CODING PROPERTIES OF $tRNA^{glu}$ OF MAMMALIAN ORIGIN: COMPARISON BETWEEN RAT LIVER AND MINIMAL DEVIATION HEPATOMA 5123C $tRNA's^{glu}$

Fabio Gonano and Giuseppe Pirrò
Istituto di Patologia Generale, Università degli Studi, Modena, Italy

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SUMMARY. 5-Methyl-2-Thiouridine has been found in rat liver $tRNA^{glu}_{,}$ but its position in the molecule has not yet been determined. Chromatography on RPC-II colums of tRNA's isolated from rat liver and Morris 5123C minimal deviation hepatoma revealed the presence of a $tRNA^{glu}_2$ that binds only to GAA and not to GAG. This finding is not consistent with the wobble hypothesis and indicates that a 2-thiouracil derivative should be in the first position of the anticodon as has been found in $tRNA^{glu}$ in yeast and E.coli. Of the two other $tRNA^{glu}$ which bind to GAA and GAG, one is absent from the minimal deviation hepatoma. This finding is discussed in relation to the possible regulatory role of $tRNA^{glu}_3$ in rat liver.

Recently Kimura-Harada et al.(1) reported that one of the $tRNA^{glu}$ isolated from rat liver contained 5-methyl-2-thiouridine. The presence of a minor base containing sulfur in a mammalian tRNA strongly suggests a similarity with $tRNA^{glu}$ from yeast and E.coli. In the latter two species, a 2-thiouridine derivative is positioned in the anticodon portion of the $tRNA^{glu}$ molecule (2,3). The presence of this odd base in the 5' - position of the anticodon loop implies a unique molecule of $tRNA^{glu}$ unable to base pair according to Crick's wobble hypothesis with G. The presence of a sulfur atom instead of oxygen in the C-2 position of 5-methyluracil does not allow the formation of a base pair with the N-3 hydrogen of uracil. Osashi et al.(3) showed that in E.coli the binding affinity of the $tRNA^{glu}_2$ containing the 2-thiouridine derivative was quite high for GAA(base pair U-A) and practically negligible for GAG(non-permissive wobble pair between G and 2-thiouracil). Also in yeast $tRNA^{glu}_{,}$ a similar base was found by Sekiya et al.

(4);the binding capacity for GAG was still present in the three $tRNA^{glu}$ species,but a fourth $tRNA^{glu}$ was specific only for GAG. In this paper we have reported the separation of a minor $tRNA_2^{glu}$ from rat liver and from a minimal deviation hepatoma (5123C from dr.Morris Series).This $tRNA_2^{glu}$ binds to GAA but not to GAG; presumably it corresponds to the $tRNA^{glu}$ reported by Kimura-Harada et al.(1).The absence of a third main $tRNA^{glu}$ in the minimal deviation hepatoma,confirming a previous finding of Gonano et al. (6), will be discussed in relation to its possible role as regulatory molecule in the liver cell.

MATERIALS AND METHODS. Crude tRNA was prepared from livers of Buffalo strain rats and from 5123C hepatoma inoculated in the leg of rats of the same strain,as described by Gonano et al.(6). The acceptor activity of crude tRNA was approximately 80 pmoles of glutamic acid per one A_{260} unit of tRNA using a partially purified synthetase(6). The separation of tRNA's^{glu} was achieved by means of a RPC-II column using a linear gradient of NaCl from 0.3 to 0.7 M.The detailsof the preparative column were described previously (6). The trinucleotides GAA and GAG were prepared according to the method of Leder et al.(7)using the dinucleotide monophosphate GpA purchased from Waldhof(Mannheim,Germany). One of us (F.G.) is grateful to dr. P.Leder for the introduction to this technique. Ribosomes were prepared according to the method of Kurland (8) and stored in liquid N_2 until used. All the radioactivity determinations were done in a Packard liquid scintillation counter model 2000,using a standard PPO-dimethyl-POPOP toluene scintillation mixture. ^{14}C-glutamic acid, specific activity 208mCi/mmole, and ^{3}H-glutamic acid, specific activity 2.06 Ci/mmole, were purchased from New England Nuclear Corp., USA.

RESULTS. Figure 1 shows the profiles obtained when ^{3}H- and ^{14}C-glutamyl-tRNA from rat liver and from 5123C hepatoma, respectively, were co-chromatographed on an RPC-II column. The presence of multiple forms of $tRNA^{glu}$ confirmed the findings of Gonano et al.(6)

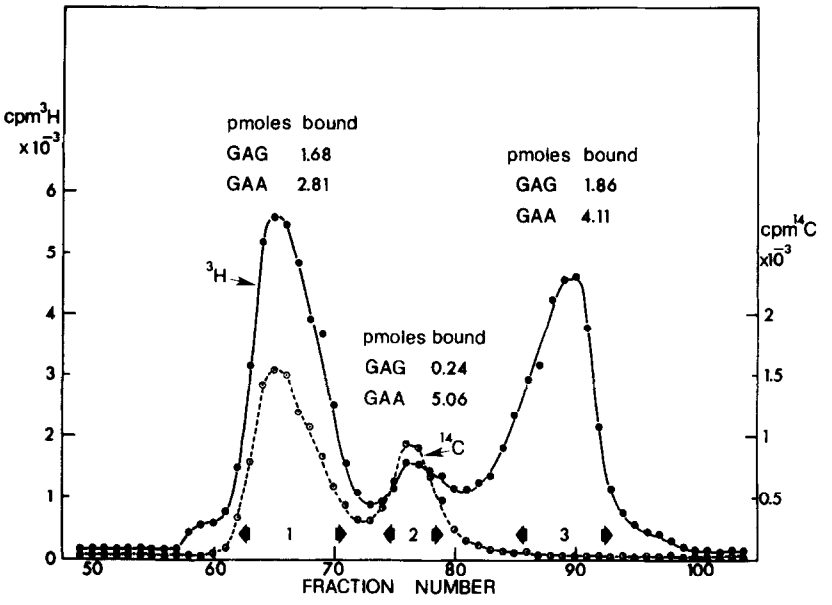

FIGURE 1.- Elution profiles obtained from a preparative RPC-II(5) column with ^{3}H-glutamyl-tRNA from rat liver (——●——) and ^{14}C-glutamyl-tRNA (- - - -⊙- - - -) from minimal deviation hepatoma 5123C. The counts per minute represent the ^{3}H and ^{14}C precipitated by cold 5% tricloroacetic acid from one ml of effluent out of 10 ml. The rest of the effluent in each tube was combined as indicated by the arrows, concentrated by means of a Diaflo UM-2 filter (Amicon Co.), dialyzed against 0.001 M sodium acetate buffer pH 4.5 and stored in small volumes at -20°C.

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of the presence of two $tRNA^{glu}$ from rat liver and one $tRNA^{glu}$ from 5123C hepatoma, corresponding to the fastest moving peak from rat liver. There was a shoulder associated with the fastest moving $tRNA^{glu}$ peak from rat liver and the corresponding one from 5123C hepatoma. In the experiment shown in Figure 1, the shoulder has been resolved as a single peak in both rat liver and 5123C hepatoma $tRNA^{glu}$. The coding properties of the three species of $tRNA^{glu}$ were studied using the Nirenberg and Leder (9) technique with triplets of known sequence corresponding to the glutamic acid code words (10). The results are illustrated in Figure 1 and in more detail in table I. $tRNA^{glu}_{1}$ and $tRNA^{glu}_{3}$ were bound to the ribosomes in the presence of both GAA and GAG. The per cent of incorporation

CODON RESPONSE OF GLUTAMYL-tRNA's

	glutamyl-$tRNA^{glu}$ source		
Template	$tRNA_1^{glu}$	$tRNA_2^{glu}$	$tRNA_3^{glu}$
GAA	2.81 (40%)	5.06 (80%)	4.11 (59%)
GAG	1.68 (24%)	0.24 (4%)	1.86 (26%)
Input	6.98	6.37	6.98

TABLE I.- The results are expressed as picomoles of glutamic acid incorporated and as the per cent of the total radioactivity (in brackets) bound to Gelman 0.45 u filters. Each reaction mixture contained the indicated picomoles of radioactivity as glutamyl-tRNA, one A_{260} Unit of ribosomes and 0.2-0.4 mumoles of GAA and GAG, respectively, at 0.01 M $MgCl_2$ concentration. All other conditions were as described previously.

with respect to the picomoles of glutamic acid attached to $tRNA^{glu}$ introduced in each assay is higher with GAA, indicating that this triplet has a higher affinity for glutamyl-$tRNA^{glu}$ than does GAG. $tRNA_2^{glu}$, which is present in both rat liver and 5123C hepatoma, does not recognize GAG. As shown in Table I, however, $tRNA_2^{glu}$ binds to GAA with a significantly higher efficiency than do the other two $tRNA^{glu}$; increasing the GAG A_{260} units in each assay didn't change the per cent of $tRNA_2^{glu}$ bound to the ribosomes.

DISCUSSION. The results reported in this paper are in accordance with some recent reports concerning the relationship of the coding properties of various species of $tRNA^{glu}$ isolated from E.coli and yeast to the presence of a 2-thiouracil derivative in the anticodon portion of the molecule. Ukita and his collaborators (2,4) demonstrated that the anticodon portion of $tRNA_3^{glu}$ from yeast contains 2-thiouridine. The results of coding experiments showed that $tRNA_3^{glu}$ is less efficiently bound to GAG than is $tRNA_1^{glu}$ which binds only

to the GAG triplet. In their initial paper, Ukita et al. reported (ref. 4, fig. 5) that an equal number of picomoles of $tRNA_2^{glu}$, $tRNA_3^{glu}$ and $tRNA_4^{glu}$ were bound to GAG. Structural studies which were performed only on $tRNA_3^{glu}$ revealed the presence of 2-thiouridine in the anticodon portion of the molecule. According to the wobble hypothesis if uracil is present in the first position of the anticodon, an ambiguous reading of two triplets $\frac{CUU}{GAG}$ and $\frac{CUU}{GAA}$ can occur. If a sulfur atom is present in the 2 position of the uracil ring, as in $tRNA_3^{glu}$ from yeast, no wobble pair is possible between 2-thiouridine and the G of the codon triplet. Ohashi et al. (3) have reported similar observations for an E.coli tRNA containing a thiobase, tentatively characterized as 5-methyl-amino-methyl-2-thiouridine, presumably also located in the first position of the anticodon portion of $tRNA_2^{glu}$. The codon response of $tRNA_2^{glu}$ from E.coli is significantly lower with GAG than with GAA (3). No reports have been published on the coding response of the other $tRNA's^{glu}$ of E.coli. Nishimura and his coworkers have reported very recently (1) that rat liver $tRNA_3^{glu}$ also contains a 5-methyl-2-thiouridine. No extensive reports have been published on the coding properties of this $tRNA^{glu}$ or on the position of the thiobase in the $tRNA_3^{glu}$ molecule.

However, Kimura-Harada (1) briefly stated that $tRNA_3^{glu}$ preferentially binds to GAA. On the basis of these data we interprete our results with $tRNA_2^{glu}$ to indicate that its capacity to bind only to GAA is due to the presence of a thiouridine in the first position of the anticodon. It is interesting to note that another case of low affinity for GAG has been described by Marshall and Nirenberg (11) for $tRNA^{glu}$ from Xenopus laevis nerula. They observed that only GAA promoted the binding to ribosomes of $tRNA^{glu}_{unfr.}$ extracted from embryos of Xenopus laevis. However, both GAA and GAG were able to stimulate the binding to the ribosomes of $tRNA^{glu}_{unfr.}$ from adult Xenopus laevis liver. It is possible that in this case, too, only the $tRNA^{glu}$ containing the 2-thiouridine is present in the embryo whereas in the

adult there may be two $tRNA^{glu}$ species, one of which contains uracil. In our case the normal rat liver and minimal deviation hepatoma showed the same pattern as far as the two first peaks of acceptor activity were concerned. The $tRNA^{glu}$ that presumably contains a 2-thiouridine derivative (peak 2 in Figure 1) is present also in the tumor tissue. As discussed in a previous paper (6), the comparison among all the tRNA's extracted from the two tissues demonstrated only few alterations in the tRNA profiles of the twenty amino acids even though the minimal deviation hepatoma is a malignant tumor of hepatic origin. A new $tRNA^{phe}$ which is present only in the hepatoma has been studied in detail (6,12,13) but no differences were found in coding properties between the two $tRNA^{phe}$ species present in the hepatoma and the one $tRNA^{phe}$ present in rat liver. We have now further characterized the disappearence of $tRNA_3^{glu}$ in the hepatoma cell as compared to the hepatocyte. Both $tRNA_1^{glu}$ and $tRNA_3^{glu}$ have the same coding properties. Therefore, the meaning of $tRNA_3^{glu}$ in the liver cell can not be explained in terms of different reading of messenger triplets. One possible explanation comes from the recent work of Jacobson (14) who suggests a possible regulatory function for special tRNA molecules. This possibility is now under investigation in our laboratory. The different chromatographic behaviour, together with identical coding properties with $tRNA_1^{glu}$ are in favour of structural variations in regions of the molecule not involved in translation processes. The more precise recognition of only one code word by $tRNA_2^{glu}$ gives to this molecule a very high fidelity in the reading of the messenger.

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