ROLE OF MODIFIED NUCLEOSIDE ADJACENT TO 3'-END OF ANTICODON IN CODON-ANTICODON INTERACTION

Kakoli Ghosh and H. P. Ghosh

Department of Biochemistry

McMaster University, Hamilton, Ontario, Canada.

Received May 26, 1970

Summary: Removal of the base Y adjacent to the anticodon of yeast $tRNA^{Phe}$ changes its coding properties. Modified $tRNA^{Phe}$ responds to the codon UUC better than the codon UUU. The codon UUU can be read efficiently only at higher Mg^{+2} conc. or in the presence of streptomycin. Binding studies show that the unmodified and the modified $tRNA^{Phe}$ can not occur next to each other on the two binding sites of a ribosome. Removal of Y changes the configuration of the anticodon loop of $tRNA^{Phe}$ such that the base (G) involved in "Wobble" changes its relative orientation to the other bases in the anticodon.

The phenylalanine transfer RNA ($tRNA^{Phe}$) of yeast and wheat germ contain a flourescent base Y of unknown structure adjacent to the 3'-end of the anticodon, 2'-OMeGAA (1,2). Occurrence of a modified adenosine next to the 3'-end of anticodon have been observed in the case of tRNA species corresponding to codons beginning with U or A (3,4). The presence of a modified nucleoside at this position in the anticodon loop suggests a possible role in the codon-anticodon interaction. Fuller and Hodgson (5) have proposed that the function of the alkylated purine in this position is to prevent the reading by the codon of any triplet other than the anticodon. It may also increase the stabilization of the stacked conformation of the anticodon such that the 5'-base ("Wobble" base) of the anticodon has the maximal degree of conformational flexibility. Previous studies involving modification or removal of the base adjacent to the anticodon suggest that this nucleoside is needed for binding of tRNA to ribosome-mRNA complex but it is not essential for charging of the tRNA (6,7,8).

The results presented in this communication show that the removal of the base Y adjacent to the anticodon of yeast $tRNA^{Phe}$ affects its coding properties. At low Mg^{+2} conc. the codon UUC is preferentially recognized. The codon UUU can be recognized only at a higher Mg^{+2} conc. or in the presence of streptomycin. These results suggest that the removal of Y from the anticodon loop changes the configuration and hence, the stacking pattern of the anticodon loop. The 5'-base, 2'-OMeG of the anticodon may now have a more rigid conformation and therefore can not "Wobble" pair with U.

Materials and Methods:

Yeast $tRNA^{Phe}$ was isolated by benzoylated DEAE - cellulose (BD-cellulose) chromatography followed by further chromatography on silicic acid coated with BD-cellulose (9,10). The acceptor activities of the $tRNA^{Phe's}$ isolated were 1.1 to 1.3 nmoles of phenylalanine per A_{260} unit. The base Y was removed by treating $tRNA^{Phe}$ with acid at pH 2.9, 37° for 4 hours and $tRNA_Y^{Phe}$ was isolated by BD-cellulose chromatography as described by Thiebe and Zachau (7). The $tRNA_Y^{Phe}$ isolated could accept 1.2 to 1.5 nmoles of phenylalanine per A_{260} unit. The preparations of $tRNA_Y^{Phe}$ were free from $tRNA^{Phe}$ as assayed by the fluorescence spectra. Flourescence spectra were determined in 0.05M cacodylate buffer, pH 7.0 containing 0.015M $MgCl_2$ (11,12). The tRNA species were charged with aminoacids using yeast aminoacyl-tRNA synthetase as described by Ghosh et al. (13). Aminoacyl-tRNA synthetase was purified from yeast as described by Nishimura et al. (14). Ribosomes were isolated from E. coli MRE600 either by the method of Gordon (15) or by the method of Erbe et al. (16). The aminoacid polymerizing factor T and G were purified from both E. coli MRE600 and E. coli B cells by a procedure involving the separation of nucleic acids by polymer phase separation followed by partial separation of the two factors T and G

by $(NH_4)_2\ SO_4$ fractionation (15). The two factors were further purified on a DEAE-Sephadex column as described by Erbe et al. (16). Polypeptide synthesis using ribopolynucleotides of repeating sequences were followed as before (13). Binding of aminoacyl-tRNA to ribosomes in the peptidyl site (P site) and aminoacyl site (A site) was followed as described by Ono et al. (17).

Results:

It was previously shown that yeast $tRNA^{Phe}$ charged homologously can transfer phenylalanine into polyphenylalanine under the direction of both poly r-U and poly r-(U-U-C), using a ribosomal system from E. coli (18). Results presented in Figure 1 show that at a Mg^{+2} conc. of 10mM Phe-$tRNA_{\gamma}^{Phe}$ fails to transfer phenylalanine into polyphenylalanine as

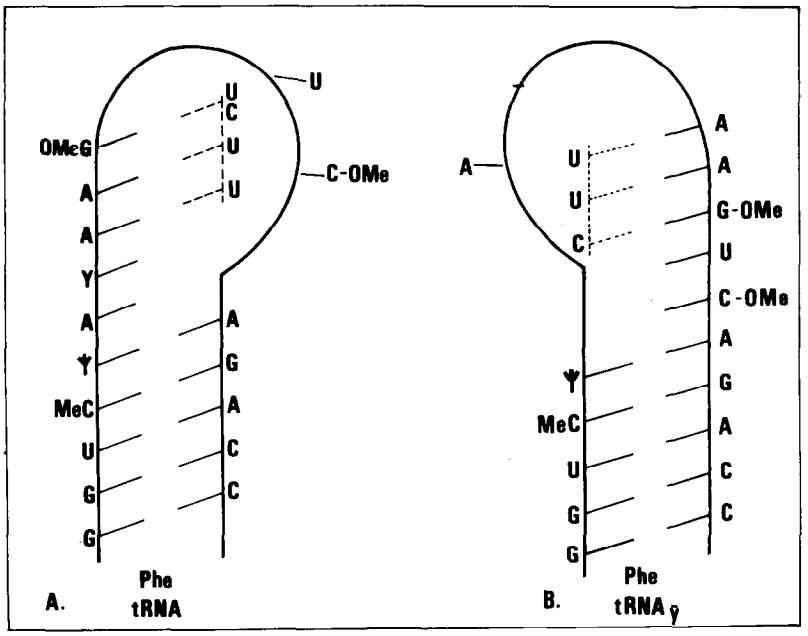

Fig. 1. Kinetics of incorporation of phenylalanine. The reaction mixture contained per ml: Tris-Cl. pH 7.5, 10 µmoles; NH_4Cl-160 µmoles: DTT-10 µmoles; GTP-1 µmole; washed ribosome-21 A_{260} units; T and G factor - 480µg; C^{14}-Phe-$tRNA^{Phe}$-350 µmoles or C^{14}-Phe-$tRNA_{\gamma}^{Phe}$-362 µmoles and Mg acetate as indicated. The template conc. was 1.0 A_{260} units of poly U or 5.8 µmoles of poly r-(U-U-C) per ml. Phenylalanine polymerized was assayed as described earlier (13).

TABLE 1

Effect of Streptomycin on Phenylalanine Incorporation

Mg^{+2} conc.	C^{14}-Phe-tRNA used	C^{14}-Phe Incorporated (pmoles/ml)					
		-poly U	+poly U	+poly U +Sm	-poly UUC	+poly UUC	+poly UUC + Sm
10 mM	$tRNA^{Phe}$	7.5	157	119	11.7	147	93
	$tRNA^{Phe}_{\overline{Y}}$	4.3	11	151	10.7	80	82
20mM	$tRNA^{Phe}$	9.4	73.5	57.5	12.4	51.4	45.7
	$tRNA^{Phe}_{\overline{Y}}$	5.5	140	178	11.3	90	89

Experimental conditions are described under Fig. 1. Streptomycin (Sm) present was 10 µg/ml. Template conc. was 1.0 A_{260} units of poly U or 5.6 nmoles of poly r-(U-U-C) per ml.

directed by poly r-U. Poly r-(U-U-C), however, is recognized by both Phe-tRNA^{Phe} and Phe-tRNA$^{Phe}_{Y}$ under identical condition. Clearly the triplet UUU is not recognized by the anticodon (2'-OMeGAA) of tRNA$^{Phe}_{\overline{Y}}$ under these conditions. Increasing the Mg^{+2} conc. to 20mM stimulates the transfer of phenylalanine from Phe-tRNA$^{Phe}_{\overline{Y}}$ directed by poly r-U by 75 fold. Polyphenylalanine synthesis from Phe-tRNA$^{Phe}_{\overline{Y}}$ in presence of poly r-(U-U-C) is, however, increased only by 2-fold. These results suggest that at higher Mg^{+2} conc. both the codons UUU and UUC are recognized efficiently by the anticodon of tRNA$^{Phe}_{Y}$.

Since streptomycin is known to induce miscoding (19), we studied the effect of streptomycin on phenylalanine incorporation from Phe-tRNA$^{Phe}_{\overline{Y}}$ directed by both poly r-U and poly r-(U-U-C). The results are shown in Table 1. Presence of streptomycin at a conc. of 10 µg/ml has no effect on the incorporation of phenylalanine at both 10mM and 20mM Mg^{+2} conc. from Phe-tRNA$^{Phe}_{\overline{Y}}$, directed by poly r-(U-U-C). Polyphenlalanine synthesis at 10mM Mg^{+2} from Phe-tRNA$^{Phe}_{\overline{Y}}$ directed by poly r-U in presence of streptomycin is, however, stimulated by 25-fold. At higher Mg^{+2} conc. no significant stimulation is observed. The above results suggest that removal of the base Y from the anticodon loop of tRNA^{Phe} produces a change in the configuration of the anticodon loop. The anticodon 2'-OMeGAA might have changed its relative orientation in the anticodon loop of tRNA$^{Phe}_{\overline{Y}}$ so that the "Wobble" base (2'-OMeG) can no longer pair with U at lower Mg^{+2} conc.

A steric hindrance between the modified tRNA molecule and the normal tRNA molecule may thus occur when they occupy the two consecutive binding sites on the ribosome-mRNA complex. We, therefore, tested the enzymatic binding of Phe-tRNA$^{Phe}_{\overline{Y}}$ at the A site of poly r-U-ribosome complex containing N-acetyl Phe-tRNA^{Phe} or N-Ac-Phe-tRNA$^{Phe}_{Y}$ at the P site. The results are presented in Table 2. The results show that C^{14}-Phe-tRNA$^{Phe}_{Y}$ can not bind to poly r-U-ribosome complex at both 10mM

TABLE 2

Binding of C^{14}-Phe-$tRNA^{Phe}_{\bar{Y}}$ to A site

Mg^{+2} conc.	Ac-Phe-tRNA in the P site	Total Ac-H^3-Phe-tRNA bound	Total C^{14}-Phe-$tRNA^{Phe}_{\bar{Y}}$ bound (p moles/ml)	Ac-H^3-Phe-C^{14}-Phe formed
10 mM	Ac-H^3-Phe-$tRNA^{Phe}$	71.2	5.4	< .05
	Ac-H^3-Phe-$tRNA^{Phe}_{\bar{Y}}$	7.0	14.0	7.8
20 mM	Ac-H^3-Phe-$tRNA^{Phe}$	72.0	11.0	< .05
	Ac-H^3-Phe-$tRNA^{Phe}_{\bar{Y}}$	23.6	22.0	20.3

The two step reaction as described by Ono *et al.* (17) was followed. In step I the reaction mixture contained per ml. 31 A_{260} units of ribosome, 4 A_{260} units of poly U, 126 pmoles of Ac-H^3-Phe-$tRNA^{Phe}_{\bar{Y}}$ or 135 pmoles of Ac-H^3-Phe-$tRNA^{Phe}_{\bar{Y}}$ and Mg acetate as indicated. Step II reaction mixture contained per ml. 0.05 µmoles of GTP, 1 µmoles of Fusidic acid, 150 µg of T, 435 pmoles of C^{14}-Phe-$tRNA^{Phe}_{\bar{Y}}$ and Mg acetate as indicated. Both reaction mixture contained 0.04M Tris-Cl, pH 7.4, 0.01M DTT and 0.16M NH_4Cl.

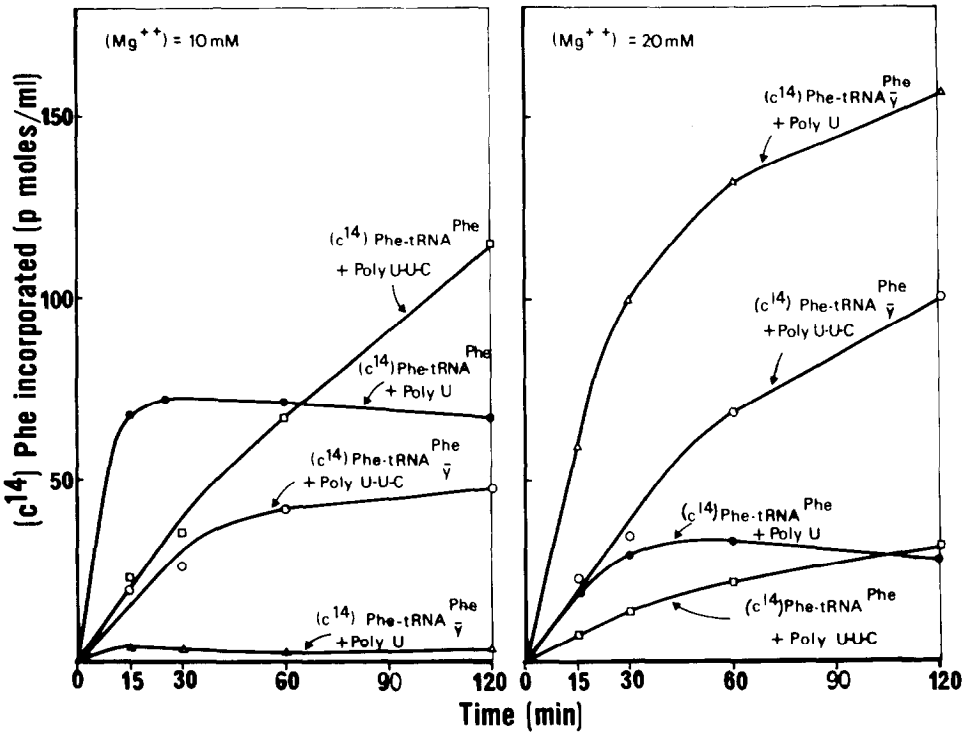

Fig. 2. Schematic diagram of the model for tRNA anticodon arm. (a) Fuller and Hodg son model for $tRNA^{Phe}$. (b) Proposed alternate model for $tRNA^{Phe}_{\bar{Y}}$. The alternate model proposes a change in the stacking pattern of the anticodon only. The helical character of the structure is considered to be the same as postulated by Fuller and Hodgson (5).

---

and 20mM Mg^{+2} when the P site of the ribosome is occupied by N-Ac-H^3-Phe-$tRNA^{Phe}$. On the other hand when the P site of ribosome-poly r-U complex is occupied by N-Ac-H^3-Phe-$tRNA^{Phe}_{\bar{Y}}$, C^{14}-Phe-$tRNA^{Phe}_{\bar{Y}}$ can bind to the A site and the dipeptide N-Ac-H^3-Phe-C^{14}-Phe is synthesized. These results strongly suggest that $tRNA^{Phe}$ and $tRNA^{Phe}_{\bar{Y}}$ have different configuration of the anticodon loops. A possible schematic diagram of the anticodon loops of $tRNA^{Phe}$ and $tRNA^{Phe}_{\bar{Y}}$ is shown in Figure 2.

Discussion

Previous workers reported that removal of the modified base adjacent to the anticodon or modification of the side-chain of this base affects

its binding capacity to the mRNA-ribosome complex and hence, its ability to transfer aminoacids into polypeptide chain (6,7,8). Our finding, however, show that removal of this modified base affects only the nature of codon recognition by the anticodon. Presumably, the conformation of the anticodon loop is changed such that two pyrimidines and the anticodon are now stacked together (Fig. 2). The "Wobble" base has no longer the flexibility needed for its "Wobble" pairing. Increasing the Mg^{+2} conc. or presence of streptomycin may increase the stability of the "Wobble" pair. It is known that streptomycin tightens the structure of ribosome and allow miscoding (20). Also Mg^{+2} conc. is known to change the conformation of the anticodon loop (12,21). The $tRNA_{\overline{Y}}^{Phe}$ molecule with the changed configuration of the anticodon loop, however, recognizes the codon UUC which involves normal base pairing. Also Phe-$tRNA_{\overline{Y}}^{Phe}$ forms the tertiary complex (T-GTP-Phe-$tRNA_{\overline{Y}}^{Phe}$) with GTP and T equally as well as Phe-$tRNA^{Phe}$, indicating that the modified base and the configuration of the anticodon loop is not recognized by T factor (unpublished observation). Our studies lend support to the Fuller and Hodgson model of anticodon loop structure and suggest that the modified base is involved in the stacking of the bases in the anticodon loop so that 5'-base of the anticodon may "Wobble". Furthermore, our results show that an alternate model of anticodon loop conformatio is possible and functionally active. Results of oligonucleotide binding to the anticodon loop of $tRNA_F^{Met}$ have already suggested an alternate conformation of the anticodon loop (22).

Acknowledgment: We thank Dr. R. H. Hall for his interest and critical discussion. This work has been supported by a grant from the Medical Research Council of Canada (grant no. MA 3514). H.P.G. is a Medical Research Council Scholar.

References

1. U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, and H. G. Khorana, Proc. Natl. Acad. Sci., U.S., 57 751 (1967).
2. B. S. Dudock, G. Katz, E. D. Taylor, and R. W. Holley, Proc. Natl. Acad. Sci., U. S., 62, 941 (1969)

3. D. J. Armstrong, W. J. Burrows, F. Skoog, K. L. Roy, and D. Söll, Proc. Natl. Acad. Sci., 63, 834 (1969)
4. H. Ishikura, Y. Yamada, K. Murao, M. Saneyoshi, and S. Nishimura, Biochem. Biophys. Res. Cmmun., 37, 990 (1969).
5. W. Fuller, and A. Hodgson, Nature, 215, 277 (1967).
6. F. Fittler, and R. H. Hall, Biochem, Biophys. Res. Commun., 25, 441 (1966).
7. R. Thiebe, and H. G. Zachau, European J. Biochem., 5, 546, (1968).
8. M. L. Gefter, and R. L. Russell, J. Mol, Biol., 39, 145 (1969).
9. I. Gillam, S. Millward, D. Blew, M. von Tigerstrom, E. Wimmer, and G. M. Tenner, Biochemistry, 6, 3043 (1967).
10. E. Wimmer, I. H. Maxwell, and G. M. Tenner, Biochemistry, 7, 2623 (1968).
11. D. Yoshikami, G. Katz, E. B. Keller, and B. S. Dudock, Biochem. Biophys. Acta, 166, 714 (1968).
12. J. Eisinger, B. Fener, and T. Yamane, Proc. Natl. Acad. Sci., U. S., 65, 638 (1970).
13. H. P. Ghosh, D. Söll, and H. G. Khorana, J. Mol. Biol. 25, 275 (1967)
14. S. Nishimura, F. Harada, U. Narushima, and T. Seno, Biochim, Biophys. Acta 142, 133 (1967).
15. J. Gordon, J. Biol. Chem., 244, 5680 (1969).
16. R. W. Erbe, M. M. Nau, and P. Leder, J. Mol. Biol., 38, 441 (1969).
17. Y. Ono, A. Skoultchi, J. Waterson, and P. Lengyel, Nature, 222, 645 (1969).
18. D. Söll, and U. L. RajBhandary, J. Mol. Biol., 29, 113 (1967).
19. J. Davies, W. Gilbert, and L. Gorini, Proc. Natl. Acad. Sci., U. S., 51, 883 (1964).
20. M. I. Sherman, and M. V. Simpson, Proc. Natl. Acad. Sci., U. S. 64, 1338 (1969).
21. K. Beardsley, and C. R. Cantor, Fred. Proc., 29, 672 (1970).
22. O. C. Uhlenbeck, J. Baller, and P. Doty, Nature 225, 508 (1970).