DIFFERENTIAL THERMAL STABILITY OF OLD AND NEW RIBOSOMAL RNA OF RAT LIVER

L. KOKILEVA, I. MLADENOVA and R. TSANEV
Biochemical Research Laboratory, Bulgarian Academy of Sciences, Sofia, Bulgaria

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1. Introduction

It is generally believed that rRNA represents an intact polynucleotide chain [1]. However, evidence has been obtained that some latent breaks in the RNA molecule are present [2], which can be revealed by heating [3]. The problem still remains whether these breaks are due to the normal metabolism of rRNA or are produced during the isolation procedure. To solve this problem it would be important to know whether old and newly synthesized cytoplasmic rRNA behave differently as far as hidden breaks are concerned.

In this communication evidence is presented that rRNA fragments produced by heating originate mainly from old rRNA.

2. Materials and methods

Three month old albino rats (150–200g) were killed by decapitation after starving for 18 hr.

Liver RNA was extracted from the total homogenate at 0° with water-saturated phenol pH 6.0, as previously described [4].

All procedures, unless otherwise stated, were carried out in the cold.

RNA was carefully deproteinized as described earlier [2]. Labelled RNA was additionally purified on Dowex 1 × 8 [5]. Fractionation of RNA was by agar-gel electrophoresis [6]. The electrophorograms and their radioautographs were scanned at 260 nm and 550 nm, respectively [7,4].

28 S and 18 S RNA fractions were obtained by preparative agar-gel electrophoresis and extraction of the corresponding zones with SDS-phenol [8].

RNA was labelled *in vivo* by intraperitoneal injection of 70–100 μCi of ^{14}C-orotic acid (sp. act. 15.11 mCi/mmole). In some experiments carrier-free ^{32}P-orthophosphate was used.

Short and long-term labelling experiments were carried out. In the first case RNA was labelled for 2 hr. In the second case the isotope was introduced three times at equal intervals during the first 18 hr and the animals were killed 10 days later.

To check the thermal stability of RNA the latter was dissolved in 0.01 M NaCl, containing 1 mM EDTA, and was heated for 5 min at 70, 80 and 90°.

3. Results and discussion

As seen in fig. 1A the labelling pattern of RNA after a two hours pulse coincides with the absorbancy profile. There is no heterogenious zone between 4 S and 18 S RNA, which is consistent with earlier results [4]. At this labelling time the newly synthesized RNA is a mixture of ribosomal RNA and DNA-like RNA [9]. The amount of the latter decreases when SDS is omitted during the extraction [4], as was the case in these experiments.

After heating, a number of RNA fractions appears between the three main RNA peaks (28 S, 18 S and 4 S). The results obtained at temperatures between 70° and 90° were essentially the same and only profiles of RNA heated at 70° are presented here.

As seen from the electrophoretic patterns (fig. 1B), the specific radioactivity of these fractions is much lower than that of the original RNA while the specific radioactivity of 28 S RNA increases. At the same time the A_{260} peak of 28 S RNA decreases. No changes are

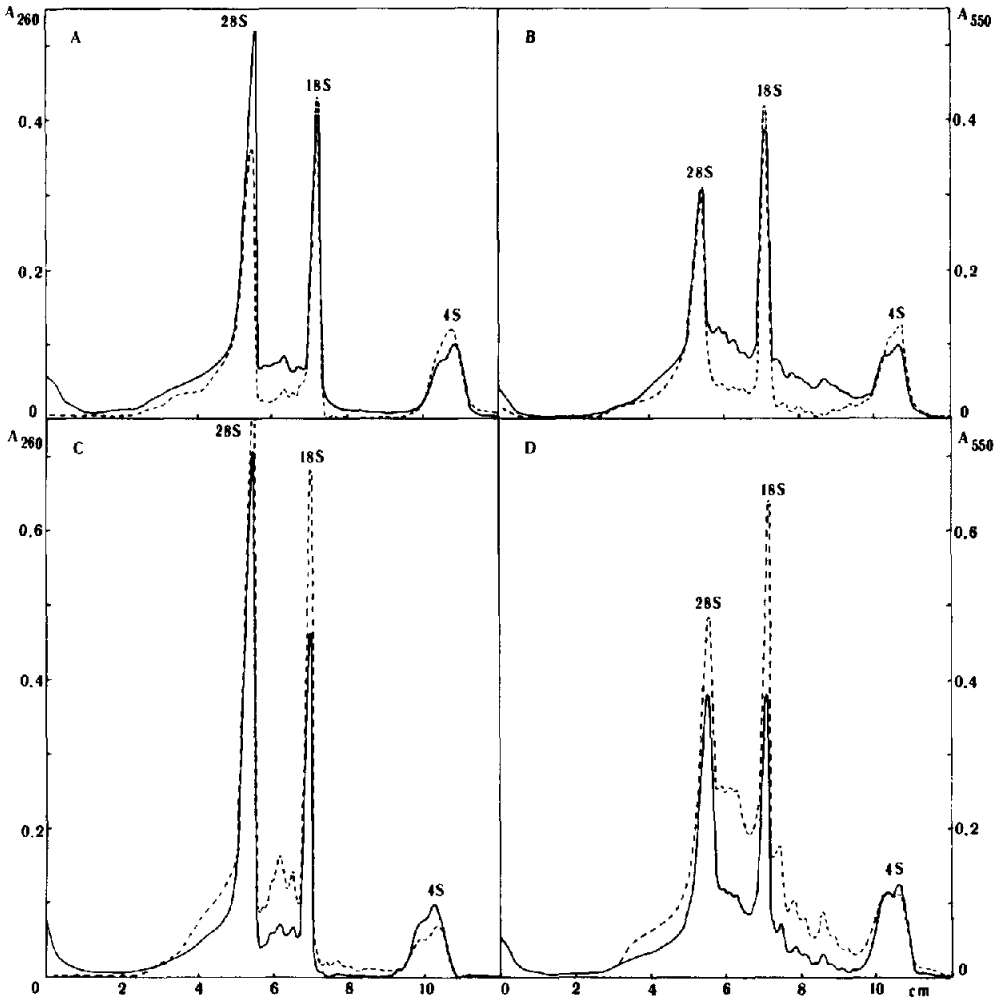

Fig. 1. Electrophoretic patterns and radioactivity profiles of short- and long-term labelled total RNA from rat liver. Direction of migration – left to right. (————) absorbance at 260 nm; (- - - - - - -) radioactivity (absorbance of radioautographs at 550 nm). (A) short-term (2 hr) ^{14}C-labelled RNA; (B) the same RNA, heated for 5 min at 70°; (C) long-term ^{14}C-labelled RNA; (D) the same RNA, heated for 5 min at 70°.

visible in the 4 S fraction. It is evident that the heating affects mainly 28 S RNA and the degradation fragments have a lower specific activity. This indicates that the newly synthesized RNA in the 28 S peak is more stable while old RNA molecules are preferentially degraded. This is confirmed by the second experimental series (fig. 1C, D). In this experiment the fragments produced by heating have a higher specific activity than 28 S RNA. This is to be expected as the labelled RNA in this long-term labelling has a base composition of rRNA [9]; the half-life of rRNA is between four and five days [10,11]; and the interval of nine days between the last ^{14}C injection and the killing of the animals is long enough to produce a chase of the newly synthesized stable rRNA molecules into the old thermally sensitive species. The labelling and A_{260} patterns in fig. 1D are the reverse of those shown in fig. 1B, confirming that new and old RNA molecules have different thermal stability.

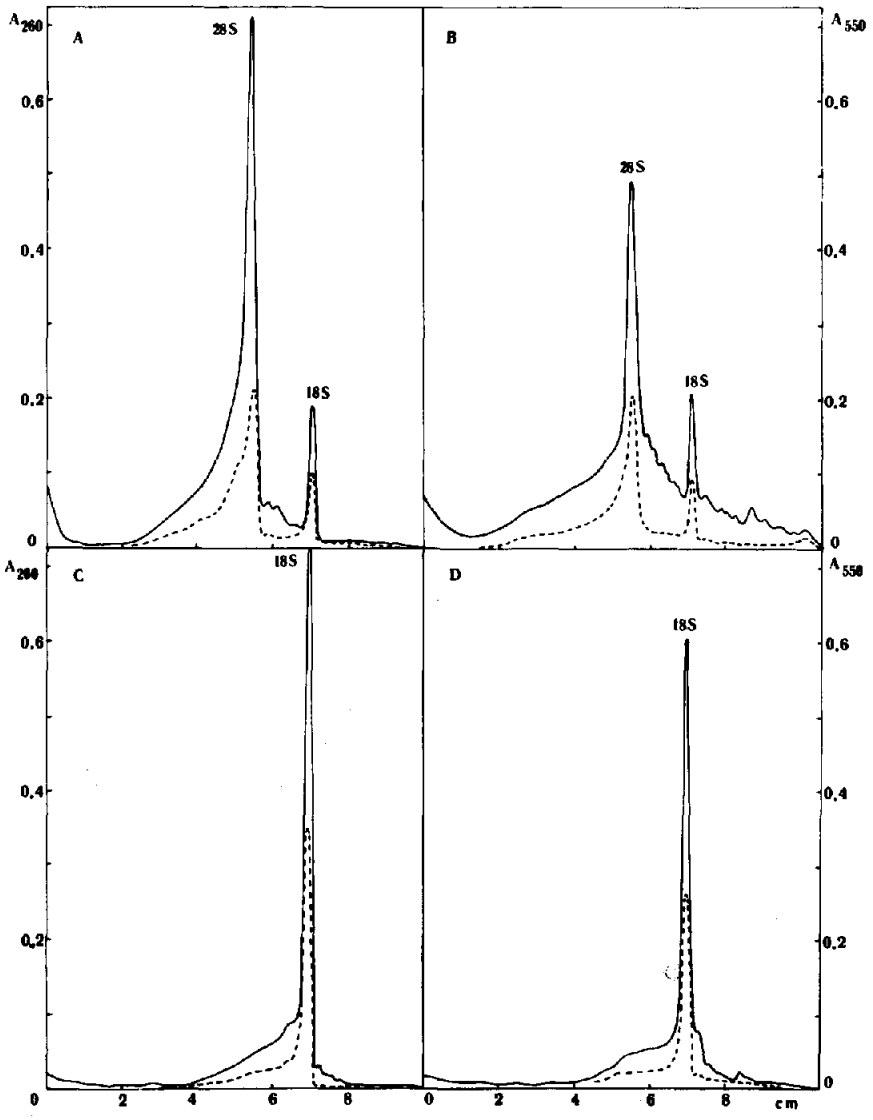

Fig. 2. Electrophoretic patterns and radioactivity profiles of ^{14}C-short-term labelled 28 S and 18 S rRNA. Legend as in fig. 1. (A) 28 S rRNA; (B) 28 S rRNA, heated for 5 min at 70°; (C) 18 S rRNA; (D) 18 S rRNA, heated for 5 min at 70°.

The same results are obtained with isolated 28 S and 18 S RNA fractions. As seen in fig. 2A, B the fragments liberated from 28 S RNA on heating have much less radioactivity after a two hours pulse, while the same fragments obtained in a long-term labelling followed by a 9 days natural chase have, on the contrary, an increased specific activity as compared with the original 28 S fraction (fig. 3A, B).

In all our experiments, 18 S RNA showed a much higher thermal stability. As seen in fig. 2C, D, only a very small amount of the 18 S peak is degraded on heating. The low specific activity of this material after a short pulse and its high specific activity after a long pulse followed by a chase shows that it also originates from old RNA molecules.

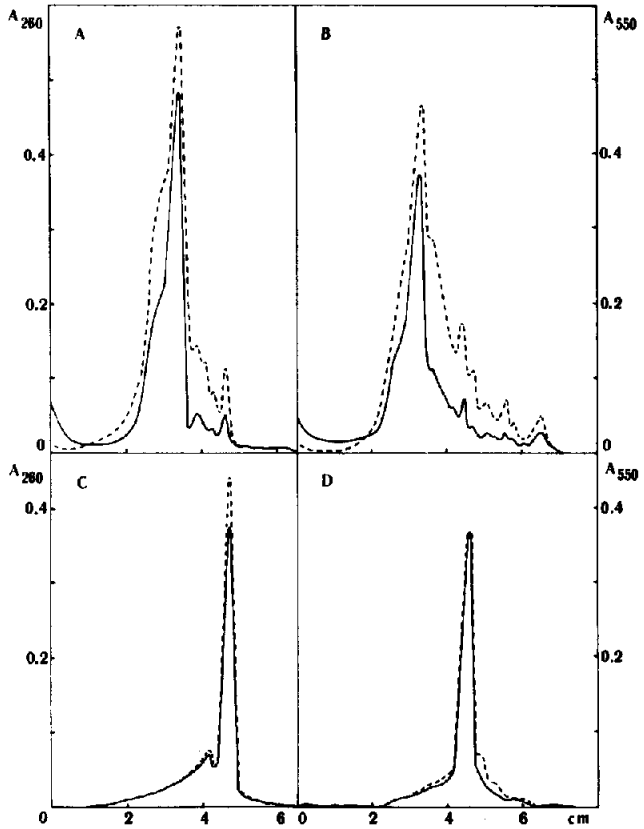

Fig. 3. Electrophoretic patterns and radioactivity profiles of long-term ^{14}C-labelled 28 S and 18 S rRNA. (A) 28 S rRNA; (B) 28 S rRNA, heated for 5 min at 70°; (C) 18 S rRNA; (D) 18 S rRNA, heated for 5 min at 70°.

Thus our data clearly show that old and new RNA species in rRNA exhibit a different thermal stability. As shown previously [2] the degradation of RNA on heating is due to latent breaks in the polynucleotide chain. The present results prove that these breaks are predominantly located in the old RNA species. The most probable explanation for this would be that the latent breaks are due to a normal catabolism of rRNA. The possibility of a preferential nuclease attack of old RNA molecules during the isolation procedure seems less probable. In a previous study it was shown that the number of hidden breaks did not decrease if RNA was isolated from rat liver directly homogenized in phenol. It would be difficult to understand why old rRNA should be more exposed to nuclease degradation during the isolation procedure.

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