EVIDENCE OF TWO SOLUBLE RNA TYPES IN EYE LENS

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In a preliminary work, we have studied the involvement of soluble lens RNA in protein biosynthesis : whatever its origin, epithelial, cortical or central, this RNA has been proved able to bind amino acids in the presence of lens pH 5 enzymes. It should be noted that only enzymes of epithelial and cortical zones are suitable (Virmaux and Mandel, 1964).

We have now examined the amount of soluble and particulate RNA, that is the 105,000 x g supernatant and pellet, in different regions of the calf lens. In order to avoid degradation of RNA during homogenization (0.3 M sucrose, 10^{-3} M $MgCl_2$) and centrifugation, polyvinylsulfate and bentonite were used as ribonuclease inhibitors (Scherrer and Darnell, 1962, Brownhill, Jones and Stacey, 1959). The different RNA's were determined by the Schmidt and Thannhauser procedure adapted for lens material (Virmaux and Mandel, 1961).

The results are given in Table 1. It appears that the supernatant fraction contains about 20 percent of the total RNA in the epithelial zone, 60 percent in the cortical region and 80 percent in the center. Thus, we note in the lens fibers of the cortex and the nucleus a much higher percentage of soluble RNA than in other mammalian cells (10 at 20 percent). For bovine whole lens, Van Heyningen (1961) has also found that most of the RNA is present in the supernatant fraction.

	Epithelium	Cortex	Nucleus
Supernatant (4 exp.)	100**	99	45
Sediment (4 exp.)	400	68	11

Table 1. Content in RNA of 105,000 x g supernatant and sediment fractions among different zones* of calf lens.

*The epithelium and the nucleus contain minor contaminants of cortical zone.

**µg RNA/100 mg protein

To test whether all of this soluble RNA is able to bind amino acids, we have incubated soluble RNA of the lens cortical zone with C^{14} amino acids of algal protein hydrolysate in the presence of lens pH 5 enzymes. After incubation, the charged S.RNA was extracted according to the method of Kirby (1956) and chromatographed on a DEAE cellulose column (Midgley, 1961) (Figure 1).

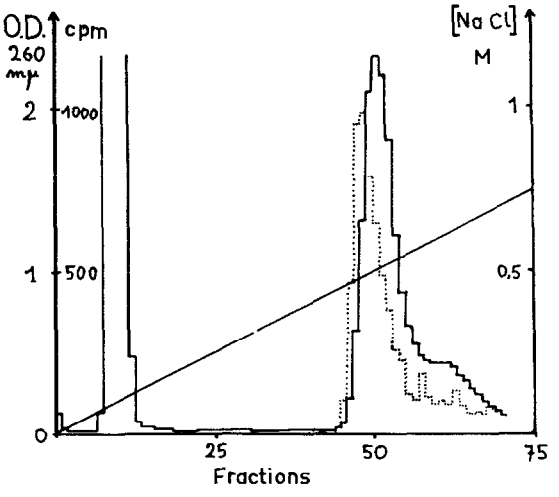

Figure 1. Chromatography on DEAE cellulose column of lens soluble RNA charged with C^{14} amino acids of algal protein hydrolysate

——— 260 mµ absorption.

..... Radioactivity after serum albumin addition and TCA precipitation. Each fraction was counted in a Packard Tri-Carb automatic scintillation counter.

The curve for absorbancy of the eluated RNA in the 0.5 M NaCl region, did not fit the curve for radioactivity : the first fractions of the S.RNA peak contained much more C^{14} amino-acyl-RNA.

In order to test whether polydispersity of S.RNA might explain the observed heterogenity and to check the assumption that only one fraction is transfer RNA, we have used molecular sieving on Sephadex.

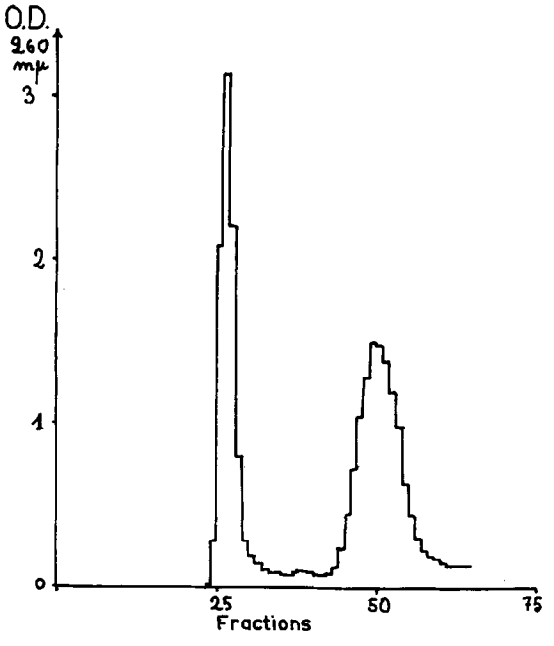

Figure 2. Chromatography on Sephadex G 100 of lens soluble RNA. Column 1.8 x 180 cm ; buffer 0.05 M NH_4CH_3COO pH 5 ; volume of fractions 5 ml ; flow rate 10-15 ml/hr ; temperature 0°.

As shown in Figure 2, the S.RNA chromatogram exhibited two peaks of polyribonucleotide material. On the average, the first peak accounted for 40 percent of the total soluble RNA. The test of amino acid acceptor activity for each of the two peaks revealed that only the second RNA was able to incorporate C^{14} amino acids (20,000 cpm/mg RNA for the second peak and 0 for the first). In the same way chromatography on Sephadex G 100 of

total soluble RNA charged with C^{14} chlorella protein hydrolysate showed the radioactivity of C^{14} amino-acyl-RNA in the second peak (Figure 3).

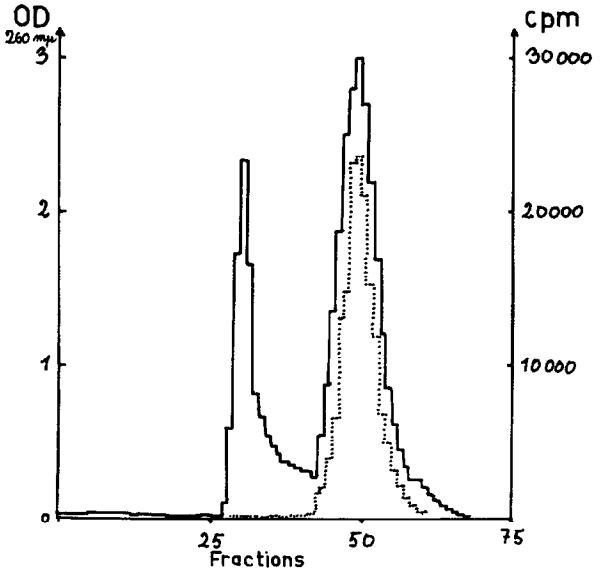

Figure 3. Chromatography on Sephadex G 100 of lens soluble RNA charged with C^{14} amino acids of algal protein hydrolysate (for conditions see figure 2).
The first peak shows a slightly decrease due to the 37° charging incubation.

——— 260 mμ absorption

..... Radioactivity

Ultracentrifugation analysis of each RNA component confirmed that they differ in their sedimentation coefficients. The S value of the first was equal to 7.2, of the second, 4.18.

The nucleotide composition of each soluble RNA fraction has been studied (Table 2). Alkaline hydrolysates were chromatographed on Dowex 1x8 column according to Breitman (1961). Estimates made from the absorbancy at 260 mμ of the pooled fractions indicated no significant differences except that pseudo-uridylic acid has been found in the 4 S RNA fraction (Table 3) and could not be detected in the 7 S RNA fraction.

	AMP	GMP	CMP	UMP
7 S	18.5*	34.5	31.5	15.5
4 S	19.0*	36.0	28.0	17.0**

Table 2. Nucleotide composition of 7 S RNA and 4 S RNA.

*moles/100 moles nucleotides. These values are only tentative because the minor nucleotides like pseudo-uridylic acid were not taken into account.

**Calculations of the moles of pseudo-uridylic acid/100 moles of uridylic acid give a value of 26 in agreement with those found for various soluble RNA's.

	Maximum		Minimum	
	pH 2	pH 12	pH 2	pH 12
Our component	260	282	237	247
Cohn 1960	263	286	233	246

Table 3. Spectral characteristic of minor component of 4 S RNA. (Wavelength, mμ).

According to its elution pattern, and its spectral characteristics the small but definite peak before uridylic acid is pseudo-uridylic.

From our results, it follows that there is a soluble RNA fraction other than transfer RNA in the cortical and central zones of crystalline lens. It differs from adaptator RNA in its molecular weight, in the absence of pseudo-uridylic acid and in its inability to bind amino acids. Its biological significance remains to be ascertained. Since this type of RNA is present in partially dedifferentiated cells, i.e. lens fibers with lowered particulate RNA content, we believe that it may result from ribosomal dissociation occurring in ageing lens cells.

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