Volume 8 Number 2 1980 Nucleic Acids Research

The nucleotide sequence of glutamate $tRNA_4$ of Drosophila melanogaster

Martin Altwegg and Eric Kubli

Zoological Institute, University of Zurich-Irchel, Winterthurerstrasse 190, CH-8006, Zürich, Switzerland

Received 17 December 1979

ABSTRACT

The nucleotide sequence of Drosophila melanogaster glutamate $tRNA_4$ was determined to be: pU-C-C-C-A-U-A-U-G-G-U-C-Ψ-A-G-D-G-G-C-D-A-G-G-A-U-A-U-C-U-G-G-C(m)-U-U*-U-C-A-C-C-A-G-A-A-G-G-C-C-C-G-G-G-T-Ψ-U-C-G-A-U-U-C-C-C-G-G-U-A-U-G-G-G-A-A-C-C-A_{OH}. A partial modified C is found at position 32 in the anticodon loop.

INTRODUCTION

The combination of molecular cloning techniques with fast sequencing methods has yielded many interesting insights into the structure and function of eukaryotic tRNA genes[1-3]. Injections of purified tRNA genes into nuclei of Xenopus laevis oocytes and "in vitro" transcription experiments with germinal vesicle extracts from Xenopus laevis oocytes make it feasible to study the transcription of these genes and the subsequent processing steps leading to the mature tRNAs[4-7]. Despite the existence of these elegant approaches the determination of the primary structure of a mature tRNA with all its modifications is still a prerequisite for biologically meaningful studies of these problems.

Drosophila melanogaster is an ideal study object for the elucidation of the structure and function of tRNA and tRNA genes of higher eukaryotes. The genes coding for tRNAs can be localized by "in situ" hybridization to polytene salivary gland chromosomes and sophisticated genetic techniques allow the manipulation of the genome. For example, it has been shown that the

genes for $tRNA_4^{Glu}$ are localized in three regions of the Drosophila melanogaster genome[8]. Two sites are of special interest. The region 56 F contains the 5 S RNA genes[9] and a putative $tRNA_4^{Glu}$ -gene deletion mutant with an altered $tRNA^{Glu}$ isoacceptor profile is localized near the region 52 F[10]. In this paper we describe the isolation and the sequencing of $tRNA_4^{Glu}$ of Drosophila melanogaster.

MATERIALS AND METHODS

Purification of Drosophila $tRNA_4^{Glu}$: Transfer RNA was isolated from adults of Drosophila melanogaster (Oregon strain) of mixed age as described[8]. About 100 A_{260} units of tRNA were then loaded on an anticodon-anticodon affinity column[11,12]. Drosophila melanogaster $tRNA_4^{Glu}$ forms a stable complex at 4° C with the immobilized yeast $tRNA^{Phe}$ (anticodon G^mAA) and is eluted by raising the temperature to 35° C and adding EDTA. The $tRNA^{Glu}$ preparation was further purified by two-dimensional polyacrylamide gel electrophoresis[13]. The gels were stained in ethidium bromide (10 ug/ml). Spots visible under UV light were cut out, eluted and ethanol precipitated. Figure 1 shows a representative picture of the tRNA species after separation by two-dimensional gel electrophoresis. Spot 1 was identified as $tRNA_4^{Glu}$ (not shown), whereas the specificity of spot 2 tRNA is not known.

Sequence analysis: Materials, enzymes and methods used were described previously[14-16]. Most of the sequence was determined with the gel sequencing method of Stanley and Vassilenko[15]. Analysis of the 5' end of each band after complete digestion with nuclease P_1 was done in two different solvent systems according to Silberklang et al.[14]. Furthermore the sequences of some "in vitro" labeled pancreatic or T_1 oligonucleotides were determined by two-dimensional homochromatography after partial digestion with nuclease P_1 or snake venom phosphodiesterase[14] or after limited hydrolysis in hot formamide (100° C, 45 min.).The very long oligonucleotide comprising the anticodon region was partially digested with RNase U_2 and Phy I RNase, respectively and

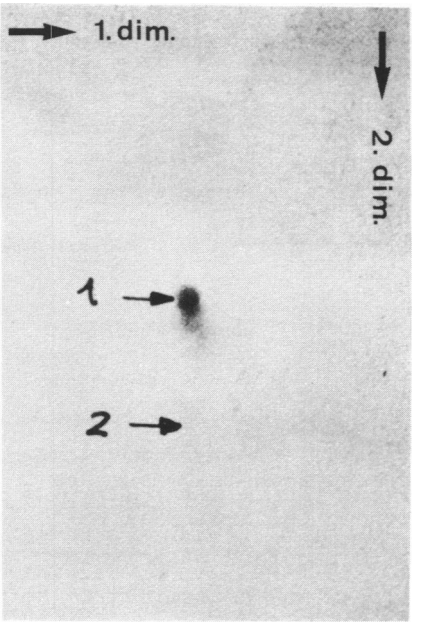

Figure 1 Two-dimensional PAGE of tRNA isolated by affinity chromatography.

analyzed on a 20%-8 M urea-polyacrylamide gel[17].

Additional information was obtained from "in vivo" uniformly labeled $tRNA_4^{Glu}$ by standard sequencing procedures[11,18].

RESULTS

Formamide digests of intact $tRNA_4^{Glu}$: Most of the sequence of $tRNA_4^{Glu}$ was determined by limited hydrolysis of the intact tRNA in hot formamide, labeling the fragments at their 5' end and analysis of 5' terminal nucleotides after electrophoresis on a 20 %-8 M urea polyacrylamide gel. The results are summarized in Fig. 2. However,this method did not give clear cut results for the last nucleotide at the 5'end of the tRNA (most probably due to a strong secondary structure maintained during the kinase reaction and/or the gel electrophoresis). The results were also not clear for the region comprising the anticodon.

Pancreatic RNase fragments: For a summary of sequenced frag-

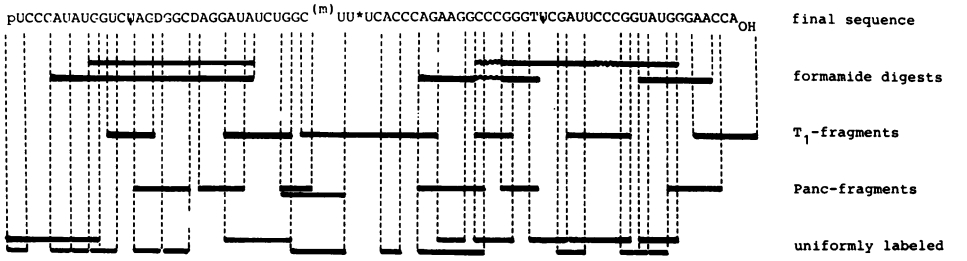

Figure 2 Compilation of sequences determined by different methods.

ments see Fig. 2.

RNase T_1 fragments: For a summary of sequenced fragments see Fig. 2. The very long fragment comprising the anticodon region was analyzed very carefully due to the lack of information from polyacrylamide gels. Its 5' end was determined to be a mixture of pC and pC^m respectively, suggesting a partial modification of nucleotide 32 as observed previously for $tRNA_2^{Phe}$ of Drosophila melanogaster[16]. The entire sequence of the fragment was determined by two-dimensional homochromatography after either partial digestion with nuclease P_1 or limited hydrolysis in hot formamide (Fig. 3a,b). Double spots were due to a heterogeneity (U-U*, see discussion) in position 34. In this heterogeneity U* was resistant to nuclease P_1 whereas U was not, resulting in a different chromatographic pattern. The jump of the terminal G in the formamide digest is not G-like, probably because the 3' terminal phosphate group has been removed by formamide. Furthermore, the fragment was digested with either RNase U_2 or Physarum-enzyme and then analyzed , together with a formamide ladder, on a 20 %-8 M urea-polyacrylamide gel (Fig. 3c).

Uniformly labeled $tRNA_4^{Glu}$: For a summary of fragments sequenced see Fig. 2.

The complete sequence of $tRNA_4^{Glu}$ of Drosophila melanogaster with its partial modification is shown in Fig. 4.

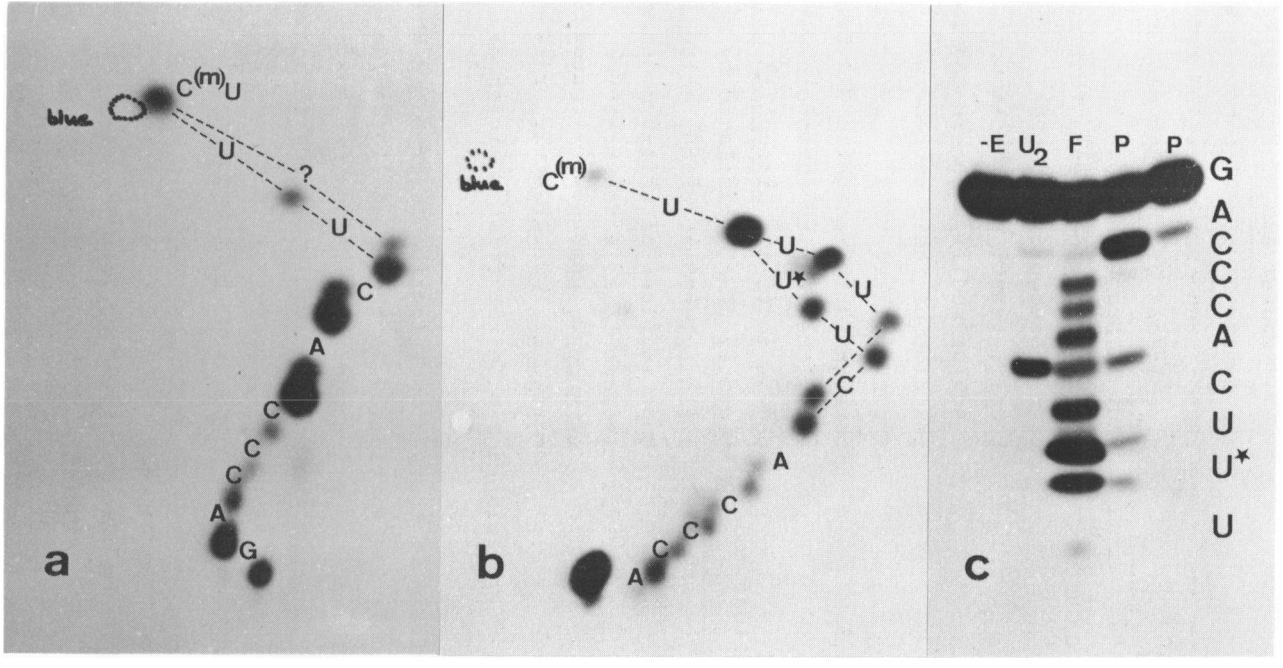

Figure 3 Determination of the sequence of the anticodon T_1-fragment. (a) and (b): Two-dimensional homochromatography of partial P_1 (a) and formamide (b) digests. (c): Sequencing gel. -E = without enzyme; U_2 = RNase U_2 ($2 \cdot 10^{-1}$ units); F = formamide ladder; P = Physarum enzyme (0.5 ul/0.05 ul).

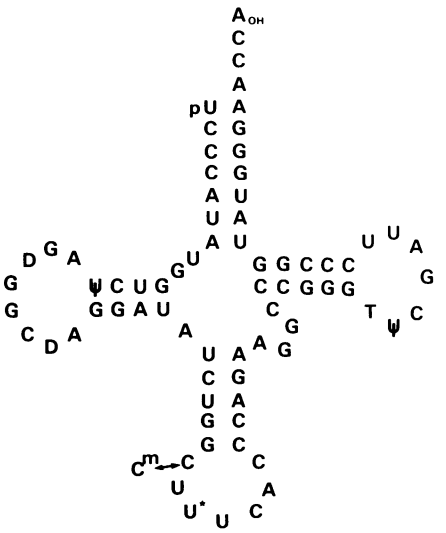

Figure 4 Cloverleaf model of Drosophila melanogaster $tRNA_4^{Glu}$ indicating the partial modification in position 32. U* = 2-thio-uridine.

DISCUSSION

Transfer RNA_4^{Glu} belongs to the family of tRNAs (together with $tRNA^{Lys}$ and $tRNA^{GlN}$) which recognize codons of the type NAA or NAG. It has been shown by ribosomal binding experiments that certain isoacceptors of $tRNA^{Glu}$ from E.coli, yeast, and rabbit contain a sulfur-modification in the first position of their anticodon[19-21]. These modified $tRNA^{Glu}$ isoacceptors can only recognize GAA triplets, the ability to wobble with GAG is lost. The Drosophila melanogaster $tRNA_4^{Glu}$ isolated by affinity chromatography belongs to this class of thio-U modified isoacceptors. After treatment with CNBr, the $tRNA_4^{Glu}$ isoacceptor cannot be aminoacylated[22,23], suggesting that it contains a s^2U in the first position of the anticodon.

The instability of the s^2U modification is probably the cause of the double spots observed in the P_1 and formamide digests of the T_1 anticodon fragment (Fig.2a and b). Enzyme P_1 is not able to cut at the modified U. This leads to a single C^mUU fragment, whereas degradation by formamide yields double spots for all the degration products up to the complete anticodon.

It is interesting to note that the C in position 32 of the anticodon loop is also partially modified as has been shown for $tRNA_2^{Phe}$ isolated from Drosophila melanogaster[16] and possesses a different sequence in this region. However, this modification was not observed in $tRNA_2^{Lys}$ from Drosophila

melanogaster[24], even though this tRNA contains the same ACU sequence in this region of the anticodon loop as $tRNA_4^{Glu}$. This is probably due to the applied sequencing methodology which renders difficult a discrimination of C and C^m. Also a m^5C is found in position 49 and/or postion 50, but again, the methodology does not permit an exact localization of this modification.

The fact that $tRNA_4^{Glu}$ recognizes only the triplet GAA implies that there must be other glutamic acid-tRNAs which are able to pair with the codon GAG. Hosbach and Silberklang (personal communication) have isolated and sequenced a plasmid containing Drosophila melanogaster $tRNA^{Glu}$ genes. These genes differ in six positions from $tRNA_4^{Glu}$. The anticodon was determined to be CUC, hence the tRNA coded by these genes is able to read the GAG codon. Grigliatti and coworkers[22] have shown that $tRNA^{Glu}$ from Drosophila melanogaster contains four isoacceptors. However, only $tRNA_4^{Glu}$ has been sequenced as of to day. It is therefore not clear, whether there are only two or whether there are more than two basic sequences coding for glutamate tRNAs.

In addition to $tRNA_4^{Glu}$, a second tRNA is retained by the $tRNA^{Phe}$ affinity column as demonstrated by 2-dimensional PAGE (Fig. 1). Considering the complementary anticodons which are able to bind to the $tRNA^{Phe}$ (G^mAA anticodon) which is fixed on the column, it can be concluded that this tRNA is probably a lysine species. Transfer RNA_2^{Lys} containing a CUU anticodon[24] is excluded; however, $tRNA_5^{Lys}$ is a possible candidate since it contains s^2U in the anticodon[23]. Although the tRNA in the second spot has been partially sequenced from the 3' end (ACC-GCUUUAAAGACACCUm^1AGCΨTGUGU...5'), its exact nature has not been determined. The assumption that it represents a $tRNA^{Lys}$ is sustained by the fact that the elution of a $tRNA^{Lys}$ is retarded on a $tRNA^{Phe}$ affinity column[12].

Since this is the first $tRNA^{Glu}$ sequence to be determined from a higher eukaryote, sequence comparisons can only be made with yeast and E.coli tRNAs. Considering the evolutionary dis-

tance between these organisms it is not astonishing that 32 nucleotides are different in E.coli $tRNA_1^{Glu}$, including an additional C in the dihydrouridine loop[25]. Twentythree substitutions are found in $tRNA_1^{Glu}$ from Schizosaccharomyces pombe[26] and 19 in $tRNA_3^{Glu}$ from Saccharomyces cerevisiae[20]. The anticodon loops are identical in all four species. Most of the differences are concentrated in the stem regions.

ACKNOWLEDGEMENTS

This investigation was supported by grant No. 3.024.76 from the Swiss National Science Foundation, the Hescheler- and the Julius Klaus-Stiftung.

We wish to express our gratitude to Dr.B.Marcum for reading the manuscript, to Drs. H.A.Hosbach and M.Silberklang for the communication of unpublished results, and to M.Kutzer for excellent technical assistance.

REFERENCES

1 Goodman, H.M., Olson, M.V., and Hall, B.D. (1977) Proc. Nat.Acad.Sci.USA 74,5433-5457.
2 Müller, F. (1978) Ph.D.Thesis, University of Zurich, Zürich.
3 Valenzuela, P., Venegas, A., Weinberg, F., Bishop, R., and Rutter, W.J. (1978) Proc.Nat.Acad.Sci.USA 75,190-194.
4 Garber, R.L., and Gage, P.L. (1979) Cell, 18,817-828.
5 Kressmann, A., Clarkson, S.G., Pirrotta, V., and Birnstiel, M.L. (1978) Proc.Nat.Acad.Sci.USA 75,1176-1180.
6 Matoccia, E., Baldi, M.I., Carrara, G., Fruscoloni, P., Benedetti, P., and Tocchini-Valentini, G.P. (1979) Cell, 18,648-648.
7 Schmidt, O., Mao, J., Silverman, S., Hovemann, B., and Söll, D. (1978) Proc.Nat.Acad.Sci.USA 75,4819-4823.
8 Kubli, E., and Schmidt, T. (1978) Nucl.Acids Res. 5,1465-1478.
9 Wimber, D.E., and Steffensen, D.M. (1970) Science 170,639-641.
10 Kubli, E., Schmidt, T.,and Egg, A.H. In: Transfer RNA (D.Söll, J.Abelson, and P.R.Schimmel, eds.) in press.
11 Altwegg, M., Vögeli, G., and Kubli, E. (1978) Rev.suisse Zool. 85,5-9.
12 Grosjean, H., Takada, C., and Petre, J. (1973) Biochem. Biophys.Res.Comm. 53,882-893.

13 Fradin, A., Gruhl, H., and Feldmann, H. (1975) FEBS Letters 50,185-189.
14 Silberklang, M., Gillum, A.M., and RajBhandary, U.L. (1979) Methods in Enzymology Vol. LIV, Part G, pp.58-109.
15 Stanley, J, and Vassilenko, S. (1978) Nature 274,87-89.
16 Altwegg, M., and Kubli, E. (1979) Nucl.Acids Res. 7,93-105.
17 Simoncsits, A., Brownlee, G.G., Brown, R.S., Rubin, J.R., and Guilley, H. (1977) Nature 269,833-836.
18 Brownlee, G.G. (1972) Determination of sequences in RNA. North-Holland/American Elsevier, Amsterdam-New York.
19 Agris, P.F., Söll, D., and Seno, T. (1973) Biochem. 12, 4331-4337.
20 Kobayashi, T., Irie, T., Yoshida, M., Takeishi, K., and Ukita, T. (1974) Biochim.Biophys.Acta 366,168-181.
21 Rudloff, E., and Hilse, K. (1975) Hoppe-Seyler's Z.Physiol. Chem. 356,1359-1367.
22 Grigliatti, T.A., White, B.N., Tener, G.M., Kaufman, T.C., Holden, J., and Suzuki, D.T. (1973) Cold Spring Harbor Symp. Quant. Biol. 38,461-474.
23 White, B.N. (1975) Biochim.Biophys.Acta 395,322-328.
24 Silverman, S., Gillam, I.C., and Söll, D. (1979) Nucl. Acids Res. 6,435-442.
25 Uziel, M., and Weinberger, A.J. (1975) Nucl.Acids Res. 2, 469-476.
26 Wong, T.W., McCutchan, T., Kohli, J., and Söll, D. (1979) Nucl.Acids Res. 6,2057-2068.