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Post-transcriptional regulation of ribosome formation in the nucleus of regenerating rat liver

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Kinetic experiments on RNA labelling *in vivo* with [^{14}C]orotate were performed with normal and 12h-regenerating rat liver. The specific radioactivities of nucleolar, nucleoplasmic and cytoplasmic rRNA species were analysed by computer according to the models of rRNA processing and nucleo-cytoplasmic migration given previously [Dudov, Dabeva, Hadjiolov & Todorov, *Biochem. J.* (1978) **171**, 375–383]. The rates of formation and the half-lives of the individual pre-rRNA and rRNA species were determined in both normal and regenerating liver. The results show clearly that the formation of ribosomes in regenerating rat liver is post-transcriptionally activated: (*a*) the half-lives of all the nucleolar pre-rRNA and rRNA species are decreased by 30% on average; (*b*) the pre-rRNA processing is directed through the shortest maturation pathway: 45S → 32S + 18S → 28S; (*c*) the nucleo-cytoplasmic transfer of ribosomes is accelerated. As a consequence, the time for formation and appearance of ribosomes in the cytoplasm is shortened 1.5-fold for the large and 2-fold for the small subparticle. A new scheme for endonuclease cleavage of 45S pre-rRNA is proposed, which explains the alterations in pre-rRNA processing in regenerating liver. Its validity for pre-rRNA processing in other eukaryotes is discussed. It is concluded that: (i) the control sites in the intranucleolar formation of 28S and 18S rRNA are the immediate precursor of 28S rRNA, 32S pre-rRNA, and the primary pre-rRNA, 45S pre-rRNA, respectively; (ii) the limiting step in the post-transcriptional stages of ribosome biogenesis is the pre-rRNA maturation.

It has been shown in previous reports that multiple pre-rRNA processing pathways operate in rat liver (Dabeva *et al.*, 1976; Dudov *et al.*, 1978). A model of pre-rRNA processing has been proposed, which envisages certain flexibility in the sequence of endonuclease cleavage at the critical sites in the precursor molecules (Dudov *et al.*, 1978). Evidence for alternative processing pathways has been obtained for many eukaryotes (Purtell & Anthony, 1975; Winikov, 1976; Niles, 1978; Batts-Young & Lodish, 1978; Long & Dawid, 1980; Bowman *et al.*, 1981), but their relation to the control of ribosome formation is still unknown.

An approach to this question is to study the changes in pre-rRNA processing under conditions of inhibited or activated ribosome biosynthesis. Characteristic changes in pre-rRNA processing in rat liver have been obtained after treatment *in vivo* with cycloheximide or fluoro-orotate (Stoyanova & Hadjiolov, 1979; Hadjiolov & Hadjiolova, 1979). In regenerating rat liver we found that the increased production of ribosomes is transcriptionally determined (Dabeva & Dudov, 1982*b*). On the other hand, the post-transcriptional control of ribosome biogenesis after partial hepatectomy is not yet clear.

It has been reported that the time required by a normal and a regenerating liver cell to make a ribosome is the same (Chaudhuri & Lieberman, 1968). Jacob *et al.* (1967) found that the half-life of 45S pre-rRNA in regenerating liver is decreased. Other authors have shown a correlation between the rate of ribosome transport to the cytoplasm and the size of the monomer pool (Rizzo & Webb, 1968; Henshaw *et al.*, 1973). However, no quantitative data on pre-rRNA processing in regenerating rat liver have been presented.

The results reported here show that the activated pre-rRNA transcription in regenerating rat liver is

Abbreviations used: pre-rRNA, precursor to rRNA; pre-rRNP, ribosomal precursor ribonucleoprotein; nuRNA, nucleolar RNA; npRNA, nucleoplasmic RNA; cytRNA, cytoplasmic RNA.

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accompanied by an acceleration of the maturation and nucleo-cytoplasmic transfer of ribosomes: the processing of pre-rRNA is directed through the shortest pathway, and the half-lives of all the nuclear pre-rRNA and rRNA species are shortened. Part of these results have been reported (Dabeva & Dudov, 1980).

Experimental

Wistar male albino rats weighing 150 ± 2 g were used. Partial hepatectomy and RNA labelling were performed as described in the previous paper (Dabeva & Dudov, 1982*b*).

Nucleolar (nuRNA), nucleoplasmic (npRNA) and cytoplasmic (cytRNA) rRNA were extracted as described (Dabeva *et al.*, 1978). RNA was fractionated in urea/agar gels (Dudov *et al.*, 1976), and the dried agar film was scanned at 260 nm, cut in 1 mm slices and the radioactivity in each sample determined (Dudov *et al.*, 1978). The specific radioactivity of each RNA species was obtained from the radioactivity in the respective electrophoretic peak and the amount of RNA in it (Dabeva & Dudov, 1982*b*).

The data for the specific radioactivities are mean values from two kinetic experiments and two to five electrophoretograms for each RNA sample.

Theory

For a steady-state system of n homogeneous RNA pools the following equations hold (see Reiner, 1974):

$$A_i \frac{dS_i}{dt} = \sum_{k=1}^{n} V_{ki}(S_k - S_i) \quad (1)$$

$$\sum_k V_{ik} = \sum_m V_{mi} (i = 1, 2, \ldots, n) \quad (2)$$

where S_i (d.p.m./μg of RNA) and A_i (mol/nucleus) are the specific radioactivity and the size of pool i respectively; V_{ji} (mol/min per nucleus) is the rate of RNA transfer from pool j into pool i.

Since A_i and S_i were experimentally determined, it was possible to evaluate the transfer rates and their errors by a method for computer analysis of tracer kinetic data described earlier (Dudov *et al.*, 1978).

The turnover rates (V_i^t) and the half-lives ($T_i^{0.5}$) of the RNA pools were obtained from:

$$V_i^t = \sum_k V_{ik}, \quad T_i^{0.5} = \ln 2 (A_i / V_i^t) \quad (3)$$

The probability (P_i^r) for endonuclease split at the critical site r in the molecules from pool i was calculated from:

$$P_i^r = V_{ir} / V_i^t \quad (4)$$

where V_{ir} is the rate of endonuclease cleavage at site r of molecules in pool i.

The rRNA cross-contamination between nucleolar, nucleoplasmic and cytoplasmic compartments was quantitatively evaluated as described previously (Dudov *et al.*, 1978).

Results

Processing and turnover of pre-rRNA in regenerating rat liver

The labelling with [^{14}C]orotate *in vivo* reveals characteristic changes in the radioactive pattern of nuRNA from regenerating liver as compared with normal liver (Fig. 1): the labelling of 45 S pre-rRNA is more intense as a result of the increased rate of transcription and the higher labelling of the free nucleotides (Dabeva & Dudov, 1982*b*). The transfer of the label from 45 S into the other rRNA species, and mainly into 32 S and 18 S, is considerably accelerated. The changes in the pre-rRNA pool sizes (Dabeva & Dudov, 1982*a*) and their labelling show that the activated production of ribosomes in regenerating rat liver is accompanied by alterations in pre-rRNA processing. To evaluate these changes quantitatively we performed detailed labelling kinetic experiments.

Nucleolar RNA was labelled *in vivo* for 20–120 min with [^{14}C]orotate and the specific radioactivities of pre-rRNA and rRNA were determined (Fig. 2). For the quantitative analysis of pre-rRNA processing in normal and regenerating liver we used also: (*a*) the data on pre-rRNA pool sizes (Dabeva *et al.*, 1978; Dabeva & Dudov, 1982*a*); (*b*) the data on the molecular weights of pre-rRNA and their arrangement in 45 S pre-rRNA (Dabeva *et al.*, 1976); (*c*) the model of pre-rRNA processing in rat liver (Dudov *et al.*, 1978), which was simplified to account for the absence of 39 S pre-rRNA from regenerating liver (Fig. 3). In the mathematical description of the models of pre-rRNA processing in normal and regenerating liver the following properties of the analysed steady-state system were taken into account: (i) no wastage of conserved sequences in pre-rRNA takes place (Dabeva & Dudov, 1982*b*); (ii) According to the topology of 45 S pre-rRNA, in the transitions 45 S → 21 S and 45 S → 18 S, two intermediate pre-rRNA species bearing the external transcribed spacer could be generated (see Fig. 3). Such precursor pools have not been found in rat liver yet, but if they exist their turnover should be very rapid, without effect on 21 S and 18 S labelling. Therefore in the model given in Fig. 3 these intermediate pools are not included.

The experimental data presented are sufficient to evaluate all the unknown transfer rates included in the model of pre-rRNA processing with the aid of the computer program described earlier (Dudov *et*

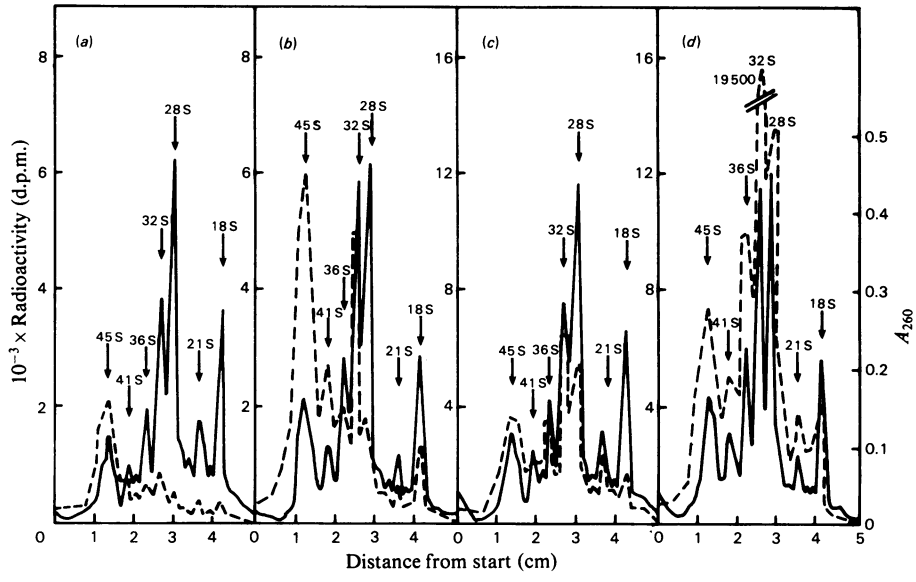

Fig. 1. *Electrophoretic pattern of nuRNA from normal and regenerating rat liver*

NuRNA was extracted from detergent purified nuclei of normal (*a* and *c*) and 12h-regenerating rat liver (*b* and *d*) as described in the Experimental section. RNA was labelled *in vivo* with [^{14}C]orotate (20μCi/100g body wt.) for 20min (*a* and *b*) or 80min (*c* and *d*). The electrophoresis was carried out in 1.5% agar gel/5M-urea at 5V/cm for 4.5h at 6°C; 2.5 A_{260} units were loaded on each track. ——, A_{260}; – – – –, radioactivity determined after cutting the dried agar film in 1mm slices and liquid-scintillation counting.

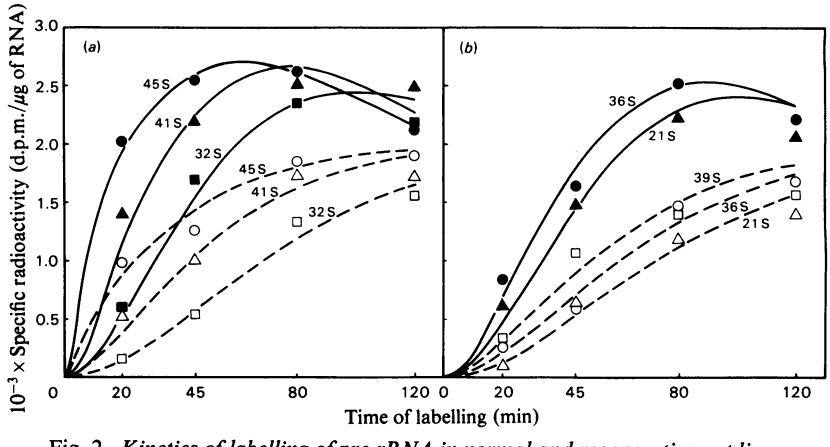

Fig. 2. *Kinetics of labelling of pre-rRNA in normal and regenerating rat liver*

RNA was labelled *in vivo* with 20μCi of [^{14}C]orotate/100g body wt. The specific radioactivities of pre-rRNA pools in normal (open symbols) and regenerating (filled symbols) rat liver were determined after electrophoretic fractionation of nuRNA as described in the Experimental section. The theoretical curves for the specific radioactivities of pre-rRNA in normal (– – – –) and regenerating (——) liver were calculated from the data given in Table 1, in correspondence with the model presented in Fig. 3. (*a*) 45S (○, ●), 41S (△, ▲) and 32S (□, ■) pre-rRNA; (*b*) 36S (○, ●), 21S (△, ▲) and 39S (□) pre-rRNA.

al., 1978). The transfer rates obtained between the pre-rRNA pools in normal and regenerating rat liver are given in Table 1. The turnover rates and the half-lives of pre-rRNA, calculated from eqn. (3), are presented in Table 2. As seen in Fig. 2, the resulting quantitative models of pre-rRNA processing fit very

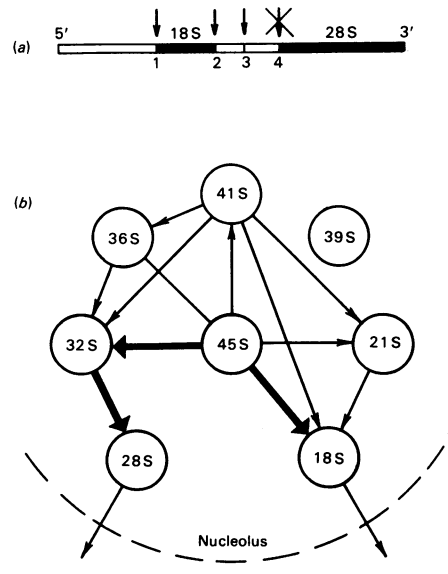

Fig. 3. *Model of pre-rRNA processing in regenerating rat liver*

(*a*) Scheme of 45 S pre-rRNA showing the location of 18 S and 28 S sequences (in black) and external and internal transcribed spacers (in white). The arrows indicate the main cleavage sites. Endonuclease splits of 45 S pre-rRNA generate: site 1, 41 S pre-rRNA; at site 2, 36 S pre-rRNA + unidentified precursor of 18 S rRNA; at site 3, 32 S pre-RNA + unidentified precursor of 21 S pre-rRNA; at site 4, 39 S pre-rRNA + 28 S rRNA. In regenerating liver, cleavage in position 4 does not occur. (*b*) Model of pre-rRNA processing in regenerating rat liver. The model is analogous to the model of pre-rRNA processing in rat liver reported previously (Dudov *et al.*, 1978). The only difference is the absence of the transitions 45 S → 39 S → 18 S and 45 S → 28 S. The transfer rates between the pre-rRNA pools, indicated with arrows, are given in Table 1. The main processing pathway is shown by thick arrows.

well to the experimental data for both normal and regenerating rat liver.

Table 1 shows that the rates of formation of all the pre-rRNA species are essentially enhanced in regenerating liver. A fact that deserves more attention is the unequal activation of 45 S pre-rRNA processing through the different intermediate precursors: the transition 45 S → 32 S is activated 4-fold, whereas the transition 45 S → 21 S is activated only 1.5-fold. Thus 32 S and 21 S pre-rRNA are not generated in equimolar amounts; less than one-third of 18 S rRNA is supplied from the pool of 21 S, the rest of it being generated directly from the pools of 45 S or 41 S. It should be noted that more than half of the 45 S molecules in regenerating liver are involved in the transition 45 S → 18 S. Although this transition is activated 6-fold, no accumulation of intermediate pre-rRNA species bearing the external transcribed spacer is observed (see Fig. 3). This finding shows that two or three of the critical splits in the 45 S pre-rRNA are accomplished simultaneously or in such a rapid succession that accumulation of the above pre-rRNA species is not possible.

The origin of all the processing pathways in normal and regenerating rat liver can be simply explained on the basis of the scheme given in Fig. 4. Depending on the number and the position of simultaneous splits the 45 S molecules are divided into five groups, A_1, A_2, A_3, A_4 and B, each one corresponding to one of the processing pathways included in the model in Fig. 3. The probabilities for processing through the different pathways, and for endonuclease attack at a given critical site, are given in Fig. 4.

The molecules from group A_1 are converted into 41 S species by a single split in position 1. All the 45 S molecules in regenerating liver and about 90% in normal liver are split at position 1, but only those that are not accessible for cleavage at position 2 or 3 are processed via 41 S species. Thus the elimination of the external transcribed spacer takes place at the first step of 45 S pre-rRNA processing.

The simultaneous cleavage at positions 1 and 2 (group A_2) generates 36 S and 18 S rRNA. The probabilities for processing via this pathway are approximately equal in normal and regenerating liver.

Group A_3 comprises molecules undergoing simultaneous splits at positions 1 and 3, which accounts for the equimolar formation of 21 S and 32 S pre-rRNA. The probability for processing through this pathway is higher in normal (0.28) than in regenerating rat liver (0.15).

The 45 S molecules from group A_4 are cleaved simultaneously at positions 1, 2 and 3, forming directly 32 S and 18 S rRNA. The probability of this occurring is very low in normal (0.07), but very high in regenerating (0.32) liver. Whereas in normal liver 32 S pre-rRNA is generated mainly from molecules of group A_3, simultaneously with 21 S, in regenerating liver it is formed exclusively simultaneously with 18 S.

Thus the observed changes in pre-rRNA processing in regenerating liver could be explained by the increased probability of simultaneous cleavage at the critical sites in 45 S molecules. As a consequence (see Table 1) the formation of 28 S and 18 S rRNA becomes more efficient in regenerating liver: the pre-rRNA processing is directed through the shortest maturation pathway 45 S → 32 S → 28 S, and 45 S → 18 S. The number of 45 S molecules processed through the other pre-rRNA intermediates

Table 1. *Transfer rates between the nucleolar pre-rRNA and rRNA pools in normal and regenerating rat liver*
The transfer rates are determined by computer analysis of the experimental kinetic data given in Fig. 2. The S.E.M. of the values, as evaluated by a computer simulation method (Dudov *et al.*, 1978), is about 35%, except for the inflow rates of 21S and 18S rRNA pools, whose S.E.M. is about 50%. The activation of the transitions is given as ratios of the values in regenerating liver to those in normal liver. The significance of the differences between transfer rates in normal and regenerating liver was calculated by the Student's t test ($n=4$, two-tailed): † $P<0.1$, †† $P<0.05$, ††† $P<0.01$; n.s., non-significant.

Transfer between pools		Transfer rates (molecules/min per nucleus)		Activation in regenerating liver
From	Into	Normal liver	Regenerating liver	
45S	41S	420	1000	2.4††
	39S (28S)*	110	—	—
	36S	270	890	3.3††
	32S	430	1680	3.9†††
	21S	350	520	1.5 n.s.
	18S	350	2050	5.9††
41S	36S, 18S	×160	×440	2.8†
	32S, 21S	×260	×560	2.1 n.s.
39S	(18S)*	110	—	—
36S	32S	430	1130	3.1††
32S	28S	1130	3570	3.2††
21S	18S	610	1080	1.8 n.s.

* These transitions do not lead to formation of mature rRNA (see the text).

Table 2. *Turnover rates and half-lives of pre-rRNA pools in normal and regenerating rat liver*
The presented values are calculated from the data given in Table 1 and the known pool sizes of pre-rRNA (Dabeva & Dudov, 1982*a*). S.E.M. values are sbout 30% for 45S, 39S, 36S and 32S pre-rRNA, 65% for 41S pre-rRNA and 50% for 21S pre-RNA. The values in parentheses are ratios of values in regenerating liver to those in normal liver.

	Turnover rate (molecules/min per nucleus)		Half-life (min)	
Pre-rRNA	Normal liver	Regenerating liver	Normal liver	Regenerating liver
45S	1240	3570 (2.9)	6.6	4.6 (0.70)
41S	420	1000 (2.4)	11.5	8.7 (0.75)
39S	110	—	16.3	—
36S	430	1330 (3.1)	19.2	13.6 (0.71)
32S	1130	3570 (3.2)	17.8	13.5 (0.76)
21S	610	1080 (1.8)	28.5	18.7 (0.66)

(45S → 41S, 36S → 32S → 28S, and 45S → 21S → 18S) is relatively diminished.

The processing via 39S pre-rRNA (group B, single endonuclease cleavage at position 4) occurs rarely in normal liver and not at all in regenerating liver. It has been suggested that this pathway does not lead to formation of mature ribosomes (Dabeva & Dudov, 1982*a*).

The results given in Table 2 show also that the enhanced rates of pre-rRNA formation in regenerating liver are accompanied by about a 30% shortening of the half-lives of the precursors. This fact demonstrates the activation of the specific endonuclease cleavage. As a result, the time for formation of 28S and 18S rRNA in the nucleolus of regenerating rat liver is decreased.

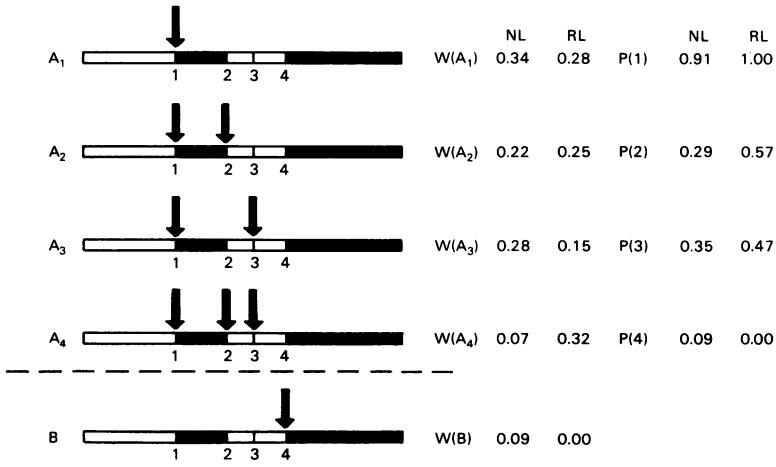

Fig. 4. *Endonuclease cleavage of 45 S pre-rRNA*

The scheme explains the origin of the parallel pre-rRNA processing pathways in rat liver. Characteristic of it is the possibility for simultaneous (or in very rapid succession) cleavage at two or three of the critical sites in the molecules. Depending on the number and position of simultaneous splits, indicated with arrows, the 45 S pre-rRNA molecules are distributed in five groups: A_1, A_2, A_3, A_4 and B. Each group accounts for one of the processing pathways presented in Fig. 3. The probabilities for 45 S pre-rRNA processing through the five pathways [$W(A_1)$, $W(A_2)$,...], and the probabilities for nuclease split at each critical site [P(1), P(2), ...] are also given. They are calculated from the data presented in Tables 1 and 2 (see the Theory section). Abbreviations: NL, normal rat liver; RL, regenerating rat liver.

Turnover of 28 S and 18 S rRNA in the nucleolus and nucleoplasm of regenerating rat liver

The specific radioactivities of 28 S and 18 S rRNA in the nuRNA, npRNA and cytRNA fractions determined after labelling for 20–120 min *in vivo* with [^{14}C]orotate are given in Fig. 5. The following features of the kinetics of rRNA labelling are well documented in both normal and regenerating liver: (i) the nuclear rRNA pools approach isotopic saturation at labelling times when the specific radioactivities of their immediate presursors are still rising (see Fig. 2); (ii) the specific radioactivity of nuclear 28 S rRNA is considerably higher than that of 18 S. Conversely, the specific radioactivity of 18 S rRNA in the cytoplasm is higher than that of 28 S. It has been shown in previous work (Dudov *et al.*, 1978) that these differences could not be due only to cross-contamination between the neighbouring rRNA compartments. That is why a model for nucleo-cytoplasmic transfer of rRNA was proposed (Fig. 6). This model envisages the nucleoplasm as a compartment including truly nuclear rRNA and rRNA associated with the nuclear membrane. The latter part of the rRNA is in kinetic equilibrium with the free ribosomes in the cytoplasm.

On the basis of this model we analysed the changes in rRNA turnover in the nucleolus and nucleoplasm of regenerating liver. The parameters of the model (transfer rates, pool sizes and half-lives) were evaluated by computer analyses of the experimental kinetic data given in Fig. 5. The values obtained for the rRNA pool sizes and their half-lives are given in Table 3. The exchange rates between the nucleoplasmic and cytoplasmic rRNA compartments are given in the legend to Fig. 6. As seen in Fig. 5, the theoretical curves calculated from the model parameters fit well to the experimental data for both normal and regenerating rat liver.

According to our results, the exchange rate of 18 S rRNA between nucleoplasmic and cytoplasmic compartments is considerably higher than that of 28 S rRNA in normal and regenerating liver. Thus the newly formed 18 S rRNA is transferred faster from nucleus to cytoplasm, its specific radioactivity in the cytoplasm being higher than that of 28 S. On the other hand, the radioactivity of nucleoplasmic 18 S rRNA is diluted more rapidly with unlabelled molecules from the cytoplasm than is the radioactivity of nucleoplasmic 28 S rRNA. The cross-contamination between nucleoplasmic and nucleolar rRNA, which is more considerable for 18 S rRNA (Table 3), decreases the specific radioactivity of rRNA in the nuRNA fraction.

The results presented in Table 3 and Fig. 6 show several characteristic changes in the turnover of nuclear rRNA in regenerating rat liver: (1) the half-lives of all the nuclear rRNA pools are

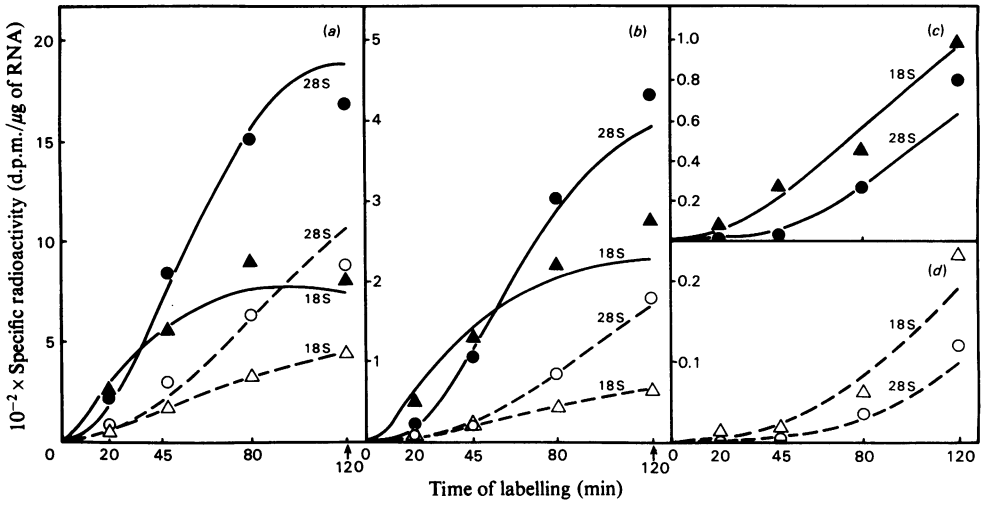

Fig. 5. *Kinetics of labelling of 28S and 18S rRNA in the nucleolus, nucleoplasm and cytoplasm of normal and regenerating rat liver*

The specific radioactivities of 28S and 18S rRNA in normal (open symbols) or regenerating (filled symbols) rat liver were determined after electrophoretic fractionation of nuRNA (*a*), npRNA (*b*) and cytRNA (*c* and *d*) as described in the Experimental section. The results presented here and in Fig. 2 were obtained in the same labelling experiments. The theoretical curves for the rRNA specific radioactivities in normal (----) and regenerating (——) rat liver were calculated on the basis of the model given in Fig. 6 and the data in Table 4. (○, ●), 28S rRNA; (△, ▲), 18S rRNA.

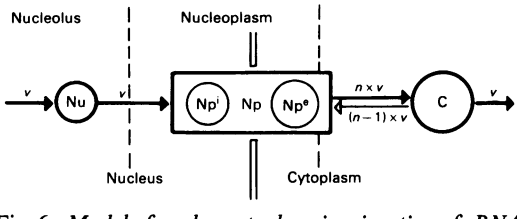

Fig. 6. *Model of nucleo-cytoplasmic migration of rRNA*

The model explains the kinetics of rRNA labelling in the nucleolus, nucleoplasm and cytoplasm of normal and 12h-regenerating rat liver and is analogous to the previous one (Dudov *et al.*, 1978). It includes the following pools of 28S or 18S rRNA: Nu, nucleolar; Np, extended nucleoplasmic, Np^i and Np^e, intranuclear and extranuclear constituents of Np; Cyt, cytoplasmic. The transfer rate v between the pools is 1240 or 3570 molecules/min per nucleus for normal and regenerating liver respectively. The values for n are evaluated by computer analysis of the kinetic data presented in Fig. 5: normal liver, $n = 5$ and 18 for 28S and 18S rRNA respectively; regenerating liver, $n = 3$ and 14 for 28S and 18S rRNA respectively. The pool sizes and their half-lives are given in Table 3.

decreased by about 50%; (2) the exchange rates between nucleoplasmic and cytoplasmic compartments are accelerated about 1.5-fold for 28S and 2.5 fold for 18S rRNA, which ensures the still faster appearance of 18S than of 28S in the cytoplasm of regenerating liver; (3) in contrast with the great accumulation of pre-rRNA, only a small increment of 28S and 18S rRNA in the nucleus of regenerating rat liver is observed. These findings indicate that a new exchange equilibrium between nuclear and cytoplasmic rRNA pools is established, resulting in a faster migration of newly synthesized ribosomes to the cytoplasm in regenerating rat liver.

Discussion

In this work we have analysed the post-transcriptional steps of ribosome biogenesis in the nucleus of regenerating rat liver. The results obtained show that the formation of ribosomes in regenerating liver is post-transcriptionally activated. The mechanisms that ensure faster formation and appearance of new ribosomes in the cytoplasm are: (i) Shortening of the half-lives of all the pre-rRNA and rRNA species in the nucleolus; (ii) channelling of the pre-rRNA processing through the shortest pathway; (iii) accelerated transfer of ribosomes to the cytoplasm. As a result, the time for maturation and nucleo-cytoplasmic transport of ribosomes in regenerating liver is decreased 1.5–2-fold as compared with normal liver (Table 4).

Table 3. *Pool sizes and half-lives of nucleolar and nucleoplasmic 28 S and 18 S rRNA in normal and regenerating rat liver*
The rRNA pool sizes were evaluated on the basis of (i) the experimental data on the specific radioactivities of 28 S and 18 S rRNA in nuRNA, npRNA and cytRNA fractions (Fig. 5) and (ii) the model of nucleo-cytoplasmic transfer of rRNA (Fig. 6). For comparison the amounts of 28 S and 18 S rRNA, extracted in the nuRNA and npRNA fractions are also given in parentheses. The designation of the pools is the same as in Fig. 6: Nu, nucleolar; Np, extended nucleoplasmic; Np^i and Np^e, intranuclear and extranuclear constituents of Np.

		10^{-4} × (Amount of rRNA molecules/nucleus)				Half-life (min)	
rRNA	Liver	Nu	Np^i	Np^e	(nuRNA, npRNA)	Nu	Np
28 S	Normal	4.0	1.7	7.8	(5.0, 8.5)	22	11
	Regenerating	5.7	2.2	9.4	(7.6, 9.7)	11	8
	Regenerating/normal	1.4	1.3	1.2	(1.5, 1.1)	0.5	0.7
18 S	Normal	1.1	4.1	8.0	(6.2, 7.1)	6	4
	Regenerating	1.3	4.3	8.4	(7.4, 6.6)	2.5	2
	Regenerating/normal	1.2	1.0	1.1	(1.2, 0.9)	0.4	0.5

Table 4. *Average time for formation and nucleo-cytoplasmic migration of ribosomes in normal and regenerating liver*
The average time ($\bar{T}$) for formation of 28 S or 18 S rRNA is calculated from the equation:

$$\bar{T} = \sum_{k=1}^{m} \frac{n_k}{N} \sum_{i=1}^{L_m} T_i^k$$

where m is the number of the parallel processing pathways leading to formation of 28 S (18 S) rRNA, L_m is the number of sequential pools in the pathway k, N is the total number of 28 S (18 S) molecules generated, n_k is the number of molecules generated *via* pathway k, T_i^k is the life-time of pool i included in pathway k. The values for m and L_m follow from the models given in Figs. 3 and 6; n_k and N are taken from Table 1; T_i^k is obtained from Table 2 as $T_i^{0.5}/\ln 2$.

	Formation in the nucleolus (min)		Formation + transfer to the cytoplasm (min)		
Liver	28 S	18 S	28 S	18 S	28 S/18 S
Normal	52	38	100	53	1.9
Regenerating	37	18	65	25	2.6
Normal/regenerating	1.4	2.1	1.5	2.1	0.7

(a) Acceleration of pre-rRNA cleavage

We found that the half-lives of all pre-rRNA pools are shortened by 30% in regenerating liver, i.e. the specific endonuclease cleavage of pre-rRNA is accelerated. There are two probable explanations of this phenomenon. The first is that the processing enzymes themselves are activated after partial hepatectomy. The second takes into consideration that in fact pre-rRNA particles undergo processing and their assembly with structural proteins is of great importance for the correct maturation (see Hadjiolov, 1980). The accelerated pre-rRNA cleavage could be explained by faster assembly of pre-rRNP particles with structural proteins. The basis for this suggestion is the observed activation of ribosomal protein synthesis in regenerating rat liver (Tsurugi *et al.*, 1972; Wu *et al.*, 1977; Nabeshima & Ogata, 1980).

(b) Alterations in the pattern of pre-rRNA processing

Our data show that pre-rRNA processing in regenerating liver is directed through the shortest pathway, which further decreases the time for ribosome formation (see Table 4).

To explain the observed alterations in the pool sizes and in the processing of pre-rRNA in regenerating liver, we developed further the model of endonuclease cleavage of 45 S pre-rRNA (Fig. 4). A new feature of the model is the possibility for simultaneous (or in extremely rapid succession) cleavage at two or three of the critical sites in 45 S pre-rRNA: site 1 is always accessible for endonuclease split, but in some molecules sites 2 and/or 3 are also cleaved. This could be connected with the fact that pre-rRNA synthesis starts with the 5′-end and RNA–protein interaction progresses simul-

taneously with the transcription. On these grounds one may expect that at the end of the transcription the 5'-end of 45S pre-rRNA will be almost completely assembled with proteins, necessary for correct processing, whereas the 3'-end should be relatively poor in such proteins. Therefore the critical sites closer to the 5'-end of the 45S molecule should be split with higher probability than those at the 3'-end, explaining the greater accessibility of site 1 for endonuclease attack. If we assume that the growing polynucleotide chain in regenerating liver interacts much faster with structural proteins, the probability for endonuclease split at site 2 and 3 should increase, more for site 2 and less for site 3. The increased probability for cleavage at positions 2 and 3 (by 97% and 34%, respectively) (see Fig. 4) in regenerating liver correlates with the higher probability for simultaneous split at positions 1, 2 and 3 and explains the activation of the transition 45S → 32S + 18S.

Experiments with different inhibitors of pre-rRNA processing have led to the conclusion that the last maturation step is critical in ribosome biogenesis (Hadjiolov, 1980). According to our model, 28S rRNA is generated only from 32S. On the other hand, 18S rRNA could be formed directly from 45S, 41S and 21S. Our results show that almost two-thirds of the 18S rRNA in regenerating liver is generated directly at the first step of 45S processing, i.e. the other intermediate pre-rRNA species are not obligatory steps in 18S formation. We can conclude that the formation of 28S rRNA is controlled at the level of 32S pre-rRNA (55S pre-rRNP), whereas the formation of 18S rRNA is most probably controlled at the level of 45S pre-rRNA (80S pre-rRNP). Experiments with cycloheximide (Stoyanova & Hadjiolov, 1979) and fluoro-orotate (Hadjiolov & Hadjiolova, 1979) in rat liver confirm this conclusion. Its validity for other eukaryotes is supported by experimental data showing an early degradation of 18S rRNA, immediately after primary pre-rRNA processing, and a late degradation of 28S rRNA in resting lymphocytes (Cooper, 1969) or fibroblasts (Abelson *et al.*, 1974), after fusion of myoblasts (Bowman & Emerson, 1977) and cycloheximide treatment of HeLa cells (Willems *et al.*, 1969).

Our model of endonuclease cleavage of primary pre-rRNA explains the origin of the multiple processing pathways in rat liver. Moreover, it reveals some new properties of pre-rRNA processing which could be also valid for other eukaryotes.

It is generally accepted that the elimination of the external transcribed spacer takes place at the first step of pre-rRNA processing (Perry, 1976; Hadjiolov & Nikolaev, 1976; Hadjiolov, 1980). The proposed model, however, does not exclude the possibility for generation of precursors bearing the external transcribed spacer, but the simultaneous splits (or following in a very rapid succession) prevent their accumulation in substantial amounts. Actually, such precursors have been identified in some eukaryotic cells (Niles, 1978; Batts-Young & Lodish, 1978; Bowman *et al.*, 1981).

The activation of the direct transition 45S → 32S + 18S in regenerating liver accounts for the non-equimolar generation of 32S and 21S pre-rRNA and for the substantial accumulation solely of 32S. A relative increase of 32S without accumulation of precursors to 18S has been observed also in phytohaemagglutinin-stimulated cells (Purtell & Anthony, 1975). In HeLa and BHK cells a 20S precursor of 18S rRNA has been identified (Weinberg & Penman, 1970; Wellauer & Dawid, 1973; Winikov, 1976), but its amount relative to that of 32S is negligible. In L cells no 20S pre-rRNA has been found initially (Wellauer *et al.*, 1974), but its presence was subsequently demonstrated (Bowman *et al.*, 1981). A 20S pre-rRNA has been found also in mouse liver (Hadjiolov *et al.*, 1974). All these results show that 32S and 20S pre-rRNA could not be generated in equimolar amounts, i.e. that the direct transition 45S → 32S + 18S may occur in different cell types.

Thus the present model could be applied for interpretation of data on pre-rRNA processing in other eukaryotes. Taking into account the strong resemblance of the cleavage sites in pre-rRNA (Winikov, 1976; Dabeva *et al.*, 1976; Wellauer & Dawid, 1975; Hadjiolov, 1980), the model is likely to be universal for eukaryotic cells.

(*c*) *Acceleration of nucleo-cytoplasmic transfer of ribosomes*

In regenerating liver as compared with normal liver, three times more ribosomes are synthesized and transferred to the cytoplasm, although the amount of their precursors in the nucleus is almost unchanged, judged by the direct measurements (Dabeva & Dudov, 1982*a*) and the labelling experiments (Table 3). The half-lives of nuclear 28S and 18S rRNA are decreased, accounting for the 2-fold faster appearance of newly synthesized ribosomes in cytoplasm (Table 4).

The model for nucleo-cytoplasmic transfer of ribosomes involves an extended nucleoplasmic pool which includes membrane-associated ribosomes. Our analyses show that the exchange rate between nucleoplasm and cytoplasm is faster for 18S than for 28S rRNA. This results correlates very well with the observed higher mobility of 18S in the cytoplasm and the slower exchange of 28S RNA in the membrane-associated polyribosomes (Borgese *et al.*, 1973; Lönn & Edström, 1976, 1977). The greater acceleration of the processing and transfer of 18S rRNA explains its still faster appearance than that of

28S in the cytoplasm of regenerating liver, as compared with normal liver (Fig. 5; Fausto & Van Lancker, 1968). The new exchange equilibrium between the nuclear and cytoplasmic pools of the ribosomal particles ensures their unrestricted (faster) transport to the cytoplasm. Consequently, the limiting step in the post-transcriptional stages of ribosome biogenesis is the pre-rRNP maturation in the nucleolus.

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