STUDIES OF ODD BASES IN YEAST MITOCHONDRIAL tRNA : II. CHARACTERIZATION OF RARE NUCLEOSIDES.

R. Martin, J.M. Schneller, A.J.C. Stahl and G. Dirheimer

Institut de Biologie Moléculaire et Cellulaire du CNRS, 15, rue Descartes, 67000 Strasbourg and Laboratoires de Biochimie et de Toxicologie, Faculté de Pharmacie, 67000 Strasbourg (France).

Received March 1,1976

SUMMARY : The nucleoside composition of tRNA from highly purified yeast mitochondria shows the presence of T, ψ, hU, m^1G, m^2G, m^2_2G, I and t^6A whereas neither m^7G, m^5C, m^3C, m^1A, i^6A and Y nor O^r-methylated nucleosides (which are common in yeast cytoplasmic tRNA) were found. The G+C content is very low (35%). The overall methylation content is 2.7% which is about half the content of yeast cytoplasmic tRNA but similar to that of E.coli tRNA. Some rare nucleosides however which are found in E.coli (s^4U, acp^3U, m^2A, m^6A, ms^2i^6A, Q) were not found in yeast mitochondrial tRNA.

INTRODUCTION : Our investigation of rare bases in yeast mit. tRNAs showed the absence of Y base and s^4U (1). The overall base composition of yeast mit. tRNA was determined from experiments done using $[^{32}P]$ labelled tRNA (2). Though some rare bases like T, ψ and hU could be detected, the separation of the nucleotides did not permit further characterization of minor components of tRNA. For this purpose, the thin layer chromatography technique developed by ROGG et al. (3) is the most suitable. We report results obtained by applying this technique to yeast mit. tRNA.

MATERIAL AND METHODS

1) Preparation of mit. tRNA from Saccharomyces cerevisiae ρ^+ IL8-8C protoplasts was as described earlier (1). $[^{32}P]$ mit. tRNA was prepared by labelling yeast cells in a low phosphate galactose medium containing 100 mCi $[^{32}P]$ orthophosphate/l (2, 4).

2) To eliminate traces of DNA fragments possibly contaminating mit. tRNA preparations, the tRNA (40 A_{260}) was incubated with 10 µg of RNase-free DNase I (Worthington-DPFF) in a 10 mM Tris-HCl (pH 7.4) buffer containing 1 mM $MgCl_2$, at 0°C for 30 min. After phenol extraction, the tRNA was precipitated with ethanol and extensively dialysed. DNase treatment was done to eliminate contamination by dA, dT, dC and dG which migrate with Am, rTm, Cm and m^2G respectively, in the nucleoside chromatography method used (3).

Abbreviations : mit. : mitochondrial ; cyt. : cytoplasmic.

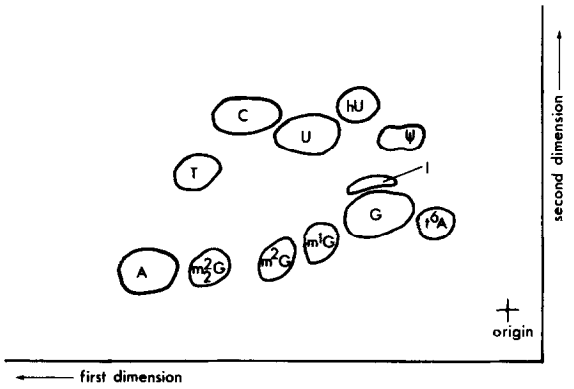

Figure 1 :

Two-dimensional thin layer chromatography of the enzymatic hydrolysate of 8 A_{260} yeast mit. tRNA on a 50 x 20 cm cellulose coated aluminium foil (DC-Alurolle Cellulose, Merck, Darmstadt). First dimension was performed by descending chromatography during 24 hrs in the solvent system I. Second dimension was performed by ascending chromatography during 4 hrs in the solvent system II.

Solvent system I : n-butanol - isobutyric acid - 25% ammonia - water (150:75:5:50, by volume).

Solvent system II : saturated ammonium sulfate - 0.1M Sodium acetate pH 6 - isopropanol (79:19:2, by volume).

3) 8 A_{260} of DNase-treated mit. tRNA were enzymatically hydrolysed at pH 7.4 and the nucleosides formed were chromatographed according to ROGG et al. (3). The spots revealed under UV light were identified by comparing their position with ROGG et al.'s chromatographic map. The nucleosides were further identified and evaluated quantitatively by their UV absorption spectra at neutral, acidic and alkaline pHs.

Nucleotide composition of $[^{32}P]$ mit. tRNA was determined by T_1 + T_2 RNase digestion and the resulting mononucleotides were separated by thin layer chromatography. The solvent systems used were : A) isobutyric acid - NH_3 25% - H_2O (66:1:33,v/v/v) (5), B) isopropanol - HCl - H_2O (68:17:15,v/v/v) (6).

4) Brewer's yeast total tRNA considered as cyt. tRNA and E.coli B tRNA were purchased from Boehringer (Mannheim) and $[^{32}P]$ orthophosphate from the C.E.A. (Saclay).

RESULTS : Figure 1 shows a typical separation of the nucleosides from a mit. tRNA hydrolysate. The nucleosides found in cyt. and mit.tRNA and in E.coli tRNA are listed in table I. The overall base composition of cold and $[^{32}P]$ mit. tRNA are in good agreement with a G+C content of 35% (2). ψ and hU are the major rare

Table I :

Nucleoside compositions of yeast mitochondrial and cytoplasmic tRNAs and of E.coli tRNA. Results are expressed as percentages of the total amount of nucleosides recovered. The values represent averages of five experiments for mit. tRNA and two experiments for cyt. tRNA and E.coli tRNA.

acp^3U : 3-(3-amino-3-carboxypropyl)uridine

Q : 7-(4,5-cis-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine.

Nucleosides	Percentages of total nucleosides		
	Yeast cyt. tRNA	Yeast mit. tRNA	E.coli tRNA
A	21.50	30.80	19.80
U	17.20	27.50	13.90
C	24.95	14.90	28.30
G	27.10	18.50	31.90
m^1A	0.65	-	-
m^2A	-	not found	not found
m^6A	-	-	0.20
t^6A	0.20	0.35	-
i^6A	0.10	-	-
ms^2i^6A	-	-	0.07
ψ	3.75	2.80	1.70
hU	present	2.60	present
s^4U	-	-	0.80
T	1.10	0.95	1.00
acp^3U	-	-	0.35
m^5C	0.80	-	0.90
m^3C	0.10	-	-
I	0.25	< 0.07	< 0.07
m^1G	0.90	0.75	0.20
m^2G	0.30	0.20	0.10
m^2_2G	0.60	0.80	-
m^7G	0.25	-	0.65
Q	-	-	0.20
Gm	0.20	-	0.25
Cm	0.30	-	0.07
G+C content	56.1	35.1	62.0
Methylated nucleosides content	5.1	2.7	2.75

nucleosides found in mit. tRNA. The following methylated nucleosides are present in mit. tRNA : T, m^1G, m^2G and m_2^2G. No O'-methylribosides are present in mit. tRNA. The degree of base methylation of yeast mit. tRNA and of E.coli tRNA is half as high compared to yeast tRNA (see table I). We found an amount of I very low as compared to that found in E.coli. Of special interest is the presence in yeast mit. tRNA of the hypermodified nucleoside t^6A (7). Using ROGG et al.'s technique, m^2A was not found, even in E.coli tRNA. Therefore, thin layer chromatography of $[^{32}P]$ mit. tRNA nucleotides, using solvents A (5) and B (6), was performed. In this system m^2Ap is easy to localize (8). However, no m^2Ap was found in yeast mit. tRNA (results not shown).

DISCUSSION : The G+C and A+U contents of mit. tRNA have been thoroughly discussed (2) and it must once more be emphasized that yeast mit. tRNA shows the lowest G+C content (35%) so far reported for tRNA. The mit. tRNA preparations appear to be pure and contamination, if any, does not exceed 4% (2). In addition specific cyt. tRNAs : $tRNA^{Tyr}$ and $tRNA^{Leu}$ (9) and $tRNA^{Phe}$ (1) are absent in these preparations.

Investigations of methylated bases in mit. tRNA from other species, e.g. hamster cells BHK-21 (10), rat liver and Morris hepatoma (11), and Neurospora crassa (12), showed that mit. tRNA is somewhat undermethylated as compared to cyt. tRNA. This is also the case for yeast mit. tRNA ; furthermore, the degree of methylation, i.e. 2.7% is the same as for E.coli tRNAs.

The pattern of methylated bases found in yeast mit. tRNA is quite different from that of BHK-21 mit. tRNA (10). Methylated A and C, found in BHK-21, are absent in yeast mit. tRNA. Their absence is consistent with the methylase specificities found in yeast mitochondria (13). While very little T is found in mit. tRNA. of BHK-21 (10) and rat liver (11), there is about one T per sequence (70-80 nucleotides) in yeast mit. tRNA. This difference may be the result of evolution of the mitochondrial system which seems to parallel that of the cytoplasm since the amount of T found in yeast cyt. tRNA is also higher than in mammalian cyt. tRNA (10,11). KLAGSBRUN reported m^2A methylase activity in HeLa cell mitochondria (14) : the modified base m^2A may therefore be present in mit. tRNA although DUBIN et al. (10) found none in BHK-21 mit. tRNA, and we detected no m^2A in yeast mit.tRNA.

Among the odd nucleosides usually adjacent to the 3'-end of the anticodon, t^6A was detected in yeast mit. tRNA. If there is one copy of this nucleotide per tRNA sequence, as expected from its position in the tRNA, then it occurs in about 25% of the yeast mit. tRNAs. This percentage of the mit. tRNA would then be expected to have anticodons terminating with U (15). Good candidates for a t^6A in their sequence are mit. $tRNA^{Arg}$, $tRNA^{Asn}$, $tRNA^{Ile}$, $tRNA^{Lys}$, $tRNA^{Met}$, $tRNA^{Ser}$ and $tRNA^{Thr}$ (15). Another nucleoside, m^1G, which is present in yeast mit. tRNA, could possibly be adjacent to the 3'-end of the anticodon in a few mit. tRNA species, as it is the case in some prokaryotic or cytoplasmic tRNA sequences (16). However, i^6A or ms^2i^6A also found adjacent to the 3'-end of the anticodon (16) could not be detected in yeast mit. tRNA.

It must be emphasized that our results do not exclude the presence of those rare nucleosides present in only one (or a few minor) species of mit. tRNA. Such modified nucleosides, which would represent less than 0.05% of the total mit. tRNA nucleosides cannot be detected by the chromatographic method used (3).

The study of rare nucleosides in mit. tRNA shows, as already noted (1,10), that this tRNA is significantly different from both eukaryotic and prokaryotic ones. Several modifying enzymes would be required to produce the number of rare nucleosides found. These enzymes, like it is the case for aminoacyl-tRNA synthetases (17) are presumably of nuclear origin because of the relatively small coding capacity of mit. DNA.

ACKNOWLEDGEMENTS : We gratefully acknowledge the technical assistance of Mrs C. Fix, M.L. Gangloff and M. Schlegel in preparing yeast mitochondria. We thank Drs G. Keith and H. Rogg for stimulating discussions. This work was partly supported by grants from the "Institut National de la Santé et de la Recherche Médicale" (contrat CRL 7310061), the "Délégation Générale à la Recherche Scientifique et Technique" (contrat 74.7.0179) and from the "Département de Biologie du Commissariat à l'Energie Atomique".

REFERENCES

1. Schneller, J.M., Martin, R., Stahl, A.J.C., and Dirheimer, G. (1975) Biochem. Biophys. Res. Commun., 64, 1046-1053.
2. Schneller, J.M., Faye, G., Kujawa, C., and Stahl, A.J.C. (1975) Nucleic Acids Res., 2, 831-838.
3. Rogg, H., Brambilla, R., Keith, G., and Staehelin, M. (1976) Nucleic Acids Res., 3, 285-295.

4. Rubin, G.M. (1973) J. Biol. Chem., 248, 3860-3875.
5. Krebs, H.A., and Hems, R. (1953) Biochim. Biophys. Acta,12, 172-180.
6. Wyatt, G.R. (1951) Biochem. J., 48, 584-590.
7. Ishikura, H., Yamada, Y., Murao, K., Saneyoshi, M., and Nishimura, S. (1969) Biochem. Biophys. Res. Commun., 37, 990-995.
8. Nishimura, S. (1972) in : Progress in Nucleic Acid Research and Molecular Biology (Davidson, J.N. and Cohn, W.E. Edt.), 12, 49-85 (Academic Press, New York).
9. Schneller, J.M., Fukuhara, H., and Stahl, A.J.C. (1975) Biochimie, 57, 1051-1057.
10. Dubin, D.T., and Friend, D.A. (1974) Biochim. Biophys. Acta, 340, 269-277.
11. Randerath, E., Chia, L.L.S.Y., Morris, H.P., and Randerath,K. (1974) Biochim. Biophys. Acta, 366, 159-167.
12. Lambowitz, A.M., and Luck, D.J.L. (1975) J. Mol. Biol., 96, 207-214.
13. Schneller, C., Schneller, J.M., and Stahl, A.J.C. (1976) Biochem. Biophys. Res. Commun., in this issue.
14. Klagsbrun, M. (1973) J. Biol. Chem., 248, 2606-2611.
15. Powers, D.M., and Peterkofsky, A. (1972) J. Biol. Chem., 247, 6394-6401.
16. Barrell, B.G., and Clark, B.F.C. (1974) in : Handbook of Nucleic Acid Sequences (Joynson-Bruvvers Ltd, Oxford).
17. Schneller, J.M., Schneller, C., Martin, R., and Stahl, A.J.C. (1976) submitted to Nucleic Acids Res..