SEPARATION OF E. COLI LEUCINE-ACCEPTOR RNA's BY COUNTERCURRENT DISTRIBUTION

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Fractionation studies have demonstrated the existence of more than one sRNA acceptor for one amino acid (Doctor, et al., 1961; Apgar and Holley, 1962; Apgar, et al., 1962; Sueoka and Yamane, 1962), and it has been shown that this provides an explanation for the observed degeneracy of the amino acid code (Weisblum, et al., 1962). Because of the important role of these molecules in protein synthesis, it is of interest to know how many sRNA's there can be for one amino acid. Some fractionation of the leucine-acceptor RNA's has already been obtained (Weisblum, et al., 1962; von Ehrenstein and Dais, 1963), and the fractions are of interest in coding experiments. Therefore, efforts were made to find countercurrent distribution conditions that would give maximum practical resolution of the different leucine-acceptor RNA's of E. coli.

The 200-transfer countercurrent distribution of E. coli sRNA described by Weisblum, et al. (1962) was carried out under conditions previously used for the fractionation of yeast sRNA (Apgar, et al., 1962). Under these conditions, leucine-acceptor activity separates into two peaks, designated as Leu I and Leu II. Further resolution of the Leu I and Leu II peaks requires considerable modification of the countercurrent distribution procedures, since conditions that favor

separation of one peak do not permit separation of the other. In order to effect maximum separation of both peaks, the change in partition coefficient of the RNA's with change in temperature has been exploited. A 1200-transfer countercurrent distribution was carried out in which the first part of the distribution was done at 28° to permit fractionation of the Leu II peak, and the remainder of the distribution was done at 23° to permit fraction of the Leu I peak. The results, shown in Fig. 1, establish that there are at least five different leucine-acceptor RNA's in E. coli.

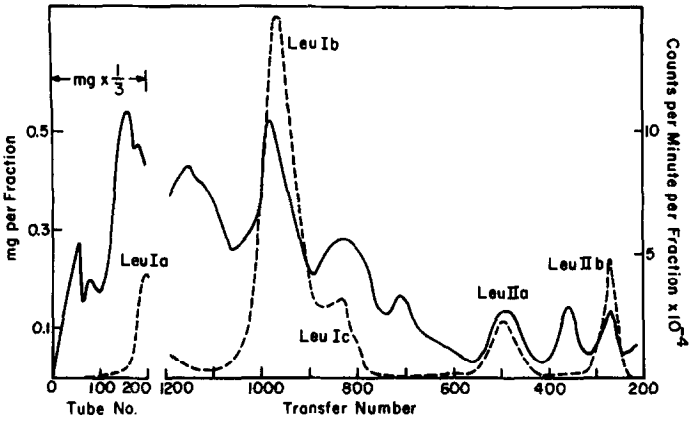

Figure 1. 1200 transfer countercurrent distribution of 100 mg. of E. coli KB sRNA (kindly furnished by Dr. Seymour Benzer). ___, mg. per fraction composed of the contents of five tubes from the distribution; - - -, counts per minute per fraction. The solvent system has been described (Holley, et al., 1963). The distribution was run in a 200-tube E-C Apparatus Company Countercurrent Fractionator[1/] using 10 ml. of each phase per tube, and with the upper phase feeding continuously. The first 585 transfers were run at 28° and the remaining 615 transfers at 23°. The upper phase running out of the machine after 200 transfers was collected in a fraction collector. Recovery of the RNA from the solvent system, and assays using E. coli activating enzyme and C^{14}-Leucine (110 mC/mM) were as previously described (Apgar, et al., 1962). Under the assay conditions, the starting RNA accepted 60,000 cpm of leucine per mg. of RNA (1.4 mμ M leucine per mg. of RNA, without correcting for non-radioactive leucine in the crude E. coli enzyme). On this basis, the Leu Ib and Leu IIb peaks can be estimated to be at least 25% pure.

1/ Trade names and company names are included for the benefit of the reader and do not infer any endorsement or preferential treatment of the product listed by the U. S. Department of Agriculture.

Acknowledgment

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