removed and the dried protein was resuspended in water and carboxymethylated by a modification of the method of Harris and Hindley (1965). The carboxymethylated protein was lyophilized, resuspended in 0.05 M ammonium bicarbonate buffer (pH 8.4), at 2 mg/ml, and digested with TPCK-treated trypsin (Kostka and Carpenter, 1964) for 5 hr at 37° at an enzyme:substrate ratio of 1:100.

The digest was lyophilized, resuspended in 10% isopropyl alcohol (2 mg/ml), and centrifuged to remove insoluble material, and the supernatant was applied to cellulose thin-layer plates for peptide mapping (Burns and Turner, 1967) employing electrophoresis at pH 2 in the first dimension and chromatography in 1-butanol–cial acetic acid–pyridine–water (15:3:12:12) in the second dimension. The presence of [^{14}C]glycine-labeled peptides was detected by autoradiography. In some cases ^{3}H-labeled peptides were identified using the method of Wilson and Spedding (1965). A cellulose acetate mixture was spread over the thin-layer plate and allowed to dry, binding the layer into a thin flexible sheet (Bieleski and Turner, 1966). After determination of the positions of the labeled peptides, they were cut out and transferred to vials for scintillation counting.

Chemicals. [^{14}C]Glycine (80 mCi/mmole), New England Nuclear Corp., Boston, Mass.; [^{3}H]glycine (1.2 Ci/mmole), Schwarz BioResearch, Orangeburg, N. Y.; pyruvic kinase, ATP, CTP, TPCK, phosphoenolpyruvate, 2-*N*-(morpholino)ethanesulfonic acid, and *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid were obtained from Calbiochem, Los Angeles, Calif.; ribonuclease, deoxyribonuclease (RNase free), and trypsin (five-times recrystallized) from Worthington Biochemical Corp., Freehold, N. J.; RNase-free sucrose and [^{12}C]amino acids were obtained from Mann Laboratories, N. Y.

Results

Transfer of Glycine into R17 Coat Protein in Vitro. Capecchi (1966) has shown that bacteriophage R17 RNA is able to direct the *in vitro* synthesis of two proteins which have been identified as the phage RNA synthetase and the coat protein. This system is convenient because the two proteins are the main products of the *in vitro* system and the coat, the major component, may be isolated substantially free of the synthetase. We have employed the R17 system in an analysis of the ability of the purified yeast glycyl-tRNAs to transfer their glycine into phage coat protein.

Preliminary Experiments. Our yeast tRNAs are not charged by the aminoacyl synthetases present in the *E. coli* S-30 extract. Hence it is necessary to precharge the yeast tRNA with amino acid using yeast synthetase before adding it to the reaction mixture. Furthermore, we found that the inclusion of Tris-acetate buffer in preincubation and incubation solutions (Capecchi, 1966) was not conducive to high levels of transfer of glycine from precharged *E. coli* or yeast tRNA. For example, transfer of glycine from precharged *E. coli* tRNA in reaction mixtures containing Tris acetate was 30–40% of the input radioactivity, and only 20% of the glycine of the input precharged [^{14}C]glycyl-$tRNA_{(yeast)}$ was transferred into protein. We tested pH 6.85 MES, TES, and cacodylate buffers. The level of transfer from yeast tRNAs was increased to 40–50% of the input with cacodylate in both preincubation and incubation mixtures. Transfer of glycine into protein synthesized in the presence of R17 RNA was up to 65% when precharged [^{14}C]-

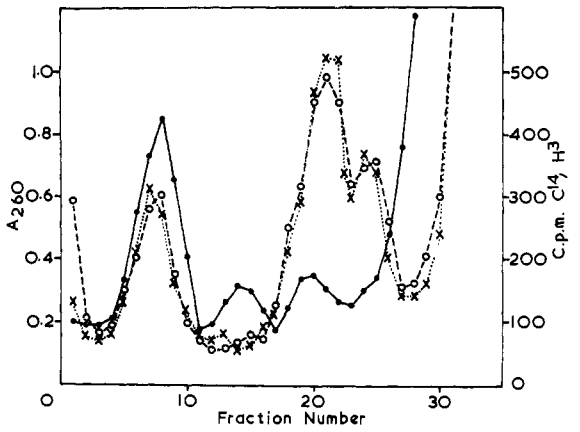

FIGURE 1: Sucrose density gradient analysis of incorporating system directed by R17 RNA. Each of the 100-μl reaction mixtures contained: 25 μl of preincubated S-30, 60 μg of R17 RNA, and 30 μl of reaction mixture (Capecchi, 1966). Tube 1 contained Tris-acetate buffer (pH 7.8), [^{14}C]glycine (5.2×10^7 cpm/μmole), 100 μg of stripped S26Rle tRNA, and 19 [^{12}C]amino acids. Tube 2 contained cacodylate buffer (pH 6.85), 100 μg of precharged glycyl-[^{3}H]tRNA from *E. coli* S26Rle (2.4×10^4 cpm), and 20 [^{12}C]amino acids. After incubation at 37° for 30 min, the tubes were chilled, and 50-μl portions were removed from each, mixed, layered onto a 5-ml 5–20% sucrose gradient, and centrifuged for 2.75 hr at 38,000 rpm at a chamber temperature of 2°. Each fraction was assayed for absorbancy at 260 mμ and for radioactivity as described in Methods. In tube 2, 57% of the added [^{3}H]glycine was transferred into TCA-insoluble material. The direction of sedimentation was from right to left. (●——●) A_{260}, (×···×) [^{14}C]glycine, and (○—○) [^{3}H]glycine.

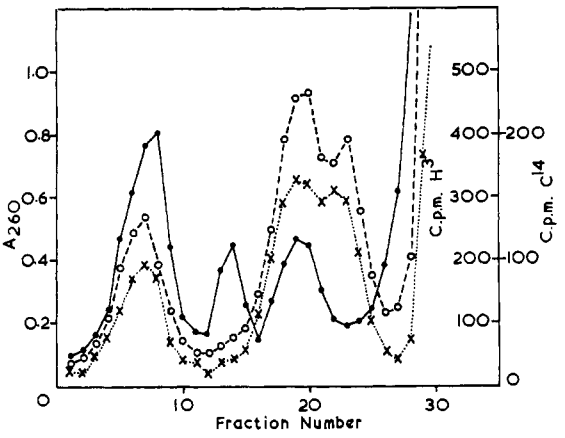

FIGURE 2: Sucrose density gradient analysis of incorporating system directed by R17 RNA using precharged RNA. Each of the two 100-μl reaction mixtures contained: 25 μl of preincubated S-30, 60 μg of R17 RNA, and 30 μl of reaction mixture containing 20 [^{12}C]amino acids. Tube 1 contained 100 μg of *E. coli* S26Rle tRNA precharged with [^{3}H]glycine (2×10^4 cpm) and 19 nonradioactive amino acids. Tube 2 had 100 μg of brewers yeast tRNA precharged with [^{14}C]glycine (2.28×10^4 cpm) and 19 nonradioactive amino acids. After 30-min incubation at 37°, 50-μl portions were removed from each tube, mixed, and centrifuged on a density gradient as described in Figure 1. A portion of the remaining reaction mixture in each tube was precipitated and its radioactivity was determined. Sixty-three per cent of the [^{3}H]glycine from the *E. coli* tRNA and 22% of the [^{14}C]glycine from yeast tRNA were transferred into TCA-insoluble material. The direction of sedimentation was from right to left. (●——●) A_{260}, (×·····×) [^{14}C]glycine, and (○– – –○) [^{3}H]glycine.

glycyl-tRNA$_{(E.\,coli)}$ was used. Neither MES nor TES gave as high a level of glycine transfer as cacodylate.

Radioactive glycine from precharged *E. coli* tRNA incubated in cacodylate buffer with S-30 and R17 RNA is incorporated into the same protein components that are labeled in experiments utilizing free radioactive glycine and noncharged tRNAs. This is shown in Figure 1, where two reaction mixtures using precharged and uncharged tRNA$_{(E.\,coli)}$ were mixed and analyzed by density gradient centrifugation.

In other experiments, we showed that precharged yeast tRNA transferred its amino acid into both the 30S and 20S protein components produced in the R17 protein-synthesizing system in the same way as did *E. coli* tRNA. Figure 2 shows a sucrose gradient profile of a mixture of two incubation solutions differing only in the source of tRNA. While the absolute incorporation of glycine from yeast tRNA is about one-third of that from *E. coli* tRNA, both 30S and 20S proteins are labeled. Similar results have been obtained using the precharged purified glycyl-tRNAs from yeast.

In our previous work (Bergquist, 1966), we examined the possibility that the label from the yeast glycyl-tRNA had been shifted to an *E. coli* glycyl-tRNA prior to incorporation, in a manner analogous to that reported for leucyl-tRNAs from *E. coli* (Yamane and Sueoka, 1964). There was no evidence for an exchange of glycine between *E. coli* tRNA and yeast tRNA.

Incorporation of amino acids by the S-30 system is stimulated threefold by the addition of *E. coli* tRNA. Incorporation of radioactive glycine was decreased substantially when precharged nonradioactive *E. coli* tRNA was used together with precharged [^{14}C]glycyl-tRNA$^{Gly}_{(yeast)}$. Presumably this effect was the result of the *E. coli* glycyl-tRNA competing with and diluting out the yeast glycyl-tRNA. This difficulty was overcome by fractionation of *E. coli* tRNA on a column of DEAE Sephadex operated at 65° (Figure 3). The fractions enriched for glycine were identified and discarded. The remaining tRNA was recombined, dialyzed to remove salt, freeze dried, and charged with nonradioactive amino acids. This glycine-depleted *E. coli* tRNA at a concentration of 0.6 mg/ml allowed maximal transfer of glycine from yeast tRNAs into R17 coat proteins. A small portion of the *E. coli* valyl- and seryl-tRNAs is removed with the bulk of the glycyl-tRNA in this process (Figure 3).

Identity of the Protein Formed When Glycine Is Transferred into R17 Coat Protein from E. coli tRNA. There are nine glycines per R17 coat subunit (Weber, 1967) and four of these are located in a large insoluble tryptic peptide composed of 38 amino acids from the amino-terminal portion of the protein (Capecchi, 1966). There are two glycines in each of two other tryptic peptides (T_1 and T_2 of Weber, 1967) and one in peptide T_6.

We prepared and fingerprinted a tryptic digest of coat protein that had been synthesized *in vivo* in the presence of [^{14}C]glycine. Only three spots were radioactive. Two of these spots (P_1 and P_2, Figure 4) were twice as radioactive as the third (P_3) and hence contained two glycine

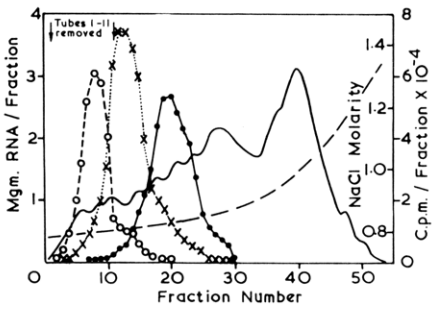

FIGURE 3: Fractionation of *E. coli* RNA on DEAE-Sephadex. *E. coli* 26Rle tRNA (100 mg) was fractionated on a 40 × 1.9 cm column of DEAE Sephadex A-25 operated at 65°. A gradient of increasing NaCl, which contained 0.02 M sodium acetate (pH 6.0) and 0.002 M EDTA, was used to elute the RNA. The RNA in the 20-ml fractions was collected and assayed as previously described (Bergquist *et al.*, 1965). Once the various acceptor species were located, tubes 1–11 were discarded, and the remaining fractions were combined and freeze dried to give the glycyl-deficient *E. coli* tRNA. Some valyl-tRNA was discarded in tubes 1–11, and also some seryl-specific tRNA (not shown). (——) Milligrams of RNA per fraction, (– – –) salt gradient, (○—○) glycine-acceptor activity, (×·····×) valine-acceptor activity, and (●– – –●) lysine-acceptance activity. Specific activity of [^{14}C]amino acids used in assays: glycine, 4 × 10^3 cpm/mμmole; valine and lysine, 6 × 10^3 cpm/mμmole.

residues. P_1 did not contain arginine or lysine, and presumably was the carboxy-terminal peptide.

In another experiment, *E. coli* tRNA was precharged with [^{14}C]glycine and incubated with S-30 and R17 RNA. The reaction mixture was centrifuged on 25-ml 5–20% sucrose gradients and the protein coat subunits synthesized *in vitro* were recovered, digested with trypsin, and mapped. The bulk of the radioactive label was located in three spots that corresponded in electrophoretic behavior and chromatographic mobility with the three glycine-containing spots observed in the *in vivo* labeling experiments. There were a number of minor radioactive spots which may have resulted from contamination of the R17 coat subunits with the synthetase produced *in vitro*. The ratio of the radioactivity in the three peptides (P_1–P_3) was 1.00:1.06:0.38. An identical autoradiograph was obtained when the *E. coli* tRNA was not precharged and [^{14}C]glycine was added to the incubation mixture. In this experiment the ratio of glycine radioactivity in the three peptides was 1.00: 0.95:0.40.

The three major radioactive peptides were eluted from the cellulose layers, hydrolyzed with 6 N HCl, and mapped on thin layers by the method of Bieleski and Turner (1966). All of the radioactivity was found to be in glycine.

Identity of the Protein Formed When Glycine Is Transferred into R17 Coat Protein from Yeast tRNAs. Glycyl-tRNA$^{Gly}_{1-3}$ were separately precharged with [^{14}C]glycine and glycyl-tRNA$^{Gly}_4$ was separately precharged with [^{3}H]glycine. Each tRNA (20–30 μg) was added separately to 1.5-ml reaction mixtures containing 375 μl of preincubated S-30 and 300–400 μg of R17 RNA. After incubation, the coat protein was separated from the bulk of the reaction mixture on 28-ml sucrose gradients.

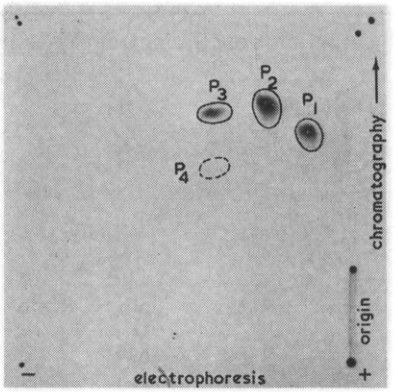

FIGURE 4: Autoradiograph of peptide map showing tryptic peptides from R17 coat protein synthesized *in vitro* that contain [^{14}C]glycine. The protein digest was applied to a band origin, electrophoresed at pH 2 in the first dimension, and chromatographed in 1-butanol–acetic acid–pyridine–water in the second dimension. The plate was exposed to Kodak RB54 X-ray film for 1 week.

The fingerprints of tryptic digests of the coat showed that the yeast tRNAs transferred their glycine into only those peptides which were labeled when *E. coli* tRNA was employed (Figure 4). However, the levels of glycine incorporation into each peptide differed from the levels determined in the all-*E. coli* system. Each glycyl-tRNA$_{(yeast)}$ transferred its amino acids into peptides 1–3 (Figure 4). Glycyl-tRNA$^{Gly}_{3\text{ and }4}$ transferred glycine into a fourth minor spot that was not present in *in vivo* labeled phage coat but was present in coat protein synthesized *in vitro* by the all *E. coli* system (peptide 4, Figure 4).

If each yeast glycine tRNA could recognize equally well all available glycine codons in the R17 message, one would expect a radioactivity ratio of 1:1:0.5 between the three soluble peptides containing glycine. Table I shows that there are significant deviations from the expected ratios. In all four experiments the ratio of radioactivity in peptide 1 to peptide 2 is much higher than expected from the known structure of the protein produced using the all *E. coli* system. In experiments using tRNA$^{Gly}_{3\text{ and }4}$ as glycine donors the ratio in peptide 3 is well below the expected 0.5.

The glycine ratios between the three peptides may have resulted from the synthesis of incomplete coat protein subunits (peptide 1 is the carboxy-terminal peptide) or from competition between the yeast tRNA^{Gly} and residual tRNA^{Gly} remaining in the *E. coli* tRNA added to the reaction mixtures. We examined this possibility by relating the glycine count ratios to the count ratios from an amino acid that was incorporated into R17 coat protein solely by transfer from an *E. coli* tRNA. Alanine occurs in peptides 1, 2, and 3 in a 3:3:1 ratio in coat protein synthesized *in vivo* (Weber, 1967). Thus the glycine:alanine ratios will be 0.67:0.67:1.0 in the three peptides. We confirmed these ratios for the *in vitro* system by synthesizing R17 coat protein in an all *E. coli* system with [^{14}C]alanine and [^{3}H]glycine added to the reaction mixture (Table II).

We then performed the experiments using precharged [^{14}C]alanyl-tRNA$_{(E.\ coli)}$ and [^{3}H]glycyl-tRNA$^{Gly}_{1\text{ (yeast)}}$.

TABLE I: Incorporation of Glycine from Yeast tRNA and from *E. coli* tRNA into Tryptic Peptides of R17 Coat Protein.[a]

Spot No.	[^{14}C]Glycyl-tRNA$^{Gly}_1$		[^{14}C]Glycyl-tRNA$^{Gly}_2$		[^{14}C]Glycyl-tRNA$^{Gly}_3$		[^{3}H]Glycyl-tRNA$^{Gly}_4$		[^{14}C]Glycyl-tRNA$_{(E.\,coli)}$		Expected Ratio from Coat Protein Synthesized *in Vivo*
	cpm	Ratio	cpm	Ratio	cpm	Ratio	cpm	Ratio	cpm	Ratio	
1	3335	1.00	856	1.00	776	1.00	1940	1.00	1670	1.00	1.0
2	5185	1.55	1263	1.49	1460	1.88	4770	2.46	1770	1.06	1.0
3	1795	0.54	482	0.57	224	0.28	520	0.27	625	0.38	0.5
4					264	0.34	690	0.35			

[a] A portion of each of the tryptic digests of the *in vitro* synthesized coat protein was mapped on thin layers (Burns and Turner, 1967). After autoradiography the ^{14}C peptides were placed in scintillation vials and counted in a scintillation spectrometer. The ^{3}H-labeled digest was counted by cutting the thin layer into squares and counting as before or by identification of the peptides by scintillation autoradiography (Wilson and Spedding, 1965).

TABLE II: Incorporation of Alanine and Glycine into Tryptic Peptides from R17 Coat Protein.

Peptide	^{14}C (cpm)	Incorp ($\mu\mu$mole)	Ratio	^{3}H (cpm)	Incorp ($\mu\mu$mole)	Ratio	^{3}H:^{14}C	Expected Ratio from Coat Protein Synthesized *in Vivo*
1. [^{14}C]Alanine and [^{3}H]Glycine Added to Reaction Mixture Containing Uncharged *E. coli* tRNA[a]								
1	13900	146.0	3.00	3625	106.8	2.00	0.73	0.67
2	13780	145.5	2.97	3661	108.0	2.02	0.74	0.67
3	3870	40.8	0.84	1329	39.0	0.76	0.96	1.00
2. Precharged *E. coli* [^{14}C]Alanyl-tRNA and Yeast [^{3}H]Glycyl-tRNA$^{Gly}_1$ Added as Amino Acid Donors.[b]								
1	6050	29.4	3.00[c]	3195	15.5	2.00[d]	0.53[e]	
2	7120	34.5	3.51[c]	5730	27.8	3.60[d]	0.80[e]	
3	1540	7.45	0.78[c]	1770	8.55	1.12[d]	1.16[e]	

[a] The specific activities of the amino acids were [^{14}C]alanine, 95 cpm/$\mu\mu$mole; [^{3}H]glycine, 34 cpm/$\mu\mu$mole. [b] The specific activity of both the *E. coli* tRNA and the yeast tRNA$^{Gly}_1$ was 4.85×10^2 cpm/$\mu\mu$mole. Alanine- and glycine-acceptor activities (750 $\mu\mu$moles) were added to the reaction mixture (total volume 1.5 ml). These figures are equivalent to 53 μg of tRNA$^{Gly}_{1\,(yeast)}$ and 990 μg of *E. coli* tRNA. A portion of the tryptic digest of the *in vitro* synthesized product was mapped on thin layers, autoradiographed, and counted as previously described. [c] This ratio was calculated by setting the number of micromicromoles of [^{14}C]alanine in peptide 1 equal to 3. [d] This ratio was calculated by setting the number of micromicromoles of [^{3}H]glycine in peptide 1 equal to 2. [e] The ^{3}H:^{14}C ratio was calculated for each peptide from the number of micromicromoles of [^{3}H]glycine divided by the number of micromicromoles of [^{14}C]alanine.

Equal amounts of the alanyl- and the glycyl-tRNAs in terms of micromicromoles of charged tRNA were added to the incubation mixtures. We made the following predictions in this experiment. The glycine:alanine ratios will be 0.67:0.67:1.0 if the glycine codons are translated with strict fidelity and equal efficiency by the yeast glycyl-tRNAs. The ratio will be lower than the calculated values if the charged but nonradioactive tRNA$^{Gly}_{(E.\,coli)}$ competes significantly with the yeast glycyl-tRNAs. The incorporation of both [^{3}H]glycine and [^{14}C]alanine into peptide 1 will be lower than into peptide 2 if incomplete coat protein subunits are synthesized, since peptide 2 is located near the middle of the coat protein.

The results in Table II show that alanine is incorporated into peptides 1–3 in approximately correct proportions. The incorporation of [^{3}H]glycine shows the same pattern observed in previous experiments. Peptide

TABLE III: Incorporation of Glycine from Pairs of Yeast tRNAs into Tryptic Peptides of R17 Coat Protein.[a]

Peptide	^{14}C (cpm)	Ratio	^{3}H (cpm)	Ratio	$^{3}H:^{14}C$[b]	Input $^{3}H:^{14}C$[c]
	1. [^{14}C]Glycyl-tRNA$^{Gly}_1$ and [^{3}H]Glycyl-tRNA$^{Gly}_2$ Added as Glycine Donors					
1	1105	1.00	1385	1.00	1.25	1.02
2	1712	1.55	2168	1.57	1.27	
3	536	0.49	641	0.46	1.19	
4						
	2. [^{3}H]Glycyl-tRNA$^{Gly}_1$ and [^{14}C]Glycyl-tRNA$^{Gly}_4$ Added as Glycine Donors					
1	1400	1.00	506	1.00	0.36	0.92
2	1385	0.99	684	1.35	0.49	
3	400	0.29	165	0.32	0.41	
4	124	0.09	83	0.16	0.67	

[a] Mapping conditions were as described in Table I. [b] $^{3}H:^{14}C$ is the ratio of tritium to carbon counts in each peptide. [c] Input ratio of $^{3}H:^{14}C$ is the total amount of radioactivity in [^{3}H]glycine:total radioactivity in [^{14}C]glycine added to the protein-synthesizing reaction mixture as precharged glycyl-tRNA.

TABLE IV: Incorporation of Glycine from Yeast and *E. coli* tRNAs into Tryptic Peptides of R17 Coat Protein.

Peptide	^{14}C (cpm)	Ratio	^{3}H (cpm)	Ratio	$^{3}H:^{14}C$[a]	Input $^{3}H:^{14}C$[b]
	1. [^{14}C]Glycyl-tRNA$_{(E.\,coli)}$ and [^{3}G]Glycyl-tRNA$^{Gly}_{2\ (yeast)}$ Added as Glycine Donors					
1	3159	1.00	522	1.00	0.17	0.92
2	3483	1.11	1225	2.35	0.35	
3	891	0.27	288	0.55	0.32	
4	316	0.10	92	0.18	0.29	
	2. [^{14}C]Glycyl-tRNA$_{(E.\,coli)}$ and [^{3}H]Glycyl-tRNA$^{Gly}_{4\ (yeast)}$ Added as Glycine Donors					
1	3670	1.00	1431	1.00	0.39	1.85
2	4180	1.14	2570	1.79	0.61	
3	1052	0.29	763	0.53	0.72	
4						

[a] $^{3}H:^{14}C$ is the ratio of tritium to carbon radioactivity in each peptide. [b] Input ratio of $^{3}H:^{14}C$ is the total amount of radioactivity in [^{3}H]glycine:total radioactivity in [^{14}C]glycine added to the protein-synthesizing reaction mixtures as precharged glycyl-tRNA.

2 has a ratio considerably greater than 1.00. The $^{3}H:^{14}C$ data show that the ratio for peptide 1 is lower than 0.67 and that the ratio for peptide 3 is rather higher than predicted. The ratio for peptide 2 was substantially higher than 0.67. This result suggests that (i) a small proportion of the subunits may be incomplete; (ii) more than two glycines are transferred into peptide 2 by tRNA$^{Gly}_{1\ (yeast)}$; and (iii) residual *E. coli* glycyl-tRNA does not compete with the yeast tRNA^{Gly} to a significant extent.

Other experiments were performed using the yeast glycyl-tRNAs in pairs. Table III shows that when [^{14}C]-glycyl-tRNA$^{Gly}_1$ and [^{3}H]glycyl-tRNA$^{Gly}_2$ are added together to the protein-synthesizing mixture, their glycine is transferred into peptides 1–3 more or less in the proportions that would be expected from the experiments using either of these tRNAs alone. The input ratio of ^{3}H-labeled tRNA to ^{14}C-labeled tRNA was 1.02, but this ratio is raised in all peptides examined. It appears that tRNA$^{Gly}_2$ has a slightly greater affinity for the glycine codons in the R17 message than does tRNA$^{Gly}_1$.

When [^{14}C]glycyl-tRNA$^{Gly}_4$ and [^{3}H]glycyl-tRNA$^{Gly}_1$ were used together in the reaction mixture, the results resembled the data obtained when *E. coli* tRNA and a yeast glycyl-tRNA were used in the synthesis of coat protein *in vitro* (see Table IV). The count ratios for [^{14}C]glycine in peptides 1 and 2 were approximately 1.0:1.0 but were 1.00:1.35 for [^{3}H]glycine. In addition, the low $^{3}H:^{14}C$ ratio for each peptide in comparison with the input $^{3}H:^{14}C$ ratio shows that tRNA$^{Gly}_4$ is able to compete very effectively with tRNA$^{Gly}_1$ for the glycine code words in the portions of mRNA specifying the sequences of peptides 1–3. The other four paired combinations of the glycyl-tRNAs have been examined. The combination tRNA$^{Gly}_{3\ and\ 4}$

gave similar results to those obtained using either tRNA alone. Glycyl-$tRNA_2$ virtually excludes the transfer of glycine from $tRNA^{Gly}_3$ into the three peptides.

$tRNA^{Gly}_1$ is superior to $tRNA^{Gly}_3$ in the transfer of glycine into coat protein, and $tRNA^{Gly}_2$ is more efficient than $tRNA^{Gly}_4$. In each of these experiments the P_1:P_2 ratio is substantially more than 1.00.

The results of the experiments using pairs of glycyl-tRNAs may be interpreted as indicating that $tRNA^{Gly}_2$ is best able to recognize the code words for glycine in the *E. coli*–R17 RNA system. It is tempting to suggest an order for the other three glycyl-tRNAs in terms of their affinity for the glycine codons but an unambiguous ranking is not possible.

The proportion of radioactivity incorporated into peptide 3 shows deviations from the expected ratio in a number of experiments (Tables II and III). For example, in the experiments using single yeast RNAs, $tRNA^{Gly}_3$ and $tRNA^{Gly}_4$ show about one-half the expected incorporation. The experiment in which precharged *E. coli* tRNA was employed also shows a low peptide 3 to peptide 1 ratio. This phenomenon is also apparent in some of the experiments using pairs of yeast glycyl-tRNAs (*e.g.*, $tRNAs^{Gly}_{2 \text{ and } 3}$ and $tRNA^{Gly}_{1 \text{ and } 4}$).

It is not easy to interpret the differing results between experiments employing single glycyl-tRNAs or pairs of tRNAs from yeast and *E. coli* glycyl-tRNA with respect to the labeling of the three peptides. It is possible that the presence of a yeast glycyl-tRNA in the *E. coli* system induces a translation error in R17 RNA readout for peptides 2 and 3.

Competition between Yeast and E. coli tRNAs in the Incorporation of Glycine into the Tryptic Peptides of R17 Coat Protein. Our yeast glycine tRNAs recognize GGpyrimidine (Bergquist, 1966). The data presented above show that translation is not terminated because the yeast tRNA is unable to provide a species of $tRNA^{Gly}$ that is essential for the translation of, for example, a GGpurine code word. The yeast tRNA may overcome this deficiency by wobble-type codon recognition (Crick, 1966). An indirect approach to the nature of the glycine codons in the R17 RNA is to perform competition experiments between yeast and *E. coli* glycine tRNAs. We assume that an *E. coli* glycyl-tRNA that recognizes GGpurine would effectively exclude a yeast $tRNA^{Gly}$ from transferring its amino acid into protein as a result of wobble-hypothesis codon–anticodon recognition.

E. coli tRNA was precharged with [^{14}C]glycine and yeast glycine tRNAs were individually precharged with [^{3}H]glycine. Preliminary small-scale experiments showed that the curves for the time course of the incorporation of [^{14}C]- and [^{3}H]glycine were of the same shape and reached plateau values after 30 min. We examined the incorporation of glycine into the tryptic peptides of coat protein synthesized after 30-min incubation. The experiments described in Table IV were conducted with equal amounts of yeast and *E. coli* glycyl-tRNAs in terms of charged acceptor molecules being added to the reaction mixtures.

Representative results from competition experiments are shown in Table IV. The data may be summarized as follows: in all experiments the expected ratio of [^{14}C]-glycine transferred from $tRNA_{(E.\ coli)}$ into peptides 1 and 2 is found but the ratio for peptide 3 is low. This is consistent with the data for *E. coli* glycyl-tRNA in Tables I and II. Similarly, the ratios of radioactivity in peptides 1–3 in these experiments, where the various [^{3}H]glycyl-tRNAs from yeast were used, also resemble the results previously obtained using the yeast glycyl-tRNAs alone or in combination (Tables II and III). The ^{3}H:^{14}C ratios for the three peptides in the various experiments in all cases are lower than the input ratios, suggesting that the yeast tRNAs do not compete very effectively with the *E. coli* glycyl-tRNAs. In almost all cases peptide 2 shows a higher ^{3}H:^{14}C ratio than the other peptides. This ratio appears to result from the observation that more glycine than expected is incorporated into this peptide from yeast glycyl-tRNAs. It is also notable that the peptide 3 to peptide 1 ratio is invariable higher for transfer of glycine from yeast glycyl-tRNA than from *E. coli* glycyl-tRNA.

The efficiency with which each yeast glycyl-tRNA is able to compete with *E. coli* glycyl-tRNAs may be compared by expressing the amount of [^{3}H]glycine as a percentage of the total amount of [^{3}H]- and [^{14}C]glycine incorporated into each peptide. Yeast glycyl-$tRNA^{Gly}_4$ is the most efficient in terms of over-all incorporation and $tRNA^{Gly}_2$ the least efficient. This result is surprising, since $tRNA^{Gly}_{2\ (yeast)}$ performs better than any of the other three yeast $tRNA^{Gly's}$ when $tRNA^{Gly}_{(E.\ coli)}$ is not present in the reaction mixture (Table III). Examination of the amount of glycine that has been transferred into the three peptides from any of the yeast glycyl-tRNAs shows that they can compete more effectively for the glycine code words in peptides 2 and 3 (30–43% of the total glycine incorporated) than in peptide 1, where 16–25% is transferred from a yeast tRNA.

Discussion

The experiments of others have been concerned with the transfer of amino acids into protein synthesized *in vitro* from tRNAs that were genetically degenerate in that they recognized synonym code words (for example, Weisblum *et al.*, 1962, 1965; von Ehrenstein and Dais, 1963; Bennett *et al.*, 1963, 1965). Our experiments have utilized glycine tRNAs that are separable by physical methods but which appear to recognize the same code words. These redundant glycine tRNAs have different primary structures, although there are considerable similarities between them in nucleotide sequences (Bergquist, 1966). We used *E. coli* ribosomes and trinucleotides in our previous experiments but our results did not provide a clear demonstration of the codon recognition pattern of the yeast glycine tRNAs (Bergquist, 1966). It might be thought that the wobble hypothesis (Crick, 1966) could provide an explanation for the multiple codon recognition of the yeast glycyl-tRNAs, but we have not been able to demonstrate the presence of inosine in any of the four tRNAs (Bergquist, 1966).

It may be argued that the four glycine tRNAs are derived from several different strains present in the brewers yeast we use for isolation of the bulk tRNA. If this were the case, then the strains would have to be

present in the same proportions from preparation to preparation, since we have observed the same ratio of glycine tRNAs in several independent preparations over a period of 3 years. Furthermore, microscopical examination of cultures of our brewers yeast did not support the suggestion that more than one strain was present.

The degree of ploidy of the organism used in a study relating to multiple copies of a particular cistron is of considerable importance. Söll *et al.* (1966) have demonstrated that redundancy of various tRNA species occurs in a haploid strain of yeast. We have not been able to ascertain whether our yeast strain is haploid or diploid. The presence of four glycine tRNAs in our preparations may merely be a reflection of the chromosome number of the chromosome number of the yeast. If this is true, one might expect the tRNAs to have much greater structural similarities than they are known to possess (Bergquist, 1966). For example, yeast $tRNA^{Gly}_3$ has a unique sequence of seven nucleotides that are not found in the other three glycine tRNAs. Furthermore, examination of the triplet binding behavior of the glycine tRNAs shows that three preferentially recognize GGU and one, $tRNA^{Gly}_4$, recognizes GGC (Bergquist, 1966).

It might be expected that there would be a correlation between structural similarity, codon recognition, and ability to transfer glycine into protein in response to natural mRNA, but such a correlation is not found. In addition, equal amounts of each of the species would be expected if the glycine tRNAs were the products of multiple alleles of the same gene. However, $tRNA^{Gly}_{1 \text{ and } 2}$ are present in approximately threefold greater amounts than $tRNA^{Gly}_{3 \text{ and } 4}$ (Bergquist, 1966). Taken together, these facts suggest that the glycine tRNAs are specified by separate cistrons rather than by alleles of the same cistron on homologous chromosomes, but conclusive proof awaits our isolation of redundant glycine tRNAs in a genetically characterized strain of yeast.

At least two phage-specific proteins are synthesized when any one of the yeast glycine tRNAs is used as glycine donor in the *E. coli* S-30 system programmed with R17 RNA. These proteins are presumed to be coat protein and RNA synthetase by analogy with the report of Capecchi (1966). Comparison of maps of tryptic digests of coat protein synthesized in the presence of glycyl-$tRNA^{Gly}_{(yeast)}$ or glycyl-$tRNA^{Gly}_{(E.\,coli)}$ showed that the subunits recovered were indistinguishable from each other or from coat protein prepared *in vivo* as judged from the pattern of radioactive spots.

Examination of the relative amounts of glycine incorporated into each peptide showed that when any one of the yeast glycine tRNAs was included in the reaction mixture, there was more radioactivity due to glycine in peptide 2 than found when *E. coli* tRNA was employed. Similar results were found in all paired combinations of the four glycyl-tRNAs except in experiments where $tRNA^{Gly}_4$ and $tRNA^{Gly}_1$ are used together (Table III). The yeast glycyl-tRNAs compete with each other for the glycine codons in the R17 RNA as shown by comparison of the differing ratios of isotopes that were added to the reaction mixtures to the ratios found in the three peptides we investigated. Yeast $tRNA^{Gly}_2$ is the most efficient in the transfer reaction but it is not possible to order the other three species as the competitive performance of any tRNA appears to depend on the tRNA with which it is paired. It is clear, however, that all four glycyl-tRNAs can recognize all glycine codons in the mRNA for peptides 1–3. Knowledge of whether these codons are the same or different awaits the determination of the base sequence of the R17 RNA, but data from competition experiments suggest that one or more of the glycine codons in peptide 1 may differ in composition from the glycine codons in the RNA specifying peptides 2 and 3 (Table IV).

The pattern of incorporation of glycine into peptide 3 is unexpected. In most of the experiments using yeast glycyl-tRNAs, the ratio of radioactivity in peptide 3 to that in peptide 1 indicated that one glycine residue was being incorporated. In all experiments where *E. coli* $tRNA^{Gly}$ was included in the reaction mixture the radioactivity measurements showed that 0.48–0.76 of a residue was incorporated in peptide 3. These results suggest that the yeast glycyl-tRNAs translate this codon with less ambiguity than does the *E. coli* tRNA. The observation that *E. coli* tRNA incorporates less than one glycine into peptide 3 resembles the ambiguous translation for leucine and other neutral amino acids in the hemoglobin α chain that has been described by von Ehrenstein (1966).

Peptide 2 also has a disproportionately high amount of [^{3}H]glycine transferred from the yeast glycyl-tRNAs in experiments involving *E. coli* $tRNA^{Gly}$ (Table IV). This result was obtained even though the total amount of [^{3}H]glycine incorporation was reduced as a result of the presence of the bacterial glycyl-specific tRNA instead of glycyl-deficient tRNA as in other experiments (Tables I–III). When taken with our results where the radioactivity in [^{14}C]alanine in each peptide was used as an internal control, we interpret these findings as indicating that more than two residues of glycine were incorporated into peptide 2 when a yeast $tRNA^{Gly}$ is present, and that this is the result of some other codon being translated as glycine. We have calculated that the mobility of peptide 2 in our mapping system would not be altered substantially if a glycine was substituted for any of the amino acids that might be replaced in this peptide. These amino acids are valine, threonine, glutamic acid, glutamine, leucine, proline, and tryptophan (Weber, 1967). Replacement of arginine would lead to loss of peptide 2 from the maps. We have shown that alanine is present in the expected amount in peptide 2 (Table II).

It is not clear why there is a number of tRNAs in yeast that recognize the same code words. Redundant tRNAs have been found in yeasts of different origin (Zachau *et al.*, 1966; Söll *et al.*, 1966) and, by quite different experimental techniques, in *Drosophila* (Ritossa *et al.*, 1966). From a genetic analysis of supersuppressor mutations in yeast, Gilmore and Mortimer (1966) have proposed that there exists several sets of tRNA genes, each of which produces a copy of the same tRNA. One of these copies could be altered to act as a nonsense suppressor without altering the over-all ability of the cell to translate the normal triplet recognized by the altered tRNA.

Our observations have a formal similarity to missense suppression. Carbon *et al.* (1966a,b) and Gupta and Khorana (1966) have demonstrated that the mechanism of missense suppression involves a genetically altered tRNA that is able to misread certain sense codons. We suggest that at least some of our redundant tRNAs may act *in vivo* like missense suppressors, having arisen in a similar manner to nonsense suppressor tRNAs in yeast (Gilmore and Mortimer, 1966; Magni and Puglisi, 1966) and in *E. coli* (Smith *et al.*, 1966; Bergquist and Capecchi, 1966). One apparent paradox in missense suppression is that the mutation to missense involves the alteration of a sense codon to another codon that is missense at the site of the change but is sense at all other sites. If it is necessary to alter a degenerate tRNA (Carbon *et al.*, 1966a,b) to read the missense code word, suppression of missense would be expected to lead to the translation of sense as missense at other positions in the genetic message. This problem could be overcome if one of a family of redundant tRNAs was modified in some way as a result of suppression so that it was able to read the missense code word as sense and the remainder of the family still recognized their appropriate codon. To fit our data, it is necessary to postulate that the altered redundant tRNA would retain the ability to recognize its former code word.

Jukes (1967) has suggested that evolutionary changes in the genetic code may have occurred by tRNA gene duplication followed by differentiation. Redundancy of tRNA would appear to be a refinement of such an evolutionary mechanism and would have high survival value in providing for the correction of missense and nonsense mutations without altering the ability of the cell to translate normal sense triplets.

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Transfer Ribonucleic Acids from *Escherichia coli* Treated with 5-Fluorouracil*

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ABSTRACT: 5-Fluorouracil (FU) is readily incorporated into the transfer ribonucleic acids (tRNAs) of *Escherichia coli*. tRNA synthesized in the presence of the analog may have up to 100% replacement of uracil by FU. The relative amounts of the other three major bases are unchanged but the relative amounts of ribothymidylic and pseudouridylic acids are reduced. Incorporation of FU into tRNA does not affect its ability to accept amino acids or to transfer phenylalanine into polyphenylalanine or lysine into polylysine in an *E. coli* S-30 system. Transfer of lysine into polypeptides synthesized under the direction of bacteriophage R17 RNA is inhibited by tRNA from FU-treated cells. This inhibition does not appear to be due to errors in codon recognition by lysyl-tRNA containing FU. There was no difference in response to a series of A,G copolymers between lysyl-tRNA containing FU and control lysyl-tRNA in the ribosome binding assay. tRNA containing FU has been separated from contaminating unsubstituted tRNA by chromatography on columns of methylated albumin kieselguhr.

tRNA containing FU has been shown to possess an altered secondary structure as judged by its thermal denaturation profile.

5-Fluorouracil is readily incorporated into the ribonucleic acids of several strains of *Escherichia coli* (Horowitz and Chargaff, 1959; Horowitz *et al.*, 1960). One consequence of incorporation of FU[1] into RNA is the production of altered proteins (Bussard *et al.*, 1960; Gros and Naono, 1961; Nakada and Magasanik, 1964).

Champe and Benzer (1962) showed that growth in the presence of FU restored the wild-type phenotype to certain rII mutants of bacteriophage T_4. They suggested that the incorporation of the analog into mRNA caused occasional base-pairing errors during message translation, FU pairing sometimes with G instead of A. Other genetical evidence also supports faulty base pairing in translation as a site of action of FU *in vivo* (Rosen, 1965; Edlin, 1965a,b). Heidelberger (1965) suggested that FU may occasionally pair incorrectly during transcription of mRNA from DNA but an attempted experimental demonstration was unsuccessful (Bujard and Heidelberger, 1966).

It is possible that coding errors leading to the production of altered proteins may be introduced by tRNA containing FU. For example, errors may occur through altered codon–anticodon interaction during translation, or by mistakes in recognition by the aminoacyl synthetases of tRNA containing FU. However, the effects of FU on tRNA have received relatively little attention other than preliminary reports in the context of other experiments (Gros and Naono, 1961; Gros *et al.*, 1962; Sueoka and Yamane, 1963). In the work reported here, we will show that tRNA synthesized in the presence of the analog may have up to 100% replacement of uracil by FU and an altered content of certain minor bases. Furthermore, we have found that tRNA from FU-treated cells accepts amino acids to loading levels indistinguishable from normal tRNA. FU-tRNA can transfer phenylalanine into polyphenylalanine and lysine into polylysine in an *E. coli* S-30 system but cannot transfer lysine into polypeptides synthesized under the direction of bacteriophage R17 RNA. The tRNA containing FU has been separated from contaminating normal tRNA that does not contain FU and shown to possess a different secondary structure.

Materials and Methods

Bacterial Growth and Media. E. coli B was usually grown in C medium (Roberts *et al.*, 1955) with 0.2%

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: FU, 5-fluorouracil; FU-tRNA, tRNA from cells treated with FU; C-tRNA, tRNA from control cells; FU-enzyme, aminoacyl-tRNA synthetases prepared from cells treated with FU; C-enzyme, aminoacyl-tRNA synthetases from control cells; A_{260} unit, the quantity of material which dissolved in 1 ml of solvent has an absorbance at 260 mμ of one measured in a cell of 1-cm path length; MAK, methylated bovine serum albumin adsorbed to Kieselguhr.