Leucyl tRNA and Leucyl tRNA Synthetase in Mitochondria of Tetrahymena pyriformis

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Recently, Barnett, et al. (1967a, 1967b) reported that Neurospora mitochondria contain 18 different tRNA's and also mitochondria specific aminoacyl tRNA synthetases. We have isolated mitochondrial and whole-cell tRNA, and their corresponding leucyl-tRNA synthetases, from the ST strain of Tetrahymena pyriformis. By a partial characterization, we find that mitochondrial leucyl-tRNA and its synthetase both differ from the extra-mitochondrial leucyl-tRNA and synthetase.

Materials and Methods

An axenic culture of a strain of Tetrahymena pyriformis previously designated ST (Suyama, 1966) was used throughout these studies. Mitochondria were prepared as previously described (Suyama, 1966). The raffinose medium (0.2 M raffinose, 1 mM potassium phosphate pH 6.2, 0.25% bovine serum albumin) used in these studies was made with sterile double-distilled water, and used either fresh or after storage at -20° C.

These mitochondrial preparations contain little or no contaminating material, as judged by plating out and by phase-contrast microscopy. Also, mitochondrial DNA isolated from this mitochondrial fraction is not contaminated with nuclear DNA.

RNA from the whole-cell homogenate was isolated by the phenol method of von Ehrenstein and Lipmann (1961). Since this RNA contained rRNA and DNA, a RNA fraction eluted at about 0.5 N NaCl from a methylated albumin coated Kieselguhr (MAK) column (Sueoka and Yamane, 1962) was collected. This tRNA fraction was then dialysed against double-distilled water and lyophilized to a powder, which was stored at -20° C. until used. This RNA is estimated to contain less than 1% of mitochondrial tRNA.

The tRNA from the mitochondrial fraction was prepared as follows. The washed mitochondria were suspended and lysed in 0.01 M acetate buffer pH 5.0

and 2% sodium dodecyl sulfate. The lysate was subjected to the same phenol treatment as the whole-cell homogenate, and the RNA thus obtained fractioned by MAK chromatography, yielding a tRNA fraction.

For preparation of the corresponding amino acyl tRNA synthetases, the following methods were used: whole-cell homogenate was centrifuged for 6 min at 500 xg, and the supernatant further centrifuged for 6 min at 6,000 xg to remove mitochondria. This supernatant was then centrifuged in a Spinco model L centrifuge for 120 min at 105,000 xg, and the supernatant thus prepared dialysed extensively against Tris-magnesium buffer (0.01 M Tris-HCl pH 7.4, 0.01 M $MgCl_2$, 1 ml/liter 2-mercaptoethanol). The dialysate was designated "post mitochondrial" enzyme and stored at -70° C.

The mitochondrial synthetase fraction was prepared by resuspending a twice-washed mitochondrial pellet in Tris-magnesium buffer (about 5 ml buffer per 100 mg mitochondrial protein), which was then homogenized by grinding with alumina powder in a Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 xg for 10 min and the supernatant used as the 'mitochondrial' enzyme after storage at -70° C.

The two tRNA's were charged with either C^{14}-L-leucine (New England Nuclear Corp., sp. act. 155 mc/mmole) or H^3-L-leucine (NEN, 170 mc/mmole) in the following 1 ml reaction mixture: 50 μmoles Tris-HCl, pH 7.5, 10 μmoles $MgCl_2$, 10 μmoles KCl, 1 μmole ATP, 0.006 μmole C^{14}-L-leucine or 0.024 μmole H^3-L-leucine, 0.10 to 0.13 ml of enzyme extract and varying amounts of tRNA. After various times of incubation at 37° C., 0.1 ml aliquot was taken, and precipitable material collected on a membrane filter and the radioactivity determined. In any case, the maximum activity was reached within 5 min incubation. For isolating charged tRNA, after incubation at 37° C., 5 ml of cold saline-phosphate buffer (0.3 N NaCl, sodium phosphate buffer pH 6.7) and an equal volume of water-saturated phenol were added. This mixture was shaken in the cold for 20 min and then centrifuged. The resulting supernatant was extracted with ether to remove phenol, and then fractionated by MAK chromatography. Differential counting of C^{14} and H^3 was done in a Packard Tricarb liquid scintillation counter.

Results and Discussion

When mitochondrial and whole-cell tRNA's are charged by mitochondrial and post-mitochondrial enzyme fractions respectively, MAK chromatography patterns such as Fig. 1A and Fig. 1B result. We find repeatedly that while whole-cell tRNA shows two equally-charged regions for C^{14}-leucine,

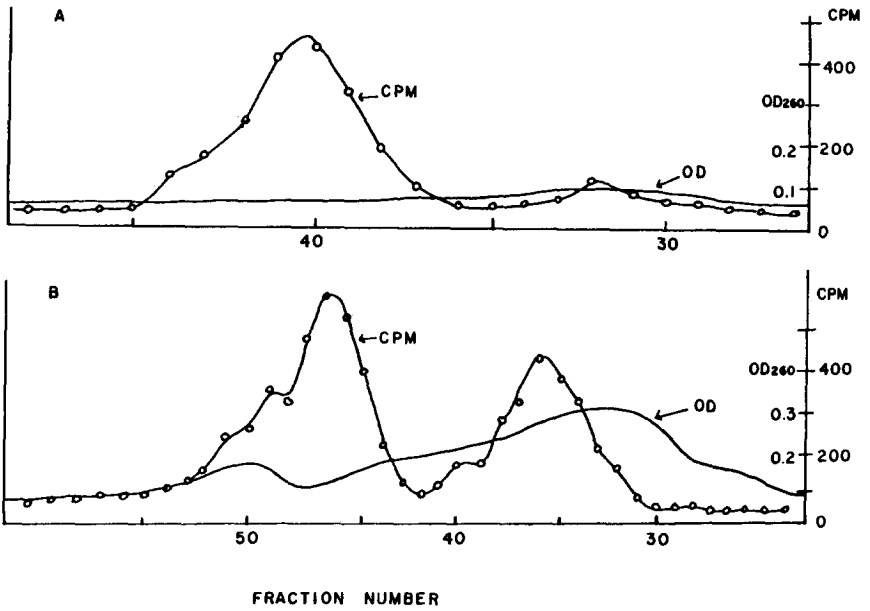

Figure 1 MAK column chromatography of mitochondrial (A) and whole-cell (B) leucyl-tRNA's, charged with mitochondrial and post-mitochondrial enzymes, respectively. A: 2.5 ml of the standard reaction mixture (see methods) containing 200 μg of mitochondrial tRNA and 0.50 ml of mitochondrial extract were incubated for 20 min at 37°. RNA was re-purified as described, applied to a MAK column, and eluted with a linear salt gradient made with 130 ml each of 0.3 N NaCl and 0.9 N NaCl, sodium phosphate buffer (0.05 M, pH 6.5). The continuous OD_{260} profile was obtained by monitoring the effluent through a flow cell (path length 1/volume = 10 mm/0.50 ml) in a Beckman DB spectrophotometer with an attached recorder. C^{14}-leucyl tRNA was precipitated by trichloracetic acid (10%) in the cold from 3 or 4 ml fractions and collected on membrane filters by suction. The filter papers were dried and radioactivity was counted in a liquid scintillation spectrometer. B: 1.0 ml of the reactive mixture containing 380 μg of whole-cell tRNA and 0.26 ml of post-mitochondrial enzyme. All operations were the same as (A), except an additional 400 μg of the tRNA was added as OD_{260} marker before MAK column fractionation.

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mitochondrial tRNA is charged with C^{14}-leucine largely in only one of these regions.

Two explanations might account for these observations. (1) If tRNA's isolated from both mitochondrial and whole-cell lysates are the same, then the post-mitochondrial enzyme extract contains two independent leucyl-tRNA synthetases, each capable of charging a different region of amino-acyl tRNA, while the mitochondrial enzyme extract contains only one. (2) If both extracts contain identical synthetases, then whole-cell tRNA contains equal amounts of at least two kinds of leucyl-tRNA, while mitochondrial tRNA lacks one. To clarify these points, we performed the following cross-

charging experiments. Mitochondrial tRNA was charged with post-mitochondrial enzyme; and, independently, whole-cell tRNA was charged with mitochondrial enzyme. Fig. 2A and Fig. 2B show the results from independent MAK chromatography of the resulting C^{14}-leucyl tRNA's. Neither whole-cell tRNA nor mitochondrial tRNA showed equal C^{14}-leucine tRNA peaks; both profiles are largely defective in the second C^{14}-leucyl tRNA peak. These results suggest that (1) mitochondrial synthetase differs from post-mitochondrial synthetase, being able to charge one peak mainly; and (2) mitochondrial tRNA differs from whole-cell tRNA by lacking one region of leucyl-tRNA.

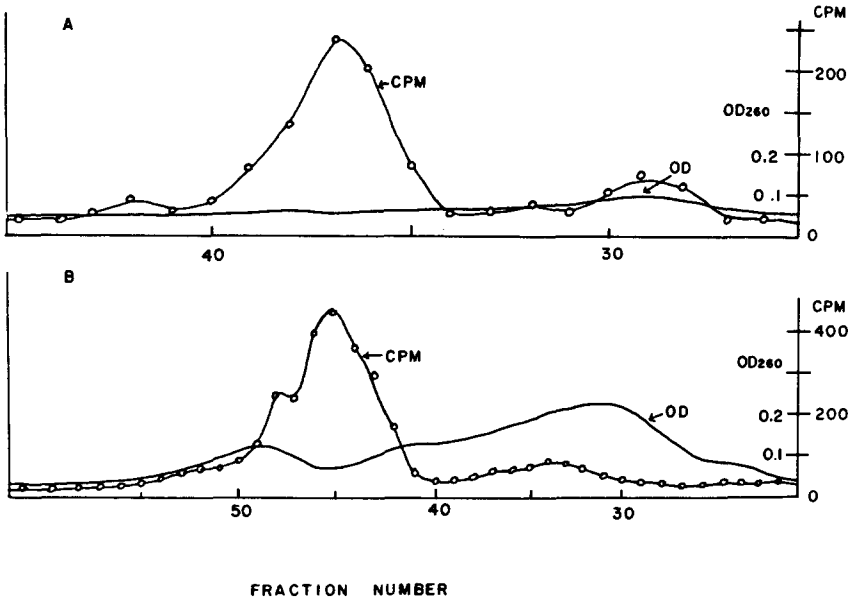

Figure 2 MAK column chromatography of mitochondrial (A) and whole-cell (B) leucyl tRNA's, cross-charged with post-mitochondrial and mitochondrial enzymes respectively. A: 2.5 ml of the reaction (see methods) mixture contained 200 μg of mitochondrial tRNA and 0.50 ml of post-mitochondrial enzyme. All other operations and conditions were the same as in Figure 1A. B: 1 ml reaction mixture containing 380 μg of whole-cell tRNA and 0.26 ml of mitochondrial enzyme was used. All other conditions and operations were the same as in Figure 1B.

At this point we attempted to distinguish further the leucyl-tRNA peak of mitochondrial tRNA from the corresponding leucyl-tRNA of the whole cell. Mitochondrial tRNA, charged with C^{14}-leucine by mitochondrial enzyme, and whole-cell tRNA, charged with H^3-leucine by post-mitochondrial enzyme, were co-chromatographed after isolation. The results of this experiment (Fig. 3) indicate a significant difference between the peaks of H^3 and C^{14}, suggesting a corresponding difference in mitochondrial and whole-cell tRNA's.

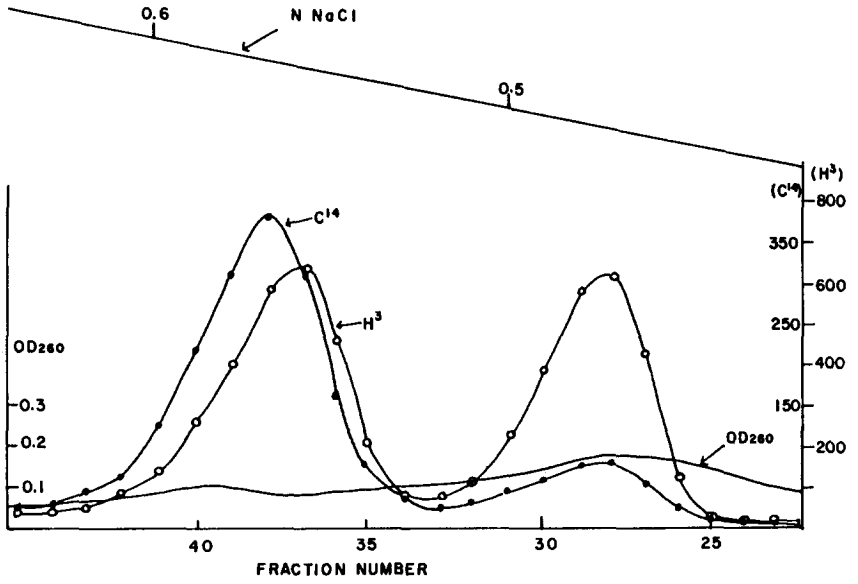

Figure 3 Co-chromatography of mitochondrial C^{14}-leucyl-tRNA and whole-cell H^3-leucyl-tRNA charged with mitochondrial and post-mitochondrial enzymes respectively. Two reaction tubes, each containing 2.5 ml of the reaction but one with 200 μg mitochondrial tRNA, C^{14}-leucine, and 0.50 ml of mitochondrial enzyme and the other with 380 μg whole-cell tRNA, H^3-leucine and 0.50 ml of post-mitochondrial enzyme, were separately incubated at 37° for 15 min. After re-purification of tRNA's, both tRNA's were mixed and co-chromatographed through a MAK column. Input count ratio of H^3 : C^{14} was 2:1.

In these analyses, the radioactivity profile of mitochondrial C^{14}-leucyl tRNA shows a minor peak coinciding with the first-eluted peak of whole-cell H^3-leucyl tRNA. This indicates that our mitochondrial tRNA preparations contain this material in much reduced amount.

Although the available evidence suggests that mechanisms of protein synthesis in mitochondria are analogous to those of bacterial systems, it is premature at present to make any conclusion as to the funtional role of the mitochondrial tRNA's, particularly in light of the soluble amino acid incorporating systems existing in E. coli (Kaji, et al. 1965) as well as in mitochondria (Kalf, 1964). Furthermore, the origin of mitochondrial tRNA is an open question. The results of DNA-RNA hybridization studies (Suyama, 1967) made with the tRNA fraction of Tetrahymena are more compatible with the idea that these tRNA's are transported into mitochondria than made within the mitochondrion.

Acknowledgement

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Literature cited

Barnett. W. E., and D. H. Brown. Proc. Natl. Acad. Sci. U. S. 57:452 (1967a).

Barnett, W. E., D. H. Brown, and J. L. Epler. Proc. Natl. Sci. U. S. 57:1775 (1967b).

Kaji, A., H. Kaji, and G. D. Novelli. J. Biol. Chem. 240:1185 (1965).

Kalf, G. F. Biochemistry. 3:1702 (1964).

Sueoka, N., and T. Yamane. Proc. Natl. Acad. Sci. U. S. 48:1454 (1962).

Suyama, Y. Biochemistry. 5:2214 (1966).

Suyama, Y. Biochemistry. 1967 (In Press).

von Ehrenstein, G., and F. Lipmann. Proc. Natl. Acad. Sci. U. S. 47:941 (1961).

Slater, (ed.), Elsevier Publ. Co., New York (1966).