CHROMATOGRAPHIC DIFFERENCES BETWEEN THE CYTOPLASMIC AND MITOCHONDRIAL tRNAs OF NEUROSPORA CRASSA[1]

David H. Brown[2] and G. David Novelli

Biology Division, Oak Ridge National Laboratory,[3]
Oak Ridge, Tennessee

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It has been demonstrated that the mitochondria of Neurospora crassa contain aspartic acid, phenylalanine, and leucine tRNAs and their respective aminoacyl-RNA synthetases that are distinct from those found in the cytoplasm (Barnett et al., 1967). In this report we show that the mitochondrial seryl-, leucyl-, and methionyl-tRNAs are chromatographically distinct from those found in the cytoplasm.

METHODS AND MATERIALS

Neurospora crassa, wild-type strain OR23-1a, was grown and harvested as described (Barnett and Brown, 1967).

Mitochondria were isolated (Hall and Greenawalt, 1964) and separated from large-scale preparations (Barnett and Brown, 1967) by sucrose-gradient zonal centrifugation in a B-IV rotor (Anderson et al., 1966). Enzyme activity was measured as ^{14}C-amino acid incorporated into tRNA by the aminoacylation reaction. The reaction mixture and the filter paper assay procedure (Bollum, 1959) were previously

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[1] Abbreviations used in this paper: tRNA, transfer RNA; RPC, reversed phase chromatography.

[2] National Institutes of Health Fellow.

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described (Barnett et al., 1967). The tRNAs were aminoacylated in 2.5 to 5.0 ml of the reaction mixture (Barnett and Brown, 1967).

To distinguish between cytoplasmic and mitochondrial tRNAs, separate reactions were carried out by using ^{3}H-amino acids for the acylation of cytoplasmic tRNA and ^{14}C-amino acids for the mitochondrial tRNA. The aminoacylation reaction was stopped by adding an equal volume of phenol after making the reaction mixture 0.2 M with potassium acetate (pH 5.0). After being shaken for 15 minutes in the cold, the samples were centrifuged, the aqueous fractions were combined, and the tRNA was precipitated by the addition of 2.5 volumes of cold 100% ethanol. The samples were held at -20°C for at least 3 hours; and the precipitated tRNA was collected by centrifugation, washed once with cold 70% ethanol, and dissolved in a small volume of 0.001 M NaCl, 0.01 M $MgCl_2$, and 0.001 M EDTA (pH 4.5). The aminoacylated tRNAs were combined, mixed with approximately 100 A_{260} units of carrier tRNA and co-chromatographed by using the reversed phase chromatographic method of Kelmers et al. (1965). Jacketed glass columns (240 x 0.9 cm) were packed with a hydrophobic diatomaceous earth (Chromosore W 120 mesh) on which dimethyldilauryl-ammonium chloride in isoamylacetate was immobilized. The columns were equilibrated with 0.01 M sodium acetate buffer (pH 4.5), containing 0.01 M $MgCl_2$, 0.05 M EDTA (disodium), and 0.4 M NaCl. The tRNAs were eluted with a 2-liter, linear NaCl gradient at 25°C at a flow rate of 2.0 ml/min. The gradients are indicated in the figures. Ten-milliliter fractions were collected in the cold. After approximately 2 A_{260} units of carrier RNA were added to each tube, the tRNA was precipitated by either adding 2.5 volumes of 100% ethanol and allowing the preparation to stand overnight at -20°C, or by adding 2 ml of 50% cold tricloroacetic acid.

The RNA was collected on Millipore filters (type HA), washed with cold 70% ethanol, and dried under a heat lamp. Radioactivity was determined by using either a Packard or Nuclear-Chicago liquid scintillation counter.

Uniformly labeled ^{14}C-L-amino acids, L-leucine 4,5-^{3}H, L-methionine-methyl-^{3}H, and DL-serine 3-^{3}H were obtained from New England Nuclear Corp.

RESULTS AND DISCUSSION

Neurospora mitochondria contain a full complement of

aminoacyl-RNA synthetases and at least three of these -- aspartyl-, phenylalanyl-, and leucyl-RNA synthetases and their respective tRNAs -- are exclusively associated with the mitochondria (Barnett et al., 1967). In this report we demonstrate that the mitochondrial leucyl-, seryl-, and methionyl-tRNAs are chromatographically distinct from their cytoplasmic counterparts.

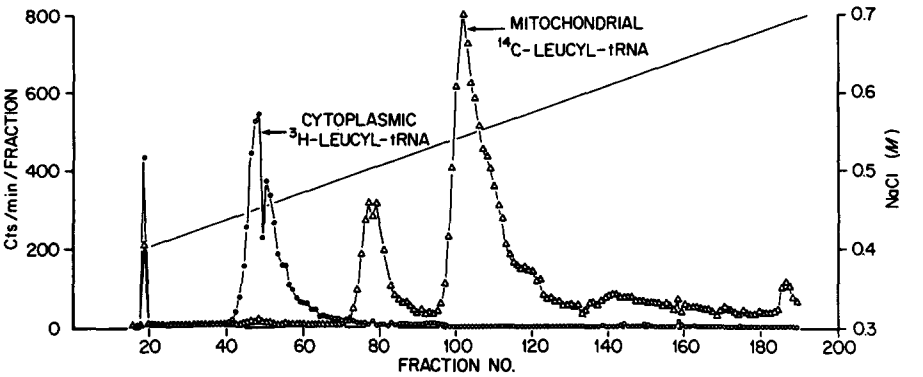

Fig. 1. Co-chromatography (RPC) of cytoplasmic ^{3}H-leucyl-tRNA with mitochondrial ^{14}C-leucyl-tRNA from N. crassa. ^{3}H, 36,000 cpm, 24 A_{260} units; ^{14}C, 102,000 cpm, 25 A_{260} units; plus approximately 100 A_{260} units of whole-cell Neurospora tRNA as carrier.

The data in Fig. 1 indicate that there are at least three different species of leucyl-tRNAs, two of which are mitochondrial in origin. Epler and Barnett (1967) have found that whole-cell Neurospora tRNA fractionated by countercurrent distribution resolves the $tRNA^{Leu}$ into at least four distinct species. As many as five species of $tRNA^{Leu}$ exist in E. coli (Kelmers et al., 1965; Apgar and Holley, 1964). The fact that only one of the cytoplasmic leucyl-tRNAs resolved could be a function of the pH at which the columns were eluted. Waters and Novelli (1967) have noted that when E. coli tRNA is chromatographed at pH 4.5 in this system, resolution is reduced to three species of leucyl-tRNA.

The difference between the cytoplasmic and mitochondrial seryl-tRNAs is clearly shown in Fig. 2. There are four distinct species, two of which appear to be mitochondrial in origin. It is of interest in this regard that three seryl-tRNAs have been reported for E. coli and five for yeast (Novelli, 1967).

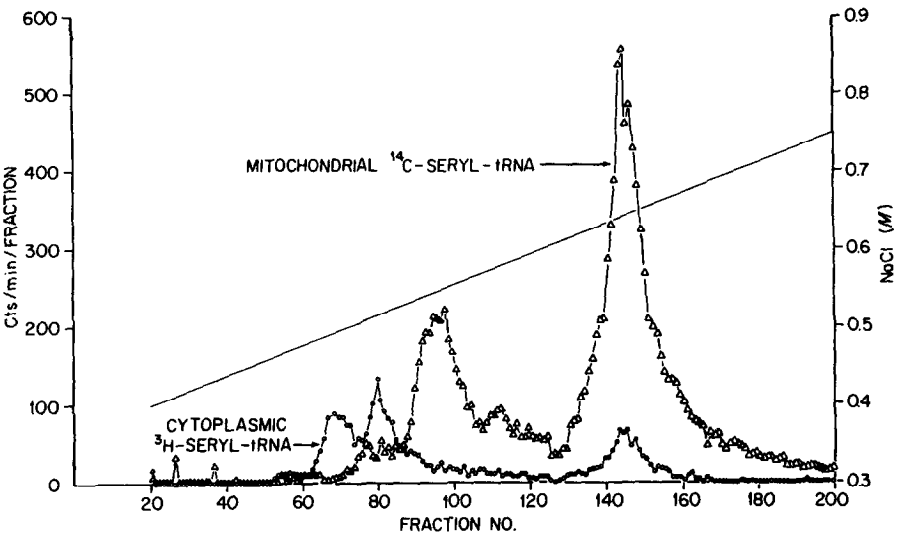

Fig. 2. Co-chromatography (RPC) of cytoplasmic ^{3}H-seryl-tRNA with mitochondrial ^{14}C-seryl-tRNA from N. crassa. ^{3}H, 18,000 cpm, 39 A_{260} units; ^{14}C, 25,000 cpm, 32 A_{260} units; plus approximately 100 A_{260} units of whole-cell Neurospora tRNA as carrier.

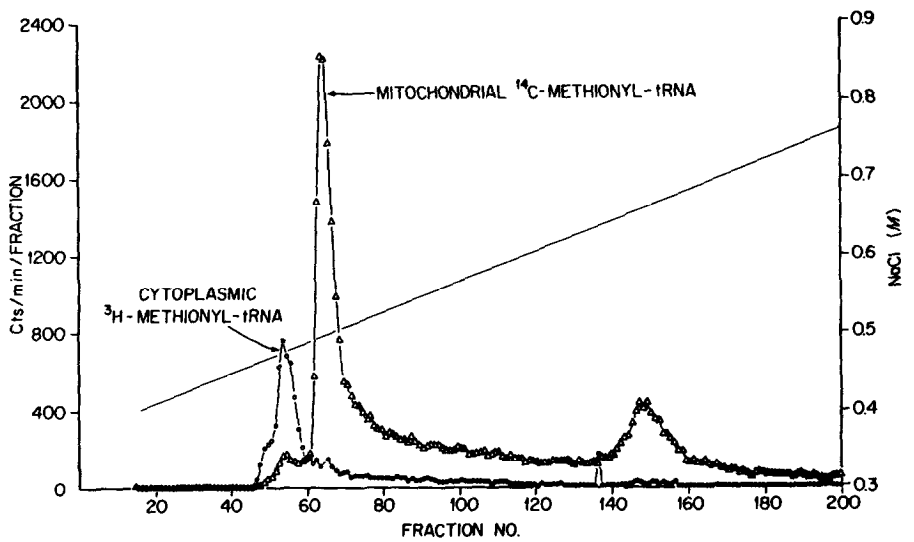

Fig. 3. Co-chromatography (RPC) of cytoplasmic ^{3}H-methionyl-tRNA with mitochondrial ^{14}C-methionyl-RNA from N. crassa. ^{3}H, 22,000 cpm, 118 A_{260} units; ^{14}C, 53,000 cpm, 32 A_{260} units; plus approximately 17 A_{260} units of whole-cell Neurospora tRNA as carrier.

Similarly, mitochondrial and cytoplasmic methionyl-tRNAs show differences upon co-chromatography (RPC). These data are shown in Fig. 3. In this case, there are three distinct species, two of which appear to be strictly mitochondrial.

These data provide additional evidence that the cytoplasm of Neurospora contains tRNAs that are chromatographically distinct from those associated with its mitochondria.

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