Volume 8 Number 7 1980 Nucleic Acids Research

Yeast mitochondrial methionine initiator tRNA: characterization and nucleotide sequence

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Received 7 March 1980

ABSTRACT

Two methionine tRNAs from yeast mitochondria have been purified. The mitochondrial initiator tRNA has been identified by formylation using a mitochondrial enzyme extract. E.coli transformylase however, does not formylate the yeast mitochondrial initiator tRNA. The sequence was determined using both ^{32}P-in vivo labeled and ^{32}P-end labeled mt $tRNA^{Met}_{f}$. This tRNA, unlike N.crassa mitochondrial $tRNA^{Met}_{f}$, has two structural features typical of procaryotic initiator tRNAs : (i) it lacks a Watson-Crick base-pair at the end of the acceptor stem and (ii) has a T-ψ-C-A sequence in loop IV. However, both yeast and N.crassa mitochondrial initiator tRNAs have a U_{11}:A_{24} base-pair in the D-stem unlike procaryotic initiator tRNAs which have A_{11}:U_{24}. Interestingly, both mitochondrial initiator tRNAs, as well as bean chloroplast $tRNA^{Met}_{f}$, have only two G:C pairs next to the anticodon loop, unlike any other initiator tRNA whatever its origin. In terms of overall sequence homology, yeast mitochondrial $tRNA^{Met}_{f}$ differs from both procaryotic or eucaryotic initiator tRNAs, showing the highest homology with N.crassa mitochondrial initiator tRNA.

INTRODUCTION

In yeast mitochondria, tRNAs corresponding to the twenty amino acids are coded for by the mitochondrial genome (1,2,3). While mitochondria are dependent on the nucleo-cytoplasmic system for numerous functions, mitochondrial protein synthesis seems to be autonomous with respect to the mitochondrial tRNAs used (4). The development of RNA sequencing techniques requiring only small quantities of tRNA have made feasible the determination of a number of *Neurospora crassa* (5,6) and yeast (7,8) mitochondrial tRNA sequences. In addition, a certain number of yeast mitochondrial tRNA genes have been sequenced (9,10,11). These tRNAs show some unusual features absent in known procaryotic and eucaryotic tRNAs. Furthermore, recent studies on yeast (11,12,13) and human (14) mitochondrial DNA show altered coding properties for mitochondrial tRNAs, suggesting the use of a different genetic code

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in these organelles.

In view of the peculiarities of known mitochondrial tRNAs their sequences merit further study. The sequence of the mitochondrial initiator $tRNA_f^{Met}$ is of particular interest since it has a specialized function. While nucleotide sequences of initiator tRNAs from a variety of organisms have been determined (quoted in ref. 15), only one from mitochondria has been published so far (5). Initiation of mitochondrial protein synthesis has been compared to that in procaryotes since formylated initiator tRNA is used (for a review see Buetow and Wood ; ref. 16). While no direct relationship has been established between the role of initiator tRNAs in the initiation mechanism and their structure, comparison of known sequences show structural features which distinguish procaryotic and eucaryotic initiator tRNAs. Procaryotic initiator tRNAs lack a Watson-Crick base pair at the end of the acceptor stem and do not have the standard pyrimidine : purine (Y11:R24) base-pair in the D-stem. In eucaryotic initiator tRNAs, the acceptor stem and D-stem follow the conventional cloverleaf, but in loop IV the distinctive A-ψ-C-G instead of the classical T-ψ-C-G(A) sequence is found. The aim of our studies was to determine whether yeast mitochondrial $tRNA_f^{Met}$ contains features of the procaryotic or eucaryotic type and to compare its overall sequence with that of other initiator tRNAs. In addition, since the only other known mitochondrial initiator tRNA, that of *N.crassa* (5) is distinctive with respect to both procaryotic and eucaryotic initiators, it was of interest to see whether mitochondrial initiator tRNAs possess unique features which distinguish themselves as a class.

MATERIALS AND METHODS

Preparation of mt $tRNA_f^{Met}$

The preparation of mt tRNAs from purified yeast mitochondria has been described elsewhere (1,7). Bulk mt tRNA (20 mg) was applied to a 0.9 x 180 cm RPC-5 column (17) and the material was eluted with a 2 l gradient from 0.35 to 0.70 M NaCl in 10 mM sodium acetate buffer (pH 4.5), 10 mM $MgCl_2$. Sepharose 4B column fractionation using a reverse salt gradient (18) was as described in Fig. 1A. Two-dimensional polyacrylamide gel electrophoresis of

mt tRNA using the system of Fradin *et al.* (19) has been previously described (1).

Aminoacylation and formylation

Crude enzyme extracts from yeast mitochondria (20) and from *E.coli* MRE600 were used as sources of both methionyl-tRNA synthetase and formyltransferase. Formylation was done in acylation media (20) containing [^{12}C]-methionine. The formyl donor, *l-10-*[^{14}C]-formyltetrahydrofolate was prepared according to Samuel *et al.* (21) using sodium [^{14}C]-formate (New England Nuclear) and formyltetrahydrofolate synthetase donated by Dr. J.C. Rabinowitz. Pure *E.coli* $tRNA^{Met}_{f}$ was purchased from Boehringer/Mannheim.

Sequencing techniques

Analysis of RNAase T_1 and RNAase A digestion products : Standard procedures (22) were used for complete RNAase T_1 and RNAase A digestions and fingerprinting of uniformly labeled mt $tRNA^{Met}_{f}$. Nucleotide compositions and molar ratios were determined as described (7). Techniques used for 5'-^{32}P-post labeling and for sequencing end-labeled oligonucleotides were those reported previously (7,23).

Preparation of 5'-^{32}P-end labeled mt $tRNA^{Met}_{f}$: Dephosphorylation of 1-3 μg tRNA was done using calf intestine alkaline phosphatase (Boehringer/Mannheim) at 2 x 10^{-3} U/μg tRNA in 10 μl 25 mM Tris-HCl pH 8 for 30 min at 50°C. After inactivation of the phosphatase, the 5' terminus was labeled as described (23) using T_4 polynucleotide kinase (donated by Dr. G. Keith) and γ-^{32}P-ATP (3000 Ci/mmole). The labeled tRNA was purified by electrophoresis on a 15% polyacrylamide gel. The 5'-end labeled tRNA and fragments were then eluted in the presence of 40 μg tRNA carrier as described (7).

Partial hydrolyses of 5'-^{32}P-labeled mt $tRNA^{Met}_{f}$: Partial hydrolyses with RNAases U_2, T_1 (Sankyo) and RNAase A (Worthington) were done at 55°C for 15 min in buffer I (20 mM sodium citrate pH 5, 1 mM EDTA, 7 M urea, 0.025% xylene cyanol, bromophenol blue) (24). Two different digestion conditions were used for each enzyme and the hydrolysates mixed before loading onto the sequencing gel. Enzyme/RNA ratios used are indicated in Fig. 2. RNAase Phy I, donated by Dr. J.P. Bargetzi, was used in buffer II (10 mM sodium acetate pH 5, 10 mM EDTA). Two incubations at 55°C, one

for 5 min, one for 20 min were mixed. Unspecific partial hydrolysis or "ladder" was obtained by incubating 5 µg RNA in 10 µl bidistilled water at 100°C for 10 min and 20 min. Samples were loaded onto thin (0.5 mm) polyacrylamide sequencing gels. Two dimensional polyacrylamide gel separation of the "ladder" hydrolysate was done according to De Wachter and Fiers (25) except that 0.5 mm thin slab gels were used and urea was omitted from the electrophoresis buffer.

Analysis of modified nucleotides : Modified nucleotides of mt $tRNA_f^{Met}$ were identified using ^{32}P-*in vivo* labeled mt tRNA, as described for mt $tRNA^{Phe}$ (7). In addition, the technique of Stanley and Vassilenko (26) was used with the following modifications : (i) partial hydrolysis of tRNA (2-5 µg) was done in hot bidistilled water (2-4 min at 80°C), (ii) after 5'-^{32}P-labeling the fragments were separated on a 15% thin polyacrylamide gel, (iii) after recovery, each fragment was completely digested with P_1 nuclease and the 5'-terminal nucleotide was identified by thin layer chromatography.

RESULTS

Purification of the two mitochondrial methionine isoacceptors and characterization of mitochondrial initiator tRNA

Yeast mt DNA codes for two heterogenic $tRNAs^{Met}$, one of which is responsable for initiation of mt protein synthesis (1,2,27). Preparative column fractionation of total mt tRNA in the RPC-5 system showed poor resolution of the mt methionine tRNAs. As shown in Fig. 1A, rechromatography on a Sepharose-4B column improved the resolution of the two methionine isoacceptors. Formylation tests using mitochondrial or *E.coli* enzyme extracts were done to identify the formylatable Met-tRNA species. Despite a somewhat low transformylase activity of the mt enzyme, the results shown in Table I indicate that mt $tRNA_2^{Met}$ corresponds to the initiator tRNA and $tRNA_1^{Met}$ to the elongating species. It is striking to note that the extent of formylation of mt $tRNA_f^{Met}$ using the *E.coli* enzyme is very low indicating that the bacterial enzyme recognizes only poorly the yeast mt initiator tRNA.

As shown in Fig. 1B, electrophoresis on a 10% polyacrylamide

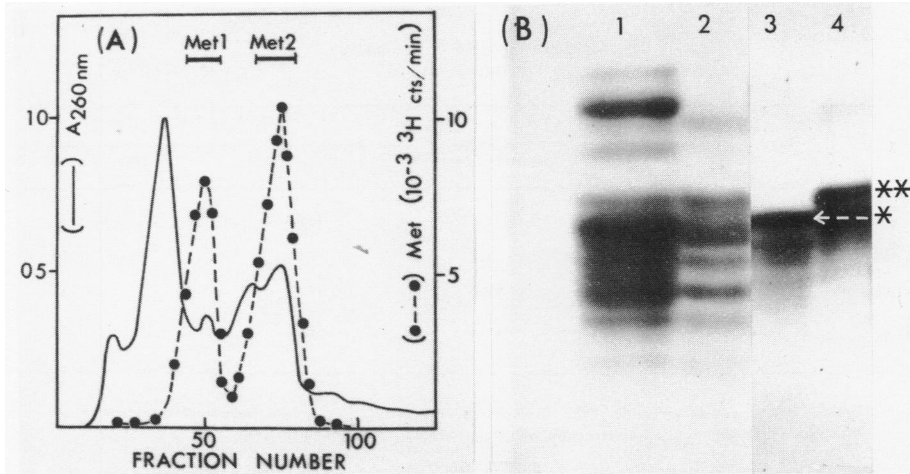

Fig. 1. Purification of yeast mitochondrial methionine tRNAs.
A : Sepharose 4B column (0.7 x 25 cm) chromatography of $tRNA^{Met}$ fraction (72 A_{260} units) from RPC-5 column in 10 mM sodium acetate buffer (pH 4.5), 10 mM $MgCl_2$, 6 mM β-mercaptoethanol, 1 mM EDTA. Elution was done in the same buffer with a gradient (2 x 200 ml) from 2 M to 1 M ammonium sulfate. Fraction volume : 2.3 ml. (———) $A_{260\ nm}$; (o---o) {^3H} Met accepting activity detected using a yeast mt enzymatic extract.
B : Separation on a 10% polyacrylamide gel (1,19) of :
1. Crude mt tRNA (60 μg)
2. mt $tRNAs^{Met}$ fraction from RPC-5 column (40 μg)
3. Met 1 fraction from fig. 1A (30 μg)
4. Met 2 fraction from fig. 1A (40 μg)
* mt $tRNA_1^{Met}$ (= $tRNA_m^{Met}$)
** mt $tRNA_2^{Met}$ (= $tRNA_f^{Met}$)

gel was used as a final purification step for both mt $tRNA_f^{Met}$ and $tRNA_m^{Met}$. Purification of mt $tRNA_f^{Met}$ - either unlabeled or ^{32}P-*in vivo* labeled - can also be achieved in a one-step procedure using two-dimensional polyacrylamide gel electrophoresis, as reported previously (1).

Analysis of mt $tRNA_f^{Met}$ RNAase T_1 oligonucleotides

After complete RNAase T_1 digestion, oligonucleotides were separated by two-dimensional homochromatography or by DEAE paper electrophoresis. Composition of the RNAase T_1 oligonucleotides was determined by analysis of uniformly ^{32}P-labeled mt $tRNA_f^{Met}$. The resulting compositions, including modified nucleotides and molar ratios are consistent with the results shown in Table II.

TABLE I

FORMYLATION OF YEAST MITOCHONDRIAL INITIATOR tRNA

tRNA	*E.coli* enzymatic extract[a] Aminoacylation	Formylation	Percentage of formylated Met-tRNA	Yeast mt enzymatic extract[a] Aminoacylation	Formylation	Percentage of formylated Met-tRNA
mt $tRNA^{Met}_1$ [b]	440	9.5	2.1	400	7.2	1.8
mt $tRNA^{Met}_2$ [b]	520	11.8	2.3	620	122.0	20.0
E.coli $tRNA^{Met}_f$	1320	816.0	61.8	800	128.0	16.0
cyt. tRNA (unfractionated	40	6.9	17.2	46	1.5	3.2

[a]Results are expressed as pmoles {^{14}C}-Met incorporated per A_{260} unit tRNA (aminoacylation) and pmoles {^{14}C}-formyl groups incorporated per A_{260} unit Met-tRNA (formylation).

[b]Recovered after Sepharose 4B column fractionation. (Fig. 1A).

TABLE II

SEQUENCE AND MOLAR RATIO OF T_1 RNAase END PRODUCTS OF YEAST mt $tRNA^{Met}_f$.

Spot N°	Sequence[a]	Molar yield[b]
t_1	pG	3.16
t_2	pU-G	0.70
t_3	pA-U-G	1.13
t_4	pC-U-(Ψ)-A-C-C-A	0.65
t_5	pU-A-A-D-U-G	0.93
t_6	pC-A-A-U-A-U-G	1.00
t_7	pU-A-U-U-A-U-U-G	0.97
t_8	pT-Ψ-C-A-A-A-U-C-G	1.03
t_9	pD-U-A-A-C-A-U-U-U-U-A-G	1.00
t_{10}	pU-C-A-U-m^{1}G-A-C-C-U-A-A-U-U-A-U-A-U-A-C-G	1.00

[a] {^{32}P} in vivo labeled oligonucleotides : nucleotide composition were determined by T_1 + T_2 RNAases hydrolysis and two-dimensional TLC. Sequence information was obtained from complementary RNAase A digestions of the oligonucleotides.
{^{32}P}-end labeled oligonucleotides : their 5'-terminal nucleotide was identified by two-dimensional TLC after complete P_1 nuclease digestions ; their sequences were deduced after homochromatography of partial P_1 ,icmease jydrolysates.

[b] determined by Cerenkov counting of the {^{32}P}-uniformly labeled oligonucleotides.

The fingerprint of ^{32}P-5'-end labeled RNAase T_1 oligonucleotides was comparable to that of *in vivo* ^{32}P-labeled RNAase T_1 digest. The oligonucleotides sequences, determined by analysis using P_1 nuclease, are shown in Table II. Localization of the modified nucleotides in these sequences is described below.

Sequencing gels

To align the RNAase T_1 products, intact ^{32}P-5'-end labeled mt $tRNA_f^{Met}$ as well as labeled fragments were partially digested as described in ref. 5. Ample overlaps and confirmation of the final sequence were obtained by different migrations of partially hybrolyzed 5'-end labeled tRNA as well as from gel sequence analysis of fragments. Knowledge of the sequences of the RNAase T_1 oligonucleotides is necessary for correct interpretation of the sequencing gels since modified nucleotides cannot be read and it is not always possible to differentiate unambiguously between C and U. Two dimensional polyacrylamide gel electrophoresis of a "ladder" hybrolysate was used to identify pyrimidine residues by the jumps produced (Fig. 2).

Localization of modified nucleosides

Analysis of uniformly labeled RNAase T_1 products showed D to be present in oligonucleotides t5 and t9, T was found in t8, ψ in t4 and t8 and m^1G in t10. In the case of t8 and t9, routine analysis of the 5' termini of post-labeled oligonucleotides gave D and rT respectively. For t5, since A-A-Dp is found in the total RNAase A digest, the sequence deduced for t5 is U-A-A-D-U-G.

In addition, application of the technique described by Stanley and Vassilenko (26) confirmed residues 2 to 65, showing D16, D20, m^1G37, T54, and $\psi55$. However, this technique could not be used to determine residues 71 to 76. The sequence of the RNAase T_1 oligonucleotide t4 corresponding to this region is C-U-A-C-C-A (see Table II). However, analysis of *in vivo* ^{32}P-labeled oligonucleotide gave 0.95 Ap, 2.75 Cp, 0.65 Up, 0.55 ψp suggesting incomplete modification of U72 to ψ.

DISCUSSION

The structure of yeast mt $tRNA_f^{Met}$ is shown in Fig. 3. Two features typical of procaryotic initiator tRNAs are found in this tRNA : (i) U1 and U72 cannot form a Watson-Crick base pair and

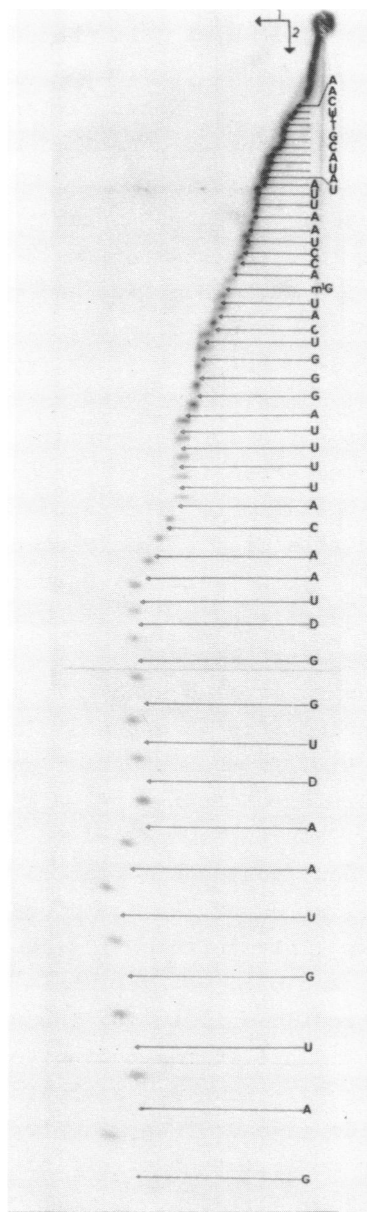

Fig. 2. Non specific partial hydrolysate (ladder) of 5'-{^{32}P}-labeled yeast mt $tRNA^{Met}_{f}$ separated according to De Wachter and Fiers (25). First dimension : 8% polyacrylamide gel, pH 3.5 ; second dimension : 15% polyacrylamide gel, pH 8.3.

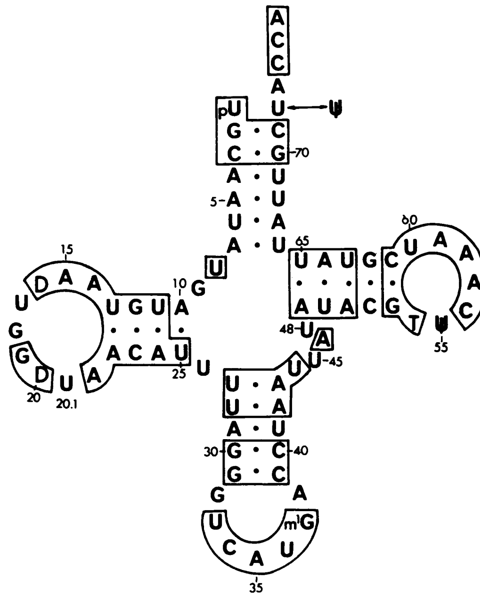

Fig. 3. Nucleotide sequence of yeast mt $tRNA^{Met}_{f}$. Residues are numbered according to (15). Boxes indicate nucleotides in common with N. Crassa mt $tRNA^{Met}_{f}$.

(ii) the loop IV sequence is T-ψ-C-A-A-A-U. However, while procaryotic initiator tRNAs lack the typical Y11:R24 base-pair in their D-stem having A11:U24, yeast mt $tRNA^{Met}_{f}$, as well as *N.crassa* mt $tRNA^{Met}_{f}$ (5), has U11:A24. But the two mt initiator tRNAs differ by the two above mentioned features, since *N.crassa* mt $tRNA^{Met}_{f}$ has (i) a base pair U1:A72 at the end of the acceptor stem and (ii) contains U-G-C in loop IV unlike any other initiator tRNA, whatever its origin.

In addition to the above, several other structural features distinguish the two mt initiator tRNAs. In the D-stem, *N.crassa* mt $tRNA^{Met}_{f}$ has only three base pairs while yeast mt initiator tRNA has four. The D-loop of yeast mt $tRNA^{Met}_{f}$ contains invariant G-G in positions 18,19 while *N.crassa* initiator tRNA has A-G. While all other initiator tRNAs contain three G:C base pairs next to the anticodon loop, both mt initiator tRNAs, as well as bean chloroplast $tRNA^{Met}_{f}$ (28), have only two G:C pairs. In the anti-

codon loop of yeast mt $tRNA_f^{Met}$, G32 is rather unusual since in all other tRNA species including initiators, a pyrimidine residue is found in this position. Both mt $tRNAs_f^{Met}$ contain m^1G on the 3' side of the anticodon, where A or modified A is ordinarily found. Some other tRNAs contain m^1G in this position (see ref. 15), but this modified nucleoside is more commonly found in mt tRNAs, since five of the eight known sequences have it.

From an evolutionary point of view, yeast mt initiator tRNA does not seem any more closely related to procaryotic than to eucaryotic initiator tRNAs since it shows only 43-55% sequence homology with procaryotic initiator tRNAs and 47-54% with eucaryotic ones. As is observed in *N.crassa*(5) the extent of homology (47%) is particularly low between yeast mt initiator tRNA and its cytoplasmic counterpart. Little similarity is observed with bean chloroplast initiator tRNA (28) whose sequence is close to that of *E.coli* (75% homology). Yeast mt $tRNA_f^{Met}$ shows the highest homology (67%) with initiator tRNA from *N.crassa* mitochondria. Although both yeast and *N.crassa* are closely related on the evolutionary scale, less sequence conservation is observed between the two mitochondrial initiator tRNAs than within the procaryotic initiator family (84-97%) or among eucaryotic ones (73-100%). From Fig. 3, it can be seen while the two mt initiators show high homology in their D-, anticodon- and "TψC"- arms, the acceptor-arms are quite different. *N.crassa* mt initiator tRNA has two more G:C pairs than yeast mt $tRNA_f^{Met}$ and is more like *E.coli* $tRNA_f^{Met}$ in this region.

One of the similarities of mt protein synthesis with that of procaryotes is the use of formyl Met $tRNA_f^{Met}$ in initiation (for a review, see ref. 16). Yeast mitochondria contain an endogeneous formyl transferase as well. Formylation has been shown to have a role in initiation factor recognition (29) and stability to peptidyl-tRNA hydrolase activity in *E.coli* (30). The yeast mt formyl transferase seems to differ from the *E.coli* enzyme since it formylates mt $tRNA_f^{Met}$ while the *E.coli* enzyme does not. The lack of formylation by *E.coli* formyl transferase is rather unusual since most initiator tRNAs, with the exception of higher plants (31,32) and *N.crassa* cytoplasm (33), are recognized by this enzyme, even if they are not formylated *in vivo*. While it is

clear that the structural elements responsible for formylation or other interactions cannot be deduced from sequence comparisons alone, the difference pointed out above between the acceptor arm structures of yeast and *N.crassa* mt initiator tRNAs may be involved in the difference in formyl transferase recognition.

From the point of view of tertiary structure of initiator tRNAs, crystallographic results show that, in *E.coli* $tRNA_f^{Met}$ (N. Woo and A. Rich, personal communication), the anticodon loop conformation is different from that of yeast cytoplasmic $tRNA^{Phe}$ (34). In addition, Wrede *et al.* have shown that the accessibility of several initiator tRNAs to S_1 nuclease differs from that of elongator tRNAs (35). It has been suggested by these authors that the three G:C pairs next to the anticodon loop in all procaryotic and eucaryotic initiator tRNAs may contribute to their distinctive anticodon conformation. An interesting feature of both mt initiator tRNAs, as well as bean chloroplast initiator tRNA is that they contain only two G:C pairs next to the anticodon loop. It would therefore be important to determine whether there is a correlation between this unique feature of organellar initiator tRNAs and their anticodon loop conformation.

ACKNOWLEDGEMENTS

We thank Dr. J.P. Bargetzi for his generous gift of Phy I RNAase and Dr. J.C. Rabinowitz for providing us with tetrahydrofolate synthetase. We are indepted to Mrs G. Issler and to Miss M. Schneider for preparing the manuscript.

This work has been supported by grants from the Délégation Générale à la Recherche Scientifique et Technique (ACC 78.17.088) and from the Institut National de la Santé et de la Recherche Médicale (CRL 79.1.154.3).

Abbreviations. mt : mitochondrial ; cyt : cytoplasmic

REFERENCES

1. Martin, R.P., Schneller, J.M., Stahl, A.J.C. and Dirheimer, G. (1977) Nucl. Acids Res. 4, 3497-3510.
2. Martin, N.C. and Rabinowitz, M. (1978) Biochemistry 17, 1628-1634.
3. Wesolowski, M. and Fukuhara, H. (1979) Mol. Gen. Genet. 170, 261-275.

4. Martin, R.P., Schneller, J.M., Stahl, A.J.C. and Dirheimer,G. (1979) Biochemistry 18, 4600-4605.
5. Heckman, J.E., Hecker, L.I., Schwartzbach, S.D., Barnett, W.E., Baumstark, B. and RajBhandary, U.L. (1978) Cell 13, 83-95.
6. Heckman, J.E., Alzner-Deweerd, B. and RajBhandary, U.L. (1979) Proc. Natl. Acad. Sci. US 76, 717-721.
7. Martin, R.P., Sibler, A.P., Schneller, J.M., Keith, G., Stahl A.J.C. and Dirheimer, G. (1978) Nucl. Acids Res. 5, 4579-4592.
8. Sibler, A.P., Martin, R.P. and Dirheimer, G. (1979) FEBS Lett. 107, 182-186.
9. Martin, N.C., Miller, P., Donelson, J., Sigurdson, C., Hartley, J., Moynihan, P. and Pham, N.D. (1979) in Extrachromosomal DNA : ICN-UCLA Symposium on Molecular and Cellular Biology, D. Cummings, P. Borst, I. Dawid, S. Weissman and C.F. Fox, Eds, Vol. 15, New York Academic Press, in press.
10. Bos, J.L., Osinga, K.A., Van der Horst, G. and Borst, P. (1979) Nucl. Acids Res. 6, 3255-3266.
11. Tzagoloff, A., Macino, G., Nobrega, M.P. and Li, M. (1979) in Extrachromosomal DNA : ICN-UCLA Symposium on Molecular and Cellular Biology, D. Cummings, P. Borst, I. Dawid, S. Weissman and C.F. Fox, Eds, Vol. 15, New York Academic Press, in press.
12. Hensgens, L.A.M., Grivell, L.A., Borst, P. and Bos, J.L. (1979) Proc. Natl. Acad. Sci. US 76, 1663-1667.
13. Fox, T.D. (1980) Proc. Natl. Acad. Sci. US, in press.
14. Barrell, B.G., Bankier, A.T. and Drouin, J. (1979) Nature 282, 189-194.
15. Sprinzl, M., Grueter, F., Spelzhaus, A. and Gauss, D.H. (1980) Nucl. Acids Res. 8, suppl. r1-r22.
16. Buetow, D.E. and Wood, W.M. (1978) in Subcellular Biochemistry, D.B. Roodyn, Ed., Vol. 5, pp 1-85, Plenum Publishing Corporation.
17. Pearson, R.L., Weiss, J.E. and Kelmers, A.D. (1971) Biochim. Biophys. Acta 228, 770-774.
18. Holmes, W.M., Hurd, R.E., Reid, B.R., Rimerman, R.A. and Hatfield, G.W. (1975) Proc. Natl. Acad. Sci. US 72, 1068-1071.
19. Fradin, A., Gruhl, A. and Feldmann, H. (1975) FEBS Lett. 50, 185-189.
20. Schneller, J.M., Schneller, C., Martin, R. and Stahl, A.J.C. (1976) Nucl. Acids Res. 3, 1151-1165.
21. Samuel, C.E., D'Ari, L. and Rabinowitz, J.C. (1970) J. Biol. Chem. 245, 5115-5121.
22. Barrell, B.G. (1971) in Procedures in Nucl. Acids Res., G.L. Cantoni and D.R. Davies, Eds, Vol. 2, pp. 751-779, Harper and Row, New York.
23. Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1977) Nucl. Acids Res. 4, 4091-4108.
24. Donis-Keller, H., Maxam, A. and Gilbert, W. (1977) Nucl. Acids Res. 4, 2527-2538.
25. De Wachter, R. and Fiers, W. (1972) Anal. Biochem. 49, 184-197.
26. Stanley, J. and Vassilenko, S. (1978) Nature 274, 87-89.
27. Martin, R., Schneller, J.M., Stahl, A.J.C. and Dirheimer, G. (1976) in Génetics and Biogenesis of Chloroplasts and Mitochondria, T. Bücher *et al.*, Eds, pp. 755-758, Elsevier, Amsterdam.

28. Canaday, J., Guillemaut, P. and Weil, J.H. (1980) Nucl. Acids Res., in press.
29. Sundary, R.M., Stringer, E.A., Schulman, L.H. and Maitra, U. (1976) J. Biol. Chem. 251, 3338-3345.
30. Schulman, L.H. and Pelka, H. (1975) J. Biol. Chem. 250, 542-547.
31. Leis, J.P. and Keller, E.B. (1970) Biochem. Biophys. Res. Commun. 40, 416-421.
32. Guillemaut, P., Burkard, G. and Weil, J.H. (1972) Phytochemistry 11, 2217-2219.
33. Gillum, A.M., Hecker, L.I., Silberklang, M., Schwartzbach, S.D., RajBhandary, U.L. and Barnett, W.E. (1977) Nucl. Acids Res. 4, 4109-4131.
34. Kim, S.H., Suddath, F.L., Quigley, G.J., McPherson, A., Sussman, J.L., Wang, A.H.J., Seeman, N.C. and Rich, A. (1974) Science 185, 435-440.
35. Wrede, P., Woo, N.H. and Rich, A. (1979) Proc. Natl. Acad. Sci. US 76, 3289-3293.