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The nucleotide sequence of histidine tRNAγ of Drosophila melanogaster

Martin Altwegg and Eric Kubli

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

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ABSTRACT

The nucleotide sequence of D.melanogaster histidine tRNAγ was determined to be: pG-G-C-C-G-U-G-A-U-C-G-U-C-ψ-A-G-D-G-G-D-D-A-G-G-A-C-C-C-C-A-C-G-ψ-U-G-U-G-m^1G-C-C-G-U-G-G-U-A-A-C-C-m^5C-A-G-G-U-ψ-C-G-m^1A-A-U-C-C-U-G-G-U-C-A-C-G-G-m^5C-A-C-C-A_{OH}. An additional unpaired G is found at the 5' end, and the T in the TψC loop is replaced by a U.

INTRODUCTION

Drosophila melanogaster $tRNA_\gamma^{His}$ belongs (together with $tRNA^{Asn}$, $tRNA^{Asp}$, and $tRNA^{Tyr}$) to a class of tRNAs decoding triplets of the type NA_C^U (N is G,A,U, or C). At least one of each of the isoacceptors of this class of tRNAs contains the hypermodified base queosine in the first position of the anticodon[1] which is inserted by the replacement of guanine in the intact tRNA[2,3]. The degree of modification changes in parallel in all four tRNAs during development[1,4,5]. This observation and the fact that the presence of the queosine base in the wobble position changes the affinity of the anticodon in anticodon-anticodon binding experiments[6] renders this class of tRNAs especially interesting. We have therefore set out to determine by post-labeling techniques the sequence of $tRNA^{His}$ isolated from D.melanogaster.

MATERIALS AND METHODS

Most of the materials, enzymes and methods used for the purification and sequence analysis of $tRNA_\gamma^{His}$ have been described[7-10]. The 3' labeling was done according to Peattie[11].

RESULTS

Purification of $tRNA^{His}$: Two-dimensional polyacrylamide gel electrophoresis resolves crude tRNA in about 50 spots, one of which was shown to contain $tRNA^{His,10}$. The RNA contained in this spot was eluted, further purified on RPC-5 and identified by aminoacylation.

Sequence analysis: The sequence at both ends of the $tRNA^{His}$ was determined by end labeling the intact molecule at either side, partial digestion in hot formamide, and two-dimensional homochromatography (Fig. 1a+b). The internal sequence was analyzed by the Stanley-Vassilenko gel method[12] (Fig. 2a). Probably due to the presence of a strong secondary structure bands were missing in the anticodon arm - extra loop region. We therefore analyzed partial digests of adjacent bands by two-dimensional homochromatography in order to obtain the sequence of this region (Fig.2b). The final sequence of $tRNA_{\gamma}^{His}$ with all its modifications is shown in Fig. 3.

DISCUSSION

This is the first $tRNA^{His}$ sequence reported from a higher eukaryote[13]. Hence, it can only be compared with the distant se-

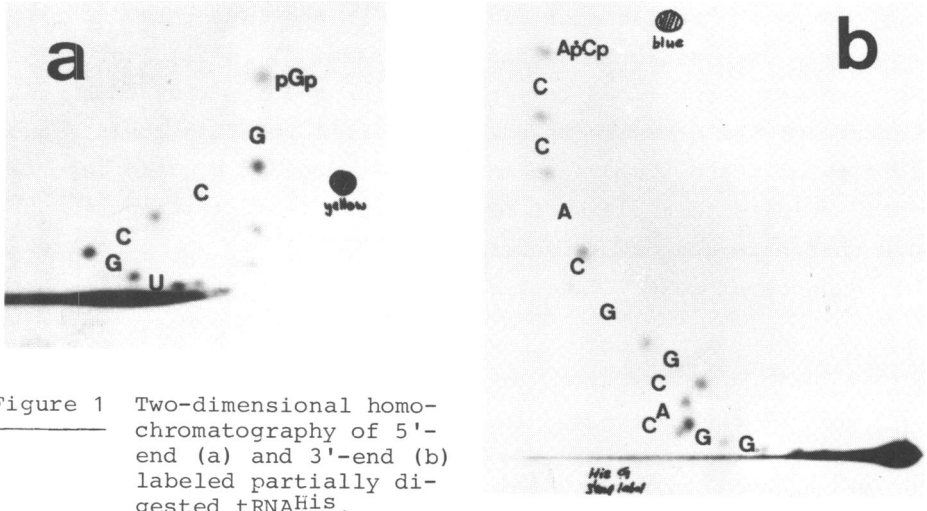
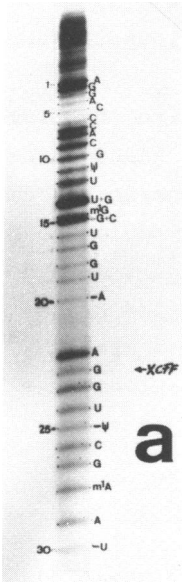


Figure 1 Two-dimensional homochromatography of 5'-end (a) and 3'-end (b) labeled partially digested $tRNA_{\gamma}^{His}$.



No.of band	sequence determined by two-dimensional homochromatography
3	GACCCCA....
4	ACCCCACG....
5	CCCCACGUU....
6	CCCACGUU....
7	CCACGUU.... / CACGUU....
8	ACGUUGU....
9	CGUUGUG....
10	GUUGUGC....
11	ΨUGUGCC....
12	UGUGC....
13	GUGCCG.... / UGCCGUG....
.	
.	
.	
17	GGUAACCCAG....

b

Figure 2 (a) Stanley-Vassilenko gel of $tRNA_{\gamma}^{His}$.
(b) Sequences obtained by two-dimensional homochromatography of partially digested material eluted from the corresponding bands.

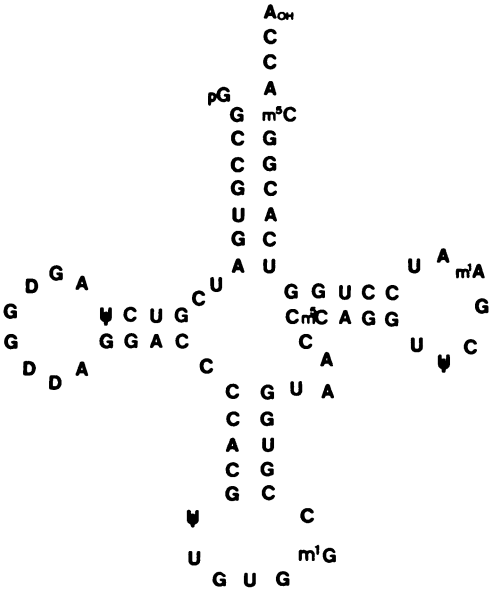


Figure 3 Cloverleaf model of Drosophila melanogaster $tRNA_{\gamma}^{His}$.

quences of $tRNA^{His}$ isolated from E.coli or yeast mitochondria[14,15]. Transfer RNA^{His} from Drosophila also contains an additional, but unpaired G at the 5' end. The TΨC loop contains a U instead of a T as found in some other tRNAs.[13] It has been claimed that the other $tRNA^{His}$ isoacceptor of Drosophila differs only by the hypermodified base queo-

sine in the anticodon[1]. This assumption is confirmed by partial sequencing results of this isoacceptor (Altwegg, M., unpublished results).

The regions 48 F and 56 F are labeled by "in situ" hybridization of [^{125}I]-tRNA^{His} to D.melanogaster salivary gland chromosomes[16]. On the other hand Dudler et al.[10] have characterized a tRNA^{His} gene(s) carrying plasmid derived from the region 48 F. However, the methods used in the above mentioned experiments do no discriminate between pseudogenes[17], silent genes and transcribed genes. The knowledge of the sequence of the mature tRNA$_\gamma^{His}$ will help in the identification of the tRNA genes actively expressed during the ontogeny of D.melanogaster.

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