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Primary structure of yeast mitochondrial DNA-coded phenylalanine-tRNA

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ABSTRACT

Mitochondrial $tRNA^{Phe}$ from Saccharomyces cerevisiae isolated by two-dimensional gel electrophoresis was sequenced by fingerprinting uniformly labeled ^{32}P -tRNA as well as by 5'-end postlabeling techniques. Its sequence was found to be : pG-C-U-U-U-U-A-U-A-G-C-U-U-A-G-D-G-G-D-A-A-A-G-C-m^2_2G-A-U-A-A-A-ψ-U-G-A-A-m^1G-A-ψ-U-U-A-U-U-U-A-C-A-U-G-U-A-G-U-ψ-C-G-A-U-U-C-U-C-A-U-U-A-A-G-G-G-C-A-C-C-A. The secondary structure we propose, in order to maximize base pairing in the ψC stem and to allow tertiary interaction between G15 and C46, excludes U50 from base pairing giving a bulge in the ψC stem.

No conclusion can be drawn concerning the endosymbiotic theory of mitochondria evolution by comparing the primary structure of mt.$tRNA^{Phe}$ with other sequenced $tRNAs^{Phe}$. This mt.$tRNA^{Phe}$ lacks some of the structural elements reported to be involved in the yeast cytoplasmic phenylalanyl-tRNA ligase recognition site and cannot be aminoacylated by purified yeast cytoplasmic phenylalanyl-tRNA ligase.

INTRODUCTION

Mitochondria have been shown to have a partial autonomy and to contain informational, transcriptional and translational macromolecules distinct from those of the nucleus or the cytoplasm. The ribosomal and transfer RNAs which are constituents of their protein biosynthesizing machinery are encoded by the mitochondrial genome (for recent reviews, see ref.1,2).

By two-dimensional polyacrylamide gel electrophoresis and column chromatography, we have shown that yeast mitochondria contain a full set of mitochondrial DNA-coded isoaccepting tRNA species corresponding to the 20 amino acids (3,4). These tRNAs have a very special base composition, the A+U content is high whereas the rare base content is low (5). This is a general feature of mitochondrial tRNAs, whatever their origins (1,2). Yeast mitochondrial $tRNA^{Phe}$ is one of the poorest in G+C and it contains neither ribothymidine (3) nor Y base and s^4U (6). It

differs from cytoplasmic $tRNA^{Phe}$ in chromatographic and electrophoretic mobility and is coded for by the mitochondrial DNA (3,6).

Phenylalanine-tRNA structures have been widely studied in procaryotes as well as in eucaryotes. Two chloroplastic $tRNAs^{Phe}$ have already been sequenced (7,8). No yeast mitochondrial tRNA structures are known. The only mitochondrial tRNA sequenced up until now is the initiator tRNA from *Neurospora crassa* (9). We therefore found it interesting to determine the sequence of yeast mitochondrial $tRNA^{Phe}$. This tRNA was purified by two-dimensional polyacrylamide gel electrophoresis (3). Owing to the small quantities of pure material obtained, either *in vivo* ^{32}P -labeled or 5'- ^{32}P postlabeled material was used for sequencing.

MATERIALS AND METHODS

General : The ρ^+ haploid yeast strain IL8-8C was kindly supplied by the Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France. T_1 and U_2 RNAases (Sankyo) were purchased from Calbiochem. Pancreatic RNAase was from Worthington Biochemicals Corporation, Calf intestine alkaline phosphatase was from Boehringer/Mannheim GmbH and Nuclease P_1 from P.L. Biochemicals. RNAase Phy I (10) was a generous gift from Dr. G. Bargetzi. T_4 polynucleotide kinase was prepared according to Panet *et al.* (11). γ- ^{32}P -ATP (300-500 Ci/mmol) was prepared by a modification of the procedure of Glynn and Chappell (12). ^{32}P -orthophosphate was purchased from C.E.A./Saclay for *in vivo* labeling and from New England Nuclear Corporation for γ- ^{32}P -ATP preparation. Cellulose thin-layer plates and cellulose acetate strips were from Schleicher and Schüll and DEAE thin-layer plates (Polygram Cel 300) from Macherey-Nagel.

Preparation of unlabeled or uniformly ^{32}P labeled $mt.tRNA^{Phe}$: Yeast cells were grown, mitochondria isolated and mt.tRNA prepared as previously described (13,14,6). Preparation of uniformly labeled ^{32}P -mt.tRNA was as in ref. 3. ^{32}P -labeled or unlabeled total mt.tRNA (100 to 150 µg per gel) was fractionated by two-dimensional polyacrylamide gel electrophoresis according to Fradin *et al.* (15), with minor modifications (4). The spot corresponding to $tRNA^{Phe}$ was eluted by grinding the gel slice in 300 µl extraction buffer (16) and by shaking for 3 hours with one volume

of water-saturated phenol. ^{32}P -labeled mt.tRNA^{Phe} was ethanol precipitated from the aqueous phase with carrier tRNA. To recover unlabeled mt.tRNA^{Phe}, the aqueous phase was lyophilised until concentrated enough to ethanol precipitate.

Sequencing techniques for uniformly ^{32}P -labeled mt.tRNA^{Phe} : Standard procedures (17) were used for Pancreatic- and T_1 RNAase digestions and fingerprinting of ^{32}P mt.tRNA^{Phe} and also for the sequence analysis of the resulting digestion products. Oligonucleotides eluted from the fingerprints were digested completely with T_1+T_2 RNAases and their nucleotide composition determined by thin layer chromatography using the following solvents (18) : 1st dimension : isobutyric acid : water : 25% NH_4OH (66 : 33 : 1), 2nd dimension : HCl : isopropanol : water (17.6 : 68 : 14.4).

5'-end labeling of tRNA digests and partial P_1 nuclease digestions : Complete T_1- or pancreatic RNAase digestions of unlabeled mt.tRNA^{Phe} and oligonucleotide labeling using T_4 polynucleotide kinase were done as described previously (19,20). The separation of the 5'-labeled oligonucleotides was by two-dimensional homochromatography (21). Sequencing of the 5'-^{32}P -labeled oligonucleotides by partial P_1 nuclease digestion and two-dimensional homochromatography fractionation were as described by Silberklang *et al.* (22).

Preparation of 5'-^{32}P end-labeled mt.tRNA^{Phe} : After heating at 60°C for 5 min, unlabeled mt.tRNA^{Phe} (1-3 µg) was dephosphorylated with calf intestine alkaline phosphatase (10^{-3} U/µg tRNA) in 10 µl 25 mM Tris-HCl, pH 8, for 30 min at 33°C. The 5'-terminus of mt.tRNA^{Phe} was labeled using the procedure described by Silberklang *et al.* (22). After lyophilization, the tRNA was dissolved in 20 µl loading solution (20 mM Na-citrate, pH 5.0, 7 M urea, 1 mM EDTA, 0.025% xylène-cyanol, 0.025% bromophenol blue (23), incubated at 60°C for 5 min, and loaded onto a 15% polyacrylamide gel containing 7 M urea and Tris-borate-EDTA buffer. The purified 5'-labeled mt.tRNA^{Phe} and its degradation products were eluted as described above.

Partial digestions of 5'-^{32}P -labeled mt.tRNA^{Phe} or subfragments and polyacrylamide gel fractionation of digestion products : Partial digestions with both T_1- and U_2 RNAases were carried out using the same conditions as Donis-Keller *et al.* (23), except that

an enzyme/tRNA ratio from 10^{-3} U/µg to 10^{-2} U/µg for T_1 RNAase, and 0.2-1 U/µg for U_2 RNAase were used. Partial digestion with pancreatic RNAase was done as described by Simoncsits *et al.* (24) using an enzyme/tRNA ratio from 0.05 to 0.1 ng/µg. Partial Phy I RNAase digestion was carried out as described (24) except that the reaction mixture contained 2.5 mM sodium acetate, 2 mM EDTA buffer (pH 5.0). Incubation was either 20 min at room temperature with a enzyme/tRNA ratio of 10^{-3} U/µg or 5 min at 50°C with an enzyme/tRNA ratio of $2 \cdot 10^{-3}$ U/µg. The "ladder" of fragments was obtained by hot formamide degradation (24) or by incubating the tRNA in 10 µl bidistilled water at 100°C, according to Winter and Brownlee (25). It was noticed that two times of incubation (15 min and 30 min) were required to obtain a good distribution of partial breaks at every phosphodiester bond. The various digests were loaded onto a 20% or 25% polyacrylamide slab gel under the conditions previously described (23). The length of the gel was 40 cm or 90 cm depending on the size of the fragments and of the region of the intact molecule to be sequenced.

RESULTS

Purification of yeast mt.tRNA^{Phe}

Two-dimensional polyacrylamide gel electrophoresis of total yeast mt.tRNA proved to be a suitable method yielding resolution of about 30 tRNA spots. Several of them are clearly resolved and pure (3,4). Among them was the spot corresponding to tRNA^{Phe} (spot 24 in fig.4 ; ref.3). 10.5 µg of pure mt.tRNA^{Phe} could be recovered from 250 µg of total mt.tRNA put on the gel. The specific radioactivity of *in vivo* ^{32}P-labeled mt.tRNA^{Phe} was about $0.5 \cdot 10^6$ dpm/µg.

Sequence analysis of yeast mt.tRNA^{Phe}

The rare nucleoside composition of mt.tRNA^{Phe} was determined using *in vivo* ^{32}P-labeled tRNA (3). It contains 3.6% ψp, 2.2% hUp, 1.1% m^1Gp and 1.2% m_2^2Gp.

Sequence analysis of the exhaustive pancreatic- and T_1 RNAase digests : Fingerprints were done on RNAase digests of uniformly ^{32}P-labeled mt.tRNA^{Phe} as well as on ^{32}P-postlabeled digests.

Fig.1a shows the two-dimensional electrophoretic separation of uniformly labeled pancreatic RNAase digestion products. Their

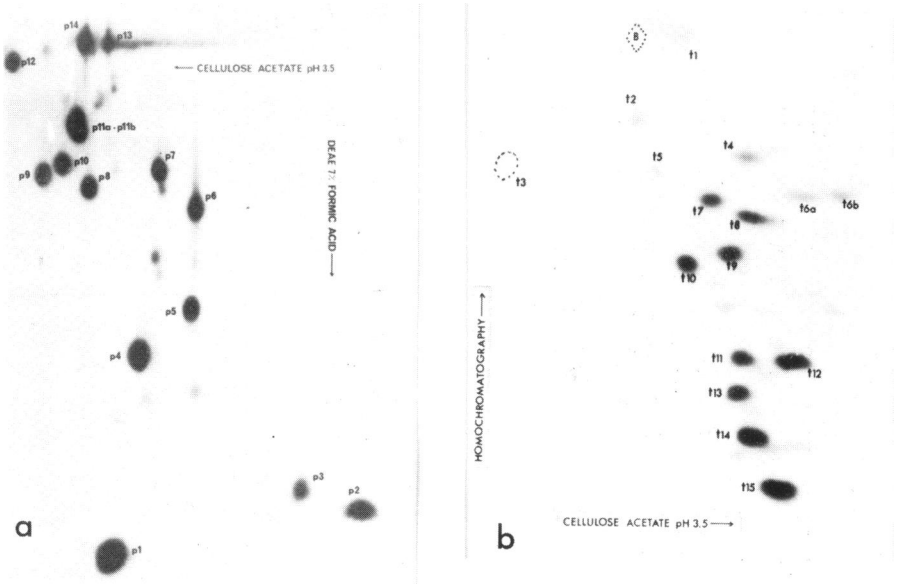

Fig. 1. Autoradiograms of the pancreatic (a) and T_1 RNAase (b) fingerprints of uniformly ^{32}P-labeled mt.$tRNA^{Phe}$.
a : Electrophoresis in the first dimension was on cellulose acetate (pH 3.5) and in the second dimension on DEAE-cellulose paper (7% formic acid).
b : Separation was by electrophoresis on cellulose acetate (pH 3.5) in the first dimension and by homochromatography on a DEAE-cellulose thin-layer plate in the second dimension. (B) surrounded by dots is blue dye marker.

nucleotide compositions and molar yields were established and the sequences of the oligonucleotides eluted from spots p5 to p12 determined by complementary T_1-RNAase digestion (Table I). In the case of oligonucleotides p13 and p14, only nucleotide composition and partial sequences could be deduced by these methods. Their sequences were established as follows : unlabeled tRNA was digested with pancreatic RNAase in presence of alkaline phosphatase and the oligonucleotides were labeled at their 5' ends with γ-^{32}P-ATP (19,20). The 5'-^{32}P-oligonucleotides were fractionated by two-dimensional homochromatography (result not shown). They were then partially digested with nuclease P_1 (22). Fig.2a shows an autoradiogram of a two-dimensional homochromatography pattern of such a digest of oligonucleotide p14 (pG-A-A-m^1G-A-ψ). Table I gives the sequences of oligonucleotides p6, p7, p13 and p14 obtained by this method.

Table I

ANALYSIS OF PANCREATIC RNAase END PRODUCTS

Spot n°	Nucleotide composition[1]	Sequence analysis	Product	Molar yield Observed	Molar yield Expected
p1	13.4Up, 0.85ψp		Up ψp	13.4 0.85	14 1
p2	3.5Cp		Cp	3.5	4
p3	1.05Ap, 0.95Cp		A-Cp	1.9	2
p4	0.95Ap, 1.05Up		A-Up	4.35	4
p5	1.15Ap, 0.9Gp, 1.05Cp	*a,d*	A-G-Cp	1.05	1
p6	3.2Ap, 0.8Gp, 1.0Cp	*a,d,e*	A-A-G-Cp	0.95	1
p7	2.9Ap, 1.1ψp	*a,d,e*	A-A-A-ψp	1.1	1
p8	0.9Ap, 0.85m_2^2G>, 1.2Up	*a,d*	m_2^2G-A-Up	0.85	1
p9	0.85pGp, 1.1Cp	*a*	pG-Cp	0.95	1
p10	0.8Gp, 1.2Up		G-Up	1.15	1
p11 a+b	3.1Ap, 2.8Gp, 1.95Up, 1.1Dp	*a,d*	A-G-Up A-G-Dp G-A-Up	3.2	1 1 1
p12	1.95Gp, 1.05Dp	*a,d*	G-G-Dp	1.05	1
p13	2.9Gp, 2.05Ap, 1.05Cp	*a,d,e*	A-A-G-G-G-Cp	0.70	1
p14	2.7Ap, 0.85m^1G>, 1.1Gp, 1.34ψp	*a,d,e*	G-A-A-m^1G-A-ψp	1.05	1

Legend to tables I and II :

1 : Determined by T_1+T_2 RNAases hydrolysis and two-dimensional TLC of the *in vivo* ^{32}P -labeled oligonucleotides.

a : Complementary T_1 RNAase digestion
b : Complementary pancreatic RNAase digestion
c : Complementary U_2 RNAase digestion
} of the *in vivo* ^{32}P -labeled oligonucleotides

d : Identification of the 5'-terminal nucleotide by two-dimensional TLC after complete P_1 nuclease digestion of the 5'- ^{32}P oligonucleotides.

e : Partial P_1 nuclease digestion of the 5'- ^{32}P oligonucleotides and two-dimensional homochromatography.

Since the larger T_1 RNAase digestion products were poorly resolved by two-dimensional electrophoresis, homochromatography (21) was found to be more suitable. Fig.1b shows the results obtained with uniformly ^{32}P -labeled oligonucleotides. Their nucleotide compositions and molar yields are shown in Table II. Several nucleotide sequences or partial sequences were obtained after pancreatic or U_2 RNAases digestion (Table II). Partial P1 digestions of the 5'-end labeled oligonucleotides were done. Fig.2b and 2c show the autoradiograms obtained for t12 (pC-U-U-U-U-A-U-A-G) and t14 (pA-U-U-C-U-U-A-U-U-A-A-G). Table II lists the sequences of t8 to t15 obtained by the "wandering spot" technique.

Direct "read off" sequencing gel : Owing to the low G+C content

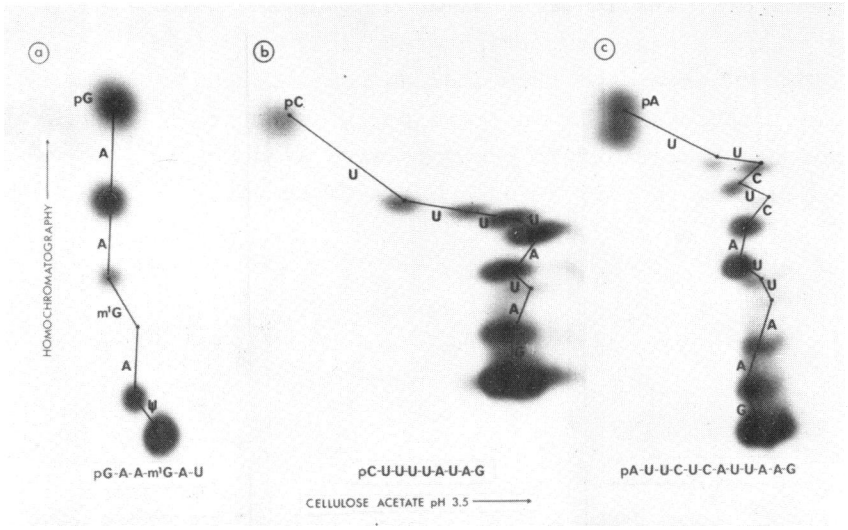

Fig. 2. Autoradiograms of the two-dimensional separation of partial P_1 nuclease digestion products from 5'- ^{32}P -labeled oligonucleotides. (a) p14 ; (b) t12 ; (c) t14 .

Table II

ANALYSIS OF T_1 RNAase END PRODUCTS

Spot n°	Nucleotide composition[1]	Sequence analysis	Product	Molar yield Observed	Expected
t1	3.85Gp+G>		Gp+G>	3.85	3
t2	1.05Cp, 0.95m_2^2G>		C-m_2^2G>	0.50*	
t3	3.1Cp, 0.9Ap	*c,d,e*	C-A-C-C-(A)	0.90	1
t4	1.15Dp, 0.85Gp		D-Gp	0.85	1
t5	1.2Ap, 0.8Gp		A-Gp	0.3**	-
t6 a+b	1.0pGp		pGp	1.0	1
t7	1.1Up, 0.9Ap, 1.0Gp	*b,d*	U-A-Gp	1.15	1
t8	1.15Up, 1.1Cp, 0.95ψp, 0.8Gp	*b,d,e*	U-ψ-C-Gp	1.05	1
t9	1.2Ap, 0.8Cp, 2.15Up, 0.8Gp	*b,c,d,e*	C-U-U-A-Gp	0.85	1
t10	2.7Ap, 1.3Dp, 0.95Gp	*b,d,e*	D-A-A-A-Gp	0.8	1
t11	3.7Ap, 2.4Up, 0.95ψp, 0.9Gp	*b,d,e*	A-U-A-A-A-ψ-U-Gp	0.45*	
t12	4.6Up, 2.2Ap, 1.1Cp, 1.15Gp	*b,d,e*	C-U-U-U-U-A-U-A-Gp	1.0	1
t13	4.1Ap, 1.8Up, 1.15m_2^2G>, 0.95Cp 0.85ψp, 0.95Gp	*b,d,e*	C-m_2^2G-A-U-A-A-A-ψ-U-Gp	0.5	1
t14	5.3Up, 4.4Ap, 1.8Cp, 0.6Gp	*b,d,e*	A-U-U-C-U-C-A-U-U-A-A-Gp	0.9	1
t15	5.7Ap, 5.4Up, 1.2Cp, 1.15m^1G> 0.8ψp, 0.95Gp	*b,d,e*	A-A-m^1G-A-ψ-U-U-A-U-U-U-A-C-A-U-Gp	0.8	1

See legend to table I

* result from uncomplete splitting of oligonucleotide t13.

** can be ascribed to degradation of some longer oligonucleotides and to the presence of some impurities.

of mt.tRNA^{Phe}, its Tm being at 28°C (26), the best dephosphorylation of the 5'-end was obtained at 33°C. After 5'-^{32}P-labeling, the tRNA was separated from degradation products by polyacrylamide gel electrophoresis. In addition to intact mt.tRNA^{Phe} molecules, some of these degradation products were sequenced.

Fig.3 show the gel autoradiogram of intact 5'-^{32}P-labeled

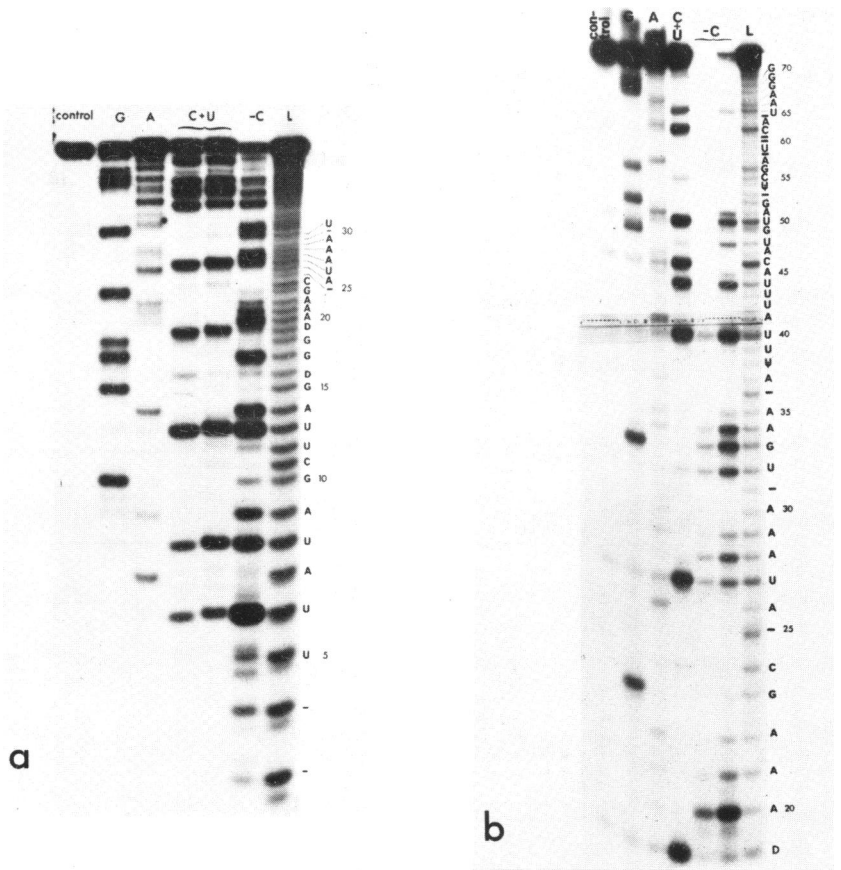

Fig. 3. Autoradiograms of the sequence analysis of intact 5'-^{32}P-labeled mt.tRNA^{Phe} on a 25% polyacrylamide gel (40x30x0.2 cm) showing residues 3-31 (a) and on a 20% polyacrylamide gel (90x30x0.2 cm) showing residues 19-70 (b). Partial enzymatic digestions were done with two different concentrations of RNAase T_1 (G), U_2 (A), pancreatic (C+U) and PhyI (-C) as described in Materials and Methods. L shows the ladder of fragments obtained by incubating the tRNA at 100°C in formamide (a) or in bidistilled water (b).

mt.tRNA^{Phe}, partially cleaved with the different enzymes (23,24) and by hot formamide (24) or bidistilled water (25) treatments, which give a "ladder" for comparison. The 5'-part of the molecule (nucleotides 5-32 ; Fig.3a) was sequenced using a 40 cm long gel (14 hours migration at 700 V) and the 3'-side (nucleotides 19-70; Fig.3b) was determined using a 90 cm long gel (72 hours migration at 1200 V). T_1-, U_2- and PhyI RNAases gave satisfactory results. The results of the digests with pancreatic RNAase were less clear since this enzyme has a strong specificity for the nucleotide following the pyrimidine residue (44). Although some pyrimidines in position 31,38,39,42,53,54,58,60,61 and 64 are difficult to distinguish on the gels (Fig.3), they can be deduced from the total T_1 RNAase digest results (see Table II).

In addition, four of the degradation products were sequenced They were shown to correspond respectively to residues 1-40 ; 41-75 ; 47-75 ; 51-75. The results obtained are consistent with those obtained with intact tRNA.

Total sequence of mt.tRNA^{Phe} : Overlapping sequences were described from sequencing gels using 5'- ^{32}P -labeled fragments and intact mt.tRNA^{Phe}. The primary structure (Fig.4) was derived from all the above results. The modified nucleotides located

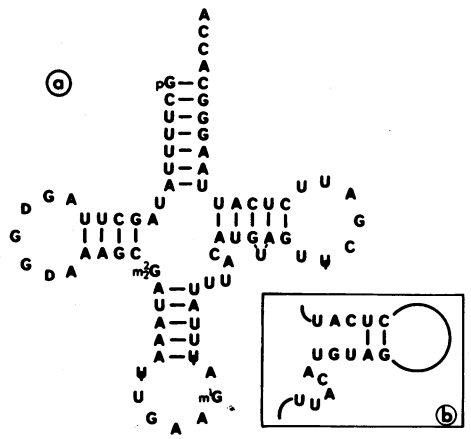

Fig. 4. Nucleotide sequence of yeast mt.tRNA^{Phe} arranged in cloverleaf form.
(a) : Representation allowing the maximum of base pairing in the ψC arm.
(b) : Conventional representation of the ψC arm.

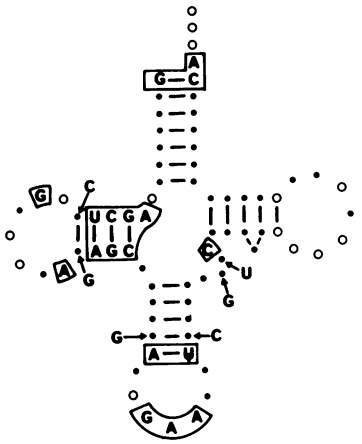

Fig. 5. Nucleotides common to all sequenced tRNAs^{Phe}. Open circles indicate invariant positions in tRNAs. Arrows show the differences between yeast mt.tRNA^{Phe} which is not aminoacylated by the yeast cytoplasmic phenylalanyl-tRNA ligase and all other tRNAs^{Phe} which are aminoacylated by this enzyme.

within the whole sequence are deduced from the results obtained with T_1- and pancreatic RNAases exhaustive digests (Tables I and II).

DISCUSSION

The G+C content of mt.tRNA^{Phe} is very low, 25 out of 75 nucleotides and the Tm is 28°C (26). These features distinguish this tRNA from all the other sequenced tRNAs whatever their origin. When the sequence is arranged in the cloverleaf form (Fig.4), the amino acid stem contains only 2 G·C pairs whereas it has 3 A·U and 2 G·U pairs. The D stem also has 2 G·C pairs in addition to 2 A·U pairs. The anticodon stem however, consists exclusively of A·U (or ψ) pairs. The ψC stem was rather difficult to deduce because when written conventionally (Fig.4b) it has only 2 base pairs : one A·U and one G·C, which are insufficient for stability (27). Moreover the A in position 47 could not pair with G15. This pairing is essential for the stability of the tertiary structure (28,29). We therefore prefer the representation in Fig.5a where the variable loop has 4 nucleotides. This type of loop has already been found in other tRNAs (30). Secondly C46 can pair with G15 and thirdly, the ψC stem has

Table III

PERCENTAGE OF SEQUENCE HOMOLOGY BETWEEN VARIOUS $tRNAs^{Phe}$

	B.subt.	B.stearo.	E.coli	Euglena chl.	Bean chl.	Yeast mt.	Yeast cyt.	Schizo. pombe	Wheat germ	Mammals
Mycoplasma	82.9	77.6	72.4	68.4	69.7	57.8	63.2	55.3	64.5	61.8
Bacillus subtilis		96.0	75.8	71.0	76.3	56.6	60.5	64.5	65.8	63.2
Bacillus stearothermophilus			76.3	72.3	75.0	56.6	56.6	60.5	61.8	63.1
Escherichia coli				70.0	78.9	63.1	63.2	56.6	64.6	71.0
Euglena chloroplasts					93.4	63.1	61.8	61.8	69.7	67.1
Bean chloroplasts						60.5	59.2	59.2	67.1	67.1
Yeast mt.							61.8	56.6	61.8	60.5
Yeast cytoplasm								64.5	84.2	77.6
Schizosaccharomyces pombe									64,5	72,4
Wheat germ										81.6

5 base pairs (3 A·U and 2 G·C), but U_{50} is excluded from base pairing and gives a bulge in the stem. Such a bulge has never been shown in a tRNA cloverleaf model but is frequent in both ribosomal and viral RNAs secondary structure models. Representation of Fig.4a permits the good positioning of all invariant and semi-invariant nucleotides.

Mt.$tRNA^{Phe}$ is only 75 nucleotides long. This distinguishes it from all other sequenced phenylalanine-tRNAs which are 76 nucleotides long (7,8,31-38). As a consequence the D-loop has a minimal size of 7 nucleotides.

Mt.$tRNA^{Phe}$ has only 7 rare nucleotides : 1 m^1Gp, 1 m^2_2Gp, 3 ψp and 2 Dp, which make it similar to prokaryotic tRNAs (31-34), but only eukaryotic $tRNAs^{Phe}$ have a m^2_2G between the D-stem and the anticodon stem (35-38). Like mycoplasma $tRNA^{Phe}$ (31), it has a m^1G in position 3' after the anticodon and it lacks ribothymidine in the ψC loop. Finally it also lacks m^7G present in the variable loop of all sequenced $tRNAs^{Phe}$ (38).

Table III shows the sequence homology between various sequenced $tRNAs^{Phe}$. Procaryotic $tRNAs^{Phe}$ have between 72.4 and 96% sequence homology and cytoplasmic $tRNAs^{Phe}$ from eucaryotes show from 64.5 to 84.2% homology. As a result of its low G+C content, mt.$tRNA^{Phe}$ shows less homology to other $tRNAs^{Phe}$ (56.6 to 63.1%) whatever the tRNA source. Chloroplastic $tRNAs^{Phe}$, on the other hand, are more similar to procaryotic $tRNAs^{Phe}$ (68.4 to 78.9%) than the mitochondrial one (56.6 to 63.1%). No conclusion concerning the

endosymbiotic theory of mitochondria evolution (39,40), can be drawn from these results as has already been noted by Heckman *et al.* (9) who sequenced the mt.initiator $tRNA^{Met}$ from *Neurospora crassa*. The comparison of the latter mt.tRNA with yeast mt.$tRNA^{Phe}$ does not show any structural feature particular to mt.tRNAs.

Mt.$tRNA^{Phe}$ has only part of the features common to the $tRNAs^{Phe}$ aminoacylated by yeast cytoplasmic phenylalanyl-tRNA ligase (Lig.Phe) (Fig.5), but it is not charged by the yeast cytoplasmic Lig.Phe (13), whereas all other sequenced $tRNAs^{Phe}$ are charged by this enzyme. Arrows in Fig.5 indicate the differences between mt.$tRNA^{Phe}$ and these other tRNAs. They are localized in three regions which have been proposed by several authors to be involved in the yeast cytoplasmic Lig.Phe recognition site (for a review,see ref.41,42). It may be that these differences are responsible for the non-charging of mt.$tRNA^{Phe}$ by the enzyme. But the other difference may be an overall modification of tertiary structure of mt.$tRNA^{Phe}$ due to the bulge in the ψC stem. Finally, it must be noted that yeast mitochondria contain a specific nuclear DNA-coded Lig.Phe which is different from the cytoplasmic Lig.Phe by its chromatographic and antigenic properties (13,43).

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Abreviations

mt. : mitochondrial ; Lig.Phe : phenylalanyl-tRNA ligase.

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