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Transfer RNA and mitochondrial information in yeast.

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Biologia molecolare. — *Transfer RNA and mitochondrial information in yeast.* Nota (*) di CLAUDIO FALCONE, LAURA FRONTALI, GIUSEPPE MACINO, CLAUDIO PALLESCHI e PAOLO SARACINO, presentata dal Socio G. MONTALENTI.

RIASSUNTO. — È stata studiata l'esistenza nei mitocondri di lievito di tRNA e aminoacil tRNA sintetasi mitocondriali differenti dai corrispondenti costituenti citoplasmatici. Costituenti specificamente mitocondriali sono stati messi in evidenza per serina e fenilalanina. Le sintetasi mitocondriali permangono in un mutante « petite » contenente solo il 3,6% di G e C e sono quindi presumibilmente di informazione nucleare. Esperimenti di ibridazione dimostrano l'esistenza sul DNA dei mitocondri « wild type » dell'informazione per il $tRNA_{ser}$ e per il $tRNA_{phe}$ mentre nel mutante l'informazione permane per il solo $tRNA_{ser}$.

The existence of a specifically mitochondrial protein synthesizing machinery including mitochondrial tRNAs different from their cytoplasmic counterparts has been well established [1]. Moreover, hybridization experiments performed in HeLa cells [2], rat liver [3] and yeast [4] have demonstrated the existence of a mitochondrial information for these tRNAs.

In yeast the existence of a mitochondrial information has been demonstrated for tRNAs specific for valine and leucine [5]. This information is sometimes retained and sometimes lost in " petite " mutants [6].

As far as specifically mitochondrial aminoacyl–tRNA synthetases are concerned, the existence of mitochondrial enzymes different from their cytoplasmic counterparts has been shown for *Neurospora crassa* [7] and rat liver mitochondria [3]. The localization of the information for these enzymes has not been established.

In this work the existence of specifically mitochondrial tRNA and aminoacyl–tRNA synthetases in yeast has been studied and the localization of the information was investigated on the basis of tRNA–mitochondrial DNA hybridization experiments and on the basis of a comparison between the mitochondrial constituents of w.t. *Saccharomyces cerevisiae* and of a mutant having a mitochondrial DNA having presumably little or no genetic information [8].

MATERIALS AND METHODS

A wild type (DM) and a " petite " (DM_1) strain were used whose characteristics have been previously described [8]. These strains were grown for 48 h in a glucose limiting medium and the harvested cells were washed and disrupted

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Abbreviations: w.t. = wild type; A = adenine; T = thymine; G = guanine; C = citosine; SSC = standard saline citrate.

with a mechanical disintegrator equipped with glass beads. The isolation of mitochondria was performed according to the method reported by Duell, Inoue and Utter [9]. The purity of mitochondrial fractions was checked by electron microscopy. For the preparation of cytoplasmic and mitochondrial tRNAs (from the supernatant and mitochondrial fractions respectively) the phenol procedure reported by Holley [10] was followed.

For the preparation of mitochondrial aminoacyl–tRNA synthetases, mitochondria were lysed with 0.2 or 0.3 % Triton X 100 in 10^{-2} M Tris buffer pH 7.4 the suspension was centrifuged at 100.000 × g for 90 min and dialyzed overnight against 10^{-2} M Tris containing $2 \cdot 10^{-3}$ M mercaptoethanol. Cytoplasmic synthetases were obtained from the cell extract after elimination of mitochondria (27.000 × g for 15 min.) and of ribosomes (100.000 × g for 90 min.) and dialysis as previously reported. Synthetases were stored at —10° C in the presence of 20 % glycerol with little loss of activity for one week.

Wild type mitochondrial DNA was obtained by the Marmur procedure from DNAase treated mitochondrial preparations. For the preparation of mitochondrial DNA from " petite " mutants, DNAase treatment was omitted and mitochondrial DNA was isolated by preparative CsCl gradient (45.000 × g for 48 h). All DNA preparations were checked for the absence of nuclear DNA by analytical CsCl gradients. For the acylation of tRNA and for the assay of aminoacyl–tRNA synthetases the method previously reported was used [11]. Incubations were performed at 30° C for 45 min.

The procedures for the denaturation of DNA and for DNA–aminoacyl–tRNA hybridization were essentially those reported by Buck and Nass [12]. RNA determinations were performed following the method of Scott, Fracastoro and Taft [13].

RESULTS

Results reported here refer to tRNAs and synthetases for phenyl–alanine and serine. The choice of these aminoacids was due to a preliminary study of the chromatographic and aminoacid–accepting properties of tRNA from mitochondria.

Chromatography of bulk yeast tRNA on hydroxy–apatite columns demonstrated the existence of multiple molecular species accepting serine and phenyl–alanine and showed differences in the elution profiles of supernatant and mitochondrial phenylalanyl– and seryl–tRNAs. Moreover, a study was performed to find out the cases in which tRNAs from mitochondria were preferentially acylated by mitochondrial synthetases.

The results of this study showed a specificity of mitochondrial synthetases towards $tRNA_{ser}$, $tRNA_{phe}$ and $tRNA_{ala}$ from mitochondria; no specificity was found towards $tRNA_{val}$ and $tRNA_{ileu}$ while no active synthetases were found in mitochondria for glycine and lysine.

In the case in which specificity indicated the existence of mitochondrial constituents it was obviously interesting to compare the existence of speci-

fically mitochondrial tRNAs and synthetases in w.t. yeast and in a " petite " mutant having very little or perhaps no mitochondrial information.

The results of this study are reported in Table I for serine and in Table II for phenylalanine. Results reported in Table I show that " petite " mitochondria contain a seryl–tRNA synthetase which preferentially acylates mitochondrial tRNA either from w.t. or from " petite " mitochondria.

TABLE I

Aminoacylation of w.t. and " petite " mitochondrial and supernatant tRNA *by mitochondrial and supernatant seryl–*tRNA *synthetases.*

For assay conditions see fig. 1. Assay mixture containing 100 μg tRNA

		d.p.m.
Aminoacyl tRNA synthetases from w.t. mitochondria . .	tRNA from petite mitochondria	3.650
Aminoacyl tRNA synthetases from supernatant	tRNA from supernatant	10.000
	tRNA from petite mitochondria	2.200
Aminoacyl tRNA synthetases from petite mitochondria	tRNA from w.t. mitochondria	3.000
	tRNA from supernatant	660
	tRNA from petite mitochondria	4.000

TABLE II

Aminoacylation of w.t. and " petite " mitochondrial and supernatant tRNA *by mitochondrial and supernatant phanylalanyl–*tRNA *synthetases.*

		d.p.m.
Aminoacyl tRNA synthetases from w.t. mitochondria . .	tRNA from w.t. mitochondria	1.650
	tRNA from supernatant	2.400
	tRNA from petite mitochondria	0
Aminoacyl tRNA synthetases from petite mitochondria	tRNA from w.t. mitochondria	2.500
	tRNA from supernatant	1.700
	tRNA from petite mitochondria	0
Aminoacyl tRNA synthetases from supernatant	tRNA from w.t. mitochondria	1.300
	tRNA from supernatant	5.500
	tRNA from petite mitochondria	0

On the other hand the supernatant synthetase preferentially acylates supernatant $tRNA_{ser}$. Results reported in Table II show that w.t. as well as " petite " mitochondrial phenylalanyl–tRNA synthetase acylate w.t. mitochondrial tRNA while they do not bind phenylalanine to tRNA from " petite " mitochondria.

The time course of acylation of supernatant and mitochondrial tRNA by mitochondrial synthetases reported in fig. 1 shows that the time used is

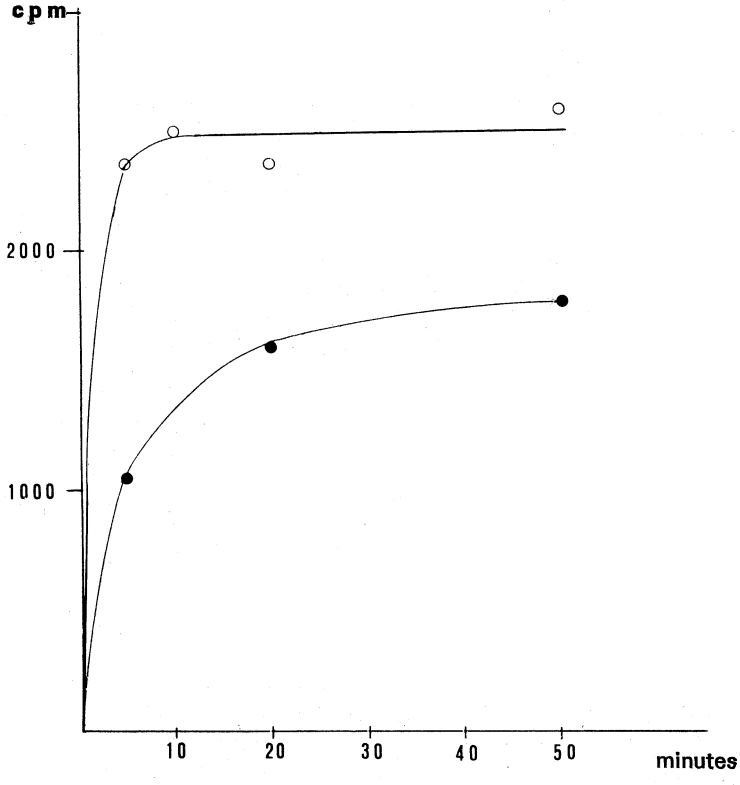

Fig. 1. – Time course of acylation of supernatant (●) and mitochondrial (○) tRNA by mitochondrial aminoacyl–tRNA synthetase.

Assay conditions are as follows: 5 μmoles ATP, 15 μmoles $MgCl_2$, 40 μmoles Tris HCl buffer, pH 7.4, 50–150 μg tRNA, 0.12 μmoles ^3H phenylalanine (spec. act. 3.48 C/mmole) and 1.6 mg enzyme protein in a final volume of 0.5 ml were incubated at 30° C for 30 min. Reaction was stopped by the addition of 3 ml of 67% ethanol containing 0.5 M NaCl. The precipitate was washed four times with 67% ethanol containing 0.5 M NaCl and dissolved in 0.5 ml of 0.2 M triethylamine. On suitable portions radioactivity was counted in a Beckman Liquid Scintillator and RNA determined by the method used by Scott *et al.* [14].

more than enough to attain a plateau in the acylation level which is only dependent on tRNA concentration. This dependence and the fact that in our conditions the synthetase is in excess is shown in Table III in which we report the increase in tRNA bound radioactivity in the presence of different amounts of mitochondrial and supernatant tRNA.

TABLE III

Aminoacylation of mitochondrial and supernatant tRNA *by mitochondrial seryl* tRNA *synthetase in the presence of different amounts of* tRNA.

	μg tRNA	c.p.m.
tRNA from mitochondria . . .	90	1.300
	180	2.800
tRNA from supernatant	40	650
	80	950

The localization of the information for these transfer RNAs has been studied by means of hybridization between denatured w.t. and " petite " mitochondrial DNA and aminoacyl–tRNA. Results are reported in figs. 2 and 3, while fig. 4 shows the absence of competition between mitochondrial and supernatant tRNAs for the sites on mitochondrial DNA.

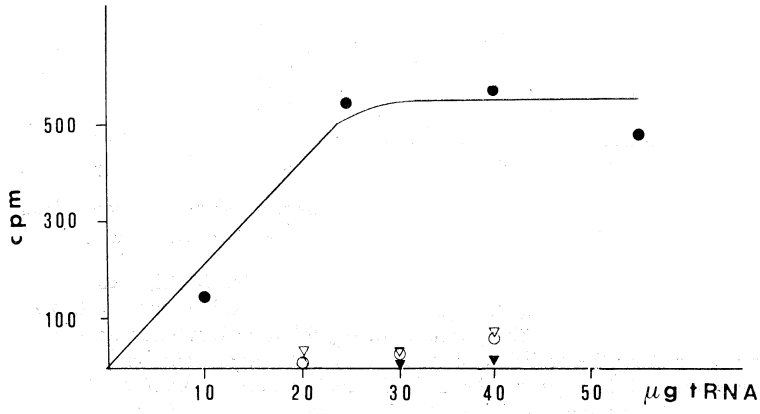

Fig. 2. – Hybridization of ^{3}H–labelled mitochondrial phenylalanyl–tRNA with w.t. and « petite » mitochondrial DNA.

● mitochondrial phenylalanyl–tRNA (660 c.p.m./μg) hybridized to filters containing 20 μg of w.t. mitochondrial DNA.

○ mitochondrial phenylalanyl–tRNA (450 c.p.m./μg) hybridized to filters containing 20 μg of « petite » mitochondrial DNA.

▲ mitochondrial phenylalanyl–tRNA hybridized to filters containing 20 μg of *E. coli* DNA.

△ mitochondrial phenylalanyl–tRNA hybridized to filters containing 20 μg of nuclear DNA.

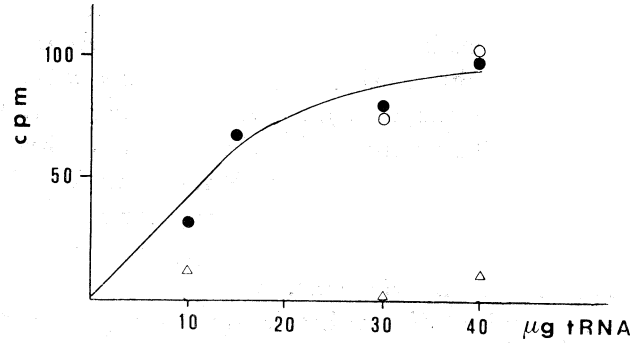

Fig. 3. – Hybridization of ^{3}H labelled mitochondrial seryl–tRNA with w.t. and « petite » mitochondrial and nuclear DNA.

Mitochondrial seryl–tRNA (239 cpm/μg) hybridized to filters containing 20 μg w.t. mitochondrial DNA (●) or 20 μg « petite » DNA (○) or 20 μg nuclear DNA (△).

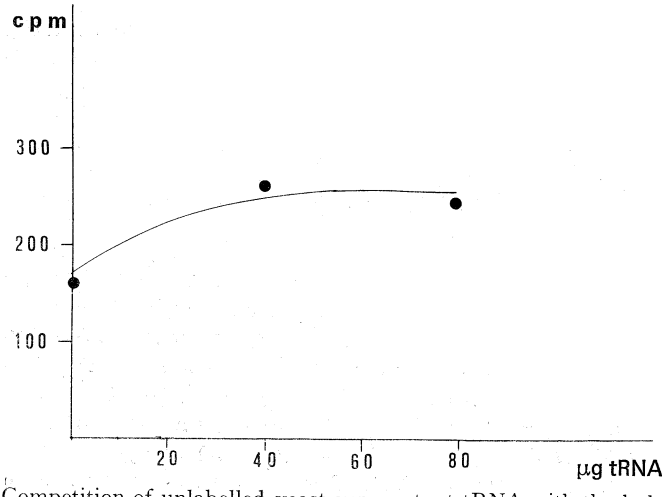

Fig. 4. – Competition of unlabelled yeast supernatant tRNA with the hybridization of ^{3}H labelled mitochondrial seryl–tRNA and w.t. mitochondrial DNA.

40 μg mitochondrial ^{3}H seryl–tRNA (spec. act. 110 cpm/μg) were hybridized with filters containing 20 μg w.t. mitochondrial DNA in the presence of increasing amounts of supernatant tRNA.

DISCUSSION

Results reported in Table I and II clearly show the existence of specifically mitochondrial tRNAs and synthetases for phenylalanine and serine. The different specificity of mitochondrial seryl–tRNA synthetase towards mitochondrial and supernatant tRNA is further demonstrated by Table III. The doubling of the amount of tRNA actually doubles the radioactivity bound to mitochondrial tRNA while it only increases by 30 % the radioactivity bound to supernatant tRNA.

The aim of this work was to investigate the localization of the information for these mitochondrial constituents. In the case of tRNAs the answer to this problem was obtained from hybridization experiments which show specific annealing between seryl– and phenylalanyl–mitochondrial tRNAs and w.t. mitochondrial DNA. With " petite " mitochondrial DNA hybridization was obtained for mitochondrial seryl–tRNA but not for phenylalanyl mitochondrial tRNA. This result is consistent with the results reported in Tables I and II: in " petite " mitochondria are actually present molecular species capable of accepting serine but not phenylalanine.

As far as aminoacyl–tRNA synthetases are concerned an answer on the localization of the information could come from the comparison between the w.t. and a " petite " mutant devoid of mitochondrial information. The DNA of our " petite " mutant (3.6 % G + C) has certainly a very low informational content.

Even if G and C were concentrated in a small informational segment of mitochondrial DNA, this segment could contain the information for a maximum of 2–3 proteins, and is therefore very improbable that the information for the synthetases studied here is just that contained in this segment. We think it therefore very probable that the information for these synthetases is of nuclear origin.

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