# KINETICS OF LABELLING OF EYE LENS RNA's

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In the course of our investigation concerning RNA's and their role in the biosynthesis of specific lens proteins (Virmaux and Mandel, 1964 - Virmaux, Mandel and Urban, 1964) we have tried to isolate the m RNA of this organ. Until now, only Scott and Bell (1965) studying chick embryo lens polysomes have reported messenger utilisation at polyribosomes level.

Our study was carried out with calf lenses. Removed from the eyes within ten minutes after the death of the animals and immediatly stored in Heuvel medium (1956) at 37°C, the lenses were incubated in this medium with $P^{32}$ (100 µC/ml) for 1/2, 1, 2, 4 and 6 hours. The epithelial zone RNA's were extracted by the phenol procedure of Kirby (1962) and chromatographed on methylated albumin column (Sueoka, 1962)(Fig.1).

The optical density curve shows three polynucleotidic peaks, a first, s RNA, then a zone containing RNA and DNA, and a last fraction of r RNA. The curve of absorbancy does not fit the curve of radioactivity which shows only two peaks, one overlapping s RNA and the second zone, and an other overlapping r RNA.

The specific activities of these different peaks, computed from chromatogramms, plotted against incubation time, are recorded in fig.2.

The diagram clearly shows that the RNA fraction eluted after r RNA (fract IIIc) possesses a high specific activity which

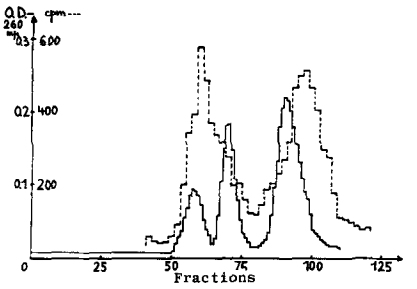


Figure 1.- Chromatography on methylated serum albumin of lens RNA's after one hour incubation.
Column 4 x 6 cm ; buffer 250 ml Tris 0.01 M NaCl 0.1 M pH 7.2 in the mixing vessel and 500 ml of Tris 0.01 M, NaCl 1.2 M pH 8.5 in the second vessel ; volume of fractions 4 ml.
———— 260 mμ absorption
-------- Radioactivity after serum albumin addition and TCA precipitation. Each fraction was counted in a Packard Tri Carb automatic scintillation Counter.

increases as a function of time and this rise is always greater than that of soluble and ribosomal RNAs.

It appears that in presence of $P^{32}$ an RNA, distinct from lens s RNA and r RNA by its pattern of elution from MAK columns, is synthesized for a very long time at a higher rate than the two other RNA's.

To test the turnover time of this RNA we have studied its specific activity during chase experiments. For this purpose, the lenses were incubated with $P^{32}$ for one hour, washed with cold inorganic phosphate and then incubated in Heuvel medium without $P^{32}$ for 1, 3, 5 or 7 hours (Fig.3).

In these experiments the incorporation of precursor into RNAs continues, and reaches, into fraction IIIc, a maximum value at about the fourth hour and then decreases slightly.

When the lenses are treated with actinomycin D (2 μg/ml) 30 minutes before incubation, the incorporation of $P^{32}$ into s RNA is less than 20 per cent and that into r RNA and fraction IIIc

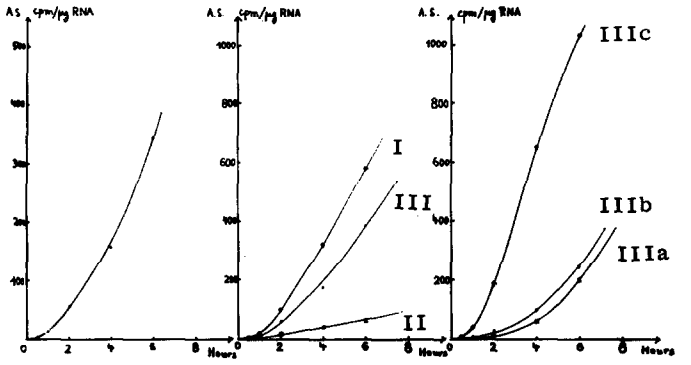


Figure 2.- Specific activities curves of epithelial lens RNAs.

A. Incorporation of $P^{32}$ into total RNA.

B. Incorporation of $P^{32}$ into the three RNA peaks separated on MAK column.

C. Incorporation of $P^{32}$ into three fractions of the third peak.
IIIa increasing branch of the third peak.
IIIb decreasing portion from O.D. maximum to O.D. half value.
IIIc decreasing portion from half value to O.D. minimum value.

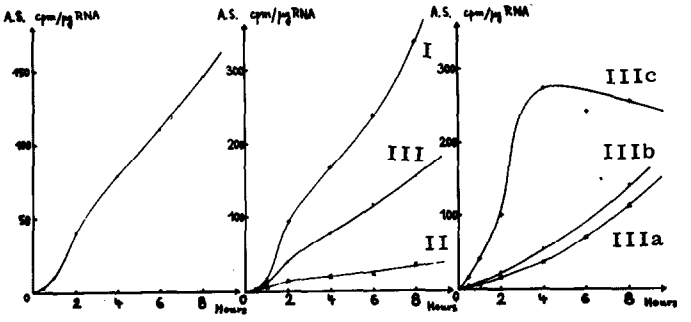


Figure 3.- Specific activities curves of epithelial lens RNAs during chase experiments.
(A, B, C : see figure 2)

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is less than 10 per cent of their control values for one hour.

The nucleotide composition of the last two fractions has been studied by measuring the amount of radioactivity of the

four nucleotides after alkaline digestion. The values are reported in table.

| | AMP | GMP | CMP | UMP | G + C/A + U | Pu/Py |
|---|---|---|---|---|---|---|
| Fraction IIIa + IIIb | 21+ | 31.2 | 32.1 | 15.7 | 1.72 | 1.10 |
| Fraction IIIc | 25.5 | 23.8 | 27.6 | 23.3 | 1.05 | 0.97 |

Table. - Nucleotide composition of fraction IIIa + IIIb (ribosomal RNA) and of fraction IIIc.
+moles/100 moles nucleotides.

The labelled r RNA fraction has a composition typical of ribosomal RNA. The ratio C + G/A + U of the fraction IIIc is approximately equal to one and this RNA has a composition similar to that of DNA.

The turnover time of this RNA fraction estimated from the specific activity of the phosphate α of the free nucleotides or from the decay time of its specific activity during chase experiments, is about 8 hours.

Our results suggest than an RNA fraction with a higher rate of labelling than that of the other fractions occurs in the lens. It is relatively stable, its nucleotide composition suggests that it has characteristics of messenger RNA. Its sedimentation pattern and its ability to stimulate protein synthesis are under investigation.

It should be noted that during a long labelling time (6 hours), such a fraction could not be detected in the cortical region of lens, which contains partially dedifferentiated cells synthesizing mainly three types of proteins : crystalline α, β, γ.

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