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Nuclear origin of specific yeast mitochondrial aminoacyl-tRNA synthetases

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ABSTRACT

Hydroxylapatite chromatographies of mitochondrial and total enzymes from a ρ^+ yeast, or from the related ρ° mitochondrial DNA-less mutant, show the occurrence in the mitochondrial enzyme of one Phe-, one Met-, one Leu-tRNA synthetase peak which elutes distinctly from the cytoplasmic counterpart and charges well mitochondrial tRNA, whereas the cytoplasmic enzyme does not. The measurement of the mitochondrial synthetases activities in various enzymatic extracts shows that they are not repressed in ρ^+ cells grown on 10% glucose and that they are concentrated in the mitochondria (Phe- and Met- tRNA synthetases) but are also present outside the mitochondria. It is concluded that yeast mitochondrial protein biosynthesis involves the nuclear coded mitochondrial specific Phe-, Met- and Leu-tRNA synthetases and that the entrance of the synthetases into the mitochondria needs no factor depending on the mitochondrial DNA.

INTRODUCTION

We reported the existence in the yeast Saccharomyces cerevisiae of mit tRNA species with a chromatographic behaviour different from that of their cytoplasmic counterparts (1, 2, 3, 6). Hybridization experiments on unfractionated (4) or fractionated (5, 6) mit tRNA species showed that they are coded by mit DNA. These results prompted us to gain similar knowledge for the mit aminoacyl tRNA synthetases. Preliminary results pointed out some differences from the cytoplasmic enzymes, when the enzymatic activity was studied as a function of the pH (2). Furthermore the mitochondrial enzymes charged more specifically the mit $tRNA^{Leu}$ species, only found in the mitochondria (3) and which is coded by mit DNA (5).

This report deals with the occurrence of specific Phe-RS, Met-RS and Leu-RS in yeast mitochondria. We wanted to check if these mitochondrial synthetases chromatograph distinctly from their cytoplasmic counterparts on hydroxylapatite and if they are present in a mutant lacking mit DNA. If so it would be a good indication that these enzymes are of nuclear origin and must be synthesized in the cytoplasm ; furthermore they should be detectable outside the mitochondria. We also checked for an eventual catabolic repression of these synthetases by glucose.

MATERIALS AND METHODS

The culture of wild type yeast ρ^+ IL8-8C was on a 1 % galactose, 1% bactopeptone, 1% yeast extract medium. The medium contained 2% galactose for the culture of the related mutant lacking mit DNA : ρ° IL8-8C/H 71 (7). The absence of ρ^+ cells in the mutant strain culture was checked by plating on glycerol and glucose mediums. In some experiments (indicated in the text) galactose was replaced by 10% glucose and the cells were harvested before the end of the log phase when the glucose was still 5% in the medium.

The preparation of mitochondria from the ρ^+ yeast protoplasts was as in ref (8). The mitochondria were further purified by centrifugation on a sucrose gradient (9). The preparation of total cell and mit enzymes are described in ref. (2) and (3). The preparation of mit tRNA was as in ref (6).

The mitochondria from the ρ° strain were prepared as the ρ^+ mitochondria but the sucrose gradient step was omitted. At the end of the preparation they were pelleted by a 30 mn centrifugation at 25 000 g in the Sorvall centrifuge ; this operation allowed us to discard a very viscous layer above the mitochondrial pellet. This layer contained no proteins. ρ° mit enzymes were prepared like the ρ^+ mit enzymes, but the Sephadex G.25 chromatography was replaced by a dialysis against the same buffer as the one used for the chromatography (see ref 2). Total enzyme from the ρ° strains was obtained by breaking the cells as for the ρ^+ cells (2).

The chromatography of about 15 mg of the enzymatic extracts was performed on 0.5 x 10 cm hydroxylapatite (Biorad) columns

at +4°C. The enzymes were usually eluted by 40 ml of a 20 mM to 0.4 M pH 7.5 K phosphate gradient in 1.37 M glycerol, 10 mM 2-mercaptoethanol, 1 mM EDTA and 400 µl fractions were collected.

Aminoacylation has been carried out as in ref (2). The 100 µl acylation medium contained : ATP 1 µmmole, $MgCl_2$ 2.5 µmoles, cysteine-HCl 7.5 µmoles, bovin serum albumin 10 µg, Tris-HCl pH 8 10 µmoles, ^{14}C aminoacids 5 nmoles i.e. 0.12 µCi. 25 µg or more of yeast total or of E. coli tRNA, or 10 µg of mit tRNA were aminoacylated at +37°C by the enzymes contained in 25 µl or less of the different chromatographic fractions and for different time intervals. Modifications of these conditions are indicated in the figures. The kinetic measurement of the enzymatic activity was carried out in a similar aminoacylation medium using ^{14}C-aminoacids (5 nmoles i.e. 0.24 µCi), 1.2 to 35 µg enzymatic proteins and 46 µg of mit tRNA (for Phe- and Leu-RS activity) or 160 µg of E. coli tRNA (for Met-RS activity) per 100 µl aminoacylation medium. The pH was raised to 8.7 for measurement of mit Leu-RS activity.

E. coli B tRNA and yeast total tRNA considered as cyt-tRNA (see specificity of mit Leu-RS) were purchased from Boehringer (Mannheim) and the ^{14}C or ^{3}H aminoacids from the C.E.A. (Saclay).

The phosphate assay was according to BRIGGS (10).

RESULTS

1) Mitochondrial and total cell Phe-RS :

a) Table I shows that both enzyme preparations charge mit- and cyt tRNA. There was no misacylation in the reaction of cyt $tRNA^{Phe}$ with the mit enzyme since RPC 5 (11) chromatographies of cyt $tRNA^{Phe}$ charged with the two enzymes produced superimposable profiles (not shown). Total cell enzyme contains mit- and cyt synthetases and the loads with this enzyme as reported in table I do not allow us to state if cyt Phe-RS charges really mit tRNA. The chromatographic fractionation of the total enzyme could show if there are several activities involved, and could allow to study their specificities.

b) Hydroxylapatite chromatography of the ρ^+ mit enzymes shows one peak eluting with 0.16 to 0.18 M phosphate (fig. 1a).

Table I : Extent of aminoacylation of E. coli-, yeast cyt- or mit tRNA, carried out with an excess of various enzyme preparations. The values are expressed as n moles of aminoacids incorporated per mg tRNA.

	tRNA							
	Phe		Met			Leu		
Enzymes	cyt	mit	cyt	mit	E.coli	cyt	mit	E. coli
ρ^+ total	1.85	2.30	0.78	1.50	0.74	2.90	2.20	2.30
ρ^+ mit.	1.25	2.60	0.56	2.40	1.30	0.30	2.60	1.85
ρ° mit.	1.25	1.85	1.10	2.10	0.90	0.00	0.96	0.74

A chromatography of ρ^+ total enzymes extracted from yeast grown on 1% galactose or 10% glucose, when tested with cyt tRNA, reveals one peak which elutes with 0.20 to 0.23 M phosphate and which shows a shoulder of enzymatic activity eluting with 0.18 M phosphate like the mit Phe-RS (fig. 1b). Furthermore, only this shoulder of enzymatic activity charges mit tRNA and the peak eluting with 0.20 to 0.23 M phosphate corresponds well to the cyt Phe-RS which was purified to homogeneity by FASIOLO et al. (12) and elutes with a similar phosphate concentration on hydroxylapatite (13).

c) Similar experiments performed with mit (fig. 1c) and total (fig. 1d) enzymes from the isogenic ρ° strain give respectively the same chromatographic profiles as for the ρ^+ mit and total enzymes. The mit Phe-RS peak is however better resolved in the ρ° total enzymes because of the lower cyt Phe-RS activity when compared to the ρ^+.

2) Mitochondrial and total cell Met-RS :

a) Total cell and mit Met-RS are able to charge cyt-, mit- and E. coli tRNA (table I). As for the Phe-RS, no answer can be given at this stage whether the charge of mit tRNA by total cell enzyme is due to one or several activities. Anyway the RPC 5 chromatography of cyt tRNA charged by mit enzymes with ^{3}H-Met does not show any additional peak as compared to cyt tRNA charged by total enzymes with ^{14}C-Met (not shown).

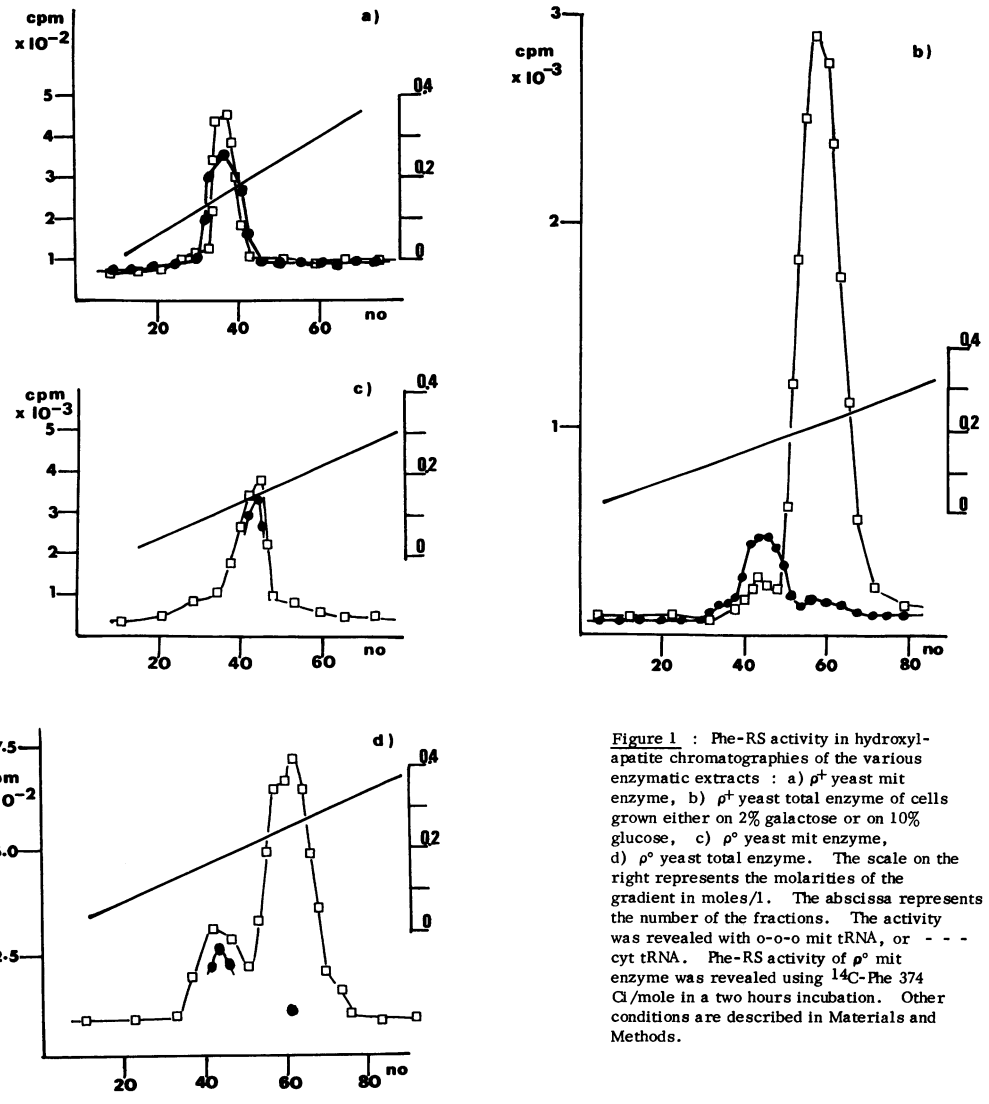

Figure 1 : Phe-RS activity in hydroxylapatite chromatographies of the various enzymatic extracts : a) ρ^+ yeast mit enzyme, b) ρ^+ yeast total enzyme of cells grown either on 2% galactose or on 10% glucose, c) ρ° yeast mit enzyme, d) ρ° yeast total enzyme. The scale on the right represents the molarities of the gradient in moles/l. The abscissa represents the number of the fractions. The activity was revealed with o-o-o mit tRNA, or - - - cyt tRNA. Phe-RS activity of ρ° mit enzyme was revealed using ^{14}C-Phe 374 Ci/mole in a two hours incubation. Other conditions are described in Materials and Methods.

b) Hydroxylapatite chromatography of ρ^+ mit enzymes reveals one peak eluting with 0.15 M phosphate (fig. 2a). This Met-RS aminoacylated cyt-, mit-, and E. coli tRNA. The chromatography of ρ^+ total enzymes extracted from yeast cells grown either in derepression conditions or with 10% glucose shows a peak with the same chromatographic and charging properties in addition to another one eluting later (0.27 M phosphate) which charges well only cyt tRNA and represents therefore cyt Met-RS (fig 2b).

c) The same experiments were performed with enzymes extracted from the ρ° strain (fig. 2c and d). Identical results were obtained.

3) Mitochondrial and total cell Leu-RS :

a) Mit Leu-RS in contrast to Phe-RS and Met-RS charges poorly cyt tRNA (table I). This is not due to a side reaction since an excess of mit enzymes does not hinder good charging of cyt tRNA by a limited amount of total enzyme. E. coli- and mit tRNA are good substrates for mit Leu-RS. The total enzyme charges well the three tRNAs (table I). We tested that there was no misacylation when E. coli tRNA is charged by mit enzymes. The RPC 5

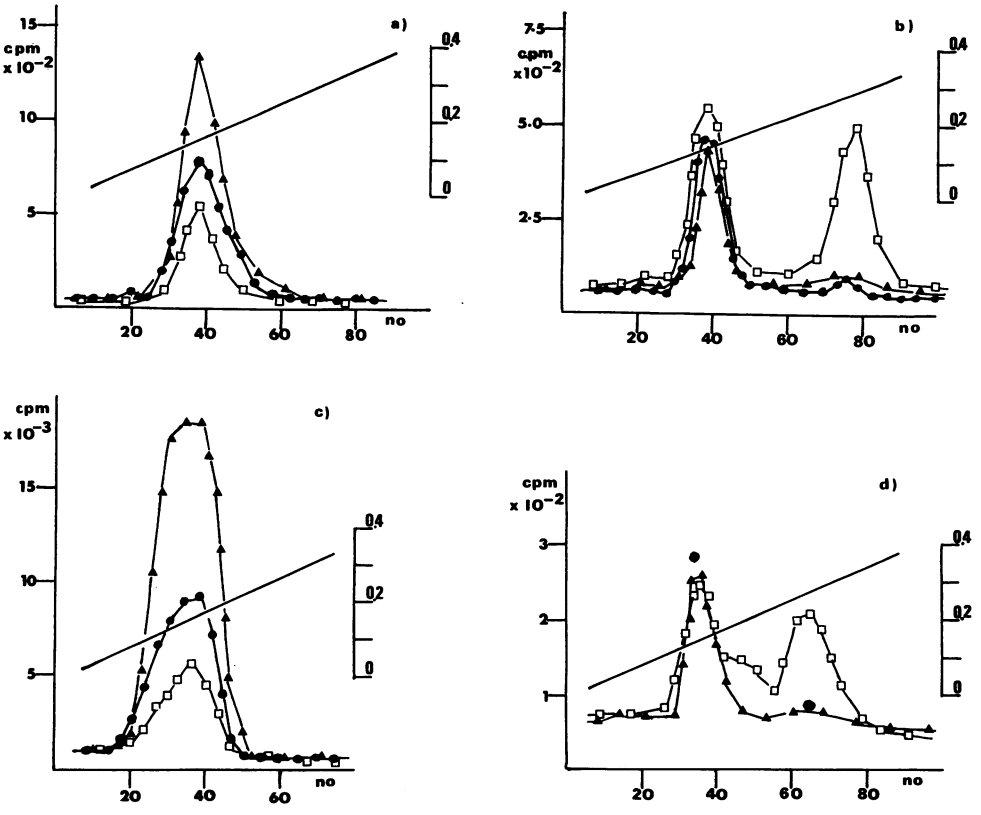

Figure 2 : Met-RS activity in hydroxylapatite chromatographies of the various enzymatic extracts : For legend see figure 1. ▲-▲-▲ Aminoacylation of E. coli tRNA. The incubation time was 40 minutes for all extracts except for the ρ° mit enzyme. Met-RS activity of ρ° mit enzymes was revealed using ^{3}H- Met 2,5 Ci/mmole in a two hours incubation.

chromatography of E. coli $tRNA^{Leu}$ charged with mit enzyme showed no additional peak to the five expected peaks revealed with E. coli enzyme (not shown). However only three out of the five peaks are charged. We studied also the activity of the enzymes as a function of pH. Figure 3 shows that mit $tRNA^{Leu}$ is efficiently charged between pH 8 and 9 and that cyt $tRNA^{Leu}$ is poorly charged by mit enzyme at all pH and chiefly at the alkaline pH.

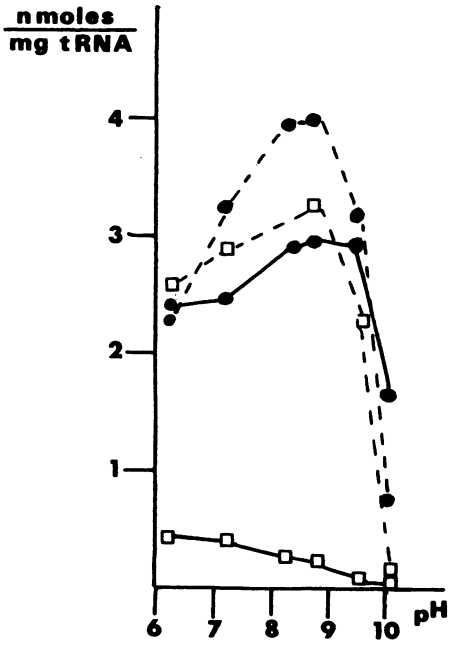

Figure 3 : Extent of aminoacylation of $tRNA^{Leu}$ as a function of pH. -□-□-□ cyt tRNA or •-•-• mit tRNA was charged by ——— mit Leu-RS or ------ total Leu-RS.

b) Hydroxylapatite chromatography of ρ^+ mit enzymes shows one peak eluting with 0.20 to 0.22 M phosphate (fig. 4a). This Leu-RS charges, as expected, mit- and E. coli-, but not cyt tRNA. When cyt tRNA is the substrate, total enzymes of ρ^+ cells grown in the presence of 1% galactose or 10% glucose show one peak of cyt Leu-RS eluting with 0.16 M phosphate (fig. 4b and c). When mit tRNA is the substrate, these total enzymes show an additional peak eluting like the mit Leu-RS. The activity of this peak is

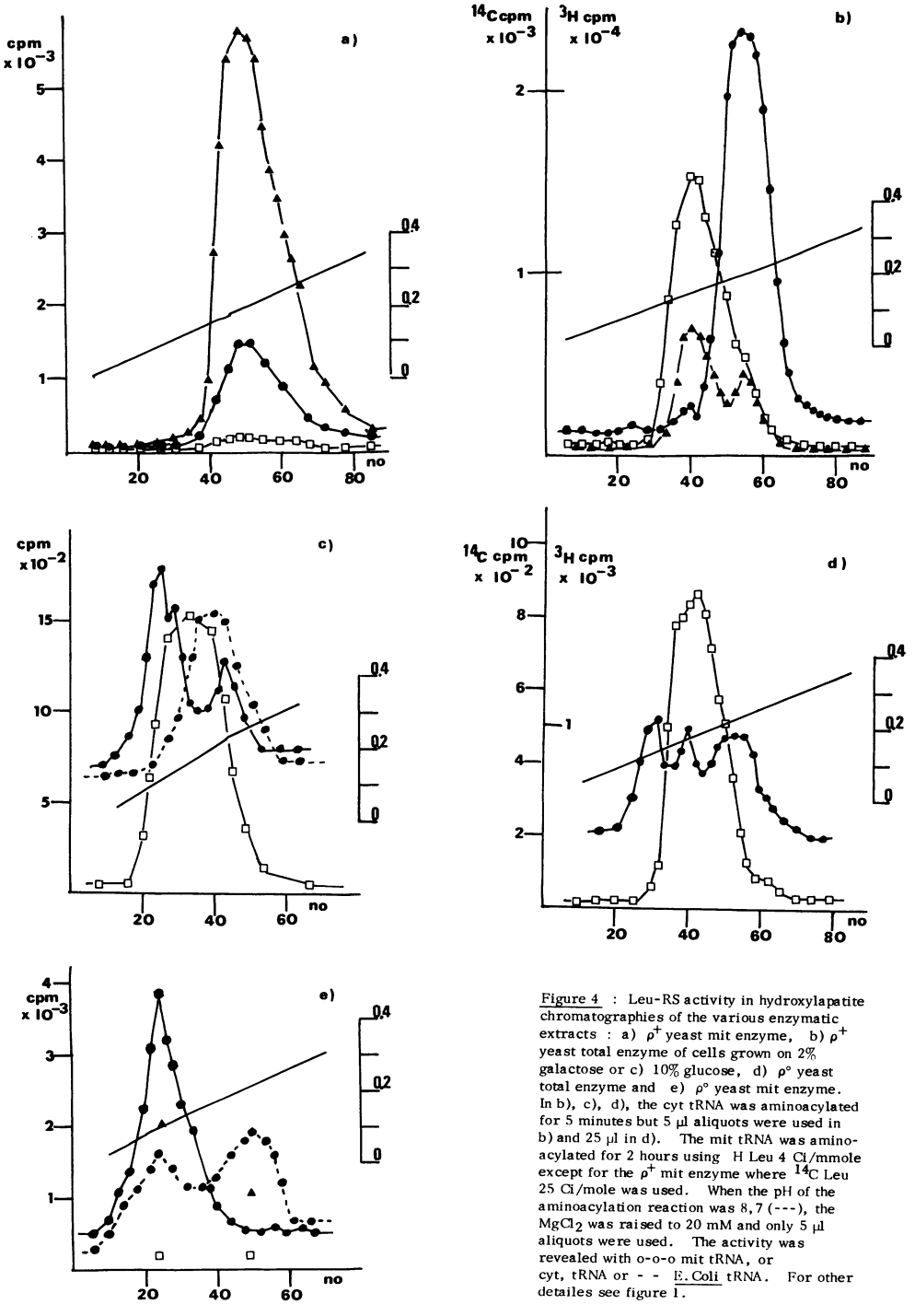

Figure 4 : Leu-RS activity in hydroxylapatite chromatographies of the various enzymatic extracts : a) ρ^+ yeast mit enzyme, b) ρ^+ yeast total enzyme of cells grown on 2% galactose or c) 10% glucose, d) ρ° yeast total enzyme and e) ρ° yeast mit enzyme. In b), c), d), the cyt tRNA was aminoacylated for 5 minutes but 5 µl aliquots were used in b) and 25 µl in d). The mit tRNA was aminoacylated for 2 hours using H Leu 4 Ci/mmole except for the ρ^+ mit enzyme where ^{14}C Leu 25 Ci/mole was used. When the pH of the aminoacylation reaction was 8,7 (---), the $MgCl_2$ was raised to 20 mM and only 5 µl aliquots were used. The activity was revealed with o-o-o mit tRNA, or cyt, tRNA or - - E. Coli tRNA. For other detailes see figure 1.

much lower in the ρ^+ cells grown on 10% glucose and can be enhanced when the aminoacylation is carried out at pH 8.7. In these cells we have however revealed an unexpected Leu-RS activity which elutes with 0.12 M phosphate when using mit tRNA as substrate. In addition, E. coli B tRNA is charged by the mit- and the cyt synthetases (fig. 4a and b).

c) Chromatography of the ρ° total enzymes gives identical results but the cyt Leu-RS activity eluting at 0.16 M is several times lower than that of the ρ^+ cells (Note that five fold less aliquots were used for testing the ρ^+ cyt Leu-RS) (fig. 4d). Furthermore, as for the ρ^+ cells grown on glucose, these enzymes show, when tested with mit tRNA, the unexpected peak eluting with 0.12 M phosphate besides a peak eluting like the mit Leu-RS. This abnormal peak is also found in the ρ° mit enzymes and when the aminoacylation is performed at pH 8.7 the peak eluting like mit Leu-RS is revealed (fig. 4e). These peaks charge E. coli - but not yeast cyt tRNA. These substrate specificities are also evidenced in Table I on unfractionated ρ° mit enzymes.

4) Mit Phe-, Met- and Leu-RS activity in various ρ^+ enzymatic extracts :

We could measure the activity of the mit specific synthetases using their specificity for mit tRNA (Phe- and Leu-RS) or for E. coli tRNA (Met-RS).

Phe- and Met-RS are concentrated into the ρ^+ mitochondria since there is four times more activity in the mitochondrial than in the total enzyme (table II). The enzymatic activities presented in table II together with the fact that we could prepare roughly ten to sixteen times more total enzyme than mit enzyme from a given ρ^+ cell mass, draw however to the conclusion that mit Phe-, Met- and Leu-RS are present outside the mitochondria. This observation was confirmed by preparing an enzymatic extract from a post- mitochondrial supernatant which showed nearly as much activity for the three synthetases as the total enzyme (results not shown). For this reason we could'nt name the post-mitochondrial enzymatic extract a pure cyt synthetase preparation and used in this work the total cell enzyme.

In order to check for an eventual repression of the biosynthesis of the mit synthetases by high glucose concentration, we

Table II : Enzymatic activity of mit Phe-, Met- and Leu-RS in various ρ^+ enzymatic extracts. Phe-RS and Leu-RS activities are measured in reaction with yeast mit tRNA, Met-RS with E. coli tRNA.

	Specific activity expressed in n moles of ^{14}C aminoacid incorporated in tRNA per mg of protein and per minute.			Amounts of proteins in the enzymatic extracts : mg/100g wet weight cells
Source of enzyme	Phe-RS	Met-RS	Leu-RS	
ρ^+ mitochondria	1.9	2.5	0.25	110
total ρ^+ cells	0.46	0.69	0.92	1800
total ρ^+ cells grown on 10% glucose	0.53	0.99	1.45	2000

measured the activities of these synthetases in total enzyme preparations from cells grown in 2% galactose or in 10% glucose media. Total enzyme from the cells grown on glucose showed no less activity than enzyme from derepressed cells (table II).

DISCUSSION

1) Occurrence of mit specific aminoacyl tRNA synthetases in yeast :

It has been reported that mit enzymes have specific charging properties different from those of the cytoplasm (14, 15, 16). In some species these enzymes were shown to have a distinct chromatographic behaviour (14, 16). We confirm these results for Saccharomyces cerevisiae as we found in the total ρ^+ enzymes, besides the cyt Phe-RS, Met-RS, and Leu-RS which charged well only cyt tRNA, chromatographically distinct Phe-RS, Met-RS and Leu-RS which charged well mit tRNA, and which were associated specifically with the mitochondria. This in turn gives the indication that our mitochondria preparations were highly purified. It must be emphasized that the mit tRNA used throughout this study was shown to be of the same purity, since only the mit DNA coded, chromatographically distinct $tRNA^{Leu}$ (5),

$tRNA^{Phe}$ (6) and $tRNA^{Met}$ (17) were found in this tRNA. This allows us to conclude that at least for these three aminoacids, mitochondrial protein biosynthesis involves only the mit DNA coded tRNA and their specific cognate synthetases. In addition, as mit Leu-RS and E. coli Leu-RS (4) charge mit- and E. coli but not cyt $tRNA^{Leu}$, mit $tRNA^{Leu}$ and some E. coli $tRNA^{Leu}$ isoacceptors must share some common features with regard to the two synthetases recognition sites.

2) Effect of growth in high glucose medium on the mit specific aminoacyl-tRNA synthetases :

It is known that several mitochondrial enzymes of yeast are repressed by glucose and induced during derepression (for a review see 18). We have checked for the presence of mit specific synthetases in yeast cells grown on 10% glucose. Our results show clearly that these enzymes were present and apparently in a slightly larger amount in ρ^+ cells grown in high glucose medium. It must be emphasized that the results found by measuring the activity of the enzymes (table II) are not always in accordance with the activity found upon chromatography of the enzymes, especially for mit Leu-RS. For this enzyme we had to use ^{3}H-Leu with a higher specific activity to test the chromatographic fractions. For mit Leu-RS too, the total enzyme of glucose grown cells (fig. 4c) showed apparently less activity than that of derepressed cells (fig. 4b) though the two enzymatic extracts were treated identically and the same amounts of enzymatic proteins were chromatographed. So mit Leu-RS revealed itself to be a somewhat unstable enzyme. Nevertheless, our results are in favour of the hypothesis that mitochondrial synthetases are not significantly affected through catabolic repression by glucose. In addition, we could also find mit Phe- and Met-RS in enzymatic extracts prepared from commercial baker's yeast grown in fermentative conditions (results not shown).

3) Nuclear origin of the mit specific aminoacyl tRNA synthetases :

Chloroplastic aminoacyl tRNA synthetases are nuclear coded (19) but very little is known about the coding origin of mit

aminoacyl tRNA synthetases. Recently BOGUSLAWSKY et al. (20) found a mit specific His-RS in a ρ° mutant lacking mit DNA. This indicates that the mit enzyme is nuclear coded but the authors could not separate mit- and cyt His-RS by chromatography. Since mit specific chromatographically distinct peaks for Phe-, Met- and Leu-RS were present in total enzyme of the isogenic ρ° strain, lacking mit DNA, the mit synthetases are certainly nuclear coded and must be synthesized on cyt ribosomes. This conclusion is strengthened by our finding of the mit Phe-RS, Met-RS and Leu-RS in ρ° mit enzymes, despite the lower activity found. For the moment, we do not fully understand the significance of the Leu-RS peak eluting with 0.12 M phosphate. We think that this peak represents some conformational or degraded form of mit Leu-RS.

4) Entrance of mit specific synthetases into the mitochondria :

The foregoing results imply that mit specific aminoacyl-RS must enter the mitochondria. As it has been proposed for other mit specific proteins (21), such proteins would be synthesized by special cytoplasmic ribosomes bound to the mitochondrial outer membrane. This implies however that such proteins should not be detectable in the extramitochondrial cytoplasm (22). We could show that the mit synthetases are present outside the mitochondria, though mit Phe- and Met-RS are concentrated inside the mitochondria. Since mit Leu-RS seems to be more unstable (see Discussion 2)), the Leu-RS enzymatic activities may be underestimated. The presence of an excess of mit synthetases in the total enzyme preparation after subtraction of that part which is associated to the mitochondria cannot be simply due to an underestimation of the latter because this would imply a more than fifty per cent loss of the mitochondria during isolation from the protoplasts. This observation has to be taken in account in the proposed explanation (21). Furthermore as mit Met-RS, like mit Phe-RS, charges cyt tRNA, this raises the possibility that some mit specific synthetases take part in the cytoplasmic protein biosynthesis.

Our data also lead to the conclusion that entrance of the synthetases into the mitochondria does not require specific products of mitochondrial protein biosynthesis, since we found

only the mit specific Phe-RS, Met-RS and Leu-RS in the mit enzymes of the mit DNA less ρ° strain which cannot synthesize these products. This conclusion together with the finding of these enzymes when the cells are grown in fermentative conditions, indicate that the synthetases must be present in the mitochondria before the derepression ; this allows aminoacylation of the functional mit tRNA just when it is synthesized.

5) The mit specific synthetases as products of genes different from those coding for cyt synthetases :

The fact that the mit enzymes have a distinct chromatographic behaviour and distinct charging properties using cyt-, mit- and E. coli tRNA as compared to the cyt synthetases, lead us to think that they are products of distinct genes and thus differ in their structure. Our results are in accordance with those of CHIU and SUYAMA (16) who found differences in mit- and cyt Leu-RS from Tetrahymena pyriformis. These authors couldn't find common antigenic determinants between mit- and cyt Leu-RS (23). The possibility that cyt- and mit synthetases would have a subunit in common cannot be fully excluded, since it was reported that the cyt- and mit Leu-RS were specified by the Leu 5 cistron in Neurospora (24).

We cannot exclude the occurrence of a mit DNA coded synthetase or synthetase subunit until all mit aminoacyl tRNA synthetases have been studied. Research on ρ° mutants will be useful to know more about the nuclear origin of other synthetases and also other proteins from the matrix. This is already under investigation, as well as the more detailed kinetic study on the purified mit enzymes.

Finally mit DNA coded RNAs interact specifically with nuclear coded proteins such as aminoacyl tRNA synthetases, but also with transformylase or ribosomal proteins... (for a review see 18). With respect to the origin of mitochondria, the specificity of these interactions may indicate that the nuclear genes for these proteins and the mitochondrial genes for RNA were probably linked in the same DNA molecule in the course of evolution.

It would be of great interest to know whether the genes for mit synthetases or/and ribosomal proteins are scattered

throughout the nuclear genome ; this would perhaps show the existence of some chromosome more specifically involved in mitochondrial functions.

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Abbreviations used : mit = mitochondrial ; cyt = cytoplasmic ; Phe-, Met-, Leu-, His-RS = aminoacyl tRNA synthetases.

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