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Study of yeast mitochondrial tRNAs by two-dimensional polyacrylamide gel electrophoresis: characterization of isoaccepting species and search for imported cytoplasmic tRNAs

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ABSTRACT

By two-dimensional polyacrylamide gel electrophoresis, yeast mitochondrial tRNA is fractionated into 27 major species. All but 6 of them migrate distinctly from cytoplasmic tRNAs. Migration of mitochondrial DNA-coded mitochondrial tRNAs shows the occurence of only one cytoplasmic tRNA in mitochondria. Several mitochondrial tRNA spots are identified on the electrophoregrams, some of them show isoaccepting species (Val, Ser, Met, Leu). It is suggested that there are sufficient mitochondrial tRNA genes on yeast mitochondrial DNA to allow mitochondrial protein biosynthesis by the mitochondrial tRNAs alone. Guanosine + Cytidine content and rare base composition are reported for some individual species. Mitochondrial $tRNA^{Phe}$ lacks ribothymidine.

INTRODUCTION

Mitochondria from various species ranging from fungi to mammals contain mit. DNA-coded mit. tRNAs (for a review, see ref. 1). However, several major problems concerning these tRNAs remain unsolved : little is known about their structure ; the small number of mit. DNA-coded heterogenic isoacceptors found, raises the question as to whether all codons can be read by mit. DNA-coded tRNAs alone. This in turn, led to the hypothesis, proposed by several authors (2, 3), that cyt. tRNAs are indispensable for mitochondrial protein synthesis. A method giving complete resolution of all tRNA species, would help to answer this question. We thought that the two-dimensional polyacrylamide gel electrophoresis of tRNA described by Fradin *et al.* (4) could be useful for this purpose. We report here the results of comparative migration of yeast total mit. tRNA and cyt. tRNA and of mit. DNA-coded tRNAs. Several mit. tRNA species including isoacceptors are identified and their base compositions are presented.

MATERIALS AND METHODS

1) Purification of mitochondria and preparation of mit. tRNA

Wild type yeast *Saccharomyces cerevisiae* ρ^+ haploid strain IL 8-8C or diploid strain IL 46 were grown as earlier described (5). Mitochondria isolation procedure from yeast protoplasts was the same as in (6) and mit. tRNA preparation was described in (7).

$[^{32}P]$-mit. tRNA was obtained by labeling the cells in a low phosphate-galactose medium containing 100 mCi $[^{32}P]$-phosphate/l (8). Extraction of mit. RNA from the purified mitochondria was followed by sucrose gradient sedimentation (6). The $[^{32}P]$-4S RNA was recovered by ethanol precipitation using ATP as carrier. The precipitate was dissolved in 0.5 ml water and ATP was removed by centrifugation on Sephadex G25 (in a 6 ml polyethylene syringe) according to the technique described by Neal *et al.* (9). The specific activity was usually 1-1.2 10^6 cpm/µg 4S RNA.

2) Aminoacylation of tRNAs

Preparation of mit. aminoacyl-tRNA ligases and aminoacylation of mit. tRNA with $[^3H]$-aminoacids were as earlier reported (5, 10, 11).

3) Polyacrylamide gel electrophoresis

Two-dimensional polyacrylamide gel electrophoresis at pH 8.3 in presence of urea and EDTA was performed at +4°C using 10% acrylamide (0.3 x 20 x 40 cm slabs) in the first dimension and 20% acrylamide (0.4 x 30 x 30 cm slabs) in the second dimension, as described by Fradin *et al.* (4). For some experiments one-dimensional runs using 10% gels were done at different temperatures.

Monitoring and quantification of tRNA bands or spots from the gels were done by the following techniques :

(i) unlabeled tRNAs : staining of the gels in the dark with 0.003% "stains all" in dimethylformamide-water (1:9, v:v) and destaining by exposure to daylight in water ;

(ii) $[^3H]$-aminoacyl-tRNAs : measuring the radioactivity by liquid scintillation, after combustion of the stained gel slices in an Oximat sample oxidizer (Intertechnique IN 4101). The identification of the different tRNA spots was carried out using bulk mit. tRNA aminoacylated with one $[^3H]$-aminoacid. In several cases isoacceptors eluted from RPC 5 columns (12) or purified tRNAs (13) were subsequently labeled and submitted to electrophoresis in presence or in absence of unlabeled bulk mit. tRNA. Stabilization of the aminoacyl-tRNA prior to electrophoresis was done by transforming

the $-NH_2$ group of the aminoacid into an -OH group by nitrous acid treatment (14).

(iii) $[^{32}P]$-tRNA : autoradiography of the gel and Cerenkov counting of the gel slices. For weak radioactivities, the slices were digested in NCS "tissue solubilizer" and counted in presence of Bray's scintillator (15).

4) Recovery of tRNA from gels

The $[^{32}P]$-mit. tRNA was eluted from isolated spots by electrophoresis onto DEAE-cellulose paper discs, as described by Adams *et al.* (16). The tRNA was recovered from the DEAE-cellulose by elution with 2M NaCl, followed by ethanol precipitation with carrier tRNA.

5) tRNA-DNA hybridization and elution of tRNA from the hybrids

$[^{32}P]$-mit. tRNA-mit. DNA hybridization procedure was as described (17), but T_1 RNAase wash was omitted. The blank filters gave values lower than 1% of the DNA filters. The filters corresponding to the hybridization plateau with tRNA excess (1.5 µg tRNA hybridized to 10 µg ρ^+ mit. DNA in a 50 µl mixture ; see ref. 17) were heated three times at 60°C for 15 min in 0.2 ml buffer (NaCl 0.3M, Na_3 Citrate 0.03M) containing 50% formamide. Formamide and electrolytes were removed from the combined solutions by Sephadex G25 filtration (9). The eluate was dried under vacuum, dissolved in 10 µl water and subjected to 2D electrophoresis in presence of unlabeled carrier mit. tRNA.

6) Nucleotide composition

The nucleotide composition of the $[^{32}P]$-mit. tRNAs isolated from polyacrylamide gels was established by $T_1 + T_2$ RNAase digestion, in presence of 0.5 A_{260} carrier tRNA, and separation on thin-layer cellulose plates (Schleicher and Schüll, 20 x 20 cm) using the following solvents : 1st dimension : isobutyric acid-water-NH_4OH (66:33:1, by vol.) (18), 2nd dimension: HCl-isopropanol-water (17.6:68:14.4, by vol.) (19). The nucleotides, revealed by autoradiography, were scraped and the radioactivity counted by liquid scintillation.

7) Materials

$[^{3}H]$-aminoacids were purchased either from Amersham/Searle or from CEA/Saclay. $[^{32}P]$-phosphate was from CEA/Saclay. Total yeast tRNA was purchased from Boehringer/Mannheim and "stains all" (2718) from Eastman/Rochester. The yeast strains were kindly supplied by the Centre de Génétique Moléculaire du

CNRS, Gif-sur-Yvette and mit. DNA was a generous gift from Dr. G. BERNARDI.

RESULTS

1) Polyacrylamide gel electrophoresis of mit. tRNA and cyt. tRNA

Electrophoresis of tRNA on 10% polyacrylamide slabs in urea and EDTA at different temperatures shows that at + 4°C mit. tRNA migrates slower than cyt. tRNA (Figure 1a), but at higher temperatures (+ 37°C) the two comigrate (Figure 1b).

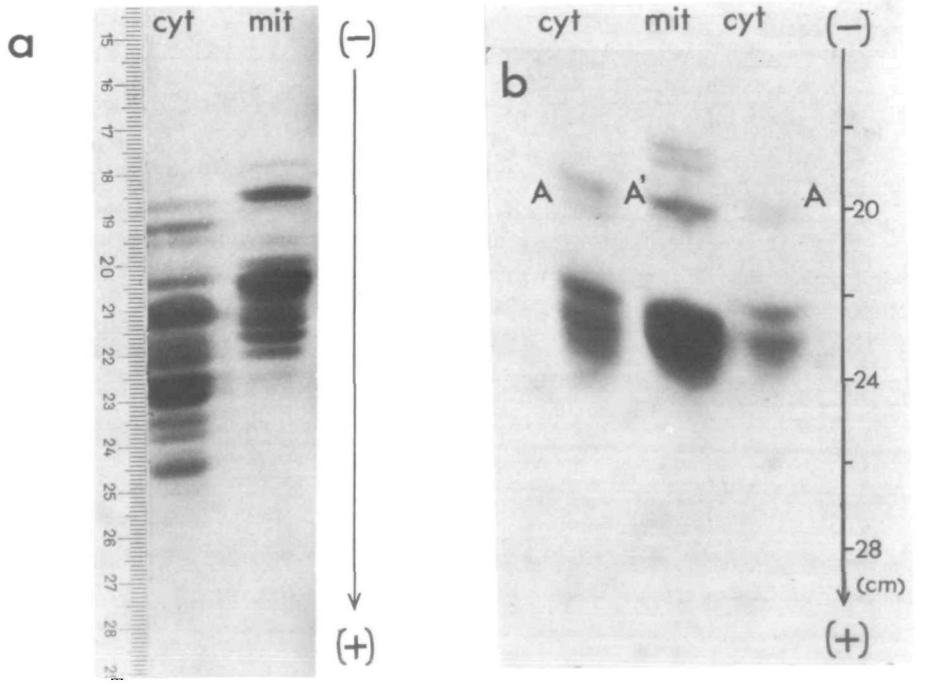

Fig. 1. Electrophoresis of cyt.- and mit. tRNA on 10% polyacrylamide gel slabs in a cold room at + 4°C for 40h at 400 V (a) or in a + 37°C room for 24h at 400 V (b).

Two-dimensional electrophoresis resolves cyt. tRNAs into more than 40 spots (Figure 2a and 2a'), as previously reported by Fradin *et al.* (4), whereas mit. tRNA gives only 26-27 major spots and a few weaker ones (Figure 2b and 2b'). No differences in the patterns are observed when mit. tRNA was prepared from two different ρ^+ strains (coelectrophoresis of $[^{32}P]$-mit. tRNA from ρ^+ IL 8-8C and unlabeled mit. tRNA from ρ^+ IL 46). However spot X is more intense and spots 9 and 11 (see fig. 4) are weaker in ρ^+ IL 46 mit. tRNA (results

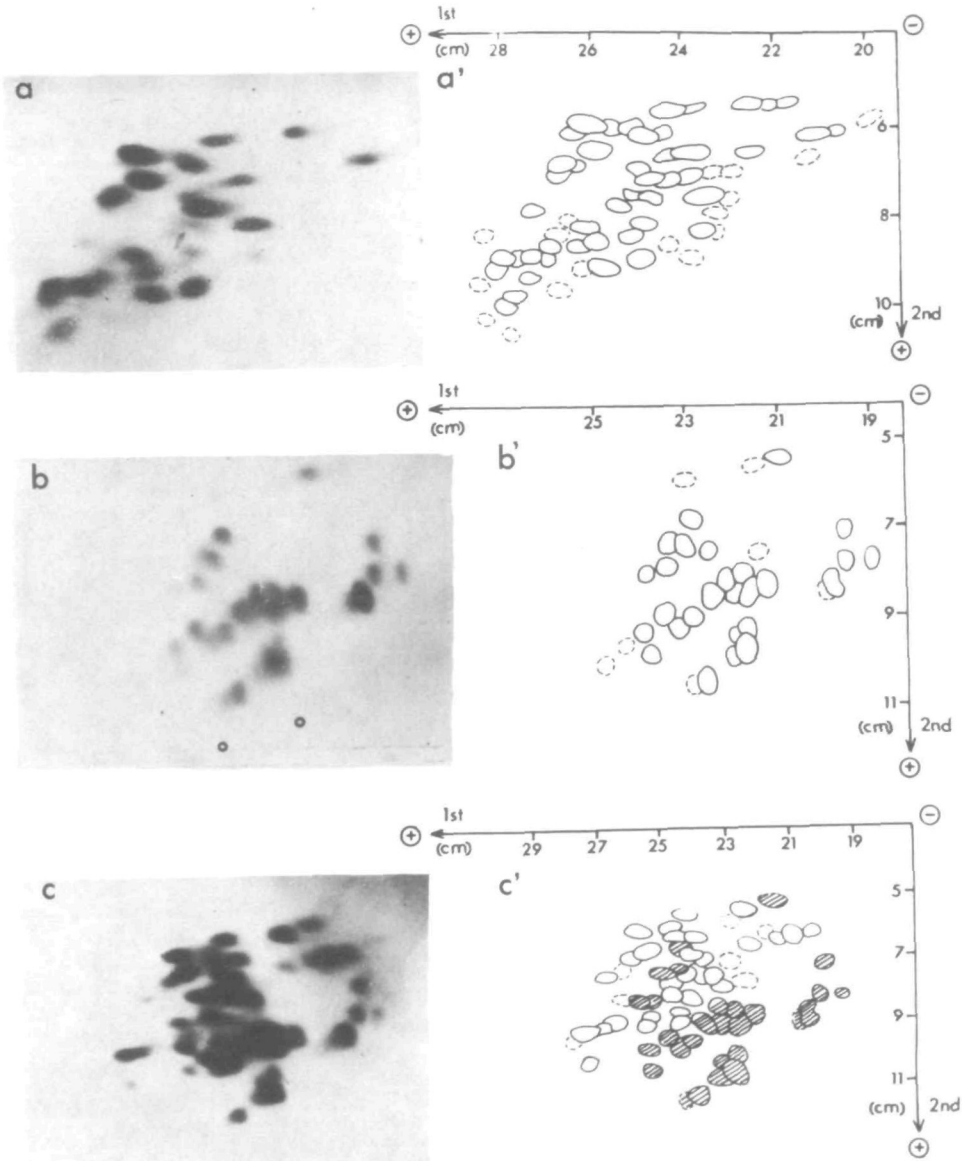

Fig. 2. 2D electrophoresis of 1.0 A_{260} cyt. tRNA (a), 1.0 A_{260} mit. tRNA (b), 2.0 A_{260} mixed cyt.- and mit. tRNA (c) and their schematic drawings (a', b' and c'). Dotted lined circles represent faint spots. The hatched spots in (c') indicate the mit. tRNA spots. The migration distances in the running gels are shown in the figure.

not shown). The two patterns of cyt. tRNA and mit. tRNA are clearly very different. Coelectrophoresis of mixed cyt.- and mit. tRNA shows that only six mit. tRNA spots are difficult to distinguish from the cyt. tRNA ones (Figure 2c and 2c'). At this stage of our study, we can assume that the only cyt. tRNAs which could be present in mit. tRNA preparations, must be located in these six spots (e.g. spots 16 to 20 and X in figure 4).

2) 2D electrophoresis of the mit. DNA-coded mit. tRNAs

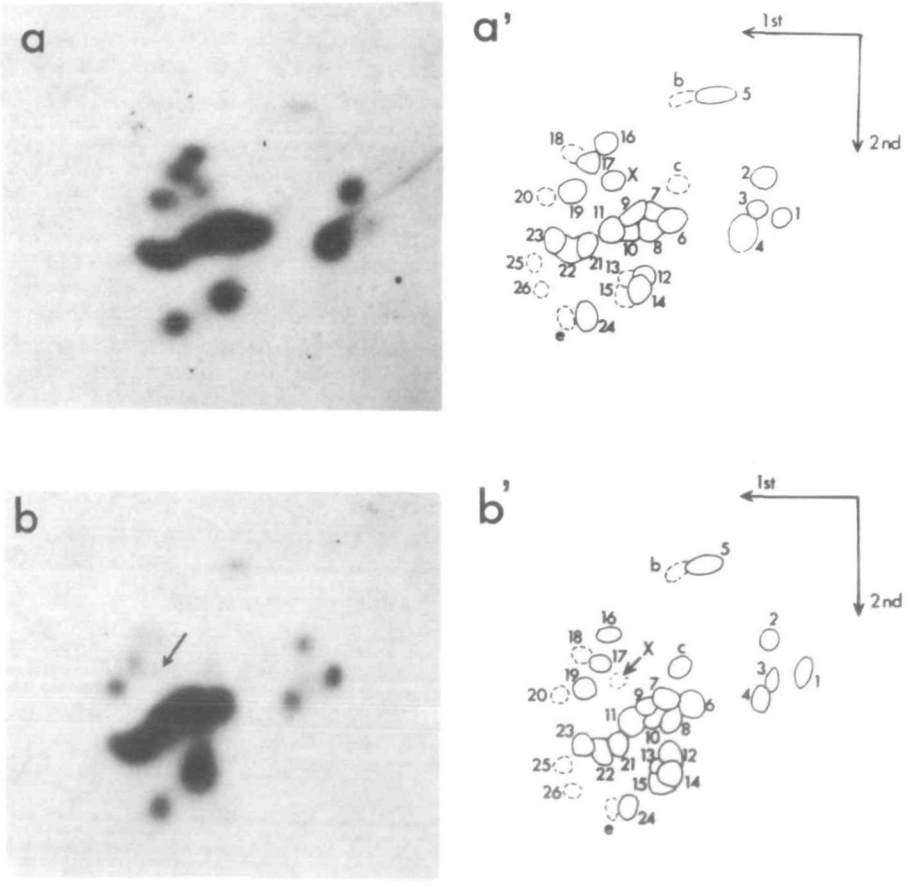

Fig. 3. 2D electrophoresis of control or non-treated [^{32}P]-mit. tRNA (a, a') and of mit. DNA-coded [^{32}P]-mit. tRNA (b, b'), in presence of unlabeled carrier mit. tRNA. (a) and (b) represent autoradiograms of the gels ; (a') and (b') give schematic drawings of the stained gels. The percentage of radioactivity in each spot is given in Table I ; spots containing less than 1.5% radioactivity (dotted circles) are not visible on the photographs. In (b), the arrow gives the location of the missing spot X. The experimental procedure is described in Materials and Methods (§ 5) and in Results (§ 2).

To determine the coding origin of all the mit. tRNA spots, especially of the six above mentioned ones, $[^{32}P]$-mit. 4S RNA was hybridized to mit. DNA with tRNA in excess. The tRNAs were then eluted from the hybrids and analyzed by 2D electrophoresis. The autoradiogram obtained (Figure 3b) was compared to the control, which consisted of non hybridized $[^{32}P]$- mit. 4S RNA treated identically (Figure 3a). The experimental procedure does not alter the migration of the $[^{32}P]$- mit. tRNAs, since they comigrate exactly with cold carrier mit. tRNA. Only one spot is lacking in the hybridized mit. tRNA pattern (spot X, figure 3b). It should be noted that this missing spot is located in the area of the six mit. tRNA spots which cannot be distinguished from the cyt. tRNA spots (see figure 2c and 2c').

TABLE I

Spot number	Percentage of total recovered radioactivity		Calculated number of genes $(= \frac{N \cdot 26}{100})$	Estimated number of genes[c]
	Total mit. tRNA[a]	Hybridized mit. tRNA[b] (= N)		
1	1.9	3.4	0.89	1
2	2.0	3.2	0.83	1
3	1.5	2.5	0.65	1
4	7.2	5.2	1.35	1 + 1 ?
5 + b	2.9	3.2	0.83	2[d]
6	4.5	6.0	1.55	2
7	5.0	4.0	1.04	1
8	4.8	5.6	1.46	2
9	6.5	6.0	1.55	2
10	3.7	3.2	0.83	1
11	7.5	8.0	2.08	2
12	2.2	4.0	1.04	1
13	1.0	3.2	0.83	1
14	8.0	6.0	1.55	2
15	1.2	1.5	0.39	1 ?
16	3.6	3.2	0.83	1
17	4.8	3.4	0.89	1
18	1.5	1.8	0.47	1
19	5.5	3.5	0.91	1
20	1.4	1.0	0.26	1 ?
21	6.5	5.0	1.30	1 + 1 ?
22	2.4	3.8	0.99	1
23	3.2	3.3	0.86	1
24	6.8	4.8	1.25	1 + 1 ?
25	1.0	1.9	0.49	1
26	0.7	1.2	0.30	1 ?
c	0.6	2.0	0.52	1
X	2.2	–	–	–
	Total number of tRNA genes		26	30 + 6 ?

a : determined from the stained pattern represented in fig. 3a'
b : determined from the stained pattern represented in fig. 3b'
c : values of the calculated number of genes ranging from 0 to 1.0 or from 1.0 to 2.0 were rounded off to 1 or to 2 respectively. Values lower than 0.4 and from 1.1 - 1.4 were somewhat ambiguous and are indicated by "?". (see Discussion § 4)
d : spot 5 contains $tRNA^{Tyr}$ + $tRNA^{Arg}$.

In the control experiment, as well as in the non treated [^{32}P]- mit.tRNA, the relative radioactivity in the different spots varies from 0.6 to 8.0% (Table I). This reflects the relative concentration of the different species in total mit. tRNA. With the mit. tRNAs eluted from the mit. DNA-tRNA hybrids, the relative radioactivity varies somewhat from spot to spot, sometimes higher and sometimes lower than in the control (see table I). The number of genes, calculated from the hybridization plateau of total mit. tRNA, was found to be 26 $\pm$ 2. Another estimate of the number of mit. tRNA genes is obtained by calculating the percent radioactivity in each gel spot (Table I) and correcting for a gene number of 26. The numbers obtained were then rounded off as indicated in the legend of Table I, and the total is found to be 30 (+ 6) (see Discussion).

3) Mapping of mit. tRNA species by 2D electrophoresis

2D electrophoresis of [^{3}H]-aminoacylated mit. tRNA allowed us to identify the spots corresponding to 20 mit. tRNA species, some of which correspond to isoaccepting species. We find (Figure 4) one isoacceptor for Arg, Gly, His, Ile, Phe and Tyr (in one experiment [^{3}H]-Tyr was also found in spots 2 and a). Two isoacceptors for Glu, Lys, Met and Leu (sometimes only

2 : Ser 2 (or Ser 3)
3 : Ser 3 (or Ser 2)
4 : Leu 1 + Leu 2
5 : Arg + Tyr
6 : Met 2
7 : Lys 2
11 : Val 2 + Val 3
12 : Met 1
14 : Ile
15 : Ser 1
16 : His 1 + His 2
18 : Val 1
X : Lys 1
21 : Val 2 + Val 3
22 : Glu ?
24 : Phe
25 : Gly ?
26 : Glu ?

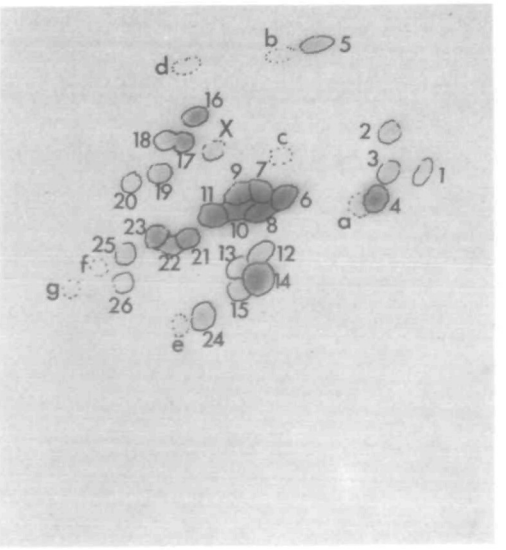

Fig. 4. Mapping of several specific mit. tRNA species by 2D electrophoresis. $tRNA_1^{Met}$ is $tRNA_m^{Met}$ and $tRNA_2^{Met}$ is $tRNA_f^{Met}$ (12). The experimentals are described in Materials and Methods.

poorly resolved) and three isoacceptors for Ser and Val, are found. In several cases precise localization of an isoacceptor was confirmed by electrophoresis of either purified tRNA ($tRNA^{Phe}$; ref. 13) or column chromatographic fractions enriched for some of them ($tRNA^{Arg}$, $tRNA^{Ile}$, $tRNA_1^{His}$, $tRNA_2^{His}$, $tRNA_1^{Leu}$, $tRNA_2^{Leu}$, $tRNA_f^{Met}$, $tRNA_m^{Met}$, $tRNA_1^{Val}$, $tRNA_2^{Val}$, $tRNA_3^{Val}$; ref. 12). No clear-cut localization of $tRNA^{Gly}$, $tRNAs^{Glu}$ and $tRNA(s)^{Tyr}$ was possible, due in part to (i) close location of spots in some regions of the pattern and (ii) some deacylation due to the rather alkaline pH and time of the runs (despite stabilization of the aminoacyl-tRNAs).

4) Nucleotide composition of mit. tRNA species

Complete nucleotide composition of some $[^{32}P]$-mit. tRNA species eluted from the gel was determined. Table II summarizes the data obtained with $tRNA^{Ser}$ (spot 2), $tRNAs^{Leu}$ (spot 4), $tRNAs^{His}$ (spot 16) and $tRNA^{Phe}$ (spot 24). The presence of those rare nucleotides, earlier reported in bulk mit. tRNA (20) is confirmed by analysis of individual species. It should be stressed that mit. $tRNA^{Phe}$ lacks ribothymidine, which is present in prokaryotic or yeast cyt. $tRNA^{Phe}$ (21).

TABLE II

Complete nucleotide composition of some $[^{32}P]$-mit. tRNA species eluted from the gel. Results are expressed as percentages of the total amount of nucleotides recovered.

Nucleotides	Percentages of total nucleotides			
	$tRNA^{Ser}$ (spot 2)	$tRNAs^{Leu}$ (spot 4)	$tRNAs^{His}$ (spot 16)	$tRNA^{Phe}$ (spot 24)
Ap	24.9	29.9	27.1	31.7
Up	32.4	27.8	25.8	31.5
Cp	17.7	17.0	18.7	13.5
Gp	16.8	15.9	18.6	15.5
t^6Ap	1.2	-	-	-
ψp	3.6	3.1	4.2	3.6
hUp	1.3	1.2	1.7	2.2
Tp	1.1	1.2	1.3	-
m^1Gp	-	1.3	0.9	-
m^2Gp	-	-	0.6	-
m_2^2Gp	-	1.3	-	1.2
pGp	1.0	1.2	1.1	1.0
G+C content	35.5	36.7	39.9	31.2

DISCUSSION

1) Size of mit. tRNA molecules

When the electrophoretic runs are done in a 37°C room (the temperature of the gel itself is appreciably higher due to ohmic heating), in presence of urea, the tRNA molecules are unfolded and it is expected that the migration reflects molecular size. As mit. tRNA and cyt. tRNA comigrate under these conditions, one can conclude that the average size of the two tRNA families does not differ. Slower migrating bands (A and A', Fig. 1b), present in both cyt.- and mit. tRNAs, may be tRNAs having long extra-loops. In yeast cytoplasm, $tRNA_1^{Leu}$, $tRNA_3^{Leu}$, $tRNA_1^{Ser}$ and $tRNA_2^{Ser}$ have 85 nucleotides and the others 75-76 nucleotides (21). In the case of yeast mitochondria, $tRNAs^{Leu}$ and $tRNAs^{Ser}$ are also found as slow-migrating species (Figure 4).

The relative slowing of mit. tRNAs in 10% gels at + 4°C, when compared to cyt. tRNAs, can be ascribed to some preferential unfolding of mit. species, due to their low G + C content (17, 20). Our results contrast with those of Dubin and Friend who concluded that BHK 21 mit. tRNAs are shorter than cyt. tRNAs (22).

2) Presence of a unique cyt. tRNA species in mit. tRNA preparations

A model for the import of numerous cyt. tRNA species in *Tetrahymena pyriformis* mitochondria has been proposed by Suyama *et al.* (3). Chromatographic studies of mit. tRNA from different origins have always led to characterization of cyt. tRNA in mit. tRNA preparations (1). We have improved the purification of yeast mitochondria so that cyt. tRNA contamination is no longer observed by column cochromatography of several aminoacyl-tRNAs : Leu-, Tyr- (23), Phe- (7), Met- (12) and others (not yet published). From 2D electrophoresis of $[^{32}P]$-mit. tRNA (ρ^+ strain IL 8-8C), purified by hybridization with mit. DNA, we show here that all but one of the mit. tRNAs are mit. DNA-coded. This tRNA is presumably one of the cyt. $tRNAs^{Lys}$ and seems to be present in a higher amount in mit. tRNA of the ρ^+ strain IL 46. The G+C content of this tRNA eluted from the gel (spot X ; Figure 3a) is about 54%, which is similar to that of the two cyt. $tRNAs^{Lys}$ (21) and much higher than the value obtained with bulk mit. tRNA (17, 20). Preliminary results (to be published elsewhere) indicate that the mit. DNA-coded $tRNA^{Lys}$ (=$tRNA_2^{Lys}$; ref. 12) is sufficient to allow mitochondrial protein synthesis. Cyt. $tRNA^{Lys}$ (= $tRNA_1^{Lys}$; ref. 12) is therefore not indispensable in mitochondrial protein synthesis. Further investigations will show whether this cyt. tRNA is a preparatory artefact or an imported species having a particular role in yeast

mitochondria.

3) Characterization of mit. tRNA species and their nucleotide compositions

The number of isoaccepting species found by 2D electrophoresis is in general agreement with chromatographic fractionation on RPC 5 columns (12). However, the two chromatographically distinct $tRNAs^{His}$ (unpublished result) map together in spot 16 (Figure 4). Such a result may well be expected since it is known that RPC 5 column chromatography can fractionate tRNA isoacceptors differing only by post-transcriptional modification (24) or even by conformational changes (13). In some cases tRNAs specific for two different aminoacids map in the same spot (for example, $tRNA^{Tyr}$ and $tRNA^{Arg}$ in spot 5, Figure 4).

All of the aminoacids specified by more than two codons have been investigated by 2D electrophoresis (Arg, Leu, Ser, Gly, Val, Ile) and/or by RPC 5 chromatography (Pro, Thr and Ala). For the last three aminoacids, 2 to 3 isoacceptors could be fractionated by RPC 5 chromatography (to be published). Although codon-dependent ribosome binding has not been done, quantitative interpretation of our data (see below) suggests that the number of isoacceptors is sufficient to allow recognition of all codons (except in the case of Arg and Gly).

Finally the two isoacceptors found for Met correspond to $tRNA_f^{Met}$ (spot 6) and to $tRNA_m^{Met}$ (spot 12) (see ref. 12) and the two $tRNAs^{Glu}$ probably correspond to $tRNA^{Glu}$ and $tRNA^{Gln}$ misacylated with Glu (25).

Investigations of mit. tRNA by 2D electrophoresis have been performed by other authors for *Locusta migratoria* (26) and by us for *Neurospora crassa* (De Vries *et al.*, to be published). Comparison with our present results leads to the following observations : (i) although, the three mit. tRNA differ strongly in their migration patterns, they show about the same number of major spots (25 to 27 spots) ; (ii) two isoaccepting species for Leu and Met were detected ; (iii) slow migrating $tRNA(s)^{Leu}$ occur in the mit. tRNA of the three organisms ; this indicates the presence of $tRNA^{Leu}$ of larger size (long extra-loop) in organellar tRNA, as is found in prokaryotic or eukaryotic $tRNA^{Leu}$ (21).

4) Quantitative analysis of the mit. tRNA migration patterns

a) Results obtained with bulk mit. tRNA

The relative amount of $\left[^{32}P\right]$-tRNA in the identified spots from bulk mit. tRNA (see Table I) fits well with the values obtained from aminoacyl-

ation tests of tRNA species specific for Leu, Phe, Met (5) and His, Ser (unpublished results). These tests confirm that $tRNA^{Tyr}$ and $tRNA^{Arg}$ are in the same spot. They point out that spot 14 (Figure 4) must contain other species beside $tRNA^{Ile}$, because 3.6% of total mit. tRNA is aminoacylated by Ile, whereas spot 14 represents 8% of total mit. tRNA (Table I) ; the same may be true for $tRNA_2^{Lys}$ in spot 7 and $tRNA_{2+3}^{Val}$ in spots 11 and 21.

Though the migration of mit. tRNA from two different ρ^+ strains are superimposable, aminoacylation tests showed some quantitative differences for tRNAs specific of Val, Lys and Tyr. These observations (though not a proof) are consistent with Prunell *et al's* hypothesis (27, 28) suggesting that (i) the differences observed between mit. DNA restriction fragments from several yeast strains should be explained by the differences in the so-called "spacer" regions rather than in the genes ; (ii) that, in yeast, each mit. DNA gene would have its own regulation unit.

b) Results obtained with mit. tRNA purified by hybridization to mit. DNA

Correlation of the relative amount of the radioactivity in the different tRNA spots with the number of mit. tRNA genes is not simple. This results from methodological difficulties such as : close location of some spots and possibility of multiple location of a single isoacceptor, due to post-transcriptional modifications or conformational changes. Further, one has to explain why some calculated gene values obtained for tRNA spots differ from whole numbers (Table I). Values higher than 1 can be explained either by a single species coded for by more than one gene ($tRNA^{Phe}$ in spot 24) or by several comigrating species, each being coded for by one gene. For values lower than 1, obviously the gene saturation was not reached : this is especially the case for spot 5 (Figure 4), which contains two tRNAs ($tRNA^{Arg}$ and $tRNA^{Tyr}$). This quantitative study shows that hybridization with mit. DNA, in apparently optimal conditions for total mit. tRNA, yields an underestimated value for some genes. This was also noted for hybridization of mit. rRNA from yeast (29) or *Neurospora crassa* (30) and for the number of tRNA genes on Hela cell mit. DNA (31). Therefore, we estimate the number of mit. tRNA genes on yeast mit. DNA to be near 33, which seems to be a more realistic value than the number of 20-26 previously published (17, 32). We suggest, as earlier pointed out for some individual mit. tRNA species (7, 23), that the mit. DNA-coded mit. tRNAs would be sufficient for mitochondrial protein synthesis, especially if one considers recent results suggesting that a specific tRNA could recognize more codons than predicted by the "wobble hypothesis" (33, 34).

In conclusion, 2D electrophoresis of tRNA appears as to be a powerful

tool, since it allows (i) a survey of individual yeast mit. tRNA species in various physiological conditions or genetic backgrounds (analysis of mitochondrial "petite"...), (ii) purification of individual species for structure analysis or for assignment of their gene location on mit. DNA, (iii) comparison of mit. tRNA from various organisms.

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REFERENCES

Abbreviations

mit. : mitochondrial ; cyt. : cytoplasmic ; 2D electrophoresis : two-dimensional polyacrylamide gel electrophoresis.

1. Avadhani, N.G., Lewis, F.S. and Rutman, R.J. (1975) Sub-Cellular Biochem. 4, 93-145
2. Borst, P. (1972) Ann. Rev. Biochem. 41, 333-376
3. Suyama, Y. and Hamada, J. (1976) in The Genetics and Biogenesis of Chloroplasts and Mitochondria, Bücher *et al.*, Edt., pp. 763-770. Elsevier, Amsterdam
4. Fradin, A., Grühl, H. and Feldmann, H. (1975) FEBS Lett. 50, 185-189
5. Schneller, J.M., Schneller, C., Martin, R. and Stahl, A.J.C. (1976) Nucleic Acids Res. 3, 1151-1165
6. Faye, G., Kujawa, C. and Fukuhara, H. (1974) J. Mol. Biol. 88, 185-203
7. Schneller, J.M., Martin, R., Stahl, A. and Dirheimer, G. (1975) Biochem. Biophys. Res. Commun. 64, 1046-1053
8. Rubin, G.M. (1973) J. Biol. Chem. 248, 3860-3875
9. Neal, M.W. and Florini, J.R. (1973) Analyt. Biochem. 55, 328-330
10. Accoceberry, B., Schneller, J.M. and Stahl, A.J.C. (1973) Biochimie 55, 291-296
11. Schneller, J.M., Accoceberry, B. and Stahl, A.J.C. (1975) FEBS Lett. 53, 44-48
12. Martin, R., Schneller, J.M., Stahl, A. and Dirheimer, G. (1976) in Genetics and Biogenesis of Chloroplasts and Mitochondria, Bücher *et al.*, Edt., pp. 755-758. Elsevier, Amsterdam
13. Martin, R., Schneller, J.M., Stahl, A. and Dirheimer, G. (1976) Proc. 10th IUB Congress, Hamburg, pp. 101.
14. Herve, G. and Chapeville, F. (1965) J. Mol. Biol. 13, 757-766
15. Bray, G.A. (1960) Analyt. Biochem. 1, 279-285
16. Adams, J.M., Jeppesen, P.G.N., Sanger, F. and Barrell , B.G. (1969) Nature 223, 1009-1014
17. Schneller, J.M., Faye, G., Kujawa, C. and Stahl, A.J.C. (1975) Nucleic Acids Res. 2, 831-838

18. Krebs, A. and Hems, R. (1953) Biochim. Biophys. Acta 12, 172-180
19. Wyatt, G.R. (1951) Biochem. J. 48, 584-590.
20. Martin, R., Schneller, J.M., Stahl, A. and Dirheimer, G. (1976) Biochem. Biophys. Res. Commun. 70, 997-1002.
21. Barrell, B.G. and Clark, B.F.C. (1974) in Handbook of Nucleic Acid Sequences, Joynson-Bruvvers Ldt, Oxford
22. Dubin, D.T. and Friend, D.A. (1972) J. Mol. Biol. 71, 163-175
23. Schneller, J.M., Stahl, A.J.C. and Fukuhara, H. (1975) Biochimie 57, 1051-1057.
24. Keith, G., Rogg, H., Dirheimer, G., Menichi, B. and Heyman, T. (1976) FEBS Lett. 61, 120-123
25. Martin, N.C. and Rabinowitz, M. (1976) in The Genetics and Biogenesis of Chloroplasts and Mitochondria, Bücher *et al.*, Edt., pp. 749-754. Elsevier, Amsterdam
26. Feldmann, H. and Kleinow, W. (1976) FEBS Lett. 69, 300-304
27. Prunell, A., Kopecka, H. Strauss, F. and Bernardi, G. (1977) J. Mol. Biol. 110, 17-52
28. Prunell, A. and Bernardi, G. (1977) J. Mol. Biol. 110, 53-74
29. Reijnders, L., Kleisen, C.M., Grivell, L.A. and Borst, P. (1972) Biochim. Biophys. Acta 272, 396-407
30. Terpstra, P. (1977) in Ph.D. Thesis, Groningen, The Netherlands
31. Angerer, L., Davidson, N., Murphy, W., Lynch, D. and Attardi, G. (1976) Cell. 9, 81-90
32. Reijnders, L. and Borst, P. (1972) Biochem. Biophys. Res. Commun. 47, 126-133
33. Mitra, S.K., Lustig, F., Akesson, B., Lagerkvist, U. and Strid, L. (1977) J. Biol. Chem. 252, 471-478
34. Weissenbach, J., Dirheimer, G., Falcoff, R., Sanceau, J. and Falcoff, E. (1977) FEBS Lett., in press.