Quantitative alterations in the nucleolar and nucleoplasmic ribosomal ribonucleic acids in regenerating rat liver

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A quantitative analysis of the nuclear pre-rRNA (precursor to rRNA) and rRNA in normal and 12h-regenerating rat liver was carried out, and the absolute amounts of the identified pre-rRNA and rRNA species in the nucleolus and nucleoplasm were determined. Characteristic changes in the pre-rRNA and rRNA pool sizes in regenerating liver are found which reveal alterations in both pre-rRNA processing and nucleocytoplasmic transition of ribosomes.

The regenerating liver is a good model system for analysis of the control mechanisms of ribosome biogenesis. The dynamics of the morphological, ultrastructural and biochemical changes in the liver induced to proliferate after partial hepatectomy have been discussed in several reviews (Bucher & Malt, 1971; Bresnik, 1971; Tsanev, 1975; Lewan *et al.*, 1977). Soon after partial hepatectomy a gradual increase of ribosome production takes place, reaching a maximum 12–18h post-operatively (Bucher & Malt, 1971). The amount of nuclear and cytoplasmic RNA increases (Tsanev & Markov, 1964). Accumulation of nucleolar RNA has been also observed (Tsukada & Lieberman, 1964; Muramatsu & Busch, 1965). Since the changes in RNA content of the different cellular compartments are directly coupled with the increased production of ribosomes in regenerating liver, their precise quantitative evaluation is significant for understanding ribosome biogenesis. The lack of quantitative data on the pool sizes of nucleolar and nucleoplasmic pre-rRNA and rRNA species has impeded so far the assessment of the rates of synthesis, maturation and nucleocytoplasmic transition of ribosomes in regenerating rat liver and, as a consequence, the study of the control mechanisms operating in the nucleus.

In the present work a quantitative analysis of nucleolar and nucleoplasmic pre-rRNA was carried out in normal and 12h-regenerating liver. A new correlation between the pool sizes of nuclear pre-rRNA and rRNA in regenerating liver was found. The results reveal alterations in both maturation and nucleocytoplasmic transition of ribosomes.

Abbreviations used: pre-rRNA, precursor of rRNA; npRNA, nucleoplasmic rRNA; nuRNA, nucleolar rRNA.

Experimental

Animals

The experiments were carried out with Wistar male albino rats weighing 150 ± 2 g. The animals were partially hepatectomized, with removal of approx. 68% of the liver (Higgins & Anderson, 1931). Control (normal) and operated animals were maintained on 5% (w/v) glucose. The animals were killed 12h after the operation, between 09:00 and 11:00h. In each experiment 7 normal and 21 partially hepatectomized rats were used.

Isolation of nuclei and fractional extraction of rRNA

Pure rat liver nuclei were isolated by the two-step hyperosmotic-sucrose/detergent method (Dabeva *et al.*, 1978).

Nucleoplasmic and nucleolar rRNA were extracted by subsequent treatment of nuclei with phenol at 4°C and 50°C as described by Dabeva *et al.* (1976), and the RNA fractions obtained are designated npRNA and nuRNA respectively.

Chemical analyses

The amounts of RNA and DNA were determined by the two-wavelength method (Tsanev & Markov, 1960) after 18h hydrolysis at 37°C with 0.3M-KOH. RNA in npRNA or nuRNA fractions was precipitated quantitatively (Dessev & Grancharov, 1973) and washed with diethyl ether/ethanol (1:1, v/v) and then ether to remove residual phenol before hydrolysis. All the results were normalized to the DNA content in the same nuclear suspension taken for extraction of RNA.

Determination of the pool sizes of pre-rRNA and rRNA

The npRNA and nuRNA were analysed by urea/agar-gel electrophoresis (Dudov *et al.*, 1976). The relative amount of RNA in each electrophoretic fraction was determined by cutting out and weighing the separate peaks from the electrophoretograms recorded at 260 nm. The pool sizes of pre-rRNA or rRNA were calculated from the relative amounts obtained and the total RNA content of npRNA or nuRNA. 32S and 21S pre-rRNA recovered in npRNA was added to the amount of nucleolar 32S pre-rRNA, respectively (see Dabeva *et al.*, 1978). All the pool sizes of nuclear 28S and 18S rRNA were corrected for cytoplasmic contaminants (Dabeva *et al.*, 1977).

Results and discussion

Changes in cellular and nuclear RNA content

Our analyses were done 12 h after partial hepatectomy, before onset of DNA synthesis (see Bucher & Malt, 1971), therefore the amount of RNA

measured in relation to DNA gives reliable evaluation of the changes in RNA pool sizes taking place in regenerating rat liver.

As shown in Table 1, 12 h after partial hepatectomy the amount of total cellular RNA increases, to 41 pg/cell. Since 95% of the cellular RNA is cytoplasmic and 82% of it is rRNA, the concentration of ribosomes per cell increases from 6.5×10^6 in normal liver to 8.4×10^6 in 12 h-regenerating liver. The increment of cellular RNA is accompanied by even faster accumulation of RNA in the nucleus of regenerating liver.

Distribution of rRNA in the nuclear compartments of regenerating rat liver

According to its electrophoretic behaviour, RNA recovered in the nuRNA and npRNA fractions was distributed into three main groups: (a) identified pre-rRNA and rRNA; (b) low-molecular weight 4–8S RNA (lmwRNA); and (c) other rRNA (Fig. 1). The analysis of the last fraction in formamide/acrylamide gels revealed that most of the RNA components in the zone 28S–8S are products of 28S-rRNA degradation (results not shown).

Table 2 shows that the nucleus of regenerating liver contains 1.48 pg of rRNA, which constitutes 43% of the total nuclear RNA: 34% of it are the identified pre-rRNA and rRNA species and the other 9% are products of rRNA degradation. About 75% of nuclear rRNA is localized in the nucleolus. Approx. 45% of the total nuclear RNA is heterogeneous nuclear RNA, which remains in the nucleus after extraction of npRNA and nuRNA. Its amount is 1.23 and 1.55 pg/nucleus in normal and regenerating liver, respectively.

The data show also that the increased amount of nuclear RNA in 12 h-regenerating liver is mainly due to accumulation of pre-rRNA in the nucleolus (78%). The contribution of the heterogeneous nuclear RNA to this increment is significantly less.

Table 1. Amount of RNA in rat liver cells and nuclei after partial hepatectomy

The amounts of RNA and DNA in liver homogenates and detergent-purified nuclei from normal and 12 h-regenerating rat liver were determined by chemical analysis (see the Experimental section). The amount of DNA is estimated as 12 pg/nucleus. The results are given as means $\pm$ s.d. for eight experiments.

Liver	RNA (pg/nucleus)	
	Total cellular	Total nuclear
Normal	32 ± 2	2.5 ± 0.2
Regenerating	41 ± 2	3.4 ± 0.3
Accumulation (%)	29	35

Table 2. Amount of rRNA in the nucleolus and nucleoplasm of normal and regenerating rat liver

The amounts of RNA recovered in the nuRNA and npRNA fractions were determined by chemical analysis. The amounts of identified pre-rRNA and rRNA (distributed in the peaks of pre-rRNA and rRNA) and of low-molecular-weight nuclear RNA (4–8S) are derived from the urea/agar-gel electrophoretograms recorded at 260 nm (see the Experimental section). The values in parentheses represent the percentage of total nuclear RNA. The mean values $\pm$ s.d. from seven independent experiments with 21 electrophoretograms for each nuclear RNA fraction are given.

Nuclear RNA fraction		Amount of RNA (pg/nucleus)			
		Total RNA in fraction	Identified pre-rRNA and rRNA	Low-molecular-weight RNA	Other rRNA
nuRNA	Normal	0.79 ± 0.08 (32)	0.51 ± 0.03 (21)	0.12 ± 0.01 (5)	0.16 ± 0.02 (6)
	Regenerating	1.28 ± 0.12 (38)	0.91 ± 0.09 (27)	0.18 ± 0.02 (5)	0.19 ± 0.02 (5)
npRNA	Normal	0.48 ± 0.09 (19)	0.21 ± 0.04 (8)	0.16 ± 0.03 (6)	0.11 ± 0.01 (4)
	Regenerating	0.57 ± 0.06 (17)	0.24 ± 0.03 (7)	0.19 ± 0.04 (6)	0.14 ± 0.02 (4)

Fig. 1. Urea/agar-gel electrophoresis of nucleolar and nucleoplasmic RNA from normal and regenerating rat liver. The nuRNA (a and b) and npRNA (c and d) fractions were extracted from detergent-purified nuclei of normal (a and c) and 12h-regenerating (b and d) liver as described in the Experimental section. The electrophoretic fractionation was carried out in 5M-urea/1.5% agar gel at 8°C for 4.5h at 4V/cm. Abbreviation: lmw, low-molecular-weight RNA.

The augmentation of nucleoplasmic rRNA species is negligible, only 14%.

Alterations in the pool sizes of pre-rRNA and rRNA in the nucleolus and nucleoplasm of regenerating rat liver

As shown in Fig. 1, the nuRNA of regenerating rat liver contains the previously identified pre-rRNA species (Dabeva *et al.*, 1976), 28S and 18S rRNA. On the other hand, some qualitative and quantitative changes in the pre-rRNA of regenerating liver are clearly visible: (a) absence of the minor 39S pre-rRNA; (b) predominance of 32S pre-rRNA and relative decrease of 21S pre-rRNA.

The electrophoretic patterns of npRNA from normal and 12h-regenerating rat liver are alike. They contain mainly 28S and 18S rRNA and small

amounts of 32S and 21S pre-rRNA. The last two components belong to the nucleolar compartment of the nucleus (Dabeva *et al.*, 1978).

The advantages of the techniques for quantitative extraction and fractionation of nuclear rRNA as well as the negligible cross-contamination between the RNA peaks in urea/agar-gel electrophoresis permit the quantitative evaluation of the changes in the pool sizes of all the pre-rRNA and rRNA species in the nucleolus and nucleoplasm. The data show (Table 3) that 12h after partial hepatectomy a new characteristic quantitative correlation between the pre-rRNA and rRNA species in the nucleolus is established. (1) The absolute pool sizes of all the pre-rRNA species are augmented. The increment of the pool sizes of 45S and 36S pre-rRNA is about 100%, that of 41S pre-rRNA 80% and that of 32S

Table 3. *Relative distribution and pool sizes of pre-rRNA and rRNA in the nucleolus and nucleoplasm of normal and regenerating rat liver*

The nuRNA and npRNA fractions from normal and 12h-regenerating rat liver were analysed by urea/agar-gel electrophoresis. The relative distribution of the pre-rRNA and rRNA components is determined from the electrophoretograms recorded at 260nm. The pool sizes of pre-rRNA and rRNA were calculated from the results given in Table 2 and from their molecular weights (Dudov *et al.*, 1976). The sum of the identified pre-rRNA and rRNA species in nuRNA or npRNA is taken as 100%. The results from 21 experiments are given as means $\pm$ s.d.

rRNA species	A_{260} (%)		$10^{-4} \times$ No. of molecules/nucleus		
	Normal liver	Regenerating liver	Normal liver	Regenerating liver	Accumulation (%)
Nucleoli					
45S	17.4 $\pm$ 3.8	20.2 $\pm$ 4.4	1.2 $\pm$ 0.2	2.3 $\pm$ 0.3	103
41S	7.5 $\pm$ 2.3	7.5 $\pm$ 2.8	0.7 $\pm$ 0.2	1.2 $\pm$ 0.4	79
39S	2.5 $\pm$ 0.8	—	0.3 $\pm$ 0.1	—	—
36S	10.2 $\pm$ 1.8	12.5 $\pm$ 2.0	1.2 $\pm$ 0.2	2.5 $\pm$ 0.3	117
32S	20.5 $\pm$ 3.3	27.8 $\pm$ 3.6	2.9 $\pm$ 0.4	6.9 $\pm$ 0.6	141
21S	8.7 $\pm$ 2.8	5.6 $\pm$ 2.7	2.5 $\pm$ 0.6	2.9 $\pm$ 0.7	16
28S	23.6 $\pm$ 3.4	19.8 $\pm$ 3.0	4.1 $\pm$ 0.7	6.2 $\pm$ 1.1	50
18S	9.4 $\pm$ 2.4	6.4 $\pm$ 2.1	4.2 $\pm$ 1.0	5.1 $\pm$ 1.2	21
Nucleoplasm					
28S	77 $\pm$ 11	80 $\pm$ 10	5.7 $\pm$ 1.5	6.5 $\pm$ 1.4	15
18S	23 $\pm$ 5	20 $\pm$ 5	4.3 $\pm$ 1.6	4.1 $\pm$ 1.6	-5

pre-rRNA about 140%. Hence, 32S pre-rRNA becomes the most prominent fraction in the nucleolar rRNA, constituting 28% of it. (2) Although the amount of 32S pre-rRNA, the immediate precursor to 28S rRNA, is most increased, that of 21S pre-rRNA, the immediate precursor to 18S rRNA, is not much affected. So the 32S/21S pre-rRNA molar ratio increases from 1.2 in normal liver to 2.4 in regenerating liver. (3) The augmentation of rRNA species in the nucleolus is less pronounced. The 28S/18S rRNA molar ratio increases from 1.0 in normal liver to 1.2 in regenerating liver. (4) One of the minor pre-rRNA components, previously designated 39S (Dabeva *et al.*, 1976), is absent from the pre-rRNA species of regenerating liver. It has been suggested that this pre-rRNA fraction (mol.wt. 2.96×10^6) is a product of direct cleavage of 45S pre-rRNA (Dudov *et al.*, 1978). Whereas in normal rat liver 39S pre-rRNA is not always clearly delineated, in rat brain it constitutes a distinct fraction (Stoykova *et al.*, 1979). After cycloheximide block of protein synthesis, 39S pre-rRNA accumulates severalfold in the nucleolus (Stoyanova & Hadjiolov, 1979; Stoyanova *et al.*, 1980). Obviously, the processing of primary 45S pre-rRNA via 39S pre-rRNA is characteristic for tissues with decreased rate of ribosome production and does not lead to formation of mature ribosomes. During liver regeneration this processing pathway is shut off.

In contrast with the profound quantitative alterations in the nucleolus, the amount of nucleoplasmic 28S rRNA is only slightly increased. No changes in 18S rRNA content can be detected. These results show that the limiting factor in

ribosome biogenesis in the nucleus of regenerating liver is the formation of the ribosomal subparticles in the nucleolus, which involves specific rRNA-protein interactions, chemical modifications and endonuclease processing. Most likely, not all of these processes are activated to the same degree, leading to accumulation of pre-rRNA. On the other hand, the unequal increment of the pool sizes of pre-rRNA species reveals essential changes in the pre-rRNA processing pathways. The 28S/18S rRNA molar ratio in the nucleus increases from 1.1 in normal liver to 1.4 in regenerating liver. This fact suggests further that the nucleo-cytoplasmic transition of the two ribosomal subparticles in regenerating liver is activated to different degrees.

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