Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6873–6877, September 1989
Biochemistry

Codon discrimination and anticodon structural context

(translation/tRNA/codon reading)

FLORENTYNA LUSTIG*, THOMAS BORÉN*, YOUSSEF S. GUINDY*, PER ELIAS*, TORE SAMUELSSON*, CHARLES W. GEHRKE†, KENNETH C. KUO†, AND ULF LAGERKVIST*

*Department of Medical Biochemistry, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden; and †Cancer Research Center, P.O. Box 1268, Columbia, MO 65205

Communicated by Peter Reichard, June 5, 1989 (received for review February 7, 1989)

ABSTRACT **Site-directed mutagenesis has been used to change the nucleotide C in the wobble position of $tRNA_1^{Gly}$ (CCC) to U. The mutated tRNA was tested for its ability to read glycine codons in an *in vitro* protein-synthesizing system programmed with the phage message MS2-RNA that had been modified by site-directed mutagenesis so as to make it possible to monitor conveniently the reading of all four glycine codons. The results showed that while the efficiency of $tRNA_1^{Gly}$ (UCC) was comparable to that of mycoplasma $tRNA^{Gly}$ (UCC) in the reading of the codon GGA, the mycoplasma $tRNA^{Gly}$ was far more efficient than the $tRNA_1^{Gly}$ (UCC) in the reading of the codons GGU and GGC. Thus, the anticodon UCC, when present in the structural context of the $tRNA_1^{Gly}$ molecule, behaved as predicted by the wobble rules while in the structural context of the mycoplasma $tRNA^{Gly}$ it read without discrimination between the nucleotides in the third codon position, in violation of the wobble restrictions. The result with the codon GGG showed that the anticodon UCC, when present in $tRNA_1^{Gly}$, was considerably less efficient in reading this codon than it was in the structural context of the mycoplasma $tRNA^{Gly}$. It would therefore seem that the anticodon UCC, when present in a certain tRNA, can be an efficient wobbler, while in the molecular environment of another tRNA it is markedly restricted in its ability to wobble.**

The genetic code can be thought of as being made up of 16 boxes, each containing four codons. All codons in a box have the same nucleotides in the first two positions; the variation between the codons is in the third nucleotide. In half of the boxes the codons have been divided up between the amino acids or between amino acids and stop words. When reading the codons of such boxes, it is obviously necessary to be able to distinguish between the nucleotides in the third codon position to avoid translational errors (1).

Half of the codons in the genetic code are contained in family boxes in which all four codons denote the same amino acid. Analysis in the laboratories of Sanger (2), Tzagoloff (3), and RajBhandary (4) of mitochondrial tRNA genes and their gene products has established that in both mammalian and yeast mitochondria the family boxes are read by only one tRNA each. Furthermore, in chloroplasts from tobacco leaves (5) and liver wort (6) the number of tRNA genes indicates that several of the codon families in these organelles would seem to be read by only one tRNA each.

Unconventional codon reading without discrimination between the nucleotides in the third codon position is not limited to chloroplasts and mitochondria but can occur also in free-living microorganisms. Kilpatrick and Walker (7) have reported that *Mycoplasma mycoides* ssp. *capri* contains only one glycine tRNA and that this tRNA has an unsubstituted U in the wobble position. We have tested this glycine tRNA in an *in vitro* protein-synthesizing system programmed with the phage messenger MS2 RNA and have found that it was almost as efficient in the unorthodox reading of the codons GGU and GGC as it was in conventional reading (8), suggesting that *M. mycoides* might, at least in the glycine case, use undiscriminating reading of the type indicated above.

Furthermore, the results of an inventory of tRNA genes and their gene products that we have undertaken in this microorganism have so far yielded results consistent with the view that several of the codon families are read without discrimination in the third codon position (9, 10).

What structural elements are important for the efficiency of this type of unconventional reading? The enhanced unconventional reading shown by the mycoplasma glycine tRNA in an *in vitro* system, derived entirely from *Escherichia coli*, might suggest that the structural context provided by the mycoplasma tRNA molecule increases the ability of the anticodon to make such unconventional codon readings. An alternative possibility would be that the presence of an unsubstituted U in the wobble position, regardless of the structural context, confers this superior ability on the tRNA. As a first approach to the elucidation of this problem, we have changed the gene for glycine $tRNA_1$ (anticodon CCC) by site-directed mutagenesis so as to substitute the nucleotide T in the gene position corresponding to the wobble nucleotide of the tRNA. In the present paper, we report the effect that this change of the wobble nucleotide has on the efficiency of the tRNA in unconventional codon reading.

MATERIALS AND METHODS

The preparation of S30 extract from *E. coli* ts48 and the purification and esterification of tRNAs were as described (8). Oligonucleotides for *in vitro* mutagenesis were obtained from Biosyntech (Hamburg, F.R.G.), from Syn-Tec (Umeå, Sweden), or from the Department of Physical Chemistry, Chalmers University of Technology (Gotëborg, Sweden). The vector pKK223-3 was from Pharmacia, and the $pGEM_1$ vector was obtained from Promega Biotec. The T7 RNA polymerase overproducing strain HMS12/pGP1-5/pGP1-1 was generously provided by Stanley Tabor and the enzyme was purified as described by Tabor and Richardson (11).

Cloning and Site-Directed Mutagenesis of the $tRNA_1^{Gly}$ (CCC) Gene. A *Pvu* II/*Eco*RI fragment from a λ transducing phage λ Gly U_{777} carrying the gene for $tRNA_1^{Gly}$ (CCC) from *E. coli* (a generous gift of C. H. Hill, Department of Biological Chemistry, Pennsylvania State University, Hershey, PA) was cloned in the M13 vector and sequenced. A subclone of ≈500 base pairs that contained the complete coding sequence was used for site-directed mutagenesis with the synthetic 20-mer oligonucleotide 5′-d(AGCTTTCCAAGCTCTATACG)-3′ essentially as described by Zoller and Smith (12). The nucleotide sequence of the mutant tRNA gene was determined by the M13 single-strand phage dideoxynucleotide chain-termination method of Sanger *et al.* (13).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The mutagenized $tRNA_1^{Gly}$ gene was cloned under the tac promoter in the expression vector pKK223-3 and the pattern of isoaccepting glycine tRNAs in cells transformed with the recombinant vector was compared to that of cells transformed with the nonrecombinant vector. Crude tRNA was purified and the glycine isoacceptor tRNAs were separated on benzoylated DEAE-cellulose as described (8).

In the experiment with nonrecombinant vector, the level of $tRNA_1^{Gly}$ was only 10–15% that of $tRNA_3^{Gly}$, while in the cells infected with the recombinant vector the concentration of mutagenized $tRNA_1^{Gly}$ was 2–3 times that of $tRNA_3^{Gly}$. Thus, the mutated $tRNA_1^{Gly}$ gene in the recombinant vector was ≈20 times overexpressed compared to the host-cell tRNA genes.

Purification and Characterization of tRNA. Crude tRNA was prepared from JM103 cells containing the pKK223-3 plasmid with a mutagenized $tRNA_1^{Gly}$ gene, and glycine isoacceptor tRNAs were separated by chromatography on benzoylated DEAE-cellulose (45–80 mesh). Fractions containing $tRNA_1^{Gly}$ (UCC) were combined, precipitated, and rechromatographed on benzoylated DEAE-cellulose (120–200 mesh) (14).

The $tRNA_1^{Gly}$ (UCC) was further purified by two-dimensional gel electrophoresis (15) and sequenced by the Gupta and Randerath direct read-out method (16). The nucleoside diphosphate corresponding to the wobble nucleotide showed the mobility expected for uridine diphosphate both in the standard chromatographic medium of that method and when analyzed by two-dimensional TLC (17).

Furthermore, in a tRNA hydrolyzate analyzed by HPLC (18) we could only detect the modified uridine phosphates present also in the wild-type $tRNA_1^{Gly}$ (CCC)—i.e., 4-thiouridine, 5-methyluridine, and pseudouridine. The result of our characterization of $tRNA_1^{Gly}$ (UCC) is shown in Fig. 1. The sequence of mycoplasma $tRNA^{Gly}$ is included for comparison.

Protein Synthesis with MS2 RNA Transcribed *in Vitro*. To be able to monitor conveniently the reading of all four glycine codons, a nearly full-size cDNA copy of the phage message MS2 RNA, kindly provided by E. Remaut (Laboratory of Molecular Biology, Rijksuniversiteit, Gent, Belgium), was used for site-directed mutagenesis of the coat protein gene. Single-stranded DNA was prepared from an M13mp18 clone containing a 754-base-pair *Xba* I/*Bam*HI fragment of MS2 cDNA (21) and site-directed mutagenesis was performed essentially as described (22).

The glycine codons corresponding to amino acid positions 13, 14, 16, 28, and 73 in the coat protein were replaced in the coat protein gene by alanine or valine codons as indicated in Fig. 2*C*. Furthermore, in four separate MS2 cDNAs, one of the glycine codons, either GGT, GGC, GGA, or GGG throughout, was introduced corresponding to amino acid positions 72, 74, 76, and 78 followed by the termination codons TAA and TAG at amino acid positions 89 and 90 (Fig. 2*C*). To suppress the translation of the replicase cistron the initiation codon ATG of that cistron was changed to ACG.

In this way, we obtained four differently modified MS2 cDNAs, one in which GGT was the only glycine codon in the coat protein gene, another that had only GGC, etc. The corresponding MS2 RNAs, when used to program the *in vitro* protein synthesizing system, would produce a coat protein fragment that could be used to monitor the reading of the appropriate glycine codon.

For transcription, the mutated MS2 cDNA fragments were inserted into the vector $pGEM_1$ containing the 3.7-kilobase MS2 cDNA fragment shown in Fig. 2. The recombinant $pGEM_1$ vectors ($pGEM_1$-MS2), each representing one of the glycine codons, were digested with *Bam*HI and the linearized DNA was transcribed with T7 RNA polymerase (23). The transcripts, which contained both the entire A protein cistron, the mutated coat protein cistron, and part of the replicase cistron (Fig. 2), were designated *Bam*HI MS2 RNA.

Conditions for the *in vitro* protein synthesis were as described (8). For the competition experiments in Table 1, the reaction mixture contained, in addition, 300 μg of *Bam*HI MS2 RNA and the competing glycyl tRNAs in concentrations of 2 μM each in a final vol of 0.25 ml. One of the tRNAs was esterified with [^{14}C]glycine (110 mCi/mmol; 1 Ci = 37 GBq) and the other with [^{3}H]glycine (1 Ci/mmol).

The coat protein fragment was purified by gel filtration on a Sephadex G25 column (1 × 25 cm). Elution was with 8 M urea/0.1 M 2-mercaptoethanol/20 mM Mops buffer, pH 7.5 (buffer A). Fractions containing the fragment were combined and it was further purified by chromatography on a CM-Sepharose column (1.5 × 3 cm). The column was first washed with 10 ml of buffer A and the fragment was then eluted with a linear gradient of NaCl using 10 ml of buffer A in the mixing chamber and 10 ml of 1 M NaCl in buffer A in the reservoir.

The synthesized coat protein fragment contains four acid to eight basic amino acids and should therefore be positively charged at most pH values. This was verified by gel electrophoresis in a pH gradient from 3 to 10 (24) with the product labeled with [^{35}S]methionine or [^{35}S]cysteine. It appeared homogenous under these conditions and when subjected to SDS/polyacrylamide gel electrophoresis (25) as shown in Fig. 3. A detailed description of the protein-synthesizing *in vitro* system will be given in a forthcoming publication.

RESULTS

Expression of the $tRNA_1^{Gly}$ (TCC) Gene. By the procedure described in *Materials and Methods* the nucleotide in the $tRNA_1^{Gly}$ gene sequence corresponding to the wobble position was exchanged for T to give the anticodon gene sequence TCC. To express the mutated gene, it was cloned in the vector pKK223-3 under the tac promoter. The $tRNA_1^{Gly}$ (TCC) gene was efficiently expressed in *E. coli* so that the level of the corresponding tRNA in the cell was ≈20 times that of the wild-type $tRNA_1^{Gly}$ (CCC). When crude tRNA prepared from cells containing the recombinant vector was chromatographed on benzoylated DEAE-cellulose, the mutated tRNA appeared as a new peak with a position on the chromatogram in between that of $tRNA_1^{Gly}$ (CCC) and $tRNA_2^{Gly}$ (NCC, where N is an unknown derivative of U). These two glycine tRNAs are for all practical purposes the only ones that could contaminate the mutated $tRNA_1^{Gly}$ fraction obtained after the purification procedure. However, because of the very considerable overexpression of the mutated $tRNA_1^{Gly}$ gene in the pKK223-3 vector, the concentration in the cell of the mutated $tRNA_1^{Gly}$ was so high compared to the concentrations of $tRNA_1^{Gly}$ (CCC) and $tRNA_2^{Gly}$ (NCC) that even if a considerable fraction of these isoacceptors still contaminated the mutated $tRNA_1^{Gly}$ after the purification, they would, nevertheless, represent only a

Mycoplasma $tRNA^{Gly}$

GCAGGUGs^{4}UAGUUUAAUGGCAGAACUUCAGCCUUCCm^{6}AAGCUGAUUGUGAGGGUFCGAUUCCCUUCACCUGCUCCA

E. coli mutant $tRNA_1^{Gly}$

GCGGGCGs^{4}UAGUUCAAUGGDAGAACGAGAGCUUUCC A AGCUCUAUACGAGGGTFCGAUUCCCUUCGCCCGCUCCA

FIG. 1. Sequences of glycine isoacceptor tRNAs (19, 20). The only sequence difference between the mutant $tRNA_1^{Gly}$ (UCC) and wild-type $tRNA_1^{Gly}$ (CCC) is in the wobble position. m^{6}A, N^6-methyladenosine; F, pseudouridine; T, 5-methyluridine; S^{4}U, 4-thiouridine.

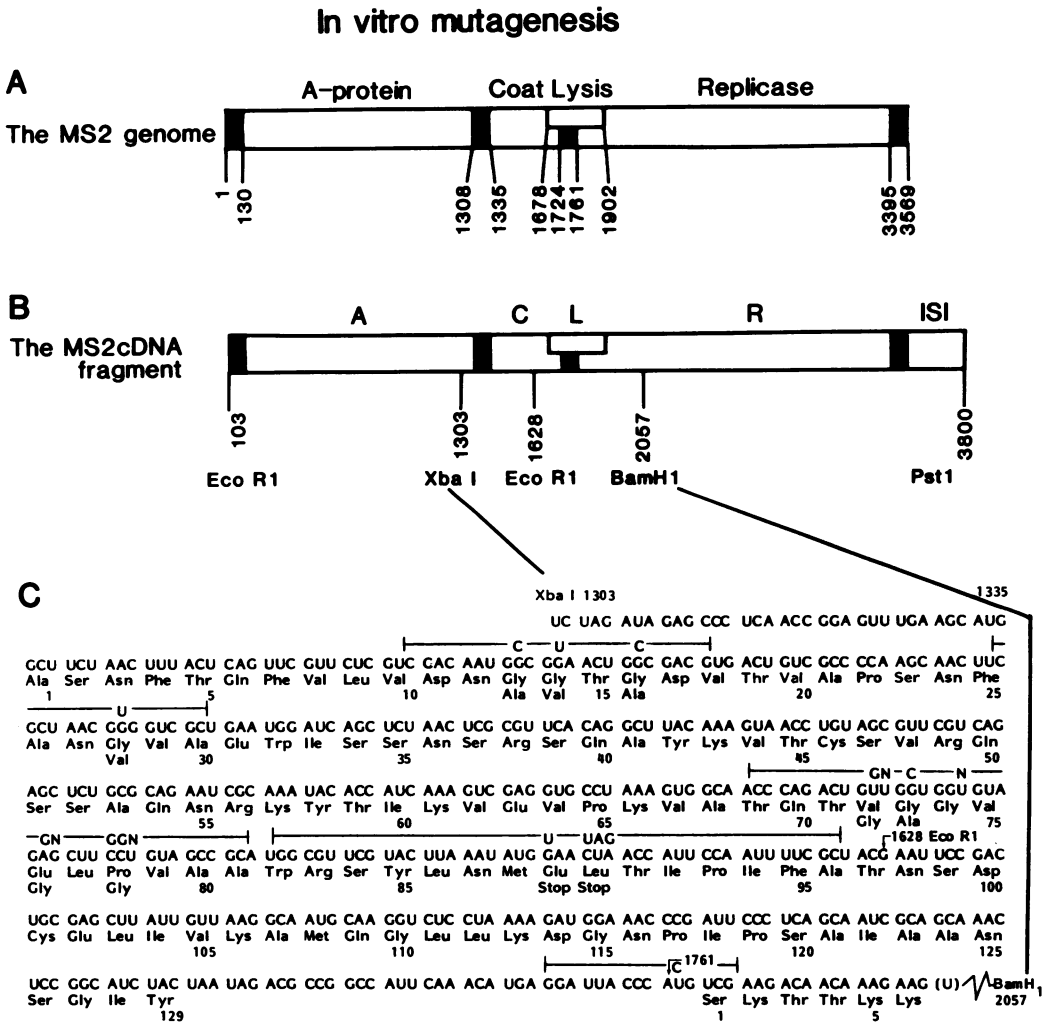

FIG. 2. Construction of the plasmid GEM$_1$-MS2. IS1, insertion sequence. For experimental details see *Materials and Methods*.

few percent of the mutated tRNA$_1^{Gly}$. This level of contamination would be of no importance for the outcome of the competition experiments described below.

Codon Reading Properties of tRNA$_1^{Gly}$ (UCC). The problem that we wanted to address was whether the structural context that the tRNA molecule provides for the anticodon could influence the ability of the anticodon to discriminate between the nucleotides present in the third position of the glycine codons. Specifically, we wanted to know whether this could possibly help to explain the ability of the mycoplasma glycine tRNA to read all four glycine codons without discrimination (8). We have therefore conducted a series of experiments in which the mycoplasma tRNA^{Gly} (UCC) competed against the

Table 1. Efficiency of glycine tRNAs in reading the glycine codons

	Relative reading efficiency			
	GGU	GGC	GGA	GGG
M. mycoides tRNA^{Gly} (UCC)/	57	59	1.2	6.9
E. coli tRNA$_1^{Gly}$ (UCC)	52	112	1.5	6.9
	111	37	0.7	9.1
	55	69	1.1	7.6
Mean	69			

To determine the relative reading efficiency of tRNA$_1^{Gly}$ (UCC) and mycoplasma tRNA^{Gly} (UCC), the tRNAs, esterified with [^{3}H]glycine and [^{14}C]glycine, respectively, were incubated together in the *in vitro* protein-synthesizing system programmed with the modified MS2 RNA messengers representing the different glycine codons described in *Materials and Methods*. By comparing the ratio of ^{3}H to ^{14}C in the coat protein fragment, synthesized in response to the modified MS2 RNA containing the appropriate glycine codon, with the isotopic ratio of the glycyl tRNAs incubated, the relative efficiency of the competing tRNAs in the reading of a certain codon could be calculated.

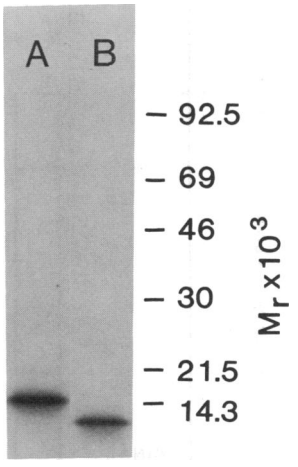

FIG. 3. SDS/polyacrylamide gel electrophoresis of full-length coat protein and the coat protein fragment (25). Full-length coat protein and the coat protein fragment were synthesized labeled with [^{35}S]methionine by translation of MS2 RNA and *Bam*HI MS2 RNA, respectively, as described in *Materials and Methods*. Lane A, full-length coat protein; lane B, coat protein fragment.

mutant $tRNA_1^{Gly}$ (UCC) for the glycine codons. It should be recalled that the wild-type $tRNA_1^{Gly}$, with the anticodon CCC, is very inefficient in the unconventional reading of GGU and GGC—i.e., it discriminates very effectively between the glycine codons as required by the wobble rules (8).

In a previous study of glycine codon reading, we have used the viral messenger MS2 RNA to program an *in vitro* protein synthesizing system, which under these conditions largely synthesized the MS2 coat protein (8). However, because of the unfavorable position of the codon GGG in the coat protein cistron, the reading properties of this codon could not be determined. For the present study, we have therefore used four different MS2 RNAs, modified by site-directed mutagenesis so as to make it possible to monitor the reading of all four glycine codons. For details the reader is referred to *Materials and Methods* and Fig. 2.

The S30 extract used in the experiments described below was prepared from an *E. coli* mutant with a temperature-sensitive glycine:tRNA ligase. The enzyme was extremely heat labile and, in our standard glycine:tRNA ligase assay, we could not detect any activity in the S30 extract. Consequently, our protein-synthesizing system was strictly dependent on added glycyl tRNA (8).

To evaluate the relative reading efficiencies of different isoacceptor glycine tRNAs, experiments were performed in which two tRNAs competed with each other for the same codon. In a competing pair, one tRNA was esterified with [^{3}H]glycine and the other with [^{14}C]glycine and the tRNAs were incubated together in equimolar concentrations in the *in vitro* protein-synthesizing system. The relative efficiency of the competing tRNAs in the reading of a certain codon could be calculated by comparing the ratio of ^{3}H to ^{14}C in the coat protein fragment synthesized in response to the modified MS2 RNA containing the appropriate glycine codon, with the isotopic ratio of the glycyl tRNAs incubated.

The yield of the translation was the same with the two tRNAs tested, as measured by the incorporation of glycine into the coat protein fragment synthesized using each tRNA alone as the only source of glycine.

The results of the competition experiments are summarized in Table 1 and the accompanying histogram (Fig. 4). The salient findings are that while the efficiency of $tRNA_1^{Gly}$ (UCC) was comparable to that of mycoplasma $tRNA^{Gly}$ (UCC) in the reading of the codon GGA, the mycoplasma $tRNA^{Gly}$ was far more efficient than the $tRNA_1^{Gly}$ (UCC) in the reading of the codons GGU and GGC. Thus, the anticodon UCC, when present in the structural context of the $tRNA_1^{Gly}$ molecule, behaved as predicted by the wobble rules. On the other hand, in the structural context of mycoplasma $tRNA^{Gly}$ it read without discrimination between the nucleotides in the third codon position in violation of the wobble restrictions (8). The result with the codon GGG showed that the anticodon UCC, when present in $tRNA_1^{Gly}$, was considerably less efficient in reading this codon than it was in the structural context of the mycoplasma $tRNA^{Gly}$.

An alternative interpretation of our results would be that both the intense discrimination and the wobble restriction shown by the mutant $tRNA_1^{Gly}$ (UCC) were caused by the presence of a modified uridine in the wobble position. This would seem unlikely, however, in view of our analysis of the wobble nucleotide, which showed that both in the Gupta–Randerath method (16) and when isolated from the ladder obtained in this method and subjected to two-dimensional TLC (17) it behaved as expected for a uridine diphosphate. Furthermore, when a hydrolyzate of $tRNA_1^{Gly}$ (UCC) was analyzed by HPLC (18) it was shown to contain only such derivative of uridine phosphate present already in $tRNA_1^{Gly}$ (CCC) (Fig. 1).

DISCUSSION

In all cases but one in which there is irrefutable evidence *in vivo* of a single tRNA reading all four codons in a family box—i.e., reading without discrimination in the third codon position—and the primary structure of the tRNA is known, it has an unsubstituted U in the wobble position (4). The only exception is the arginine tRNA that reads the codon family CGN in yeast mitochondria and has an A in this position (26). The ubiquity of unsubstituted U in this type of unconventional codon reading *in vivo* is of course suggestive and it has been argued that in the mitochondrion U in the wobble position can form stable base pairs with both U and C in the third codon position (4). The other possibility, that family codons ending in U or C may be read by a two-out-of-three mechanism in the mitochondrion, has also been suggested

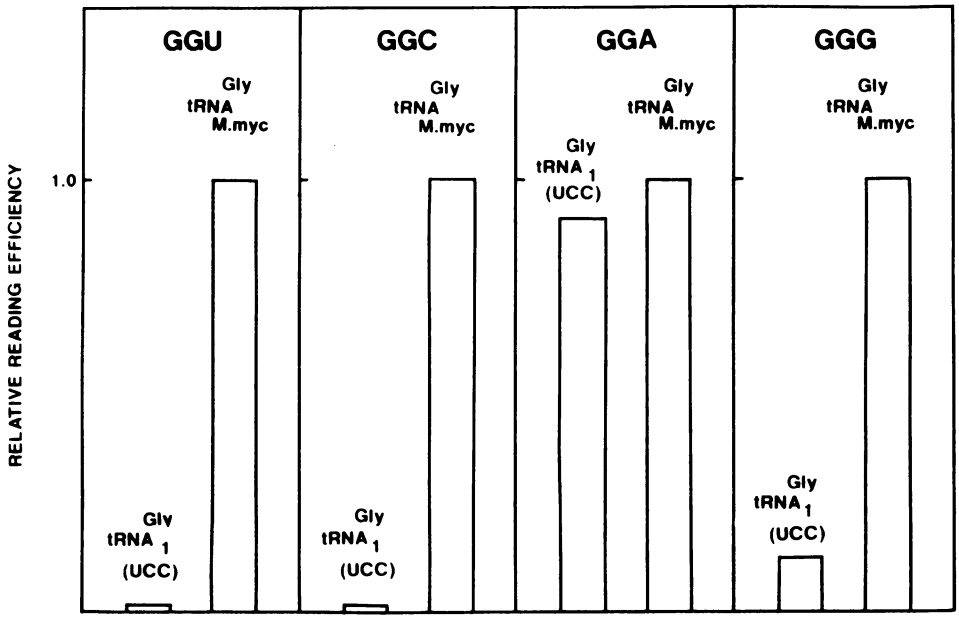

FIG. 4. Histograms showing relative reading efficiencies of the competing mycoplasma $tRNA^{Gly}$ (UCC) and the mutated $tRNA_1^{Gly}$ (UCC). The reading efficiencies are given as mean values, using the highest efficiency as an arbitrary unit. For further details see Table 1 and *Materials and Methods*.

(3), in which case the ubiquitous U in the wobble position would have been selected for in evolution because it forms the least objectionable mispairs (27).

It is not possible yet to decide what mechanism operates in unconventional codon reading without discrimination in the third codon position. However, it is possible to address the question of what role the wobble nucleotide and the structural context provided by the tRNA molecule play in this type of reading. As a first approach toward this goal, we have taken advantage of our previous finding that in our *in vitro* protein-synthesizing system $tRNA_I^{Gly}$ (CCC) from *E. coli* is remarkably inefficient in the unconventional reading of the codons GGU and GGC (8). Thus, $tRNA_I^{Gly}$ (CCC) and the mycoplasma $tRNA^{Gly}$ (UCC) can be said to represent the two extremes of unconventional codon reading efficiency. We have used site-directed mutagenesis to exchange a C in the wobble position of the gene for $tRNA_I^{Gly}$ (CCC) for T, so that the resulting tRNA would have U in this position. When analyzed by methods designed to detect nucleotide modifications, the mutant $tRNA_I^{Gly}$ was indeed found to contain an unsubstituted U in the wobble position.

When the $tRNA_I^{Gly}$ (UCC) was tested for its ability to read the glycine codons in our *in vitro* protein-synthesizing system, its efficiency was comparable to that of mycoplasma $tRNA^{Gly}$ (UCC) in the reading of the codon GGA while, on the other hand, it was far less efficient than mycoplasma $tRNA_I^{Gly}$ (UCC) in reading the codons GGU and GGC. In other words, the anticodon UCC, when present in the structural context of the $tRNA_I^{Gly}$ molecule, read the codons GGU, GGC, and GGA with discrimination between the nucleotides in the third codon position, entirely as required by the wobble rules. On the other hand, when present in the mycoplasma $tRNA^{Gly}$, the same anticodon did not discriminate between the glycine codons (8).

In this context, it should be emphasized that the efficient reading of GGA by the mutant $tRNA_I^{Gly}$ (UCC) makes it very unlikely that its poor reading of GGU and GGC could be explained by it being generally inefficient in translation.

The result of the experiments with the codon GGG, in which reading by the anticodon UCC would involve a wobble interaction, showed that this anticodon, when present in $tRNA_I^{Gly}$, was considerably less efficient in reading GGG than it was in the structural context of the mycoplasma $tRNA^{Gly}$. It would thus seem that this anticodon, when present in a certain tRNA molecule, can be an efficient wobbler while the same anticodon in the molecular environment of another tRNA is markedly restricted in its wobbling interactions.

Yarus and coworkers (28, 29) have provided evidence for what they call an extended anticodon. They find that the efficiency of a nonsense suppressor tRNA is dependent on the relation of the anticodon, in particular the so-called cardinal nucleotide in the 3′ position of the anticodon, to the structure of the rest of the anticodon loop and stem. Thus, the extended anticodon hypothesis deals with the influence of the anticodon structural context on the efficiency of a codon reading that is entirely according to the wobble rules. The phenomenon that we are primarily interested in, reading without discrimination between the nucleotides in the third codon position, is obviously very different from nonsense suppression. Nevertheless, there is a similarity in the sense that the structural context of the anticodon has a decisive influence on its reading properties.

Concluding Remarks. In this paper, we have shown that the anticodon UCC may be strongly influenced by its structural context in the tRNA molecule, both with regard to its ability to discriminate between the nucleotides occupying the third position of the glycine codons and also in terms of its ability to wobble with G. These results would seem to argue for a new concept in codon reading, which questions the supremacy of the anticodon in the selection of the correct codon and instead emphasizes the importance of the interplay between the anticodon and the rest of the tRNA molecule.

The expert technical assistance of A.-M. von Essen and M. Olsson is gratefully acknowledged. This investigation was supported by a grant from the Swedish Medical Research Council.

1. Crick, F. H. C. (1966) *J. Mol. Biol.* **19,** 548–555.
2. Barrell, B. G., Anderson, S., Bankier, A. T., de Bruijn, M. H. L., Chen, E., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R. & Young, I. G. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3164–3166.
3. Bonitz, S. G., Berlani, R., Coruzzi, G., Li, M., Macino, G., Nobrega, F. G., Nobrega, M. P., Thalenfeld, B. E. & Tzagoloff, A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3167–3170.
4. Heckman, J. E., Sarnoff, J., Alzner-Deweerd, B., Yin, S. & RajBhandary, U. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3159–3163.
5. Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B. Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. & Sugiura, M. (1986) *EMBO J.* **5,** 2043–2045.
6. Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S.-i., Inokuchi, H. & Ozeki, H. (1986) *Nature (London)* **322,** 572–574.
7. Kilpatrick, M. W. & Walker, R. T. (1980) *Nucleic Acids Res.* **8,** 2783–2786.
8. Samuelsson, T., Axberg, T., Borén, T. & Lagerkvist, U. (1983) *J. Biol. Chem.* **258,** 13178–13184.
9. Samuelsson, T., Elias, P., Lustig, F. & Guindy, Y. S. (1985) *Biochem. J.* **232,** 223–228.
10. Samuelsson, T., Guindy, Y. S., Lustig, F., Borén, T. & Lagerkvist, U. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3166–3170.
11. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1074–1078.
12. Zoller, M. J. & Smith, M. (1983) *Methods Enzymol.* **100,** 468–500.
13. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
14. Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E. & Tener, G. M. (1967) *Biochemistry* **6,** 3043–3056.
15. Garel, J. P., Garber, R. L. & Siddiqui, M. A. Q. (1977) *Biochemistry* **16,** 3618–3624.
16. Gupta, R. C. & Randerath, K. (1979) *Nucleic Acids Res.* **6,** 3443–3458.
17. Silberklang, M., Prochiantz, A., Haenni, A.-L. & RajBhandary, U. L. (1977) *Eur. J. Biochem.* **72,** 465–478.
18. Gehrke, C. W., Kuo, K. C. & Zumwalt, R. W. (1983) in *The Modified Nucleosides of Transfer RNA II: A Laboratory Manual of Genetic Analysis, Identification and Sequence Determination,* eds. Agris, P. F. & Kopper, R. A. (Liss, New York), pp. 59–91.
19. Kilpatrick, M. W. & Walker, R. T. (1980) *Nucleic Acids Res.* **8,** 2783–2786.
20. Hill, C. W., Combriato, G., Steinhart, W., Riddle, D. L. & Carbon, J. (1973) *J. Biol. Chem.* **248,** 4252–4262.
21. Devos, R., van Emmelo, J., Contreras, R. & Fiers, W. (1979) *J. Mol. Biol.* **128,** 595–619.
22. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488–492.
23. Samuelsson, T., Borén, T., Johansen, T.-I. & Lustig, F. (1988) *J. Biol. Chem.* **263,** 13692–13699.
24. Pharmacia Fine Chemicals (1982) *Isoelectric Focusing: Principles and Methods* (Pharmacia Fine Chemicals, Uppsala, Sweden), pp. 118–122.
25. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
26. Sibler, A.-P., Dirheimer, G. & Martin, R. P. (1986) *FEBS Lett.* **194,** 131–138.
27. Lagerkvist, U. (1981) *Cell* **23,** 305–306.
28. Yarus, M., McMillian, C., III, Cline, S., Bradley, D. & Snyder, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5092–5096.
29. Yarus, M. (1982) *Science* **218,** 646–652.