CHROMATOGRAPHIC DIFFERENCES BETWEEN THE CYTOPLASMIC AND THE MITOCHONDRIAL t-RNA OF SACCHAROMYCES CEREVISIAE

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SUMMARY Mitochondrial and cytoplasmic t-RNAs have been isolated from Saccharomyces cerevisiae and acylated with an enzyme of the same strain. Mitochondrial arginyl-, lysyl-, and phenylalanyl-t-RNAs are chromatographically distinct from those found in the cytoplasm, whereas mitochondrial isoleucyl t-RNA contains only one species identical with the main cytoplasmic fraction.

The presence of transfer ribonucleic acid (t-RNA) in purified mitochondria of the yeast Saccharomyces cerevisiae has been shown by Wintersberger (1) in 1965. On the other hand Linnane and al. (2, 4) have been able to differentiate in yeast the mitochondrial and the cytoplasmic protein-synthesizing systems by the use of antibiotics. These works suggested that the cytoplasmic and the mitochondrial t-RNAs might contain differences in their structures. Comparisons between the t-RNAs of cytoplasmic and mitochondrial origin by chromatographic methods have been achieved by Brown and Novelli (5) in Neurospora crassa. Studies of the t-RNAs acylated by cytoplasmic or mitochondrial enzymes have been described by Suyama and Eyer (6) in Tetrahymena pyriformis, by Lietman (7) and by Buck and Nass (8, 9) in rat liver. These experiments have been able to show important differences between the two kinds of t-RNA.

In the present report we want to discuss the differences

between mitochondrial and cytoplasmic t-RNAs in Saccharomyces cerevisiae. We labeled the t-RNAs with radioactive amino-acids and compared their chromatographic behaviour using reversed phase chromatography, with the Weiss and Kelmers technique (10).

MATERIAL AND METHODS The yeast cells (wild type, strain Y. F. was a gift of the Laboratoire de Génétique physiologique, Gif-sur-Yvette) were grown on a glucose-limiting medium and the mitochondria were isolated after mechanical disruption of cells (11). Cytoplasmic and mitochondrial t-RNAs were extracted by the phenol method, purified and assayed for their amino-acids acceptor activity, as described elsewhere (12).

Aminoacyl t-RNAs were obtained by the method of Von Ehrenstein and Lipman (13) with minor modifications : A final volume of 2 ml of the reaction mixture contains : TRIS-HCl pH 7.2 200 µM ; ATP 10 µM ; $MgCl_2$ 20 µM ; phosphoenolpyruvate 20 µM ; pyruvate kinase 50 µM ; uniformly labeled L-amino-acids 100 m-µM (C. E. A., Saclay : labeled with ^3H 1 Ci/mM ; with ^{14}C 0.1 Ci/mM) ; cytoplasmic or mitochondrial t-RNA 2 mg ; freshly prepared enzyme from the same yeast strain 2 mg.

The enzyme has been prepared as previously described (12).

After 30 min. incubation at 37° C, the pH of the reaction medium is lowered to 5.0 by addition of 50 µl acetate buffer 1.0 M pH 4.5, the amino-acyl-t-RNAs are purified by shaking with 1.5 volume of water-saturated phenol during 30 min. at 4° C. Following centrifugation, the aqueous phase is collected and the phenolic phase is extracted twice with distilled water for 20 min. The joint aqueous phases are washed twice 2 ml ethylether. Amino-acyl-t-RNAs are precipited by addition of 3 volumes of 95 % (v/v) ethanol during 12 hours at -20° C. The precipitate is centrifuged, dissolved in a minimum of distilled water and dialysed against 3 x 5 l distilled water at 4° C. Cytoplasmic t-RNAs are acylated with ^3H-labeled aminoacids and mitochondrial t-RNAs with ^{14}C- labeled aminoacids.

The cytoplasmic and mitochondrial amino-acyl-t-RNAs are dissolved in 10 ml acetate buffer 0.010 M pH 4.5, $MgCl_2$ 0.005 M, NaCl 0.30 M. Commercial purified yeast t-RNA (Boehringer, Manheim) is added as carrier. The mixture is applied to a reversed phase chromatographic column and fractionated by the method of Weiss and Kelmers (10) at 25° C, using a NaCl linear gradient from 0.30 to 0.80 M in acetate buffer, 0.010 M pH 4.5, $Mg\ Cl_2$ 0.005 M (total elution volume 2 liters). The rate flow is 1 ml/min. ; 10 ml fractions are collected. This system is used for the separation of arginyl-, isoleucyl- and lysyl-t-RNAs.

For the phenylalanyl-t-RNA we have replaced Freon 214 by isoamyl acetate and Aliquat 336 by Aliquat 204, following the procedure of Dirheimer and Kuntzel (14), the other experimental conditions were the same as described above.

The absorbance at 260 nm of each fraction is determined. The fractions are then slowly dessiccated at 70° C in radioactivy counting vials ; 10 ml of toluene containing 4 g p. liter of Omnifluor (NEN Chemicals, Frankfurt-a.-Main) are added to the residue. The radioactivity is measured in a Packard scintillation spectrometer. Presence of salts decreases the counting efficiency by a maximum of 5 % for ^{3}H and by a maximum of 7 % for ^{14}C.

RESULTS The chromatographic behaviour of the t-RNAs acylated with 4 different aminoacids : arginine, isoleucine, lysine, phenylalanine is shown in the figures 1 to 4. The choice of those aminoacids was made after preliminary incorporation experiments of labeled aminoacids (12).

The cochromatography of arginyl-t-RNA (figure 1) shows a sharp difference in behaviour between mitochondrial and cytoplasmic t-RNAs. Mitochondrial arginyl-t-RNA is eluted in two fractions before the two major peaks of cytoplasmic t-RNA. The major mitochondrial fraction is eluted at the same time as a minor fraction of cytoplasmic t-RNA.

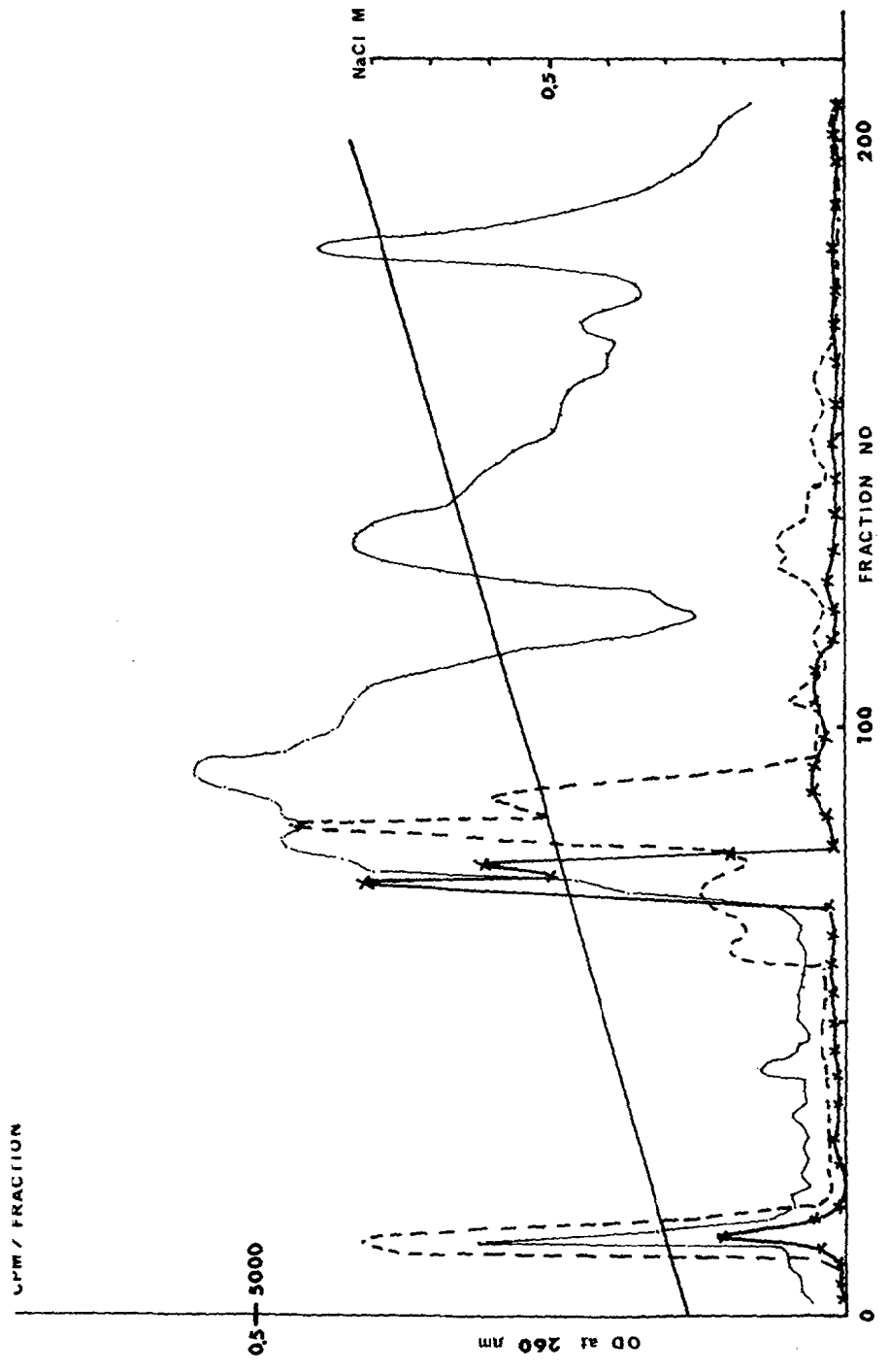

Figure 1 : Reversed phase cochromatography of cytoplasmic and mitochondrial arginyl-t-RNAs from yeast Saccharomyces cerevisiae. Cytoplasmic ^{3}H-arginyl-t-RNA : 25 A_{260} units, 120,000 CPM. Mitochondrial ^{14}C-arginyl-t-RNA : 100 A_{260} units, 60,000 CPM. Commercial yeast t-RNA as carrier 475 A_{260} units. ^{3}H-CPM per fraction (.---.--.) ; ^{14}C-CPM per fraction (x————x) ; absorbance at 260 nm (————).

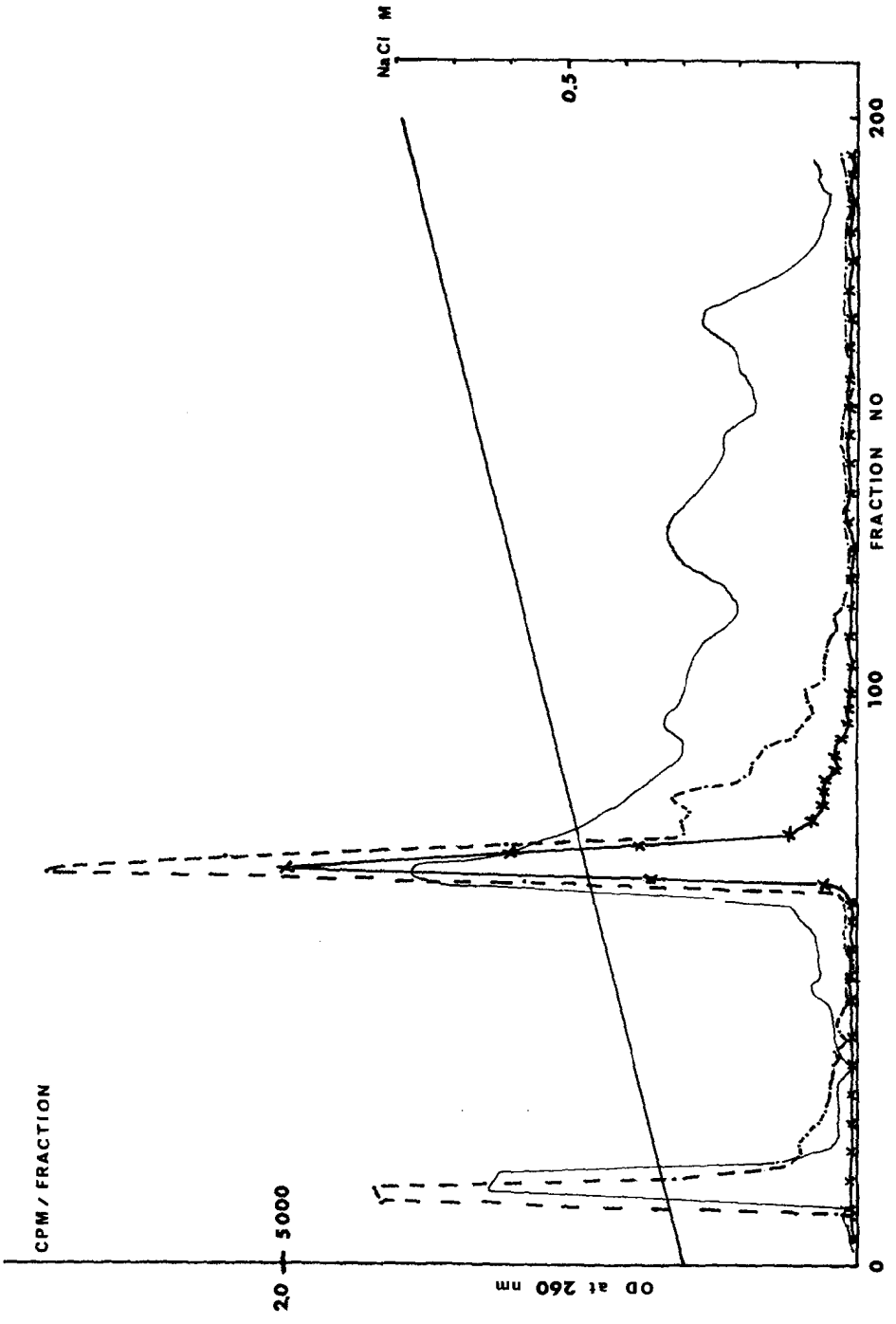

Figure 2 : Reversed phase cochromatography of cytoplasmic and mitochondrial isoleucyl-t-RNAs from Saccharomyces cerevisiae. Cytoplasmic ^{3}H-isoleucyl-t-RNA : 60 A_{260} units, 129,000 CPM, Mitochondrial ^{14}C-isoleucyl-t-RNA : 110 A_{260} units, 55,000 CPM. Commercial yeast t-RNA as carrier 650 A_{260} units. ^{3}H-CPM per fraction (.--.--.), ^{14}C-CPM per fraction (x————x) ; absorbance at 260 nm (————).

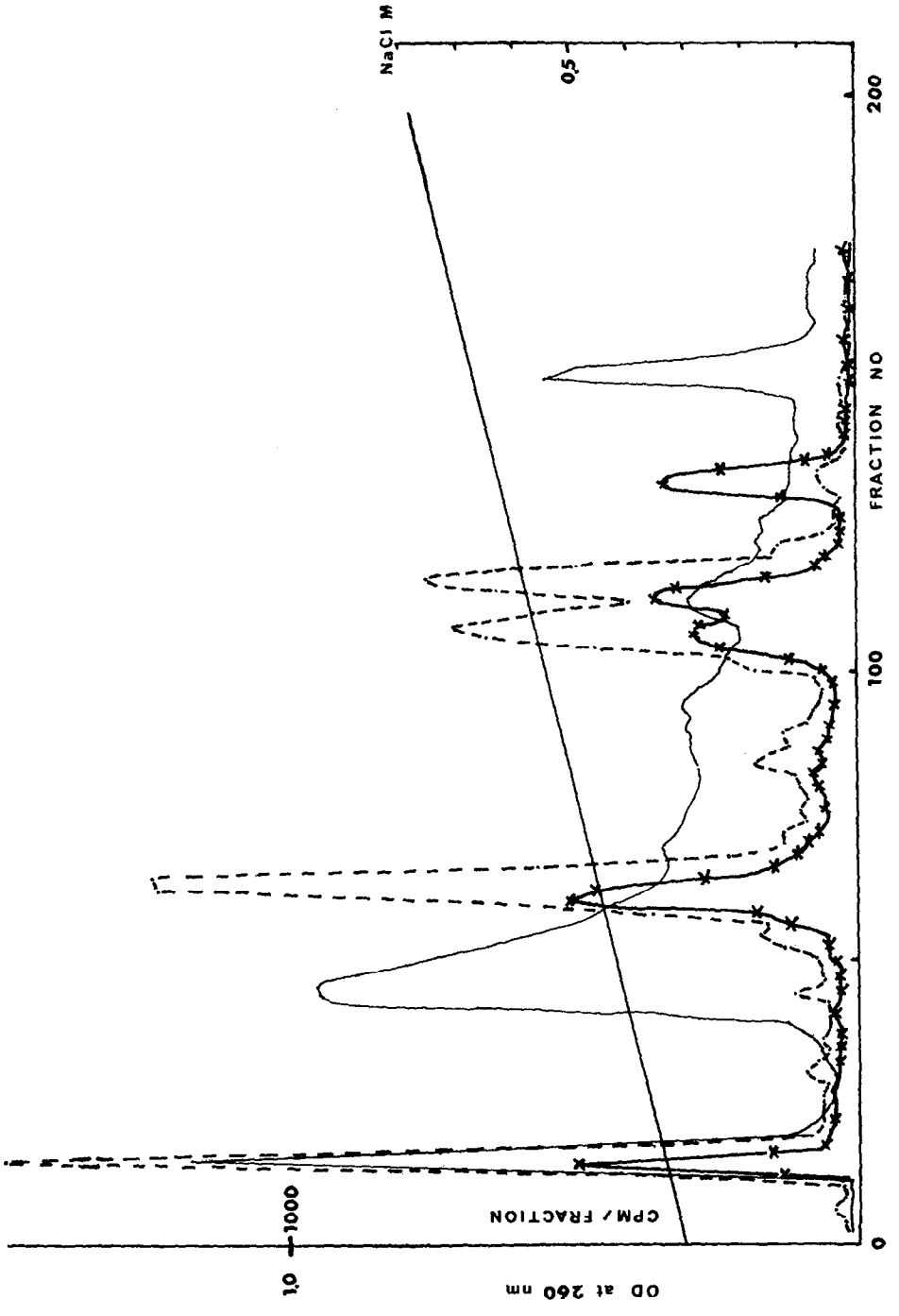

Figure 3 : Reversed phase cochromatography of cytoplasmic and mitochondrial lysyl-t-RNAs from Saccharomyces cerevisiae. Cytoplasmic ^{3}H-lysyl-t-RNA : 45 A_{260} units, 90,000 CPM. Mitochondrial ^{14}C-lysyl-t-RNA : 74 A_{260} units ; 40,000 CPM. Commercial yeast t-RNA as carrier 250 A_{260} units. ^{3}H-CPM per fraction (.--.--.) ; ^{14}C-CPM per fraction (x————x) ; absorbance at 260 nm (————).

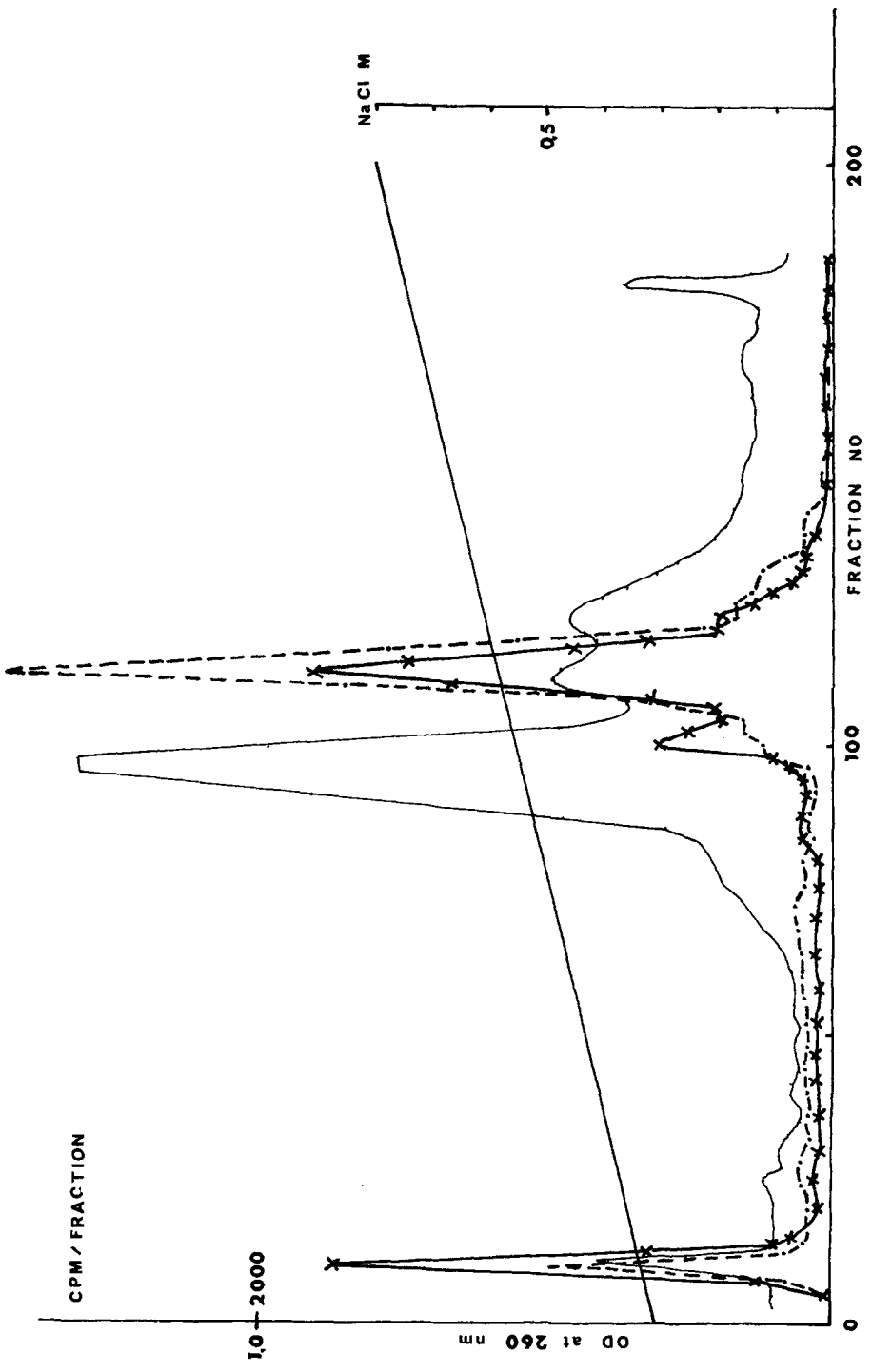

Figure 4 : Reversed phase cochromatography of cytoplasmic and mitochondrial phenylalanyl-t-RNAs from Saccharomyces cerevisiae. Cytoplasmic ^{3}H-phenylalanyl-t-RNA : 27 A_{260} units, 80,000 CPM. Mitochondrial ^{14}C-phenylalanyl-t-RNA : 46 A_{260} units, 47,000 CPM. Commercial yeast t-RNA as carrier 300 A_{260} units. ^{3}H-CPM per fraction (.-.-.-.) ; ^{14}C-CPM per fraction (x———x) ; absorbance at 260 nm (———).

The behaviour of the isoleucyl-t-RNAs can be seen in figure 2. Mitochondrial t-RNA is eluted in only one peak along with the major cytoplasmic t-RNA fraction. The later eluted cytoplasmic fraction is absent in the mitochondrial t-RNA.

There are five distinct fractions of cytoplasmic lysyl-t-RNA and four fractions of mitochondrial lysyl-t-RNA (figure 3). The first two mitochondrial t-RNA peaks seem to fit with fractions also present in the cytoplasmic t-RNA ; the second cytoplasmic fraction does not exist in mitochondrial t-RNA ; the third mitochondrial peak is eluted slightly before the fourth cytoplasmic peak and the last mitochondrial fraction is much more larger than the corresponding cytoplasmic fraction. The last two mitochondrial t-RNA fractions behave like specific mitochondrial species.

In preliminary chromatographic experiences of the phenylalanyl-t-RNA with the Weiss and Kelmers method, the mitochondrial t-RNA was not eluted even when very high NaCl molarity was used. For this reason we used an isoamyl acetate column to achieve the separation (figure 4). Cytoplasmic t-RNA shows only one important fraction, whereas the mitochondrial t-RNA is eluted in two fractions : the larger of these fits with the cytoplasmic peak and is preceded by a small fraction which is specific for mitochondria.

DISCUSSION The comparison of the four chromatographic profils shows the presence of mitochondrial t-RNA fractions which are eluted at the same time as the corresponding cytoplasmic t-RNA fractions. This cytoplasmic fraction is sometimes a minor component (arginyl- and lysyl-t-RNAs). There is often a correspondance between the cytoplasmic and the mitochondrial t-RNA peaks (isoleucyl-, lysyl-, phenylalanyl-t-RNAs). But for three aminoacyl-t-RNAs we were able to show specific mitochondrial fractions (arginyl-, lysyl-, phenylalanyl-t-RNAs). The mitochondrial isoleucyl-t-RNA is eluted in only one fraction (figure 2) which coincides with the main cytoplasmic t-RNA peak.

In preliminary experiments (12) we wanted to obtain the maximal amino-acid incorporation into the total t-RNA ; but each t-RNA species has probably different optimal conditions for maximal amino-acid charging. Our present experiments do not therefore lead to quantitative results. Their principal aim was to differentiate qualitatively the mitochondrial and the cytoplasmic amino-acyl-t-RNA species from Saccharomyces cerevisiae.

It is noteworthy to remember that we used a total yeast amino-acyl-t-RNA synthetase for amino-acid incorporation into mitochondrial t-RNAs. A purified mitochondrial enzyme might probably lead to slightly different results, but our efforts to prepare this enzyme fraction have been unsuccessful until now.

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