STUDIES OF ODD BASES IN YEAST MITOCHONDRIAL tRNA : ABSENCE OF THE FLUORESCENT "Y" BASE IN MITOCHONDRIAL DNA CODED $tRNA^{Phe}$, ABSENCE OF 4-THIOURIDINE

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SUMMARY : Reversed phase chromatography of mitochondrial [^{3}H] Phe-tRNA from Saccharomyces cerevisiae shows only one peak which elutes distinctly from cytoplasmic [^{14}C] Phe-tRNA. Mitochondrial $tRNA^{Phe}$ from this peak hybridizes specifically with ρ^+ and a ρ^- mitochondrial DNA. Search for rare bases in mitochondrial tRNA shows the absence of the eukaryotic "Y" base and of the prokariotic s^4U base.

INTRODUCTION : [^{3}H] aminoacyl-tRNA-DNA hybridization studies in yeast provided evidence that mit. DNA was able to code for at least fourteen tRNAs (1). Among them [^{3}H] Phe-tRNA could be hybridized to mit. DNA from ρ^+ ("grande") (2) and some ρ^- ("petite") strains (3). $tRNA^{Phe}$ is of some special interest with respect to the fluorescent "Y" base. This hypermodified base occurs in yeast cyt. $tRNA^{Phe}$ in a position adjacent to the 3'-hydroxyl end of the anticodon (4). It is not found in E. coli $tRNA^{Phe}$ (5). Its presumed function in yeast cyt. $tRNA^{Phe}$ seems to be facilitation of the formation of precise codon-anticodon base pairing by stabilizing the three-dimensional structure of the anticodon loop (6). These results prompted us to look for such a fluorescent base in yeast mit. tRNA. For this purpose we checked for the presence of mit. DNA coded $tRNA^{Phe}$ after reversed-phase chromatography. We also looked for the presence of

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Abbreviation : mit. : mitochondrial ; cyt. : cytoplasmic ; nuc. : nuclear ; s^4U : 4-thiouridine.

4-thiouridine (s^4U) in mit. tRNA, as this base, in contrast to the "Y" base, is present in E. coli (7) and absent in eukaryotic tRNAs.

MATERIALS AND METHODS :

1) The wild type Saccharomyces cerevisiae strain IL8-8C ρ^+ and the derived mutant strain ρ° IL8-8C/H71 a "petite" lacking mit. DNA (8) were grown as described earlier (9). Mitochondria isolation procedure from yeast protoplasts was the same as in (10). These mitochondria were further purified by a sucrose gradient step (11).

2) Mit. RNA was prepared after phenolic extraction as in (10) and mit. rRNA precipitated with 2M NaCl. The supernatant (mit. tRNA) was further purified by a Sephadex G100 chromatography. Cyt. tRNA was extracted from ρ° cells. Total yeast tRNA was purchased from Boehringer-Mannheim. Mitochondrial and cytoplasmic enzymes were prepared as in (9).

3) Aminoacylation of tRNA and chromatography on RPC5 (12) were as previously described (9). [^{3}H] Phe (20 Ci/mM) was purchased from Amersham-Searle and [^{14}C] Phe (200 mCi/mM) from CEA-Saclay.

4) Hybridization of mit. [^{3}H] Phe-tRNA ρ^+ isolated peak or of ^{3}H Phe-tRNA ρ° with mit. DNA $^+$ IL8-8C or ρ^- IL8-8C/D21 (a mit. mutant) and nuc. DNA ρ° IL8-8C/H71 was performed according to ref. (13).

5) Fluorescence spectra were recorded on a FICA 55 spectrofluorimeter. For these measurements the tRNA solution contained 20 mM Tris-HCl pH 7.4 and 10 mM $MgCl_2$.

RESULTS : 1) Chromatographic separation of mit. $tRNA^{Phe}$: Figure 1 shows the cochromatography of cyt. [^{14}C] Phe-tRNA ρ° with mit. [^{3}H] Phe-tRNA in the RPC5 system. Mit. $tRNA^{Phe}$ is eluted earlier from the column than ρ° $tRNA^{Phe}$ and it does not contain cyt. $tRNA^{Phe}$.

2) Hybridization of [^{3}H] Phenylalanyl-$tRNA^{Phe}$: Figure 2 shows the hybridization with mit. DNA ρ^+ of mit. [^{3}H] Phe-tRNA obtained in a preparative scale chromatography using the RPC5 system. This peak could be hybridized to saturation. It must be noticed that the hybridization of this species could be increased about three times with the "petite" ρ^- IL8-8C/D21 mit. DNA (see Table I). This table shows also that no significant hybridization could be obtained with nuc. ρ° DNA. Cyt. [^{3}H] Phe-tRNA could not be hybridized either to mit. ρ^+ or ρ^- D21 DNA. A very low level of hybridization, if significant, is obtained with nuc. ρ° DNA.

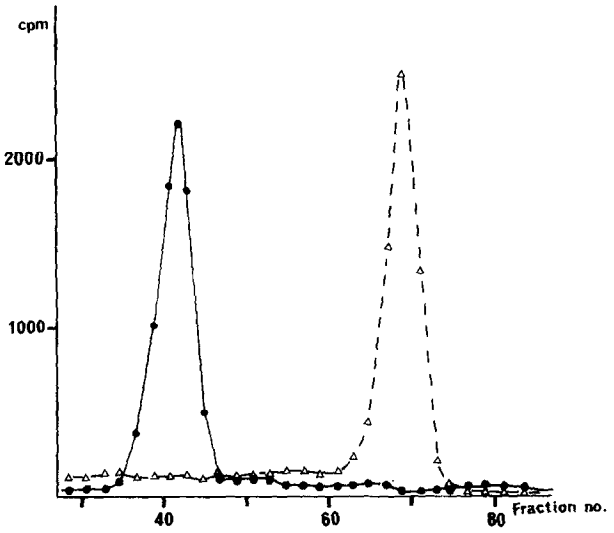

Figure 1. Co-chromatography of cyt. [^{14}C] Phe-tRNA (Δ ---Δ) and of mit. [^{3}H] Phe-tRNA (●——●). The chromatography was performed on a RPC5 column (0.5 x 100 cm) and the tRNAs were eluted using a 0.35 to 0.75 M NaCl gradient in 10 mM AcONa pH 4.5, 10 mM $MgCl_2$ buffer (total volume of the gradient : 400 ml ; volume of the fractions : 2.7 ml).

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3) Search for a "Y" base in mit. tRNA : Figure 3 shows the fluorescence-emission spectrum of total yeast tRNA (12 A_{260} units/ml) when excited at 315 nm. The peak of fluorescence-emission at 450 nm was found to be specific for the "Y" base in yeast cyt. $tRNA^{Phe}$ (14). As shown in this figure, the fluorescence-emission of a ten fold dilution could be detected. At a similar concentration (11 A_{260} units/ml) mit. total tRNA does not fluoresce. It must be emphasized that mit. tRNA was found to be aminoacylated (extent of aminoacylation) 1.5 to 2 times more efficiently with [^{14}C] Phe than cyt. tRNA when using the homologous enzymes. We checked also for a possible modified "Y" base. Such bases are known to be present in tRNAs from other species than yeast ; for a review see (15). We found no fluorescence by varying excitation wavelengths and measuring the whole emission spectra.

4) Search for the s^4U base in mit. tRNA : LIPSETT et al. (7)

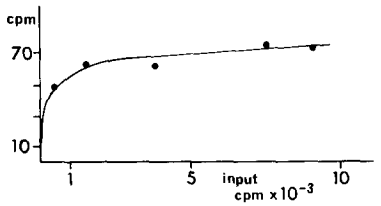

Figure 2. Hybridization of mit. [^{3}H] Phe-tRNA (isolated peak) to filters containing 10 µg mit. DNA ρ^+. Blank filters (0,17,43,72, 121 cpm) were substracted from the experimental values.

TABLE 1

[^{3}H] Phe-tRNA input		mit. DNA ρ^+ 10 µg (-blank)	mit. DNA ρ^- 10 µg (-blank)	nuc. DNA ρ° 10 µg (-blank)	Blank
mit. ρ^+	4500 cpm	90	242	1	41
(")	11300 cpm	72	226	18	121
cyt. ρ°	5000 cpm	11	19	28	28

TABLE 1 :
Comparative hybridizations of mit. ^{3}H Phe-tRNA (isolated peak) and cyt. ^{3}H Phe-tRNA (unfractionated) to the different DNAs. Values are expressed in cpm.

have reported that absorbance of E. coli tRNA at 336 nm was due to the presence of s^4U. We therefore studied the UV spectrum of a very concentrated solution (72 A_{260} units/ml) of yeast mit. tRNA. No peak was found at 336 nm (see figure 4).

DISCUSSION : 1) Absence of the cytoplasmic $tRNA^{Phe}$ species in mitochondria : As reported earlier (16) separation of cyt. and mit. $tRNA^{Phe}$ could be obtained in the RPC2 system. However the

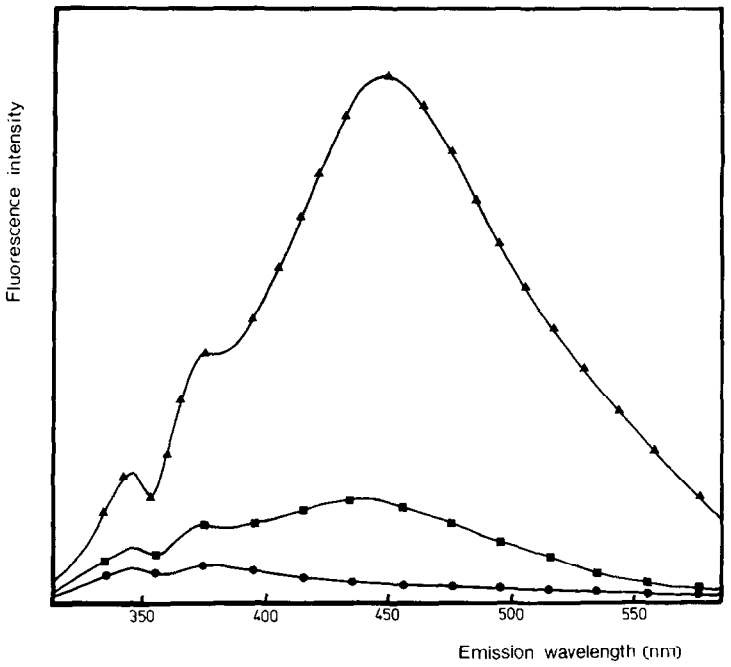

Figure 3. Uncorrected fluorescence emission spectra of yeast unfractionated tRNAs at a concentration of 12.4 A_{260}/ml (▲——▲), of 1.24 A_{260}/ml (■——■) and of yeast mit. unfractionated tRNAs at a concentration of 11 A_{260}/ml (●——●). The excitation wavelength was 315 nm.

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elution profile presented here using the RPC5 system shows a clearer separation. Further, no cyt. $tRNA^{Phe}$ species contaminates the mit. tRNA and vice-versa. This shows that earlier preparations of mit. tRNA were more contaminated with cyt. tRNA. This mit. $tRNA^{Phe}$ peak seems homogenous as it could not be further resolved using other chromatographic steps.

2) Mitochondrial origin of isolated [^{3}H] Phe-tRNA fractions : Results of experiments show that mit. $tRNA^{Phe}$ species can give specific hybrids with mit. DNA. The absence of hybridization of mit. DNA with cyt. $tRNA^{Phe}$ provides informations relative to the specificity of the hybrids. The "petite colonie" ρ^- D21 mutant that was used in this work is known to have retained a mit. $tRNA^{Phe}$ gene (3). The hybridization of mit. Phe-tRNA

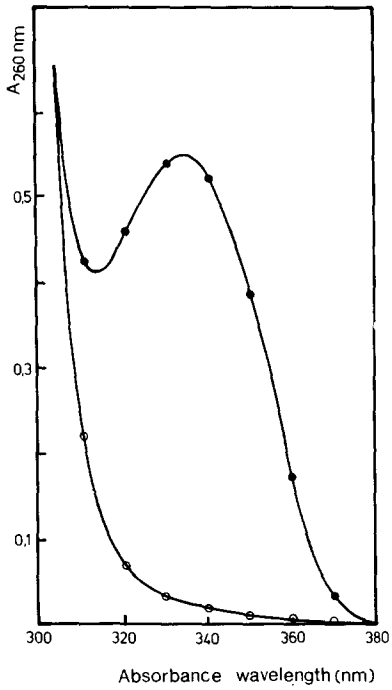

Figure 4. Absorption spectra of E. coli unfractionated tRNAs (●——●) and yeast mit. unfractionated tRNAs (o——o). These spectra were obtained at the following tRNA concentration : E. coli, 84 A_{260}/ml ; yeast mit., 72 A_{260}/ml.

(unfractionated) with this DNA was three times higher than the level obtained with the wild type ρ^+ mit. DNA. The increased levels of hybridization have been explained by the reduced genome complexity of the ρ^- D21 DNA and possibly by intramolecular amplification of the genes. Our experiments with a more purified mit. $tRNA^{Phe}$ species are in good agreement with the results of CASEY et al. (3).

3) Absence of "Y" base and s^4U : Preliminary studies (result not shown) indicated that mit. $tRNA^{Phe}$ had no hydrophobic behaviour when chromatographed on BD-cellulose (17) and lead us to consider the absence of the hypermodified "Y" base. Results presented here confirm this hypothesis. Such an observation had already been reported for Neurospora mit. tRNA and Euglena chloro-

plastic tRNA (18) though the authors did not perform hybridization experiments to know the transcriptional origin of $tRNA^{Phe}$. Experiments with mammalian or plant mit. tRNA would be necessary for the generalization of this observation. FINK et al. (19) reported the occurence of a "Y" base in rat liver mit. $tRNA^{Phe}$ but they did not perform fluorescence measurements. Furthermore, BUCK and NASS (20) showed the presence of cyt. tRNA species in the rat liver mit. tRNA preparations ; this may account for FINK et al's finding (19). As this base is not present in E. coli tRNA it was concluded that mit. tRNA resembles to prokaryotic tRNA (18). However we did not detect the presence of s^4U in yeast mit. tRNA. This base was found in both chicken liver and mouse tumor mit. tRNA (21), but this has been critisized by BORST (22).

The chromatographic behaviour of mit. $tRNA^{Phe}$ and his hybridization properties exclude that cyt. $tRNA^{Phe}$ takes part in mitochondrial protein biosynthesis. The same situation has been found for mit. $tRNA^{Leu}$ and mit. $tRNA^{Tyr}$ (13). The absence of an eukaryotic "Y" base and of the prokaryotic s^4U in mit. tRNA may be the result of symbiosis with the cell sap or may reflect a more primitive prokaryotic origin.

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