

Preliminary Notes

Occurrence of the fifth nucleotide in soluble ribonucleic acid in yeast

In 1957, DAVIS AND ALLEN¹ discovered an as yet unidentified nucleotide in a preparation of yeast RNA. This fifth nucleotide is concentrated in a definite RNA fraction which is not precipitated by 1 M NaCl in the procedure of CRESTFIELD, SMITH AND ALLEN². The present paper reports that the RNA in the soluble fraction of the cell as fractionated by differential centrifugation of a cell homogenate is especially rich in this nucleotide.

Yeast cells (*Saccharomyces cerevisiae*, Strain Kaneka) grown anaerobically in synthetic medium³ were harvested, ground with quartz sand for 20 min at 2°, and suspended in the medium of CHAO AND SCHACHMAN⁴. Differential centrifugation of the suspension yielded the following subcellular components: cell-wall fraction, pellet of $2,240 \times g$, 5 min; large granules, pellet of $10,000 \times g$, 20 min; 80 S particles, pellet of $105,000 \times g$, 90 min; and $105,000 \times g$ supernatant. The nucleotide composition of the RNA's contained in these fractions was determined by Dowex-1 ion-exchange chromatography after alkaline hydrolysis as described previously⁵. As seen in Table I, the composition of the RNA of the 80-S particles and of large granules is very similar, whereas that of the supernatant RNA is significantly different. The chromatogram demonstrated the presence of a small but definite peak just before that of uridylic acid (Fig. 1). This peak is absent or insignificantly small in RNA's of the other subcellular fractions. The spectral data on this substance, taken at acidic, neutral, and alkaline pH's, agreed closely with those reported by DAVIS AND ALLEN¹ for what they called "the fifth nucleotide".

Comparison of the present data with those shown by MIURA *et al.*^{6,7} on 1 M or 2 M NaCl-insoluble and soluble fractions of yeast RNA made it clear that the RNA in the 80-S particles and large granules has a nucleotide composition similar to that of NaCl-insoluble fraction, while the supernatant RNA is closely related to the NaCl-soluble RNA. Moreover, the amount of supernatant RNA in the cell (14.3 %) is just about equal to that of 2 M NaCl-soluble fraction (15 %) in their RNA preparation. These facts together with the localization of the fifth nucleotide strongly suggest that

TABLE I
NUCLEOTIDE COMPOSITION OF RNA'S OF SUBCELLULAR COMPONENTS OF *Saccharomyces cerevisiae*

Fraction	Distribution of RNA in cell (%)	Molar ratio; adenyllic acid = 10.0			
		Guanylic	Cytidylic	Uridylic	5th nucleotide of DAVIS AND ALLEN
Whole cell	100	11.0	7.5	10.4	trace
Cell wall	6.4	—	—	—	—
Large granules	7.7	10.3	7.6	10.4	not detectable
80-S particles	69.0	10.7	7.3	10.6	trace
Supernatant	14.3	12.5	11.9	10.1	1.4

Abbreviation: RNA, ribonucleic acid.

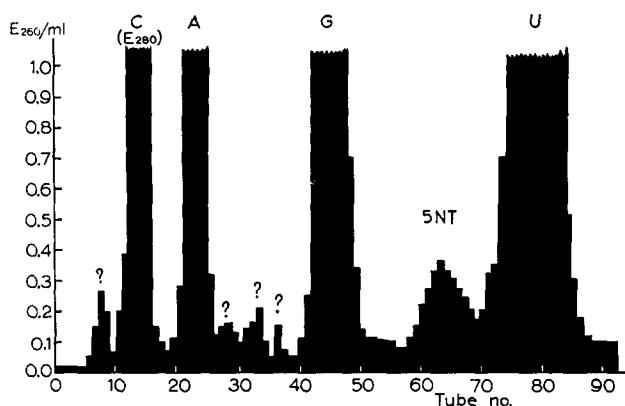


Fig. 1. Chromatographic separation of individual mononucleotides of RNA in soluble fraction of *S. cerevisiae*. C, cytidylic acid; A, adenylic acid; G, guanylic acid; U, uridylic acid; 5 NT, "the fifth nucleotide" of DAVIS AND ALLEN¹.

the NaCl-soluble RNA obtained by DAVIS AND ALLEN¹ and MIURA *et al.*^{6,7} is the so-called soluble RNA in yeast cells.

In mammalian liver cells, the soluble RNA has been separated into several fractions differing in the metabolic activities⁸⁻¹⁰. We have no data at present on the heterogeneity of yeast supernatant RNA, and further fractionation of this with special reference to the closer localization of the fifth nucleotide is now being made. The rather restricted occurrence of this special nucleotide in supernatant RNA might have some relation to the known specific function of soluble RNA in the transfer of activated amino acids to the microsomal ribonucleoproteins. In this connection, it is worthwhile to add our recent observations which revealed the presence of a considerable amount of the fifth nucleotide in the RNA precipitable at pH 5 from the $105,000 \times g$ supernatant of rat-liver homogenate. On the other hand, only a trace of this nucleotide was detected in the microsomal RNA.

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