IN VITRO AUTODEGRADATION OF RIBOSOMAL RNA
FORMATION OF SPECIFIC FRAGMENTS WITH ENDONUCLEOLYTIC CLEAVAGES

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SUMMARY: When incubated *in vitro* with EDTA, ribosomal RNA in fetal rat liver polysomes was degraded to produce fragments some of which have molecular weights similar to those of *in vivo* degradation intermediates of 28S ribosomal RNA. The degradation occurred endonucleolytically and was inhibited by SDS and liver "cell sap".

INTRODUCTION

In a previous paper (1), we reported that polysomes of rat liver cells contained four kinds of minor RNA components whose molecular weights were between those of 28S and 18S ribosomal RNA. Since then, some investigators have reported the presence of similar minor RNA components in various types of eukaryotic cells (2-6), and from the several lines of evidence, it has been concluded that they were the intermediates in the degradation of 28S RNA in the cells (2-4). More recently, we showed that liver polysomes of fetal rats younger than 17th day contained little, if any, of the minor components and that their appearance was age-dependent (7).

In this communication, we report that under the appropriate condition, ribosomal RNA in fetal rat liver polysomes is degraded *in vitro* to produce fragments including those which have similar molecular weights to those of *in vivo* degradation intermediates without the addition of any other cellular fractions. This fact may indicate that the ribosomes possess mechanisms by which their own ribosomal RNA is degraded.

MATERIALS AND METHODS

Polysomes were prepared from 17th day fetal rat livers (7). Polyacrylamide gel electrophoresis and quantitation of RNA in the gel were performed

Abbreviations: SDS, sodium dodecylsulfate; *p*CMB, *p*-chloromercuric benzoate.

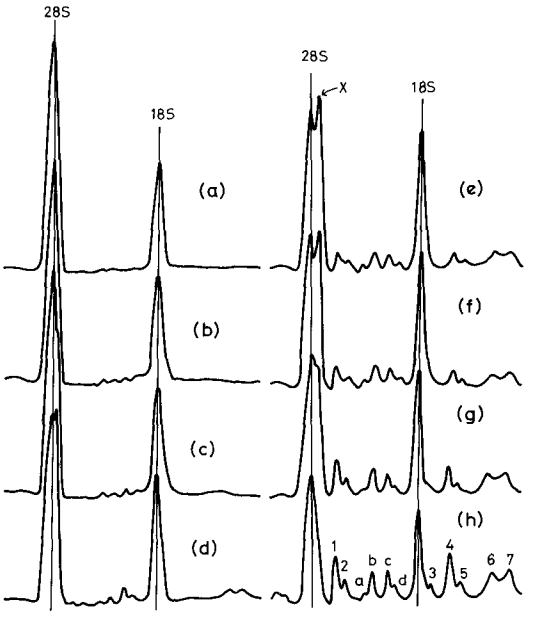

Fig. 1. Effect of EDTA on the *in vitro* autodegradation of fetal rat liver ribosomal RNA. Fetal rat liver polysomes (5.4 $A_{260\ nm}$ units) were incubated at 37 °C for 45 min in 0.1 ml of a buffer containing 25 mM Tris-HCl, pH 7.6 and EDTA as indicated. Reaction was stopped by the addition of 0.2 volumes of 10 % SDS and chilling. After the addition of 0.5 volumes of three fold strength buffer E (1) containing 24 % sucrose, an aliquot of the mixture was applied to 2.8 % polyacrylamide gel electrophoresis (1, 7).
(a) Original sample without the incubation; (b) 0 mM EDTA; (c) 0.2 mM EDTA; (d) 0.5 mM EDTA; (e) 1.0 mM EDTA; (f) 2.0 mM EDTA; (g) 5.0 mM EDTA; (h) 10.0 mM EDTA.

as described earlier (1, 7). "Cell sap" was prepared by passing the adult rat liver post-microsomal supernatant through Sephadex G-25 equilibrated with 2 mM EDTA-25 mM Tris-HCl, pH 7.6.

Other methods are given in the legends.

RESULTS

As shown in Fig. 1, the *in vitro* autodegradation of fetal rat liver ribosomal RNA occurred depending on EDTA. No degradation was observed when the incubation mixture contained Mg^{2+} even after prolonged incubation time. With the increase of the EDTA concentration, minor fragments appeared and amounts of them increased except fragment X (Fig. 1e). The fragment X appeared when the EDTA concentration was between 1 and 5 mM and disappeared when the concentration of EDTA was 10 mM. The degradation did not occur at 0 °C

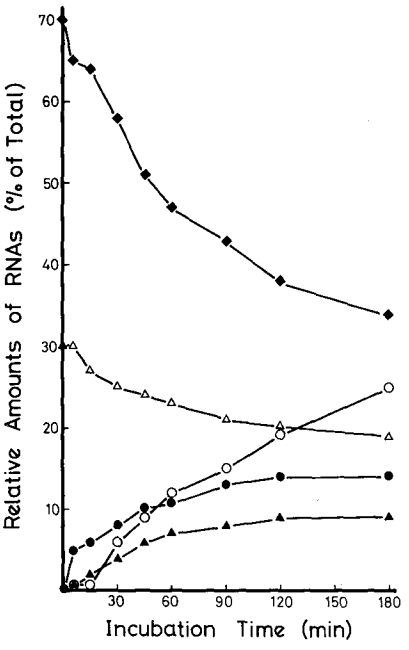

Fig. 2. Time course of the *in vitro* autodegradation of fetal rat liver ribosomal RNA. Fetal rat liver polysomes were incubated in a buffer containing 25 mM Tris-HCl, pH 7.6 and 2 mM EDTA at 37 °C. At the indicated time, reaction was stopped by the addition of SDS and chilling, and RNA was analyzed as described in the legend to Fig. 1. Quantitative determination of each separated RNA was performed as described previously (7).
◆——◆, 28S+X; △——△, 18S; ▲——▲, 1+2; ●——●, a+b+c+d; ○——○, 4+5+6+7.

with any of the concentration of EDTA tested.

The apparent molecular weights of the fragments designated as shown in Fig. 1e and 1h are compared in Table I with those of the four minor RNA components which are thought to be *in vivo* degradation intermediates of 28S ribosomal RNA. As seen from this table, the molecular weights of the fragments a, b, c and d, which were produced with *in vitro* autodegradation of ribosomes, were very similar, if not identical, to those of *in vivo* degradation intermediates.

The time course of the *in vitro* autodegradation is shown in Fig. 2. The relative amounts of the fragments whose molecular weights are between 28S and 18S ribosomal RNA reached plateau within a fairly short period of

Table I. Apparent molecular weights of the RNAs produced with *in vitro* autodegradation of fetal rat liver ribosomal RNA

RNA species	Molecular Weights ($\times 10^{-5}$)	
	in vitro products	*in vivo* products
28S	17.5	17.5
X	16.4 ± 0.08	
1	14.1 ± 0.20	
2	13.1 ± 0.26	
a	11.5 ± 0.20	11.4 ± 0.15
b	10.5 ± 0.08	10.4 ± 0.13
c	9.42 ± 0.16	9.32 ± 0.12
d	8.68 ± 0.13	8.48 ± 0.15
18S	7.00	7.00
3	6.70 ± 0.05	
4	5.70 ± 0.07	
5	5.28 ± 0.08	
6	4.09 ± 0.08	
7	3.59 ± 0.05	

The molecular weight of each RNA species was estimated by assuming that the electrophoretic mobility is inversely related to the logarithm of the molecular weight (8, 9); the molecular weights of the two rat liver ribosomal RNAs were used as references (10). Each value is representative of three to five individual determinations.

Table II. Release of acid soluble materials during the *in vitro* autodegradation of fetal rat liver ribosomal RNA

Incubation Time (min)	Acid Soluble Materials Released	
	$A_{260\ nm}$ unit	% of input
0	0	0
30	0.126	1.04
60	0.162	1.33
90	0.162	1.33

Fetal rat liver polysomes (12.15 $A_{260\ nm}$ units) were incubated in 0.5 ml of a buffer containing 2 mM EDTA and 25 mM Tris-HCl, pH 7.6 at 37 °C. At the indicated time, reaction was stopped by the addition of 3 volumes of 6.67 % perchloric acid, and amount of UV-absorbing materials in the acid soluble fraction was determined. The value obtained from the sample without the incubation was regarded as zero.

incubation, while those of the fragments smaller than 18S ribosomal RNA increased continuously after a short lag.

These results indicate that our preparation of fetal rat liver polysomes have ribonucleases which cleave ribosomal RNA into specific fragments including those similar to *in vivo* degradation intermediates.

There are many reports describing ribosome-bound ribonucleases in various types of cells (11-17), although it is not clear whether the ribonucleases are adsorbed contaminants or structural protein(s) of ribosomes. We therefore examined whether the autodegradation activity of the fetal rat liver polysomes was removed by washing the particles with a high salt buffer (500 mM KCl; 10 mM $KHCO_3$; 5 mM $MgCl_2$; 50 mM Tris-HCl, pH 7.6), since the treatment is said to remove the loosely bound proteins from the particles (18, 19). Gel electrophoretical pattern of RNA of washed polysomes after the incubation in the buffer containing 2 mM EDTA was indistinguishable from that of non-treated polysomes (data not shown), suggesting that the ribonucleases bound to the fetal rat liver polysomes were very tightly integrated to ribosomes or they were structural proteins of ribosomes.

Recently, Apirion proposed a hypothesis that degradation of cellular RNA was started by endonucleolytic cleavages which were followed by exonucleolytic digestion (20). We therefore tested whether the fragments derived *in vitro* were produced endonucleolytically or exonucleolytically. The data presented in Table II suggest that there had occurred little exonucleolytic digestion, since very little acid soluble UV-absorbing material was released after the incubation for 90 min when about one-third of 28S and 18S RNA was degraded as shown in Fig. 2.

Ceri and Maeba (17) examined the effect of certain reagents on the *in vitro* autodegradation of *E. coli* ribosomal RNA and found that SDS and the SH-reagent $HgCl_2$ inhibited the autodegradation completely. We also examined the effect of some reagents including liver "cell sap" on the autodegradation of fetal rat liver ribosomal RNA. As shown in Fig. 3, SDS inhibited completely

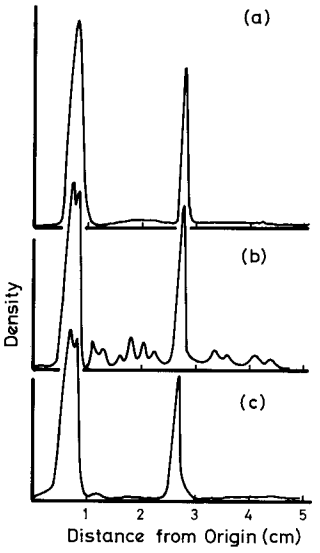

Fig. 3. Effect of inhibitors on the *in vitro* autodegradation of fetal rat liver ribosomal RNA. Fetal rat liver polysomes were incubated in the buffer containing 2 mM EDTA as described in the legend to Fig. 1 in the presence of (a) 2 % SDS, (b) 10^{-5} M *p*CMB or (c) "cell sap" equivalent to 0.28 mg of protein.

the degradation of ribosomal RNA (Fig. 3a), however, unlike the case of *E. coli* ribosomes, *p*CMB, a well known SH-reagent, had no effect on the degradation (Fig. 3b). Rat liver "cell sap" inhibited the formation of the fragments except "X" (Fig. 3c). The inhibitory effect of the "cell sap" may be ascribed to the cytoplasmic ribonuclease inhibitor of liver cells (21).

The properties of the fragment X remain vague, however, it should be produced by a mechanism different from that concerned with formation of other fragments.

DISCUSSION

While there is a wealth of information, at present, on the synthesis of ribosomal RNA in animal cells (see ref. 22, for review), comparatively little is known about the degradation of the RNA. Many kinds of ribonucleases are said to be present and they may play specific roles in the metabolism of RNA in the cells (23). There have been many reports which assert that certain preparation of ribosomes had activities to hydrolize their own ribosomal RNA

(11, 12, 15, 17) or exogenously added RNA (13, 14) when incubated *in vitro* under appropriate conditions. Little is known, however, whether the ribonucleases contained in ribosomal preparations are responsible for the degradation of ribosomal RNA *in vivo*.

As described in this communication, the ribonuclease bound to the polysomes of fetal rat liver is endoribonuclease which is stimulated by EDTA and is inhibited by SDS or liver "cell sap". It is not clear whether the stimulative effect of EDTA on the enzyme is a result of the action on the enzyme itself or the alteration of the ribosomal structure or both. The similarity between the molecular weights of the fragments produced during the *in vitro* autodegradation and those of *in vivo* degradation intermediates suggests that the ribonucleases bound to fetal rat liver polysomes may play some roles in the degradation of ribosomal RNA *in vivo* and that the particle itself has a machinary by which its own ribosomal RNA is degraded. However, the facts that fetal rat liver polysomes contained no *in vivo* degradation intermediates (7) and that there was no increase in the amounts of these intermediates when adult rat liver polysomes were incubated in the presence of 2 mM EDTA (data not shown) indicate that the expression of the ribosome-bound ribonuclease in the liver cells is controlled by some unknown mechanism.

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