FRACTIONATION OF YEAST MITOCHONDRIAL $tRNA^{Tyr}$ AND $tRNA^{Leu}$

Jean-Marie SCHNELLER, Bernard ACCOCEBERRY and André J. C. STAHL

Laboratoire de Biochimie, U.E.R. des Sciences Pharmaceutiques, Université Louis Pasteur, 3, rue de l'Argonne, 67083 Strasbourg Cedex, France

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1. Introduction

We earlier reported the existence of mit isoacceptor tRNA in *Saccharomyces cerevisiae* with a different chromatographic behaviour from that of the corresponding cytoplasmic isoacceptors for: Arg–, Lys–, Phe–, Val– tRNA [1,2]. Recently mit $tRNA^{His}$ has been separated from the cytoplasmic isoacceptor [3]. However Tyr– and Leu– tRNA which were not fractionated till now are of special interest. It is known that $tRNA^{tyr}$ plays an important role as suppressor tRNA in procaryotes [4] and in yeast [5]. Several isoacceptors of $tRNA^{Leu}$ are present in yeast [6] and leucine is one of the major components of the mitochondrially synthesized peptides [7]. Unfractionated yeast Tyr– and Leu– tRNA could be hybridized to mit DNA [8,9].

We tried to separate the mit Leu– and Tyr– tRNA isoacceptors. Among the different peaks found by two types of reversed phase chromatography yeast mit $tRNA^{Tyr}$ and $tRNA^{Leu}$ showed each, one peak not found in cyt tRNA. This peak in the case of $tRNA^{Leu}$ could be more specifically acylated by the mit aminoacyl–tRNA synthetase.

2. Materials and methods

Saccharomyces cerevisiae haploid strain IL 8-8 $C\rho^+$, and the derived strain H 71 ρ°, a gift from Dr H. Fukuhara of the Centre de Génétique Moléculaire du C.N.R.S. (Gif-sur Yvette) were used. ρ° is a mit DNA-less mutant.

Cellular culture, isolation and purification of the mitochondria on sucrose gradient, tRNA and aminoacyl–tRNA synthetase extraction from the cytoplasm and the mitochondria have been described earlier [2,10]. The method for the preparation of the mit aminoacyl–tRNA synthetases was improved as follows: β-mercaptoethanol at a final concentration of 10 mM is added only at the end of the mitochondria lysis. Several mitochondrial enzyme preparations, stored as the $(NH_4)_2\ SO_4$ precipitate at −20°C, are dissolved together, chromatographed on a Sephadex G-25 column and only the active fractions passed onto the DEAE cellulose column. In this way a more concentrated enzymatic extract (10 mg/ml) is obtained with a ratio D.O. 280/D.O. 260 = 1.7, and a measurable catalytic activity at pH 7.5. Cyt tRNA, completely free from mit tRNA, is extracted from the ρ° strain.

Assay conditions of the acceptor activity of the tRNA for the aminoacids and preparation of aminoacyl–tRNA for chromatography have been reported [2]. Reversed phase chromatography RPC 5 [11] was done at room temperature on columns 200 × 1 cm, with a 2 l linear gradient elution from 0.35 M to 0.85 M NaCl in the buffer: $MgCl_2$ 10 mM, acetic acid–Na acetate 10 mM, pH 4.5. 10 ml fractions were recovered and counted for radioactivity as described [12]. Some chromatography was done on RPC 5 in the presence of 6 M urea at pH 3.0 [13]: columns of 150 cm × 0.5 cm are eluted with 500 ml of a 6 M urea, 0.25 M to 0.35 M NaCl gradient. 2.5 to 3 ml fractions are recovered, precipitated at −20°C in the presence of carrier tRNA by 2.5 volumes of

Abbreviations: mit tRNA = mitochondrial tRNA; cyt tRNA = cytoplasmic tRNA.

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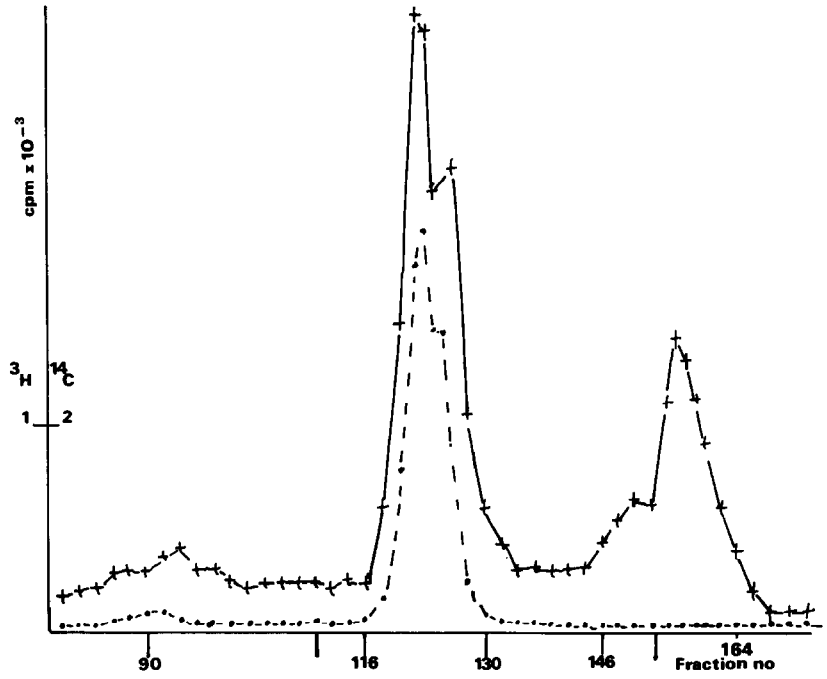

Fig.1. Reversed phase cochromatography of mit [^{3}H]Tyr–tRNA (+——+) and cyt [^{14}C]Tyr–tRNA (•- - -•).

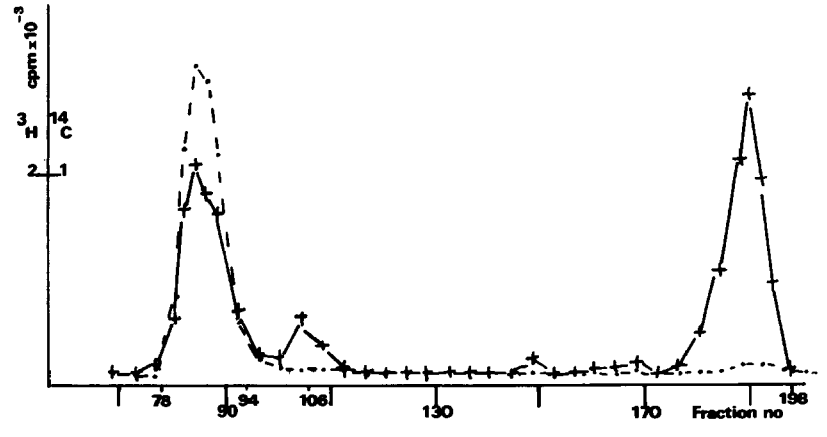

Fig.2. Reversed phase cochromatography in the presence of 6 M urea of mit [^{3}H]Tyr–tRNA (+——+) and cyt [^{14}C]Tyr–tRNA (•- - -•).

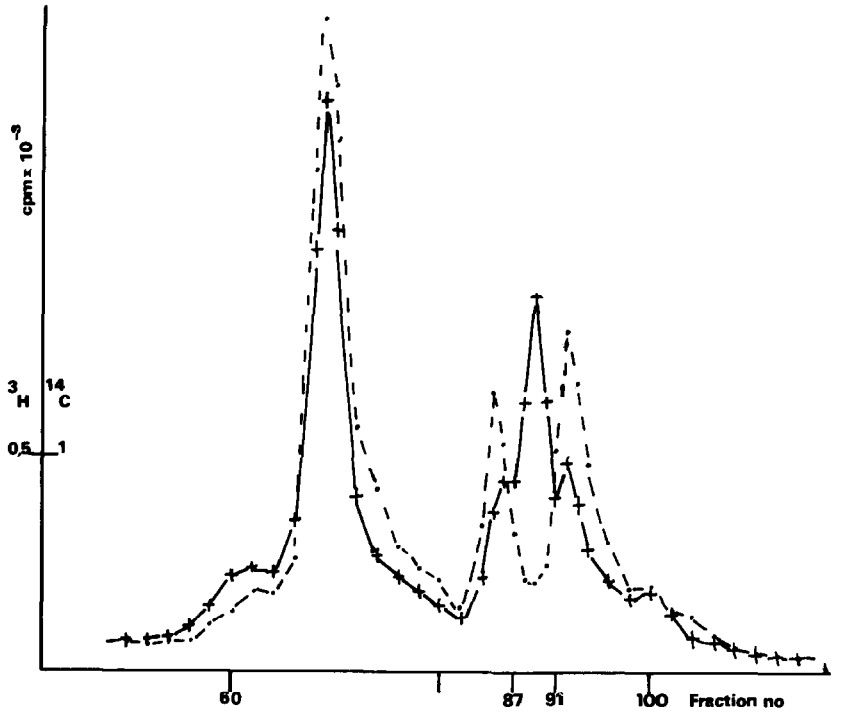

Fig.3. Reversed phase cochromatography of mit [^{3}H]Leu–tRNA (+——+) and cyt [^{14}C]Leu–tRNA (•- - -•).

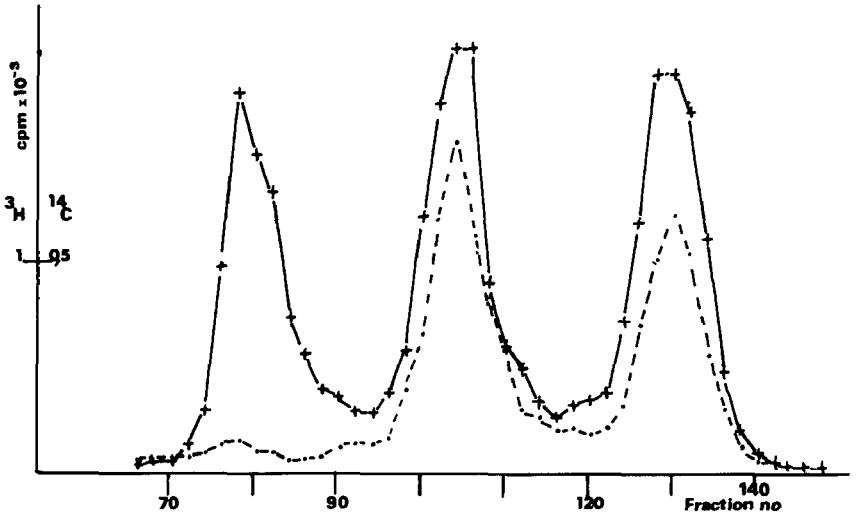

Fig.4. Reversed phase cochromatography in the presence of 6 M urea of mit [^{3}H]Leu–tRNA (+——+) and cyt [^{14}C]Leu–tRNA (•- - -•).

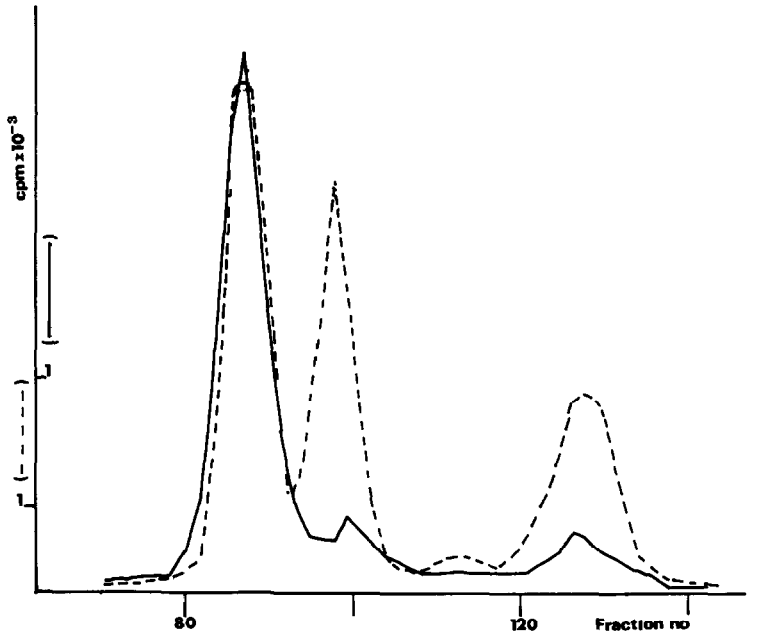

Fig.5. Reversed phase chromatography in the presence of 6 M urea of mit tRNA loaded with [^{3}H]Leu in the presence of cytoplasmic enzyme at pH 7.5 (- - -) or of mitochondrial enzyme at pH 8.7 (——). The two chromatographies were done on the same column.

95% ethanol, centrifuged, transferred with minimum water into the radioactivity counting vials and counted as in [12].

3. Results and discussion

3.1. *Tyrosyl–tRNA*

The cochromatography in the normal RPC 5 system of cyt [^{14}C]tyrosyl–tRNA (ρ° strain) and mit [^{3}H]tyrosyl–tRNA loaded with cytoplasmic enzyme is shown in fig.1. The mit tRNA shows one peak in common with the cyt tRNA (fractions 116–130). A supplementary broad peak (fractions 146–164) is present only in the mit tRNA. The two peaks show the beginning of a doubling.

The cochromatography in the presence of urea of cyt [^{14}C]tyrosyl–tRNA loaded with the cytoplasmic enzyme and the mit [^{3}H]tyrosyl–tRNA loaded with the mitochondrial enzyme can be seen in fig.2. The presence of two main mitochondrial fractions is again found (fractions 78–94 and 170–198) one of which chromatographes with the cyt tRNA species (fractions 78–94). A third small mit peak is sometimes seen (fraction 106). The presence of urea improves to great deal the separation of the main peaks, which are now symetric.

3.2. *Leucyl–tRNA*

The cochromatography of cyt [^{14}C]leucyl–tRNA (ρ° strain) and mit [^{3}H]leucyl–tRNA loaded with the homologous enzymes at pH 7.5 is shown in fig.3. There are at least three main fractions in the cyt tRNA (fractions 60–80, 80–87, 91–100) and a further peak appears in the mit tRNA (fractions 87–91) between the second and the third major peaks of cyt tRNA.

Figure 4 shows the RPC 5 cochromatography in the presence of urea of cyt [^{14}C]leucyl–tRNA from the ρ° strain and the mit [^{3}H]leucyl–tRNA loaded with the cytoplasmic enzyme. The specific mitochondrial peak (fractions 70–90) is well separated from the main cytoplasmic isoacceptors (fraction 90–120, 120–140).

We checked for the appearance of an extra peak in cyt tRNA, charged after renaturation using the conditions described by Lindahl et al. [14]. The

chromatographic profile in the normal RPC 5 system, of renatured cyt tRNA was essentially superimposable on the one shown in fig.3.

In an other study which will be described elsewhere we were able to show that the extent of aminoacylation for mit $tRNA^{Leu}$ with mitochondrial synthetase is optimal at pH 8.7. Under these conditions the cyt $tRNA^{Leu}$ is poorly charged with the mitochondrial enzyme, but it is still well charged with the cytoplasmic enzyme. Figure 5 shows the superposition of the chromatographic profiles: obtained when the mit $tRNA^{Leu}$ was charged in presence of the mitochondrial enzyme at pH 8.7 or in the presence of the cytoplasmic enzyme at pH 7.5. This figure shows that the mitochondrial enzyme charges specifically the $tRNA^{Leu}$ isoacceptor which is only found in the mitochondria.

In this study we show that the tRNA preparations, even when the mitochondria have been well purified on a surcrose gradient, contain always peaks which chromatograph like the cytoplasmic isoacceptors. A contamination by cyt tRNA during the isolation of the mitochondria can therefore not be excluded entirely. Besides of those peaks we found Leu– and Tyr– tRNA isoacceptors which are only present in the mitochondria; these species could be best separated from the others when the reversed phase chromatography was carried out in the presence of urea. At the moment, we cannot state if the mitochondrial isoacceptors are unique. For leucine this mitochondrial isoacceptor was more specifically recognized by the mitochondrial aminoacyl–tRNA-synthetase. tRNA–DNA hybridization studies are in progress in our laboratory to know the transcriptional origin of the different yeast mit tRNA isoacceptors as was done for mit $tRNA^{Leu}$ isoacceptors in *Tetrahymena* [15].

Acknowledgements

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