NUCLEOTIDE SEQUENCES IN TWO SERINE-ACCEPTOR RNA COMPONENTS

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Heterogeneity in biological activity among individual amino acid acceptor RNA's (s-RNA) of the same species has been demonstrated by several investigators (Berg et al., 1961; Doctor et al., 1961; Sueoka and Yamane, 1962; Apgar et al., 1964; Goldstein et al., 1964). An explanation for some of the degeneracy of the amino acid code was based on the heterogeneity of the s-RNA itself when it was shown, with the cell-free E. coli system of Nirenberg and Matthaei (1961), that two components of leucyl-s-RNA preparations responded to different polynucleotides (Weisblum et al., 1962; Bennett et al., 1963; von Ehrenstein and Dais, 1963; Yamane and Sueoka, 1963). Such a differential response might be due to different nucleotide sequences. Marked heterogeneity in nucleotide sequence has been demonstrated for s-RNA preparations specific for different amino acids (Holley et al., 1963; Armstrong et al., 1964; Doctor et al., 1963). This report describes an application of the same techniques (Doctor et al., 1963) to two serine-specific s-RNA components.

Several grams of s-RNA from Baker's yeast (Holley, 1963) were fractionated by a 200-transfer countercurrent distribution as described previously (Doctor et al., 1963). Fractions with serine-acceptor activity were pooled and redistributed for 300 transfers in a solvent system of 1.9 M Na-K phosphate, pH 6.0:isopropanol:formamide (100/45/10, v/v) (Apgar et al., 1962). Three fractions with serine acceptor activity were thus obtained. The amount of serine I RNA was too small for further purification on this scale. The two larger peaks (serine II and III, respectively) were separately pooled and redistributed twice in the same solvent system as above for 600 and then for 800 transfers. After the last distribution, the serine-acceptor activity and the weight of serine RNA were in good agreement for each component. With both serine-RNA components separately freed of other s-RNA activities, it remained to be shown that they still differed from each other. The two serine-RNA preparations were therefore mixed together and redistributed. Separation was obtained as shown in Fig. 1. The two fractions, combined as marked, were then dialyzed and precipitated with alcohol (Doctor et al., 1963). Aliquots of both fractions were used for the determination of base ratios with ribonuclease T_2 (Rushizky and Sober, 1963). The remainder of each serine-RNA was digested with pancreatic ribonuclease. After separation by mapping (Rushizky and Knight, 1960), the major mono-, di-, tri- and tetranucleotides were determined and calculated as moles, assuming a molecular weight of 30,000 for each serine-RNA (Table 1). The insoluble oligonucleotides that remained at the origin during mapping (core material) were eluted with N KOH for 24 hrs. at 23°. Assuming a 30% increase in A_{260} due to hyperchromicity,

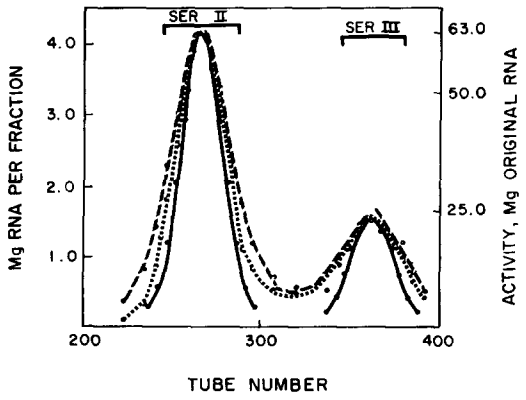

Figure I

Redistribution (650 transfers) of 32 mg of serine II and 16 mg of serine III s-RNA (from 15 g of unfractionated s-RNA) in the phosphate system described in the text. (----), mg RNA per fraction; (.....), serine acceptor activity calculated as mg of original RNA; (_____), theoretical distribution curve (Craig and Craig, 1956) for components with partition coefficients of 0.78 (ser II) and 1.26 (ser III), respectively. The peak fractions were pooled as indicated, and assayed for serine acceptor activity with rat liver aminoacyl RNA synthetases (Holley et al., 1961). While 1 mg of unfractionated yeast s-RNA incorporated 1.80 mμmoles of serine, the purified fractions incorporated 26.9 (ser II) and 27.4 (ser III) mμmoles of serine. Assuming a molecular weight of 30,000, the s-RNA preparations were thus 81% pure. When assayed with C^{14} algal protein hydrolysates and C^{12} serine, no incorporation in excess of that of the blank was observed. If lost, terminal adenosine would have been reintroduced during the enzymatic assay of the s-RNA preparations (Holley et al., 1961).

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24 and 26% of the total absorbance applied to the maps were thus recovered in the core fraction.

TABLE 1. Major mono- to tetranucleotides in two serine-RNA components*

(Moles per mole of s-RNA)**

	Serine II RNA	Serine III RNA
Cp	10.60	11.00
ApCp	0.60	0.49
ApApCp	0.78	0.81
GpCp	3.90	3.82
(ApGp)Cp	1.30	1.04
ApApUp	0.78	0.88
GpGpCp	1.33	1.48
(ApApGp)Up	0.52	0.44
(ApGp)GpUp	1.06	2.32
(ApGp)Up	1.37	1.09
GpGpUp	1.25	1.30
ApUp	1.27	0.52
GpUp	2.60	2.28
pseudo Up	0.26	0.25
Up	8.60	7.80

* Serine II- and serine III-RNA were mapped in triplicate and duplicate, respectively. Amounts of 2-3 mg of each RNA were hydrolyzed with pancreatic ribonuclease, mapped, and the separate spots eluted with ribonuclease T_2 in order to hydrolyze oligonucleotides to mononucleoside-3'-phosphates to avoid hypochromicity corrections. The A_{260} for 1 mg/ml of unhydrolyzed s-RNA of neutral pH in water was taken as 24.0. The reproducibility of the determination is better than $\pm$ 5% for mono-,

The results indicate few variations between the two serine-RNA preparations. There is no difference in the base ratios, both containing Cp 27.7; Ap 20.8; pseudo Up 3.3; Up 19.2; and Gp 28.7%. However, hydrolysis with pancreatic ribonuclease indicates that serine II RNA contains more ApUp and less (ApGpGp)Up than serine III RNA (Table 1). Digestion of (ApGpGp)Up with RNase T_1 (Sato and Egami, 1952) followed by mapping revealed no ApUp sequences, thus ruling out the presence of GpGpApUp in either serine-RNA preparation, but not distinguishing between GpApGpUp and ApGpGpUp.

At the present level of examination, an explanation of the variation in distribution coefficient of the two s-RNA preparations is not apparent. However, the results do show that in addition to

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di-, and trinucleotides, and better than $\pm$ 8% for tetranucleotides (Doctor et al., 1963). The material in the spots was identified by spectrophotometry and by comparison with similar maps obtained with RNA from TMV (Rushizky and Knight, 1960). This identification does not distinguish between corresponding minor and major bases in oligonucleotides. Terminal adenosine can not be determined by the mapping procedure since it moves off the paper during electrophoresis.

** Assuming a molecular weight of 30,000 for both serine-RNA's. The deviation of the molar amounts from whole numbers may be due to the presence of minor bases with different extinction coefficients, as well as to selecting an erroneous value for the molecular weight. For example, if a molecular weight of 23,000 were used, the purity of the serine RNA's would be reduced to 62%.

specificity for the same amino acid, serine, both components are very similar with respect to the major mono-, di-, tri- and tetranucleotides in pancreatic ribonuclease digests. This is in marked contrast to the corresponding values obtained for s-RNA preparations specific for different amino acids (Holley et al., 1963; Armstrong et al., 1964; Doctor et al., 1963).

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