CODING PROPERTIES OF NEUROSPORA MITOCHONDRIAL AND CYTOPLASMIC LEUCINE TRANSFER RNA's*†

J. L. Epler and W. Edgar Barnett

Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

Received June 20, 1967

INTRODUCTION

One of the more thoroughly examined cases of multiple tRNA's for a single amino acid and their coding properties has involved Escherichia coli leucine tRNA's (Weisblum, Gonano, von Ehrenstein, and Benzer, 1965; Bennett, Goldstein, and Lipmann, 1965). Neurospora also contains multiple leucine tRNA's with coding properties similar to those of E. coli (Barnett and Epler, 1966a; see also Table I).

It has recently been shown that Neurospora contains tRNA's and synthetases uniquely associated with the mitochondria as well as those found in the cytoplasm (Barnett and Brown, 1967; Barnett, Brown, and Epler, 1967). Utilizing these mitochondrial and cytoplasmic tRNA's and enzymes, we have observed that mitochondrial leucyl-tRNA responds to only UC-containing polymers in ribosomal binding; whereas cytoplasmic leucyl-tRNA's respond to UC-, UG-, and (ambiguously) to U-containing polymers.

*Research sponsored by the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

†The following abbreviations and symbols are used: tRNA, transfer RNA; AA-tRNA, aminoacyl-tRNA; Leu, leucine; U, uracil; C, cytidine; G, guanine; A, adenine; X, degenerate 3'-terminal position; poly U, polyuridylic acid; poly UC, randomly ordered copolymer of uridylic and cytidylic acid; poly UG, copolymer of uridylic and guanylic acid; A^{260}, absorbancy at 260 mμ; and DEAE, diethylaminoethyl.

TABLE I

Polynucleotide specificity of leucyl-tRNA's in ribosomal binding

^{14}C-Leu-tRNA	$[Mg^{++}]$	Polymer (25 mμmoles nucleotide)			
		None	U	UC (2:3)	UG (2:1)
E. coli*	0.03 M	0.78	3.15	3.28	3.84
Neurospora* whole cell	0.03 M	0.58	1.40	2.12	0.69
Neurospora† cytoplasm	0.01 M	0.55	1.04	1.82	0.96
Neurospora† mitochondria	0.02 M	0.19	0.07	0.92	0.12

Values shown are in μμmoles of ^{14}C-L-Leu-tRNA bound to (*) 2.5 A^{260} units of Neurospora ribosomes (see also Barnett and Epler, 1966a) or (†) 4.0 A^{260} units of E. coli A-19 ribosomes in 30 minutes at 20°C except for ^{14}C-Leu-tRNA from mitochondria where a 5-minute incubation was used. Additions of ^{14}C-Leu-tRNA's were: E. coli, 13.3 μμmoles as 0.26 A^{260} units; whole cell, 6.4 μμmoles as 0.79 A^{260} units; cytoplasm, 5.0 μμmoles as 0.48 A^{260} units; and mitochondria, 3.7 μμmoles as 0.36 A^{260} units.

METHODS

Neurospora crassa, wild-type strain OR23-1a, was used. Cultures for mitochondrial preparations were grown as described (Barnett and Brown, 1967). Cultures for ribosome preparations were grown on minimal medium (Westergaard and Mitchell), 1947) containing 1 percent glucose and 0.05 percent casamino acids. E. coli, strain A-19, grown in 3XDM medium (Guthrie and Sinsheimer, 1960) was used.

Neurospora tRNA was prepared by phenol extraction, and countercurrent distribution was accomplished by using Holley's methods (Holley, Apgar, Everett, Madison, Merrill, and Zamir, 1963). E. coli tRNA was purchased from General Biochemicals. ^{14}C-AA-tRNA's were prepared as described (Barnett and Epler, 1966a) and assayed by the filter-paper disc method (Bollum, 1966).

Mitochondrial and cytoplasmic fractions of Neurospora were prepared as described previously (Barnett et al., 1967).

Neurospora and E. coli crude enzyme fractions were prepared, and partially purified leucine aminoacyl-tRNA synthetases were obtained from the mitochondrial and cytoplasmic enzyme fractions of Neurospora by using DEAE-Sephadex (A-25) chromatography (Barnett et al., 1967; Barnett and Epler, 1966b).

AA-tRNA-ribosomal binding was assayed with the cellulose nitrate filter technique of Nirenberg and Leder (1964). The reaction mix contained 10.0 µmoles Tris-acetate buffer, pH 7.2; magnesium acetate as noted; 5.0 µmoles potassium chloride; 2.5 A^{260} units Neurospora ribosomes or 4.0 A^{260} units of E. coli A-19 ribosomes; ^{14}C-AA-tRNA; and polyribonucleotide in a total volume of 0.1 ml. Ribosomes were treated with DNase, preincubated, and washed three times as described by Nirenberg (1964). Radioactivity of the dried filter was measured in a Packard Tricarb liquid scintillation spectrometer. Polyribonucleotides were obtained from Miles Chemical Corporation. Uniformly labeled L-leucine-^{14}C (231 µc/µmole, New England Nuclear Company) was used throughout.

RESULTS AND DISCUSSION

Fractionation of whole-cell Neurospora tRNA by countercurrent distribution has resolved multiple leucine tRNA's when acylated by a whole-cell Neurospora enzyme preparation (Fig. 1; Barnett and Epler, 1966a). However, we have recently shown that the mitochondrial leucyl-tRNA synthetase specifically acylates mitochondrial leucine tRNA (Barnett et al., 1967). In Fig. 1, mitochondrial leucine tRNA (assayed with the mitochondrial synthetase) is resolved as a single peak contributing to Leucine II of whole-cell tRNA.

Observations on code word recognition of leucyl-tRNA's in E. coli has pointed to the occurrence of two types: (1) tRNA's coded by CUX, where the 3' position X may be A, G, C, or U; and (2) a tRNA coded by UUX, where X may be G or A (Marshall, Caskey, and Nirenberg, 1967). In addition, another of the leucyl-tRNA's of E. coli responds, ambiguously, to poly U, especially at high magnesium levels (Weisblum et al., 1965; Bennett et al., 1965). Similarly, it was shown that Neurospora utilizes separate leucyl-tRNA's, responding preferentially to polymers containing the above codons. In Fig. 1, the polymer preferences of the various fractions are noted (see also Barnett and Epler, 1966a).

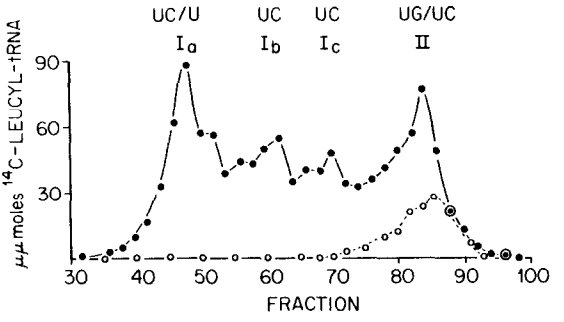

Fig. 1. Transfer countercurrent distribution of 230 mg of Neurospora (whole cell) transfer RNA. Samples (0.1 ml) were assayed for leucine acceptor activity as described in Methods by using: (1) ●——●, an unfractionated aminoacyl-RNA synthetase preparation, and (2) O---O, a mitochondrial synthetase preparation. General polymer preferences of the various fractions charged with leucine-^{14}C are noted (see also Barnett and Epler, 1966a).

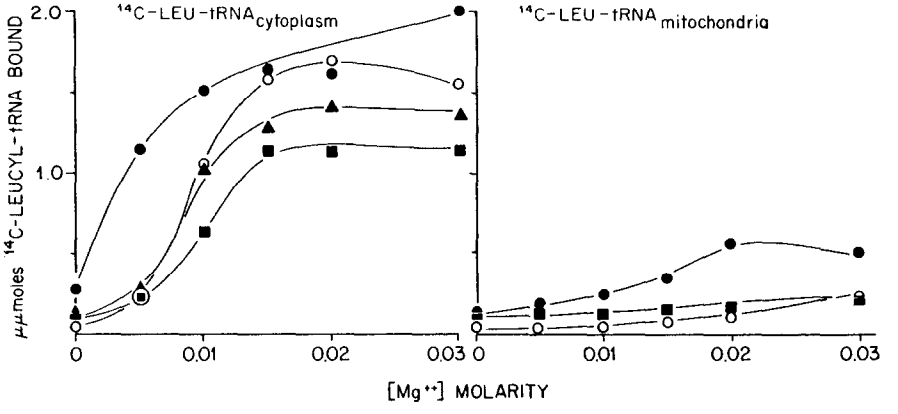

Fig. 2. Effect of magnesium concentration on ribosomal binding. Reactions were terminated at 30 minutes and assayed by the filtration method of Nirenberg and Leder (1964). Each reaction mix contained the components listed in Methods with 4.0 A^{260} units E. coli A-19 ribosomes and 25 mμmoles base of the respective polymer. ^{14}C-leucyl-tRNA was added as: (1) cytoplasmic, 5.0 μμmoles as 0.48 A^{260} units; and (2) mitochondrial, 3.7 μμmoles as 0.36 A^{260} units. Symbols used are: ●——●, plus poly UC (2:3); O——O, plus poly U; ▲——▲, plus poly UG (2:1); and ■——■, no addition.

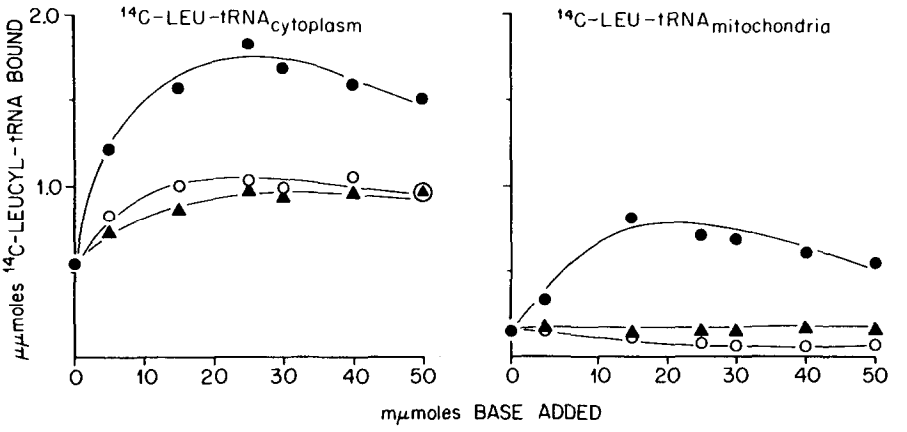

Fig. 3. Effect of polymer concentration on ribosomal binding. Reactions were terminated at 30 minutes as given in Fig. 2 except that polymers were added as shown.

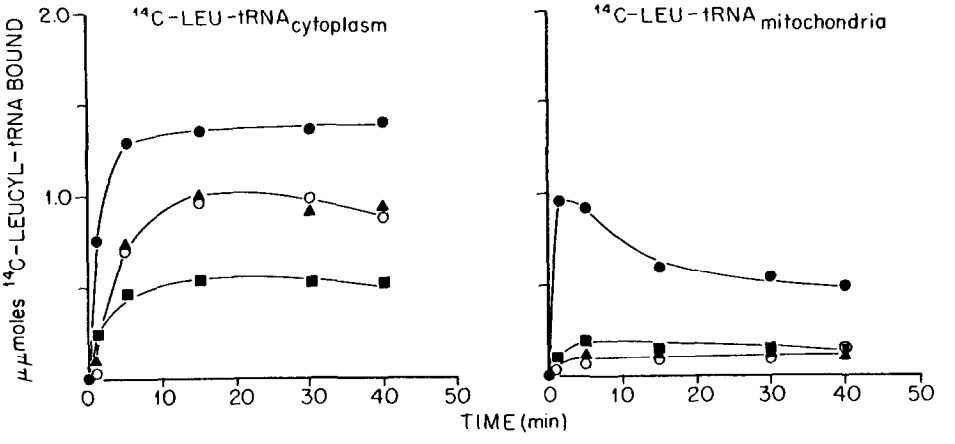

Fig. 4. Kinetics of ribosomal binding. Reactions were terminated at the time shown and contained the components listed above with 25 mμmoles of base added.

Table I summarizes the binding properties of whole-cell Neurospora tRNA when acylated with an unfractionated enzyme preparation. Comparative results with the E. coli system are also shown. Coding responses to poly U, UC, and UG are evident. However, when isolated cytoplasmic and mitochondrial enzymes are used to charge their respective tRNA fractions, it is clear that mitochondrial leucyl-tRNA responds to only poly UC. Furthermore, Figs. 2, 3, and 4 illustrate that neither magnesium

concentration, polymer concentration, nor time of incubation, respectively, influence this specificity. Thus, Neurospora cytoplasmic leucyl-tRNA's respond to UC-, UG-, and, ambiguously, to U-containing polymers; whereas mitochondrial leucyl-tRNA responds to only UC-containing polymers.

A more detailed examination of the coding properties of the mitochondrial tRNA's is now in progress.

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