CHAIN LENGTH OF RIBOSOMAL RNA EXTRACTED FROM MAMMALIAN CELLS

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1. Introduction

It is believed that the polynucleotide chains of the two ribosomal RNA (rRNA) species are intact [1]. On the other hand, the conversion of the heavier rRNA component into the lighter one has been observed [2-5], suggesting that it might consist of subunits. In agreement with such a conclusion the chain length of rRNA of *E. coli* [6,7] and of wheat germs [8,9] was found to be much shorter (1300–1500 nucleotides) than expected from its sedimentation behaviour. However, no systematic study on this problem has been made and no data concerning the chain length of rRNA from animal cells are available.

In this study the chain length of rRNA of rat liver and Ehrlich ascites tumor (EAT) was determined with special attention to the methods of RNA extraction and deproteinization. Under most careful isolation a maximum mean chain length of about 1600 nucleotides was found for 28S+18S rRNA.

2. Methods

All procedures, unless otherwise stated, were carried out in the cold. The phenol was redistilled, pH 5.0, and contained 0.05% 8-hydroxychinoline. The 0.14 M NaCl contained 0.05% PVS.

2.1. *Extraction of RNA*

1. Rat liver RNA. Three-months old albino rats (150–200 g) were used. Eighteen hours before killing the animals were only given water. Four modifications of the phenol method were used: 1a) cold phenol procedure [10] using livers of animals killed by decapitation; 1b) as in 1a but the animals were anesthetized with ether, the livers frozen in liquid nitrogen and the powdered tissue added (1 : 10 w/v) to the phenol-0.14 M NaCl mixture; 1c) cold-phenol SDS procedure using only the cytoplasmic fraction [11]; 1d) hot phenol procedure (65°, 5 min) [12].

2. Ascites cell RNA. EAT cells were harvested 7 days after transplantation to albino mice. The modifications 1a and 1d were used.

2.2. *Deproteinization of RNA*

The aqueous layer was made 0.5% with SDS, shaken for 2 min and deproteinized four times with an equal volume of phenol. RNA was precipitated with two volumes of ethanol containing 1.0% K-acetate and cooled at −25°C. The precipitate was dissolved in 0.14 M NaCl containing 0.2% SDS and 0.001 M EDTA, pH 5.0. The solution was treated three times with phenol and two times with chlorophorm (30 min each time). After the last deproteinization 1–2 mg/ml macaloid [13] was added to the aqueous layer, the suspension was shaken for 30 min and centrifuged for 20 min at 100000 × *g*.

2.3. *Separation and purification of rRNA*

The supernatant was made 2 M with NaCl and kept at −10°C overnight. The precipitate was dissolved in H_2O (5–7 mg RNA/ml) and the traces of 4S RNA removed on a Sephadex-G 200 column. RNA was precipitated once more with 2M NaCl.

2.4. *Preparation of dye-linked RNA*

The 3′-OH end of RNA was oxidized by periodic acid and bound to 2-hydroxynaphthoic acid hydrazide as described by Zamecnik [14] using

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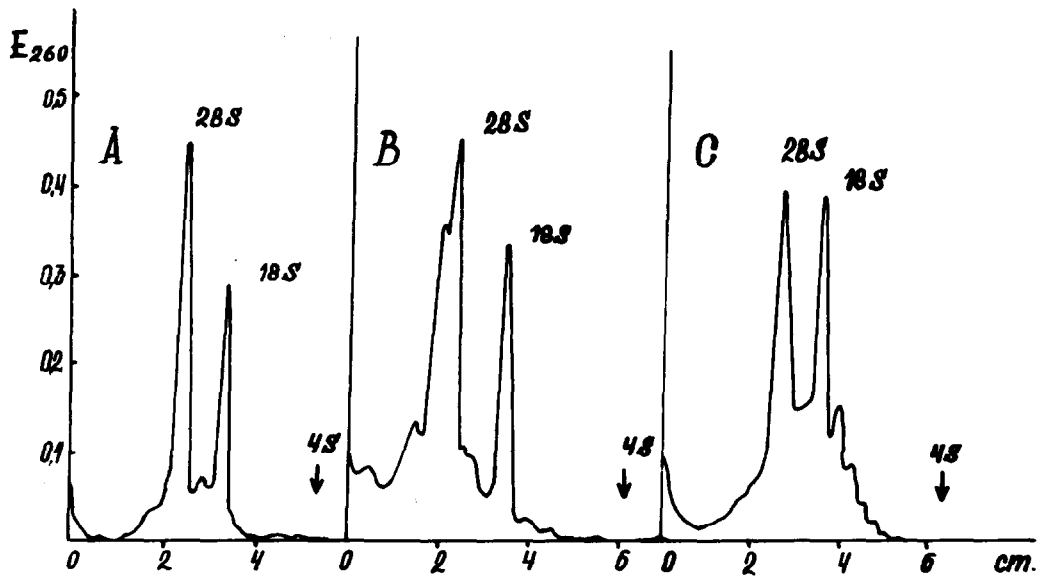

Fig. 1. Electrophoretic profiles of rat liver dye-linked rRNA isolated by different methods: A) cold phenol treatment (see section 2, 1a); B) hot phenol treatment (see section 2, 1d) ; C) as in A but after incubation of the homogenate at 37°C for 30 min.

glycolmonoacetate as solvent instead of 2-methoxyethanol. The modified RNA was dissolved in 0.01 M phosphate buffer pH 7.0 (about 5 mg RNA/ml) and the solution clarified by centrifugation. An ice-cold 1.0% solution of freshly prepared diazotized aniline sulphate was added to make 2 mg diazonium salt per 5 mg RNA. After 10 min two volumes of ethanol were added to obtain RNA as a pink-coloured precipitate. The absorption maximum of the RNA-linked dye was at 512 mμ [15]. The molecular weight (Mw) of a single RNA chain was calculated by the formula:

$$Mw = A \times 306 \times 42.5/13.2$$

where 306 is the Mw of the dye, 42.5 and 13.2 are the experimentally determined weight-equivalents of 1 optical density unit of RNA at 260 mμ and of RNA-coupled dye at 512 mμ, A is the ratio between the extinctions of dye-linked RNA at 260 mμ and 512 mμ [15].

Agar gel electrophoresis and scanning of the dry electrophoregrams were as described earlier [16,17].

3. Results

All preparations of rRNA gave the electrophoretic pattern of nondegraded RNA consisting of the two fractions 28S and 18S [18]. No changes in this pattern were observed in dye-linked RNA (fig. 1A).

The mean Mw of 28S+18S rRNA is given in the table. The maximum value obtained was 5.3×10^5. Lower values $(3.05–3.3) \times 10^5$ were found in the case of RNA isolated by hot phenol although this RNA showed the same two major 28S and 18S components (fig. 1B).

RNA from homogenates incubated at 0°C for 30 min did not show any changes in its electrophoretic pattern and in its Mw. However, the incubation of the homogenate at 37°C for 30 min led to a partial degradation of the rRNA as seen from its electrophoretic profile (fig. 1C). At the same time a decrease of its Mw was observed (table 1 and fig. 2). No additional coupling of dye with this RNA was observed after its treatment with acid phosphomonoesterase (EC 3132, Worthington) at pH 5.0 for 40 min at 40°C (40 μg enzyme per mg RNA).

Table 1

Cells	Method	Treatment of homogenate	Number of experiments	Mw $\times 10^{-5}$ ±m **	Number of nucleotides per chain ***
Rat liver	cold phenol 1a *	–	6	5.25 ± 0.1	1624
	cold phenol 1a *	0°, 30 min	2	5.20	1610
	cold phenol 1a *	37°, 30 min	2	1.72	533
	cold phenol liquid N_2 (1b *)	–	2	5.05	1565
	cold phenol cytoplasm (1c *)	–	3	5.10 ± 0.1	1577
	hot phenol 1d *	–	3	3.30 ± 0.1	1020
EAT	cold phenol 1a *	–	2	5.03	1560
	hot phenol 1d *	–	1	3.05	944

* See section 2.
** Standard deviation of the mean.
*** The mean molecular weight of the nucleotides in rRNA was assumed to be 323 on the basis of its base composition.

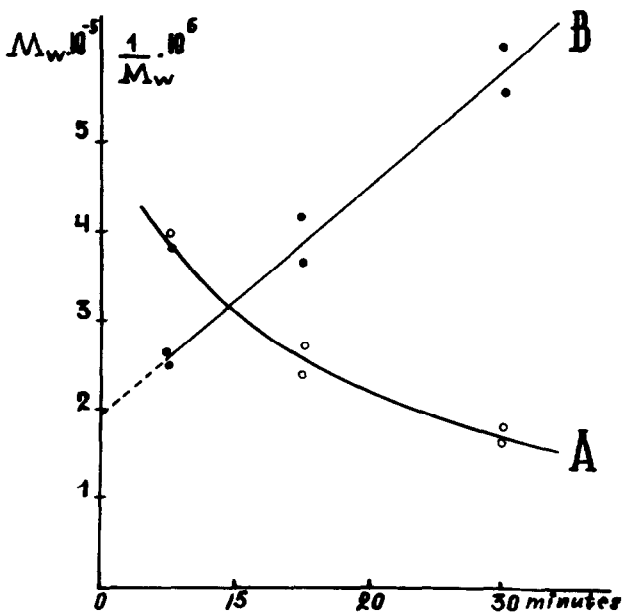

Fig. 2. Changes in the Mw of rat liver rRNA during the incubation of the homogenate at 37°C. Abscissa – time; ordinates Mw (curve A) and 1/Mw (curve B).

4. Discussion

Because of the twofold precipitation of RNA from 2 M NaCl, the additional Sephadex-G 200 filtration and centrifugation at 100000 × *g* neither 4S RNA nor polysaccharides could be present in our preparations to influence the results. Enzymic activities usually present in RNA preparations were also efficiently eliminated by the careful deproteinization. No changes in the RNA electrophoretic profile were observed after a 24 hr incubation at 37°C, pH 5.0. If this deproteinization procedure was not followed the Mw of RNA progressively decreased on storage. The method used in this study gave the same value for the Mw of rRNA extracted from *E. coli* as reported by others [6].

Our results show that the chain length of both rat liver and EAT rRNA are only the half of what should be expected for an equimolar mixture of 28S and 18S molecules. Thus a mean Mw of about 5×10^5 seems to be characteristic of rRNA of quite

different origin: microbial [6,7], plant [8,9] and animal cells.

The question arises whether these results were not caused by breakdown of rRNA in the course of its preparation. Breaks produced by the known endonucleases would not interfere with the Mw estimation since they lead to 3′-phosphorylated ends which are resistent to periodate oxydation [19]. However, the combined action of an RNAase and a phosphomonoesterase would result in the formation of fragments with 3′-OH ends. The action of enzymes during the preparation of RNA seems rather unlikely because of the following reasons: a) some of the methods used (1b) are hardly compatible with the action of enzymes; b) the Mw of RNA did not decrease even after incubation of the liver homogenate at 0°C for 30 min; c) when 1/Mw of RNA extracted from homogenates incubated at 37°C for 5, 15 and 30 min was plotted against time a straight line was obtained (fig. 2). The extrapolation to zero time gave a Mw of 5.1×10^5, which is the same as directly found. No 3′-phosphorylated ends were present in this RNA as can be concluded from the results with phosphomonoesterase treatment. The conclusion can be drawn that all fragments produced during the incubation of liver homogenates at 37°C have free 3′-OH groups. This is probably due to the abundance of phosphomonoesterases in the liver homogenate [20].

All these experiments come to prove that the value of about 5.0×10^5 for Mw of rRNA is not due to a degradation of RNA during its preparation but reflects the state of rRNA in the living cell. This value should correspond to an RNA molecule of about 16S. On the other hand the electrophoretic pattern of this RNA exhibits the two major rRNA components 28S and 18S in a ratio 2.5 : 1 as expected for an equimolar mixture of RNA species with a Mw of 1.7×10^6 for 28S and 6.7×10^5 for 18S [1]. No material lighter than 18S is observed on the electrophoregrams. The small amount of fast moving material in the case of partly degraded RNA could not account for the estimated Mw of 1.75×10^5. This supports the idea [21] that a few breaks in the polynucleotide chain may not change the macromolecular behaviour of RNA in separation techniques.

Two hypotheses may be put forward: a) rRNA represents a molecular population with a mean Mw of the order of 5.0×10^5 but due to secondary interactions the two rRNA species are formed; b) rRNA is synthesized as molecules corresponding to 28S and 18S but later on 3′-OH-ending breaks in the polynucleotide chain appear.

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