The primary structure of yeast mitochondrial tyrosine tRNA

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The mitochondrial tyrosine tRNA from *Saccharomyces cerevisiae* has been sequenced. It has two interesting structural features: (i) it lacks two semi-invariant purine residues in the D-loop which are involved in tertiary interactions in the yeast cytoplasmic $tRNA^{Phe}$; (ii) it has a large variable loop and therefore resembles procaryotic $tRNAs^{Tyr}$ rather than eucaryotic cytoplasmic ones.

Mitochondria (yeast) *Mitochondrial $tRNA^{Tyr}$* *Primary structure of tRNA* *Mitochondria evolution*

1. INTRODUCTION

Comparison of the primary structures of yeast mitochondrial (mt) tRNAs to those of procaryotic or eucaryotic cytoplasmic counterparts showed a low level of sequence homology [1–5]. However, some mt tRNAs exhibit structural features which are otherwise unique to the procaryotic tRNAs. In particular, the yeast mitochondrial methionine-initiator tRNA resembles the procaryotic ones in that it has an unpaired 5′-terminal residue and the T-ψ-C sequence in loop IV [4]. Another family of tRNAs, in which structural features can be distinguished readily between procaryotes and eucaryotes, is that of the tyrosine-tRNAs which have a long variable loop in procaryotes and a short one in the cytoplasmic tRNAs of eucaryotes [6]. Thus, determination of the nucleotide sequence of yeast mt $tRNA^{Tyr}$ could provide interesting information concerning mitochondria evolution. Here we show that this tRNA has a 14 nucleotides-long variable loop and therefore falls into the procaryotic class by this criterion.

2. MATERIALS AND METHODS

Total mt tRNA from *Saccharomyces cerevisiae* (strains IL 8-8C and IL 46) mitochondria was prepared as in [7]. The mt $tRNA^{Tyr}$ was isolated using two-dimensional polyacrylamide gel electrophoresis [7,8] and identified by aminoacylation using [^{3}H]tyrosine (30–40 Ci.mmol^{-1}, CEA/Saclay) and a preparation of yeast mitochondrial aminoacyl-tRNA synthetases [9]. For the sequence determination 3 postlabelling methods were utilized:

(i) Analysis of 5′-^{32}P-labelled oligonucleotides by partial P_1 nuclease digestion followed by homochromatography [1].

(ii) Read-off sequencing gels using either 5′- or 3′-^{32}P-labelled tRNA [1–4].

(iii) The technique developed in [10] with the modifications reported in [2] as indicated in fig.1.

The chemicals, enzymes, thin-layer cellulose plates, and other materials used in experiments were as in [1–5]. γ-[^{32}P]ATP (3000 Ci.mmol^{-1}) and α-[^{32}P]ATP (400–600 Ci.mmol^{-1}) were from Amersham/Searle.

3. RESULTS

3.1. *Purification of mt $tRNA^{Tyr}$*

Two-dimensional polyacrylamide gel electrophoresis of mt tRNA yields one spot showing tyrosyl-acceptor activity [7,11]. Hybridization of the end-labelled $tRNA^{Tyr}$ to Sau 3A restriction fragments of mt DNA allowed us to localize its gene on a 5.7 kilobase fragment which also con-

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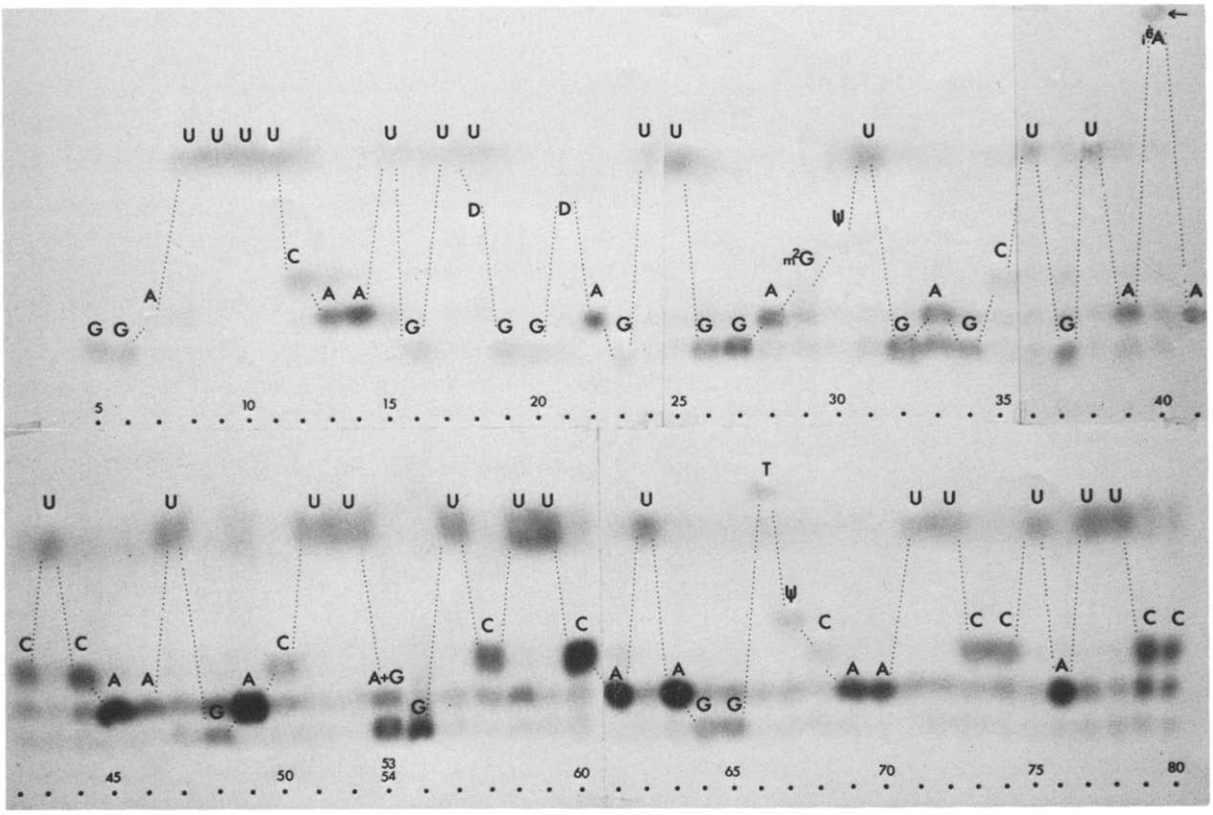

Fig.1. Thin-layer chromatography of the 5′-^{32}P-labelled termini corresponding to residues 5–80 in the sequences of mt tRNA^{Tyr}. 2 μg of tRNA were incubated at 80°C for 2 min in 10 μl of bidistilled water. After 5′-^{32}P-labelling, the digestion products were separated by polyacrylamide gel electrophoresis. Fragments were eluted from the gel and totally digested with nuclease P_1. The resulting mononucleosides 5′-^{32}P-phosphate were separated on cellulose plates using the following solvent: 2-propanol/conc. HCl/H_2O 68:17.6:14.4 (by vol.).

tains the genes for tRNA$_1^{Ser}$, tRNA$_2^{Arg}$, tRNA^{Ala}, tRNA^{Ile}, tRNA^{Asn}, and tRNA$_m^{Met}$ [11]. Thus this tRNA is transcribed from the tRNA^{Tyr} gene which maps in the *oxi*1 region of mtDNA [12]. Recently, in another yeast strain (*S. cerevisiae* TR3-15A), a second mt tRNA^{Tyr} isoacceptor was found [13] to map in the *oxi*2-P region of mt DNA. This isoacceptor could not be detected in mt tRNA from our yeast strains.

3.2. *Sequence analysis*

The sequences of the 5′-labelled oligonucleotides present in T_1 RNAase and in pancreatic RNAase hydrolysates of mt tRNA^{Tyr} were determined by partial digestion with P_1 nuclease and homochromatography. Their sequences, listed in table 1, could be aligned into a unique primary structure by analysis of either 5′- or 3′-labelled

Table 1

Sequences of oligonucleotides longer than trinucleotides in ribonuclease digests of yeast mt tRNA^{Tyr}

T_1 RNase digestion products	Pancreatic RNase digestion products
t_1: pT-U^a-C-A-A-U-U-C-C-U-A-U-U-C-C-C-U-U-C-A-C-C-A	p_1: pG-G-A-G-G-G-A-U
t_2: pU-A-A^a-A-C-U-C-A-A-U-G	p_2: pG-G-A-G^a-U^a
t_3: pA-U-U-U-U-C-A-A-U-G	p_3: pA-G-G-U / pA-G-G-U^a
t_4: pU-C-U-U-C-A-U-A-G	
t_5: pA-C-U-U-A-G	p_4: pG-A-G-C
t_6: pA-G^a-U^a-U-G	p_5: pA-A^a-A-C

[a] Modified nucleotides

$tRNA^{Tyr}$ on read-off sequencing gels (not shown). These results were completed and confirmed using the partial degradation method described in [10]. This is illustrated in fig.1 where the separation by thin-layer chromatography of the ^{32}P-labelled 5′-termini corresponding to residues 5–80 in mt $tRNA^{Tyr}$, is shown. In addition, the modified nucleotides were identified by two-dimensional thin-layer chromatography in the presence of non-radioactive marker nucleotides. The total nucleotide sequence of mt $tRNA^{Tyr}$, as arranged in the cloverleaf form, is shown in fig.2.

4. DISCUSSION

The mt $tRNA^{Tyr}$ contains 88 nucleotides, 7 of which are modified: D in positions 18 and 21, ψ in positions 30 and 67, m^2G in position 29, i^6A in position 40 and rT in position 66. Although not easily visible in fig.1, the uridine residue in position 18 is only partially modified to D_{18}. The G + C content of mt $tRNA^{Tyr}$ (41%) is one of the highest reported for a yeast mt tRNA.

The most interesting structural feature of this molecule is that it has a large variable loop (14 nucleotides) similar to that in procaryotic $tRNAs^{Tyr}$ (13 nucleotides) but different from those of yeast, *S. pombe* and *T. utilis* cytoplasmic $tRNAs^{Tyr}$ (5 nucleotides) [6]. In contrast to the latter $tRNAs^{Tyr}$, *Neurospora crassa* mt $tRNA^{Tyr}$ has a variable loop of 16 nucleotides [14] and *Paramecium primaurelia* mt $tRNA^{Tyr}$, deduced from its gene sequence, contains 13 nucleotides in this loop [15]. On this criterion, mt $tRNAs^{Tyr}$ from fungi or from protozoa fall distinctly in the procaryotic class. However, like the cytoplasmic $tRNAs^{Tyr}$, the mammalian mt $tRNAs^{Tyr}$, deduced from their gene sequences, have a small variable loop (4 nucleotides) [16,17]. This supports the hypothesis of an evolutionary origin of animal mitochondria independent from fungal mitochondria, as proposed by others on the basis of mt rRNA gene sequences comparison [18].

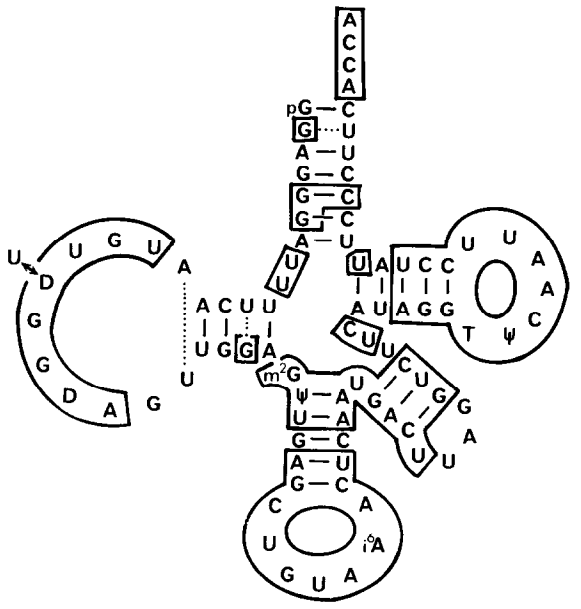

Fig.2. Cloverleaf structure of yeast mitochondrial $tRNA^{Tyr}$. Residue U_{18} which is uncompletely modified to D is indicated by an arrow. Residues which are in common with *N. crassa* mt $tRNA^{Tyr}$ [14] are in boxes.

Yeast mt $tRNA^{Tyr}$ contains all the invariant residues found for tRNAs active in the elongation step of protein synthesis. However, it lacks two semi-invariant nucleotides – in position 15 and 24 (position 21 in the numbering of the generalized cloverleaf [6]) – which are involved in tertiary interactions in the 3-dimensional structure of yeast cytoplasmic $tRNA^{Phe}$ [19]. These two positions, although usually occupied by a purine, have a uridine in mt $tRNA^{Tyr}$. The only other known tRNA which also has a U in position 15 is *N. crassa* mt $tRNA^{Tyr}$ [14]. In the tertiary structure of yeast $tRNA^{Phe}$ [19], the residue G_{15} interacts with residue C_{48} (at the end of the variable loop) by 'transpairing' between the two bases. This 'trans base-pair' is not possible in *N. crassa* and yeast mt $tRNAs^{Tyr}$, unless it takes place between the G residue present in position 16 in both tRNAs and the C residue at the end of the variable loop (C_{60} in the case of yeast mt $tRNA^{Tyr}$).

Concerning its sequence homologies with other sequenced $tRNA^{Tyr}$, table 2 shows that the yeast mt $tRNA^{Tyr}$ clearly resembles its procaryotic counterparts (49–52%) more than its eucaryotic cytoplasmic counterparts (37.5–39%). The highest degree of homology is observed with *N. crassa* mt $tRNA^{Tyr}$ (64.5%), the main differences between these two tRNAs being located in the acceptor- and D-stems (see fig.2). Finally, little homology is found with the human mt $tRNA^{Tyr}$ (37.5%). This supports the concept of the possible different

Table 2

Sequence homologies[a] between *S. cerevisiae* mt $tRNA^{Tyr}$ and other sequenced $tRNAs^{Tyr}$ (or their genes)

Procaryotes [6]		Mitochondria		Eucaryotes [6] (Cytoplasm)	
B. stearothermophilus	52	*N. crassa* [14]	64.5	*S. cerevisiae*	37.5
B. subtilis	49	*Paramecium primaurelia* [15]	47	*S. pombe*	39
E. coli	52	Human [16]	37.5		

[a] Values correspond to percentages of common residues. The 3′-terminal C-C-A sequence present in all tRNAs has not been taken into account for the calculations

evolutionary origin of fungal and mammalian mitochondria [18].

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REFERENCES

[1] 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.
[2] Sibler, A.P., Martin, R.P. and Dirheimer, G. (1979) FEBS Lett. 107, 182–186.
[3] Sibler, A.P., Bordonné, R., Dirheimer, G. and Martin, R.P. (1980) C.R. Acad. Sci. Paris (Série D) 290, 695–698.
[4] Canaday, J., Dirheimer, G. and Martin, R.P. (1980) Nucl. Acids Res. 8, 1445–1457.
[5] Sibler, A.P., Dirheimer, G. and Martin, R.P. (1981) FEBS Lett. 132, 344–348.
[6] Sprinzl, M. and Gauss, D.H. (1982) Nucl. Acids Res. 10, r1–r55.
[7] Martin, R.P., Schneller, J.M., Stahl, A.J.C. and Dirheimer, G. (1977) Nucl. Acids Res. 4, 3497–3510.
[8] Fradin, A., Grühl, H. and Feldmann, H. (1975) FEBS Lett. 50, 185–189.
[9] Schneller, J.M., Schneller, C., Martin, R.P. and Stahl, A.J.C. (1976) Nucl. Acids Res. 3, 1151–1165.
[10] Stanley, J. and Vassilenko, S. (1978) Nature 274, 87–89.
[11] Sibler, A.P. (1980) Thesis, Université Louis Pasteur, Strasbourg.
[12] Wesolowski, M., Monnerot, M. and Fukuhara, H. (1980) Curr. Genet. 2, 121–129.
[13] Wesolowski, M., Palleschi, C., Agostinelli, M., Frontali, L. and Fukuhara, H. (1981) FEBS Lett. 125, 180–182.
[14] Heckman, J.E., Alzner-DeWeerd, B. and RajBhandary, U.L. (1979) Proc. Natl. Acad. Sci. USA 76, 717–721.
[15] Seilhamer, J.J. and Cummings, D.J. (1981) Nucl. Acids Res. 9, 6391–6406.
[16] Anderson, S., Bankier, A.T., Barrell, B.G., De Bruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. and Young, I.G. (1981) Nature 290, 457–464.
[17] Bibb, M.J., Van Etten, R.A., Wright, C.T., Walberg, M.W. and Clayton, D.A. (1981) Cell 26, 167–180.
[18] Küntzel, H. and Köchel, H.G. (1981) Nature 293, 751–755.
[19] Kim, S.H., Suddath, F.L., Quigley, G.J., McPherson, A., Sussman, J.S., Wang, A.H.J., Seeman, N.C. and Rich, A. (1974) Science 185, 435–440.