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Quantitative Analysis of Rat Liver Nucleolar and Nucleoplasmic Ribosomal Ribonucleic Acids

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rRNA from detergent-purified nuclei was fractionated quantitatively, by two independent methods, into nucleolar and nucleoplasmic RNA fractions. The two RNA fractions were analysed by urea/agar-gel electrophoresis and the amount of pre-rRNA (precursor of rRNA) and rRNA components was determined. The rRNA constitutes 35% of total nuclear RNA, of which two-thirds are in nucleolar RNA and one-third in nucleoplasmic RNA. The identified pre-rRNA components (45 S, 41 S, 39 S, 36 S, 32 S and 21 S) are confined to the nucleolus and constitute about 70% of its rRNA. The remaining 30% are represented by 28 S and 18 S rRNA, in a molar ratio of 1.4. The bulk of rRNA in nucleoplasmic RNA is represented by 28 S and 18 S rRNA in a molar ratio close to 1.0. Part of the mature rRNA species in nucleoplasmic RNA originate from ribosomes attached to the outer nuclear membrane, which resist detergent treatment. The absolute amount of nuclear pre-rRNA and rRNA components was evaluated. The amount of 32 S and 21 S pre-rRNA (2.9×10^4 and 2.5×10^4 molecules per nucleus respectively) is 2–3-fold higher than that of 45 S, 41 S and 36 S pre-rRNA.

Recent evidence reveals that post-transcriptional regulatory mechanisms may play an important role in the control of ribosome biogenesis in eukaryotes (see Warner, 1974; Hadjiolov & Nikolaev, 1976). Yet these mechanisms, in particular the intranucleolar formation of ribosomes and their migration through the nucleolus, nucleoplasm and cytoplasm, have not been clarified (see Hadjiolov & Nikolaev, 1976). A major obstacle to such studies is the limited information on the amount of pre-rRNA and rRNA species (and their respective ribonucleoprotein particles) in the nucleolus and nucleoplasm compartments of the nucleus (see Busch & Smetana, 1970). Several factors, e.g. contamination of nuclei by cytoplasmic ribosomes (Penman, 1966; Sadowski & Howden, 1968; Whittle *et al.*, 1968; Smith *et al.*, 1969), leakage of nuclear components during isolation and detergent purification of nuclei (Penman *et al.*, 1966; Chatterjee & Weissbach, 1973; Dessev *et al.*, 1973), cross-contamination between the nucleolus and nucleoplasm compartments (see Loening & Baker, 1976), interference by highly labelled heterogeneous nuclear RNA (Penman *et al.*, 1966; Weinberg & Penman, 1968), degradation of RNA (Hadjiolov *et al.*, 1965; Dabeva & Tsanev, 1966; see Bramwell, 1976) and others, contribute to make uncertain the quantitative analyses of intranuclear compartmentation of rRNA species. In the present work a quantitative analysis of the intranuclear compartmentation of liver pre-rRNA and rRNA species was carried out under conditions in which interference by the above factors was minimized. The results obtained reveal a characteristic pattern in the compartmentation of nuclear pre-rRNA and rRNA, which may serve as a basis for the analysis of the formation and the fate of ribosomes in hepatocytes.

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

Experimental

Isolation of nuclei

Pure rat liver nuclei were isolated by a two-step hyperosmotic-sucrose/detergent procedure based on the original method of Chauveau *et al.* (1956). The nuclear fraction obtained at the first step of this procedure was purified by treatment with Triton X-100.

The experimental animals, weighing about 150g, were killed by cervical dislocation, the livers dissected out, rinsed in cold 0.9% NaCl and immediately processed further at 4°C. Livers (10g) were homogenized in 20ml of 2.3M-sucrose in TM buffer

[0.01 M-Tris/HCl (pH 7.0)/0.01 M-$MgCl_2$] with a glass/Teflon Potter–Elvehjem homogenizer. The homogenate was filtered through nylon bolting cloth (200 mesh), adjusted to 30ml with the same sucrose solution and mixed. It was then layered over 8ml of buffered 2.3 M-sucrose (as above) and centrifuged at 0°C for 30min at 25000rev./min in the SW 27 rotor of a Beckman L5-50 ultracentrifuge. The supernatant was aspirated, the tube walls were cleaned and the nuclear pellet was rinsed with 0.25 M-sucrose in TM buffer.

The nuclear pellet (from 10g of liver) was suspended in 20ml of 0.5 M-sucrose in TM buffer plus 0.1% Triton X-100, by four strokes of a loose-fitting Potter–Elvehjem homogenizer. The suspension was layered over 10ml of 1 M-sucrose in TM buffer (without Triton X-100) and centrifuged for 5min at 2000*g* in the cold to obtain detergent-purified nuclei. In experiments where pool sizes of pre-rRNA were determined, detergent purification of nuclei was carried out in the presence of 0.1mg of dextran sulphate/ml to minimize degradation or processing of pre-rRNA (Dabeva *et al.*, 1976*c*).

Nuclei isolated at low ionic strength by the above method do not cluster and are easily suspended in 0.25 M-sucrose, which permits their accurate counting in a haemocytometer.

Isolation of nucleoli

Nuclei from 5g of liver were suspended in 10ml of 0.5 M-sucrose, containing 0.01 M-Tris/HCl (pH 7.0), 1 mM-$MgCl_2$ and 0.1mg of dextran sulphate/ml. The nuclei were disintegrated by sonication for 15s in an MSE 60 W ultrasonic disintegrator. Under these conditions they were completely disintegrated, whereas nucleoli were stabilized by the presence of Mg^{2+} (Higashinakagawa *et al.*, 1972). The suspension was layered over 10ml of 1 M-sucrose (in the same medium) and centrifuged for 10min at 10000*g*. The sediment constitutes the nucleolar fraction, and the supernatant is the 'nucleoplasmic' fraction.

Isolation and fractionation of nuclear RNA

Fractional extraction of RNA species from purified nuclei was achieved by sequential treatment with phenol at different temperatures (Hadjiolov *et al.*, 1974; Dabeva *et al.*, 1976*a*). The extraction was carried out with a 1:1 (v/v) mixture of 0.1 M-Tris/acetate buffer (pH 5.2) and phenol, saturated with the same buffer and containing 0.1% 8-hydroxyquinoline. The first extraction step was carried out for 20min at 4°C and the RNA obtained is designated 4°C npRNA. In the second step, the resulting interphase layer was extracted for 15min at 50°C and the RNA fraction obtained is designated 50°C nuRNA. To obtain all the rRNA of the nucleus quantitatively, the 50°C extraction was repeated.

The nucleoli obtained from 10g of liver were suspended in 10ml of a 1:1 (v/v) mixture of 0.1 M-Tris/acetate (pH 5.0) and phenol, saturated with 0.1 M-sodium acetate (pH 5.0), containing 0.1% 8-hydroxyquinoline. The mixture was homogenized in a loose homogenizer and the extraction of RNA carried out as above for 15min at 50°C. The total nucleolar RNA extracted in the water phase constitutes the nuRNA fraction.

The 'nucleoplasmic' fraction (see above) was mixed with 0.1vol. of 1.0 M-sodium acetate (pH 5.0) and 0.33vol. of 0.1 M-Tris/acetate (pH 5.0). This addition minimizes further extraction of DNA and heterogeneous nuclear RNA. The suspension was mixed with an equal volume of phenol (as above) and the RNA was extracted for 20min at 4°C. The RNA obtained in the water phase constitutes the npRNA fraction.

The RNA fractions extracted in the water phase were further deproteinized by three successive treatments with phenol in the presence of 0.5% sodium dodecyl sulphate (not added to npRNA), then phenol/chloroform (1:1, v/v) and finally chloroform, and the RNA from the final water phase was precipitated with 2vol. of 96% (v/v) ethanol/1% potassium acetate at −10°C overnight. The precipitate was collected by centrifugation for 15–20min at 5000*g*, dissolved in a small volume of 0.01 M-NaCl/0.01 M-EDTA and reprecipitated with 96% ethanol. The RNA fractions were stored under ethanol at −20°C. The npRNA fraction was purified further by treatment with deoxyribonuclease I and passage through a Dowex 1 (formate form) column (Mackedonski *et al.*, 1972).

Contamination by cytoplasmic rRNA

Contamination of detergent-purified nuclei by cytoplasmic rRNA was evaluated by carrying out the liver-homogenization step in the presence of a long-term labelled cytoplasmic fraction and determining the radioactivity present in npRNA and nuRNA fractions (Dabeva *et al.*, 1977). This technique gives lower estimates for contamination by cytoplasmic rRNA, since the ribosomes attached *in situ* to the outer nuclear membrane are not expected to be labelled.

Urea/agar-gel electrophoresis of RNA

This was carried out by the method of Dudov *et al.* (1976). Since the relation concentration/A_{260} for RNA in dried agar films follows the Lambert–Beer law (Tsanev & Staynov, 1964), the amount of RNA in each fraction is given by the enclosed area of the respective peak. This amount was determined by

recording the A_{260} of the electrophoretograms on Bristol board, cutting out the separate peaks and weighing each peak with an accuracy of 0.1 mg. Alternatively, the area of each peak was calculated by using the formula for the area of an isosceles triangle.

Labelling experiments

To study the labelling of the separate nuclear RNA fractions, the RNA was labelled *in vivo* by intraperitoneal injection of 25 μCi of [^{14}C]orotate (sp. radioactivity 18.94 mCi/mmol) and/or 50 μCi of [^{3}H]orotate (sp. radioactivity 7 Ci/mmol). For the determination of specific radioactivity see the following paper (Dudov *et al.*, 1978).

Chemical analyses

The amount of RNA and DNA was determined by the two-wavelength method of Tsanev & Markov (1960).

Materials

Analytical-grade reagents were used throughout. Dextran sulphate (mol.wt. 5×10^5) was from Pharmacia, Uppsala, Sweden. Triton X-100 was from BDH Chemicals, Poole, Dorset, U.K.; PPO (2,5-diphenyloxazole), dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] and agar no. 1 were from Koch–Light Laboratories, Colnbrook, Bucks., U.K.; [^{14}C]- and [^{3}H]-orotate were from NAEC Institute for Isotopes, Budapest, Hungary.

Results

General characteristics of detergent-purified nuclei

The integrity and purity of isolated nuclei is a basic requirement in studies on rRNA compartmentation. In this work we use rat liver nuclei isolated by a hyperosmotic-sucrose technique and purified further by Triton X-100 treatment. Electron-microscopic characterization of these nuclei reveals that Triton X-100 treatment completely removes both nuclear membranes, but the integrity of nuclei is preserved (Sadowski & Steiner, 1968; Laval & Bouteille, 1973; Aaronson & Blobel, 1974; Dabeva *et al.*, 1976*a*, 1977). Analysis of the labelling of the RNA removed by Triton X-100 treatment of nuclei (Table 1) shows that it is about 3-fold lower than that of nucleoplasmic rRNA extracted from detergent-purified nuclei. Both findings indicate the absence of appreciable leakage of nuclear rRNA during the procedure of isolation and detergent treatment of nuclei. The RNA/DNA ratio of liver nuclei, isolated by our procedure (eight experiments, mean $\pm$ S.D.), is 0.205 ± 0.004 before and 0.195 ± 0.006 after Triton X-100 treatment, showing the removal of about 5% of total nuclear RNA, a value in good agreement with previous studies (Whittle *et al.*, 1968; Tata & Baker, 1974). Although by both electron-microscopic and chemical criteria detergent-purified rat liver nuclei appear free of contamination by cytoplasmic ribosomes, we have shown (Dabeva *et al.*, 1977) that some of the ribosomes associated with the nuclear membrane still contaminate this nuclear fraction. Therefore we consider our nucleoplasmic rRNA fraction as consisting of (*a*) true intranuclear rRNA and (*b*) rRNA from ribosomes associated with the outer nuclear-membrane, adhering to nuclei after detergent purification. The attempted determination of these two pools of nucleoplasmic rRNA is described in this and the following paper (Dudov *et al.*, 1978).

Table 1. *Labelling of RNA associated with the outer nuclear membrane of rat liver nuclei*

The RNA from the outer nuclear membrane (RNA_{0nm}) remaining in the supernatant after treatment of isolated nuclei with 0.1% Triton X-100 is precipitated and its amount and radioactivity determined. The specific radioactivity of 4°C npRNA and total nuclear RNA obtained from the nuclei after detergent purification is given for comparison. The RNA in each experiment is from three rats and is labelled *in vivo* with 10 μCi of [^{14}C]orotate for 120 min.

Expt. no.	$10^{-3}\times$ Specific radioactivity (c.p.m./mg of RNA)		
	RNA_{0nm}	4°C npRNA	Total nuclear RNA
1	11.0	64.0	303.1
2	24.5	63.4	301.0
3	14.5	63.4	280.7
4	21.0	53.1	259.0
Average	17.7	61.0	286.0

rRNA components in the nucleolar and nucleoplasmic compartments of the nucleus

The quantitative analysis of RNA components in the nucleolar and nucleoplasmic compartments of the nucleus depends critically on the separation methods used. Therefore we carried out comparative studies by two independent techniques: (*a*) isolation of nucleoli and selective extraction of rRNA from the nucleolar (nuRNA) and nucleoplasmic (npRNA) fractions obtained and (*b*) differential extraction of rRNA by subsequent treatment of nuclei with phenol at 4°C (4°C npRNA) and 50°C (50°C nuRNA).

Electron microscopy of isolated nucleoli revealed that they display a characteristic structural pattern, but are contaminated by disrupted chromatin material, because, in order to minimize leakage of nucleolar components, we used milder conditions of isolation. Since the observed chromatin contaminants

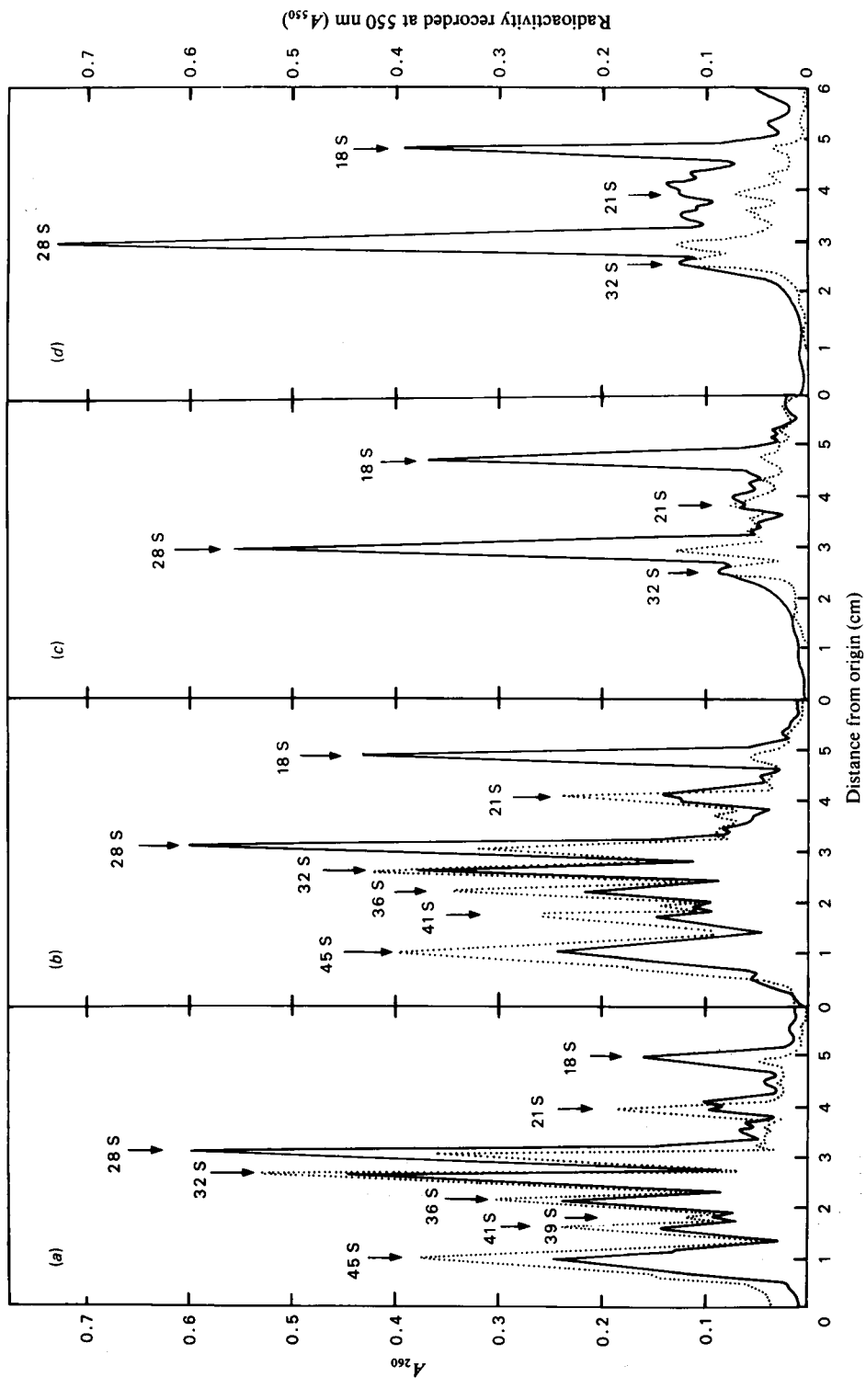

Fig. 1. *Urea/agar-gel-electrophoresis pattern of pre-rRNA and rRNA components in nucleolar and nucleoplasmic RNA fractions*
RNA is labelled *in vivo* for 60 min with 25 μCi of [^{14}C]orotate/100 g body wt. Nuclei are isolated and purified by Triton X-100 treatment. Nucleolar [nuRNA (*a*) and 50°C nuRNA (*b*)] and nucleoplasmic [npRNA (*c*) and 4°C npRNA (*d*)] RNA fractions are extracted from detergent-purified nuclei as described in the Experimental section. ——, A_{260}; · · · ·, radioactivity recorded from the radioautogram at 550 nm after 1 day (*a* and *b*) or 6 days (*c* and *d*) exposure.

are unlikely to contain a substantial amount of rRNA species, most of the RNA extracted from isolated nucleoli may be considered truly nucleolar. This is evidenced by the urea/agar-gel electrophoresis pattern of the nuRNA fraction (Fig. 1*a*), characterized by the presence of all known liver pre-rRNA species (Dabeva *et al.*, 1976*b*) and a noticeable preponderance of 28 over 18 S rRNA. The urea/agar-gel electrophoresis pattern of the rRNA extracted from the separated nucleoplasmic fraction (Fig. 1*c*) shows that most of the rRNA in this compartment is 28 and 18 S rRNA, although small amounts of 32 and 21 S pre-rRNA are also present.

The urea/agar-gel electrophoresis patterns of the 50°C nuRNA and 4°C npRNA fractions shown in Figs. 1(*b*) and 1(*d*) reveal that the 50°C nuRNA fraction contains all pre-rRNA species plus mature 28 and 18 S rRNA and corresponds to nuRNA, whereas the 4°C npRNA fraction contains mainly 28 and 18 S rRNA and corresponds to npRNA. The only noticeable difference between the two nuclear-fractionation techniques is the markedly higher peak of 18 S rRNA in 50°C nuRNA, as compared with nuRNA, a finding most likely reflecting the incomplete extraction of 18 S rRNA at 4°C and low pH (Dabeva, 1976).

The high relative amount of pre-rRNA species in both nucleolar RNA fractions strongly suggests that the hazard of pre-rRNA processing and degradation during isolation and fractionation of nuclei (Vesco & Penman, 1968) is minimized. Also, fractionation of nuclear RNA by phenol treatment offers the advantage of a quantitative recovery of pre-rRNA and rRNA species in the 4°C npRNA and 50°C nuRNA fractions and the virtual absence of contamination by heterogeneous nuclear RNA containing poly(A) (Dabeva *et al.*, 1976*a*). Therefore potential losses of rRNA species during the procedure of isolation of nucleoli (Muramatsu, 1970) may be independently assessed.

The similar distribution of pre-rRNA and rRNA components in the nucleolar and nucleoplasmic fractions obtained by the two methods used is corroborated by the analysis of their labelling presented in Table 2. The results obtained demonstrate that pre-rRNA and rRNA components in either nuRNA or 50°C nuRNA display comparable specific radioactivities (except for the lower labelling of 18 S rRNA in 50°C nuRNA; see above). The labelling of 28 and 18 S rRNA in npRNA and 4°C npRNA is also comparable, although higher values are found in the npRNA fraction, most likely owing to partial disruption of nucleoli during the isolation procedure (Muramatsu, 1970). Further, the labelling of 28 and 18 S rRNA in both nucleolar fractions is severalfold higher than in the respective nucleoplasmic fractions, a finding that supports their belonging to distinct nuclear compartments.

Table 2. *Specific radioactivity of the pre-rRNA and rRNA in the nucleolar and nucleoplasmic RNA fractions*

[^{14}C]Orotate (25μCi/100g body wt.) is injected intraperitoneally 40min before death. Rat liver nuclei are isolated and the nucleoplasmic (npRNA and 4°C npRNA) and nucleolar (nuRNA and 50°C nuRNA) RNA fractions extracted. RNA is fractionated by urea/agar-gel electrophoresis and the specific radioactivity of the separate pre-rRNA and rRNA is determined as described in the following paper (Dudov *et al.*, 1978).

rRNA species	10^{-3} × Specific radioactivity (c.p.m./A_{260} unit)			
	4°C npRNA	50° nuRNA	npRNA	nuRNA
45 S	—	44.8	—	44.1
41 S	—	40.9	—	34.4
36 S	—	26.5	—	27.5
32 S	—	24.5	—	25.4
21 S	—	24.6	—	25.0
28 S	1.3	9.3	2.4	9.7
18 S	2.2	4.4	2.8	7.9

Taken together, the above results demonstrate that: (*a*) nuclear rRNA may be extracted quantitatively and reproducibly in two distinct fractions corresponding to the nucleolus and nucleoplasm compartments of the nucleus; (*b*) the methods of nuclear fractionation used permit the isolation of nucleolar and nucleoplasmic RNA fractions under conditions where degradation and/or processing of pre-RNA and rRNA species are minimized. The similarity of fractionation achieved by two independent techniques supports the view that it reflects the compartmentation of nuclei *in situ* and makes possible quantitative studies on the distribution of pre-rRNA and rRNA.

Distribution of pre-rRNA and rRNA in nucleolar and nucleoplasmic RNA fractions

The high resolving power of urea/agar-gel electrophoresis and the virtual absence of RNA degradation minimizes cross-contamination and permits the quantitative determination of pre-rRNA and rRNA species by direct measurement of their A_{260}. This approach eliminates the difficulties encountered when saturation-labelling techniques are used, in particular when experiments with intact animals are carried out.

As shown above, all pre-rRNA species are found in the nucleolar compartment of the nucleus. However, the presence of small amounts (about 10%) of 32 and 21 S pre-rRNA in both nucleoplasmic fractions required further evidence to certify their intranuclear location. For this purpose, we carried out double-labelling experiments ([^{14}C]orotate for 5h and [^{3}H]orotate for 40min) and determined the

$^3H/^{14}C$ labelling ratio of 32 and 21 S pre-rRNA in 50°C nuRNA and 4°C npRNA fractions. The results (not shown) reveal that these two pre-rRNA species display similar $^3H/^{14}C$ ratios in both nuclear fractions and therefore may be considered as belonging to the nucleolar compartment. On the other hand, the $^3H/^{14}C$ ratio for 28 and 18 S rRNA in the nucleolar fraction is higher than in the nucleoplasmic fraction, thus confirming the existence of separate nucleolar and nucleoplasmic pools. Further, to gain information on the quantity of true intranuclear rRNA in our npRNA fractions we introduced a correction based on the evaluation of the amount of cytoplasmic ribosomes adhering to nuclei after detergent purification. As shown (Dabeva *et al.*, 1977), the correction used yields upper limits for true nucleoplasmic rRNA.

The results of our distribution studies are presented in Table 3 and allow the following conclusions: (*a*) the sum of pre-rRNA species constitute about 50% of the total nuclear rRNA and 70% of the nucleolar rRNA; (*b*) the amount (in mol) of 32 and 21 S pre-rRNA is 2–3 times that of 45, 41 and 36 S pre-rRNA; (*c*) total nuclear rRNA is characterized by a slight excess of 28 over 18 S rRNA (molar ratio 1.1). This excess is more pronounced in nuRNA, where the 28S/18 S rRNA molar ratio is 1.4, whereas in npRNA this ratio is close to 1.0.

Amount of pre-rRNA and rRNA in the nucleolus and the nucleoplasm

Determination of the absolute amount of rRNA species in the nucleus depends on the accuracy with which total RNA and rRNA are assayed. Several factors, such as incomplete extraction or degradation of nuclear RNA (heterogeneous nuclear RNA in particular), may contribute to distort the results of such analyses. We determined the total amount of liver RNA as 2.5±0.3 pg per nucleus (seven independent analyses, mean±S.D.). This value is in agreement with previous authors (Muramatsu *et al.*, 1963; Busch & Smetana, 1970) and may be used as a

Table 3. *Distribution of pre-rRNA and rRNA species in the nucleolar and nucleoplasmic RNA fractions of detergent-purified rat liver nuclei*

The separate nucleolar and nucleoplasmic RNA fractions are obtained from detergent-purified rat liver nuclei and fractionated by urea/agar-gel electrophoresis (see the Experimental section). The amount of the separate pre-rRNA and rRNA species is determined from their A_{260} and the sum of identified species in each RNA fraction is taken as 100. The values for total nuclear rRNA are calculated on the basis of the results for 50°C nuRNA and 4°C npRNA. The amount of 39 S pre-rRNA in this Table is not given and it is distributed between 41 S and 36 S pre-rRNA. The amount of 28 and 18 S RNA is calculated after the correction which evaluates the cytoplasmic contaminants. The designation of the separate pre-rRNA and rRNA species and assigned molecular weights are from Dabeva *et al.* (1976*b*).

RNA fraction	No. of expts.	rRNA species	$10^{-6}\times$ Mol.wt.	A_{260} (% ± S.D.)	Amount (mol% ± S.D.)
nuRNA	8	45 S	4.61	17.9 ± 4.2	7.4 ± 1.7
		41 S	3.25	10.1 ± 2.7	5.9 ± 1.6
		36 S	2.60	11.7 ± 1.7	8.6 ± 1.2
		32 S	2.15	23.2 ± 3.9	20.6 ± 3.5
		21 S	1.05	8.7 ± 2.5	15.8 ± 4.5
		28 S	1.74	22.1 ± 3.1	24.2 ± 3.4
		18 S	0.68	6.2 ± 2.0	17.4 ± 5.6
50°C nuRNA	32	45 S	4.61	18.5 ± 3.7	7.2 ± 1.5
		41 S	3.25	9.5 ± 0.9	5.2 ± 0.5
		36 S	2.60	10.3 ± 1.2	7.1 ± 0.8
		32 S	2.15	19.3 ± 2.5	16.0 ± 2.0
		21 S	1.05	8.9 ± 1.8	15.1 ± 3.1
		28 S	1.74	23.9 ± 3.5	24.5 ± 3.6
		18 S	0.68	9.5 ± 2.2	24.9 ± 5.7
npRNA	8	28 S	1.74	71.2 ± 5.2	49.1 ± 3.6
		18 S	0.68	28.8 ± 2.7	50.9 ± 4.8
4°C npRNA	31	28 S	1.74	77.1 ± 10.8	56.8 ± 8.0
		18 S	0.68	22.9 ± 4.6	43.2 ± 8.6
Total nuclear rRNA	31	45 S	4.61	13.0 ± 2.6	4.5 ± 0.9
		41 S	3.25	6.7 ± 0.7	3.3 ± 0.3
		36 S	2.60	7.3 ± 0.8	4.5 ± 0.5
		32 S	2.15	13.5 ± 1.7	10.0 ± 1.3
		21 S	1.05	6.3 ± 1.3	9.6 ± 2.0
		28 S	1.74	39.7 ± 4.5	36.4 ± 4.1
		18 S	0.68	13.5 ± 2.1	31.7 ± 5.0

Table 4. *Amount of rRNA in the nucleolar and nucleoplasmic fractions of purified rat liver nuclei*

The analyses are carried out with detergent-purified rat liver nuclei. The total amount of nuclear RNA (taken as 100) and the amounts of RNA recovered in the separate nucleolar and nucleoplasmic RNA fractions are determined by chemical analysis (see the Experimental section). The amount of identified pre-rRNA and rRNA (distributed in the peaks of pre-rRNA and rRNA), other rRNA (outside the separate peaks and located in the zone between 18 S and 28 S rRNA and heavier than 45 S pre-rRNA) and low-molecular-weight nuclear RNA is derived from their A_{260} recorded from urea/agar-gel electrophoretograms. All values are expressed as percentage of total nuclear RNA. The values in parentheses represent the absolute amount of RNA in pg/nucleus. The total absolute amount of nuclear RNA is 2.5 ± 0.3 pg/nucleus (mean from seven experiments ± S.D.). The cytoplasmic rRNA contaminants are taken into consideration and the values presented are the mean values obtained after the correction.

	Amount of RNA (% of total nuclear RNA)			
Nuclear RNA fraction	Total RNA in fraction	Identified pre-rRNA+ rRNA	Other rRNA	Low-molecular-weight RNA (4 S–18 S)
(*a*) 4°C npRNA+50°C nuRNA	49.6 (1.24)	28.4 (0.71)	6.8 (0.17)	14.4 (0.36)
4°C npRNA	20.4 (0.51)	8.4 (0.21)	3.6 (0.09)	8.4 (0.21)
50°C nuRNA	29.2 (0.73)	20.0 (0.50)	3.2 (0.08)	6.0 (0.15)
(*b*) npRNA+nuRNA	52.0 (1.30)	28.8 (0.72)	7.6 (0.19)	15.6 (0.39)
npRNA	25.6 (0.64)	9.6 (0.24)	4.8 (0.12)	11.2 (0.28)
nuRNA	26.4 (0.66)	19.2 (0.48)	2.8 (0.07)	4.4 (0.11)

Table 5. *Amount of pre-rRNA and rRNA molecules in rat liver nuclei*

The estimates are based on data for the distribution of pre-rRNA and rRNA species between the nucleolar and nucleoplasmic fractions (Table 3) and the absolute amounts of rRNA recovered in these fractions (Table 4). The values for 28 S and 18 S are corrected on the basis of the results obtained with the nuRNA and npRNA fractions and data on the contamination of detergent-purified nuclei with cytoplasmic ribosomes (see the Experimental section). The amount of RNA in pg per nucleus is calculated for each pre-rRNA and rRNA species and the number of molecules present is determined on the basis of the molecular weights given in Table 3. The results from 31 experiments are given as means ± S.D.

	10^{-4} × No. of molecules per nucleus		
rRNA species	Whole nucleus	Nucleoli	Nucleoplasm
45 S	1.2 ± 0.2	1.2 ± 0.2	—
41 S	0.9 ± 0.2	0.9 ± 0.2	—
36 S	1.2 ± 0.2	1.2 ± 0.2	—
32 S	2.9 ± 0.4	2.9 ± 0.4	—
21 S	2.5 ± 0.5	2.5 ± 0.5	—
28 S	9.6 ± 1.5	3.7 ± 0.7	5.9 ± 1.3
18 S	8.7 ± 1.7	2.6 ± 0.9	6.1 ± 1.5
Pre-rRNA/rRNA	0.5	1.4	—
32 S/21 S	1.2	1.2	—
28 S/18 S	1.1	1.4	1.0

reliable basis for estimates of the amount of RNA in npRNA and nuRNA fractions. Quantitative analyses of these RNA fractions by urea/agar-gel electrophoresis allow an evaluation of the amounts of rRNA and low-molecular-weight RNA to be made (Table 4). The results show that rRNA in the liver nucleus constitutes about 35% of total nuclear RNA. About 30% is identified as known pre-rRNA and rRNA species, and the remaining 5% is accounted for by discrete RNA components in the zone between 28 and 18 S rRNA, most likely representing 28 S rRNA-degradation products (Aaij *et al.*, 1971). The remaining 65% of the total nuclear RNA is represented by low-molecular-weight RNA (15%) and heterogeneous nuclear RNA (about 50%). A comparison of the distribution of rRNA species between the nucleolus and nucleoplasm compartments reveals that the nucleolus contains about two-thirds, and the nucleoplasm contains at most one-third of the total nuclear rRNA species.

The above quantitative analyses allow an evaluation of the absolute amount of pre-rRNA and rRNA molecules in liver nuclei, as well as their distribution between the nucleolar and nucleoplasm compartments (Table 5).

Discussion

The results obtained in this work add information to the controversial subject of intranuclear compartmentation of rRNA species (see Hadjiolov & Nikolaev, 1976). It is evident that even detergent-purified liver nuclei still contain a substantial amount of rRNA, probably originating from the outer nuclear membrane and contributing to the nucleoplasmic rRNA fraction (Dabeva *et al.*, 1977). Therefore further evidence is needed before the intranuclear existence of ribosomes and polyribosomes (Sadowski & Howden, 1968) is ascertained. Our

results rather support the view (Penman, 1966) that only nascent ribosomes are found in the nucleus. This does not mean that all 28 and 18 S rRNA found in nuclei are of cytoplasmic origin (see Bramwell, 1976). In fact, after partial correction for contaminating cytoplasmic ribosomes we estimate that about 35% of the nuclear RNA is ribosomal, including a substantial amount of mature rRNA species. The amount of 28 and 18 S rRNA in the nucleolus and the nucleoplasm is comparable, which shows that an about 20-fold higher concentration of these rRNA molecules is found in nucleoli. It is also noteworthy that the 28 S/18 S rRNA molar ratio in liver nuclei is only 1.1 (see also Weinberg & Penman, 1968). Therefore the faster appearance of small ribosomes in the cytoplasm could hardly be explained by a vanishingly small pool in the nucleus, and alternative explanations for this phenomenon must be sought (see Dudov *et al.*, 1978).

Our results provide evidence that two-thirds of the total nuclear rRNA species are confined to the nucleolus, with more than 60% of the molecules being pre-rRNA species. Comparison of the amounts of liver pre-rRNA and rRNA with those reported for HeLa cells (Weinberg & Penman, 1968) reveal that they are of the same order, an observation suggesting that ribosome formation in animal cells follows a similar pattern even with respect to the quantitative correlations involved in pre-rRNA processing. We consider that the quantitative data on nucleolar and nucleoplasmic pre-rRNA and rRNA obtained in the present work may be a basis for quantitative studies on ribosome formation in liver cells and an analysis of the regulatory factors involved.

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