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Transcriptional control of ribosome production in regenerating rat liver

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Kinetic experiments of labelling *in vivo* with [^{14}C]orotate of cellular free UMP and/or UTP, nucleolar, nucleoplasmic and cytoplasmic rRNA in normal and 12h-regenerating rat liver were performed. The specific-radioactivity curves obtained were analysed by computer and the rates of synthesis of precursor rRNA (45S pre-rRNA) and cytoplasmic 28S and 18S rRNA calculated. (*a*) The rates of synthesis of 45S pre-rRNA in normal and regenerating rat liver are 1400 and 3700 molecules/min per nucleus respectively; (*b*) the average rates of formation of mature 28S and 18S rRNA are identical with the rates of synthesis of 45S pre-rRNA in both normal and regenerating rat liver. Thus the synthesis of rRNA in 12h-regenerating rat liver is activated 2.7-fold. The analysis of rRNA synthesis in isolated nucleoli also shows a 2.7-fold stimulation of transcription in regenerating liver. It is concluded that all the 45S pre-rRNA molecules synthesized are processed and transferred as 28S and 18S rRNA in the cytoplasm, i.e. degradation (wastage) of newly synthesized ribosomes in the nucleus does not occur in both normal and regenerating rat liver. Thus the enhanced production of ribosomes in regenerating rat liver is regulated only at the transcriptional level.

The regenerating liver is a good model for studying the regulation of ribosome biosynthesis in animal cells. To estimate the role of transcriptional and post-transcriptional control in the production of ribosomes in regenerating rat liver, it is necessary to determine the rate of synthesis of rRNA precursor and the rate of formation of mature ribosomes. The comparison of these two parameters in normal and regenerating rat liver in labelling kinetics experiments encounters considerable difficulties (see Bucher & Malt, 1971). The latter stem from the profound changes in the total cellular metabolism taking place after partial hepatectomy and especially from the alterations in the pools of the free nucleotides, pre-rRNA and rRNA. It has been shown that: (*a*) livers from partially hepatectomized rats show an increased accumulation of radioactive precursors, which results in an increase of the specific radioactivity of RNA (Ord & Stocken, 1973; Muramatsu & Busch, 1965; Yngner *et al.*, 1979); (*b*) the kinetics of labelling of the free UTP and CTP differ substantially in normal and regenerating liver (Bucher & Swaffield, 1965, 1966, 1969; Yngner *et al.*, 1979); (*c*) significant changes occur in the pool sizes of nucleolar and nucleoplasmic pre-rRNA and rRNA (Dabeva & Dudov, 1982).

For the above reasons, the published data on the rate of pre-rRNA synthesis and the rate of ribosome formation in regenerating rat liver vary in a wide range. It has been reported that rRNA transcription in regenerating liver exceeds that in normal liver 2–4 times (Tsukada & Lieberman, 1964; Jacob *et al.*, 1967; Loeb & Yeung, 1975; Glazer, 1977). On the other hand, data have been presented that the rate of ribosome production is enhanced 10–12-fold (Chaudhuri *et al.*, 1967; Chaudhuri & Lieberman, 1968). Therefore it has been suggested that the increase in ribosome production is a result of both increased rate of pre-rRNA synthesis and more efficient utilization of 45S pre-rRNA (Chaudhuri & Lieberman, 1968; Rizzo & Webb, 1972).

On the basis of labelling kinetics experiments we have determined the absolute rate of synthesis of pre-rRNA and the rate of formation of ribosomes in normal and regenerating liver. The results show that the synthesis of 45S pre-rRNA in 12h-regenerating liver is enhanced 2.7-fold. Comparing the rates of synthesis of pre-rRNA and of mature rRNA, we conclude that degradation of newly synthesized

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

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ribosomes in the nucleus occurs neither in normal nor in regenerating rat liver. Thus the enhanced production of ribosomes in regenerating liver is regulated only at the level of transcription. Part of these results have been reported (Dabeva & Dudov, 1980).

Theory

When labelled orotate is used as a precursor, the specific radioactivity of RNA, $S(t)$, is:

$$S(t) = \alpha^u S^u(t) + \alpha^c S^c(t) \quad (1)$$

where S^u and S^c are the specific radioactivities of UMP and CMP in RNA respectively, and α^u and α^c are the contents of UMP and CMP in RNA.

If we designate:

$$\beta(t) = \alpha^u + \alpha^c \frac{S^c(t)}{S^u(t)} \quad (2)$$

eqn. (1) can be written:

$$S(t) = \beta(t) S^u(t) \quad (3)$$

On the other hand, the specific radioactivity of cellular free UTP, $U(t)$, is directly related to the specific radioactivity of UMP in RNA (see Reiner, 1974; Dudov *et al.*, 1978) (where k is the fractional turnover rate of the RNA pool):

$$\frac{dS^u(t)}{dt} = k[U(t) - S^u(t)] \quad (4)$$

Thus, from eqns. (3) and (4) one obtains the following equation for the specific radioactivity of RNA:

$$\frac{dS(t)}{dt} = k[\beta(t) U(t) - S(t)] + \frac{S(t)}{\beta(t)} \cdot \frac{d\beta(t)}{dt} \quad (5)$$

where the effect of RNA base composition and CMP labelling is included via $\beta(t)$.

When $U(t)$, $S(t)$, $\beta(t)$ and RNA pool size (A) are known, the rate of synthesis (V) and the half-life ($T^{0.5}$) of RNA can be calculated from eqns. (5) and (6):

$$V = kA, \quad T^{0.5} = k^{-1} \cdot \ln 2 \quad (6)$$

The above equations were used for computer evaluation of the rate of synthesis of 45 S pre-rRNA.

The rate of formation of mature rRNA (V') was obtained from the total radioactivity in rRNA [$R(t)$] and the specific radioactivity of 45 S pre-rRNA [$S(t)$]:

$$\frac{dR(t)}{dt} = V' S(t) \quad (7)$$

The above equation does not include the specific radioactivity of rRNA, which is negligibly small in comparison with the specific radioactivity of 45 S pre-rRNA for the labelling time intervals studied.

The computer analysis of the experimental kinetic data and the evaluation of the rates of synthesis and their errors were performed by the algorithms described previously (Dudov *et al.*, 1978).

Experimental

Animals

The experiments were performed with Wistar male albino rats weighing 150 ± 2 g. Partial hepatectomy was performed as described (Dabeva & Dudov, 1982), 12 h before the animals were killed. Labelling *in vivo* was with 20 μCi of [^{14}C]orotate/100 g, given intraperitoneally 20–120 min before death. Seven normal and 21 partially hepatectomized animals were used for each period of labelling.

Cell fractionation and RNA extraction

Pure nuclei were isolated by the two-step hyperosmotic-sucrose/detergent method described by Dabeva *et al.* (1978). For isolation of cytoplasmic rRNA 10 ml of the initial homogenate was diluted with 60 ml of 0.01 M-Tris/HCl (pH 7.6)/0.004 M-$MgCl_2$. Triton X-100 was added to 1% (v/v) final concn., the homogenate was shaken for 1 min and the nuclei were sedimented by centrifugation for 10 min at 2000 *g* in the cold. To the post-nuclear supernatant SDS was added (0.5% final concn.) and the cytoplasmic RNA was extracted by the phenol method (Hadjiolov *et al.*, 1974).

The nucleolar (nuRNA) and nucleoplasmic (npRNA) RNA were extracted from the detergent purified nuclei as described elsewhere (Dabeva *et al.*, 1976).

Isolation of nucleoli

Detergent-purified nuclei from 5 g of liver were suspended in 10 ml of 0.5 M-sucrose/2 mM-$MgCl_2$ containing 0.1 mg of dextran sulphate/ml and disintegrated by sonication for 90 s in an MSE 60 W ultrasonic disintegrator at −15 °C. The nucleolar suspension was centrifuged for 15 min at 3000 *g* through a cushion of 1 M-sucrose in the same medium.

Determination of radioactivity in cellular free UTP and UMP

To determine the specific radioactivity of free UMP, equal pieces from each liver were combined and homogenized in ice-cold 0.3 M-$HClO_4$. The extraction of acid-soluble nucleotides and the separation of uracil nucleotides were done by two-dimensional paper chromatography (Zhivkov,

1970). The specific radioactivity was calculated as d.p.m./μg of UMP.

The specific radioactivity of cellular free UTP and UMP were compared in independent experiments: the livers were frozen *in situ*, excised, weighed, pulverized and processed further as described above.

Determination of radioactivity in rRNA pools

The specific radioactivity of total nucleolar RNA (d.p.m./μg of RNA) was determined after alkaline hydrolysis in 0.3 M-KOH. The specific radioactivity of UMP fraction (d.p.m./μg of UMP) was determined after fractionation of mononucleotides by the method of Katz & Comb (1963).

The total cellular radioactivity in 28S or 18S RNA (d.p.m./nucleus) was obtained by summing the radioactivity incorporated in 28S or 18S rRNA (mature rRNA and the corresponding segments in pre-rRNA) in the nucleolar, nucleoplasmic and cytoplasmic compartments of the cell. The nuRNA, npRNA and cytRNA were fractionated electrophoretically (Dudov *et al.*, 1976), the dried agar films were scanned at 260nm and cut in 1mm slices and the radioactivity (R_i) in the separate pre-rRNA and rRNA species was determined (Dudov *et al.*, 1978) as shown in Fig. 1. The radioactivity per nucleus incorporated into 28S or 18S species in the whole nuRNA, npRNA or cytoplasmic RNA fractions (R^x; x$\equiv$28S or 18S rRNA) was calculated by the equation:

$$R^x = \frac{\text{amount of RNA in whole fraction/nucleus}}{\text{amount of RNA/gel}} \cdot \sum_i \frac{(\text{mol.wt.})^x}{(\text{mol.wt.})^i} R_i \qquad (8)$$

(for x$\equiv$28S: i = 41S, 36S, 32S pre-rRNA and 28S rRNA; for x$\equiv$18S: i = 41S, 21S pre-rRNA and 18S rRNA); where the amount of RNA/nucleus was determined as described by Dabeva & Dudov (1982), and molecular weights of pre-rRNA are from Dudov *et al.* (1976).

The specific radioactivity of 45S pre-rRNA (d.p.m./μg of RNA) was calculated from the radioactivity in the peak and its A_{260} (under specified conditions 1mm^2 of the area under the A_{260} peak corresponds to 0.094μg of RNA).

The specific radioactivities of free UMP, UMP in nuRNA, 45S pre-rRNA and the radioactivity in cellular 28S and/or 18S rRNA are mean values of three independent labelling kinetics experiments, where for each labelling period three to five electrophoretic or chromatographic analyses were carried out.

Synthesis of rRNA in vitro

RNA polymerase A was assayed *in vitro* by incubating isolated nucleoli in 25mM-Tris/HCl (pH 7.9) / 50mM-$(NH_4)_2SO_4$ / 7mM-$MgCl_2$ / 6mM-NaF/0.05% β-mercaptoethanol/12.5% glycerol/0.5mM-EDTA containing 1μg of α-amanitin/ml, 0.3mM each of ATP, GTP and CTP, and 0.017mM-[^{3}H]UTP (sp. radioactivity 2Ci/mmol) in a final volume of 0.120ml, containing 10μg of nucleolar DNA at 20°C. Samples (0.020ml) were taken every 5min and processed further as described by Hadjiolov & Milchev (1974). Enzyme activity was estimated in the absence or presence of 150μg of heparin/ml. DNA content was determined by the method of Burton (1956).

Materials

Analytical-grade reagents were used throughout. ATP, GTP, CTP and α-amanitin were obtained from Sigma, St. Louis, MO, U.S.A. [^{3}H]UTP (sp. radioactivity 39Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and [6-^{14}C]orotate (sp. radioactivity 37mCi/mmol) was from NAEC Institute for Isotopes, Budapest, Hungary.

Results

It has been shown that 12h after partial hepatectomy the amount of nuclear rRNA is increased by 60% and that of cytoplasmic rRNA by 25–30% (Dabeva & Dudov, 1982). The pool size of 45S pre-rRNA doubles, reflecting the significant increase of pre-rRNA synthesis. To answer the question whether the increased production of ribosomes is regulated only transcriptionally or whether a post-transcriptional control of the production of ribosomes plays an essential role in regenerating rat liver, the rate of synthesis of 45S pre-rRNA and the rate of formation of cytoplasmic ribosomes in normal and regenerating liver were determined and the obtained values were compared.

Rate of synthesis of 45S pre-rRNA

The rate of synthesis of 45S pre-rRNA in normal and regenerating rat liver was determined directly on the basis of the following experimental data: (*a*) the pool size of 45S pre-rRNA (Dabeva & Dudov, 1982); (*b*) the kinetics of labelling of cellular free UTP; (*c*) the kinetics of labelling of 45S pre-rRNA; and (*d*) evaluation of the influence of CMP labelling and of RNA base composition on the specific radioactivity of 45S pre-rRNA [function $\beta(t)$ in eqn. (5)].

In the course of the kinetic experiments we estimated the specific radioactivity of free UMP in the same liver used for isolation of nuclei and for

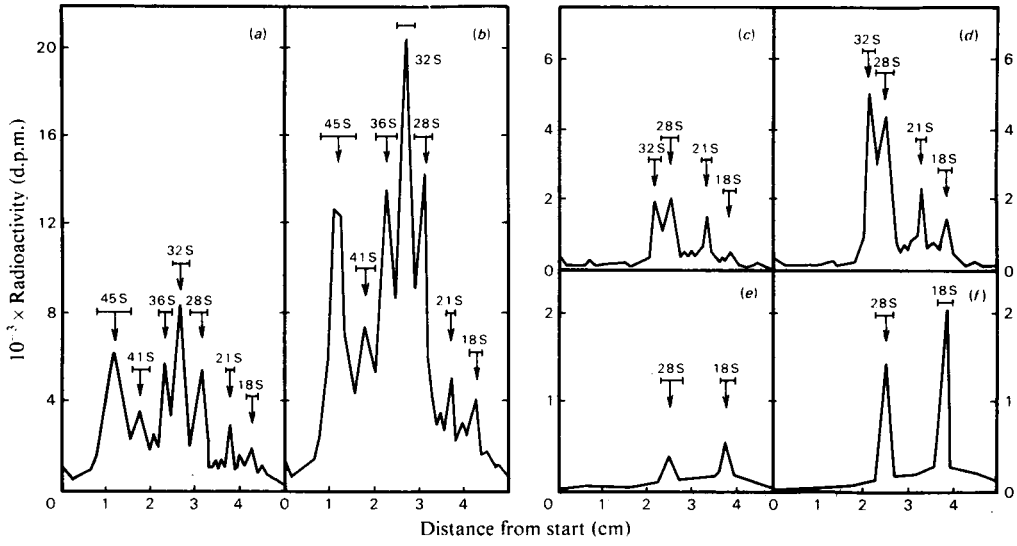

Fig. 1. *Labelling of pre-rRNA and rRNA in the nucleolar, nucleoplasmic and cytoplasmic compartments of normal and regenerating rat liver*

RNA was labelled *in vivo* for 80 min with 20 μCi of [^{14}C]orotate/100 g body wt. The nuRNA (*a*, *b*), npRNA (*c*, *d*) and cytoplasmic RNA (*e*, *f*) from normal (*a*, *c*, *e*) and regenerating (*b*, *d*, *f*) rat liver were extracted and fractionated electrophoretically in urea/agar gels as described in the Experimental section. The dried agar films were cut in 1 mm slices and the radioactivity in each slice determined. The radioactivity incorporated into individual pre-rRNA and rRNA species was obtained by summing the radioactivity corresponding to the indicated zones. Each track is loaded with 100 μg of RNA. The amounts of RNA (pg/nucleus) extracted in this experiment are (for normal and regenerating liver respectively): nuRNA, 0.8 and 1.22; npRNA, 0.63 and 0.76; cytoplasmic RNA, 25 and 32.

RNA extraction. The specific radioactivities of free UTP and UMP were compared in separate experiments. The results showed that a 20 min pulse with [^{14}C]orotate was sufficient for equilibration of the specific radioactivities of free UMP, UDP and UTP.

The kinetics of labelling of free UMP and 45 S pre-rRNA are quite different in normal and regenerating liver (Fig. 2). In normal liver the UMP specific radioactivity increases rapidly during the first 20 min, but reaches a plateau between 45 and 120 min. The corresponding curve for regenerating liver rises abruptly, reaches a maximum after 45 min and then declines. Since the endogenous pool of uridine phosphates doubles 12 h after partial hepatectomy (Bucher & Swaffield, 1969), these data show that the turnover rate of uracil nucleotides is about 4 times higher in regenerating liver than in normal.

The influence of CMP labelling and RNA base composition on the specific radioactivity of 45 S pre-rRNA was taken into account by the function $\beta(t)$ (eqn. 5), obtained as a ratio between the specific radioactivity of nuRNA and the specific radioactivity of UMP in it (see eqn. 3), assuming that $\beta(t)$ is identical in nuRNA and 45 S pre-rRNA by reason of the relatively high turnover rates of all the nucleolar pre-rRNA and rRNA species (Dudov *et al.*, 1978). As seen in Fig. 2, in the short periods of labelling, $\beta(t)$ tends to 0.21 when $t \to 0$. This result agrees with the experimentally determined percentage of UMP in 45 S pre-rRNA (Dabeva & Tsanev, 1968) and with the theoretical curve of $\beta(t)$ (predicted by eqn. 2). Whereas the values for $\beta(t)$ change insignificantly in normal liver, in regenerating liver $\beta(t)$ increases appreciably with time, reflecting a more intense incorporation of the label into CMP of RNA, most probably as a result of stimulated CTP synthetase activity (Genchev & Mandel, 1976).

The best theoretical fit to the experimentally determined specific-radioactivity points corresponds to the rates of synthesis and half-lives of 45 S pre-rRNA in normal and regenerating rat liver given in Table 1. It should be noted that the synthesis of 45 S pre-rRNA in 12 h-regenerating liver is enhanced 2.7-fold and its pool is doubled. This result shows that the turnover time of 45 S pre-rRNA is shortened by 25%. Thus the rates of both synthesis and processing of 45 S pre-rRNA are accelerated in regenerating liver.

Rate of formation of 28 S and 18 S rRNA

To determine the rate of formation of cytoplasmic 28 S and 18 S rRNA it is necesary to know

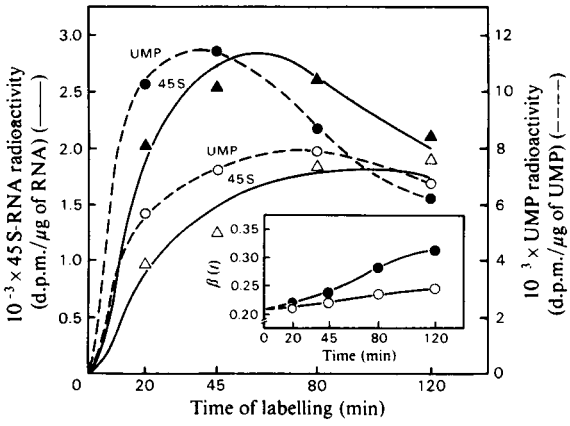

Fig. 2. *Kinetics of labelling of cellular free UMP and 45 S pre-rRNA in vivo*

The experimental points for the specific radioactivities of cellular free UMP (○, ●) and of 45 S pre-rRNA (△, ▲) in normal (open symbols) and 12 h-regenerating (filled symbols) rat liver are obtained as described in the Experimental section. (----), Experimental curves for free UMP obtained after polynomial approximation; (——), theoretical curves for 45 S pre-rRNA corresponding to the rate of synthesis of 1380 and 3670 molecules/min per nucleus in normal and regenerating rat liver respectively. The experimental data for $\beta(t)$ are given in the insert [for determination of $\beta(t)$ see the text].

the specific radioactivity of their immediate precursors, nuclear 28 S and 18 S rRNA. As shown previously, the detergent-purified nuclei are still contaminated by cytoplasmic ribosomes (Dabeva *et al.*, 1977), and precise estimation of the specific radioactivities of truly nuclear 28 S and 18 S rRNA is not possible. For this reason the rates of synthesis of 28 S and 18 S rRNA were calculated on the basis of the specific radioactivity of 45 S pre-rRNA and the total radioactivities in cellular 28 S and 18 S rRNA (see the Theory section, eqn. 7). The total radioactivity in 28 S or 18 S rRNA was determined as described in the Experimental section (eqn. 8), and it included the radioactivity incorporated in 28 S or 18 S sequences of all the pre-rRNA species. The latter could not be neglected, because of their higher labelling and the great differences between the pool sizes of the nucleolar pre-rRNA in normal and regenerating liver.

The kinetic data obtained for the labelling of 28 S and 18 S rRNA are given in Fig. 3. The calculated rates of formation of 28 S and 18 S rRNA in normal and regenerating liver are presented in Table 2; the obtained rate of formation of 28 S rRNA is lower than that of 18 S rRNA in both normal and regenerating liver. However, the differences in the rates of 45 S pre-rRNA synthesis and the rates of 28 S or 18 S rRNA formation are statistically insignificant (see Table 2). The calculated average

Table 1. *Rate of synthesis and half-life of 45 S pre-rRNA in normal and regenerating rat liver*

The fractional turnover rate (k), the half-life and the rate of synthesis of 45 S pre-rRNA in normal and 12 h-regenerating liver are calculated from the experimental data on specific radioactivities of free UMP and 45 S pre-RNA, taking into account the CMP labelling and the base composition of pre-rRNA [function $\beta(t)$ in Fig. 2]. Values are means ± S.E.M. evaluated by a computer simulation method described previously (Dudov *et al.*, 1978). Student's t test (two-tailed, $n = 3$ independent experiments) was applied to assess the significance of the differences between normal and regenerating liver. The pool sizes of 45 S pre-rRNA are from Dabeva & Dudov (1982).

Liver	k (min^{-1})	Pool size (molecules/nucleus)	Half-life (min)	Rate of synthesis (molecules/min per nucleus)
Normal	0.117 ± 0.024	11800	6.0 ± 1.3	1380 ± 360
Regenerating	0.157 ± 0.035 ($P < 0.2$)	23900	4.5 ± 1.0	3670 ± 1000 ($P < 0.05$)

Table 2. *Rates of formation of 28 S and 18 S rRNA in normal and regenerating rat liver*

The rates of formation (means ± S.E.M.) of 28 S and 18 S rRNA in normal and 12 h-regenerating liver are evaluated by computer on the basis of the experimental data on specific radioactivities of 45 S pre-rRNA and total radioactivities incorporated into 28 S and 18 S rRNA sequences (Fig. 3). Statistical significance (Student's t test, two-tailed, $n = 3$) of the differences in the rates of rRNA formation between normal and regenerating liver is indicated by $^{*}P < 0.1$ and $^{**}P < 0.05$. The rates of 28 S or 18 S rRNA formation obtained are not significantly different from the rate of 45 S pre-rRNA synthesis in both normal and regenerating liver (Table 1): $\dagger P > 0.9$, $\dagger\dagger P > 0.5$.

	Rates of formation (molecules/min per nucleus)		
Liver	28 S rRNA	18 S rRNA	Mean value
Normal	1080 ± 390††	1440 ± 500†	1260 ± 450††
Regenerating	3220 ± 1160*,††	4200 ± 1480**,††	3710 ± 1330*,†
Activation (fold)	2.98	2.92	2.95

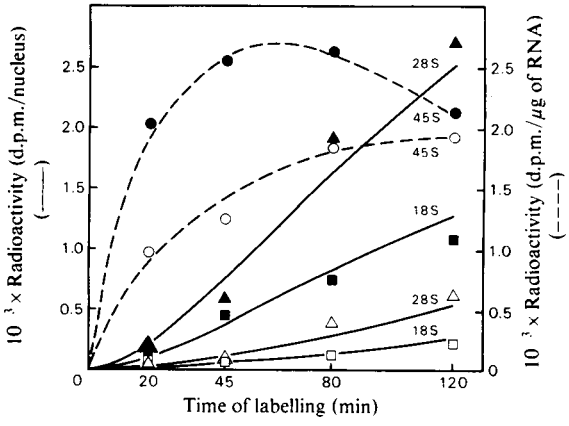

Fig. 3. *Kinetics of labelling of cellular 28S and 18S rRNA in vivo*

The experimental points for the total radioactivity incorporated into 28S (△, ▲) and 18S (□, ■) rRNA in normal (open symbols) and regenerating (filled symbols) rat liver are obtained as described in the Experimental section. The experimental points for the specific radioactivity of 45S pre-rRNA (○, ●) are the same as in Fig. 2. (----), Experimental curves for 45S pre-rRNA obtained after polynomial approximation; (——), theoretical curves for 28S and 18S rRNA corresponding to the rates of rRNA formation given in Table 2.

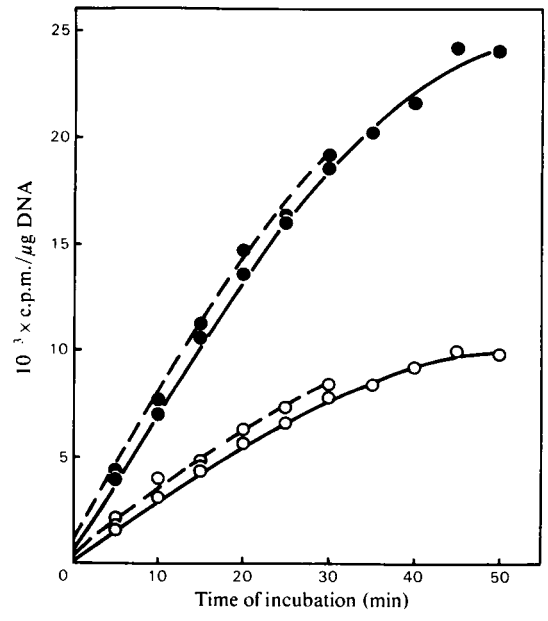

Fig. 4. *Kinetics of incorporation of [³H]UTP in isolated nucleoli*

Nucleoli from normal (○) and 12h-regenerating (●) rat liver were incubated in the presence (——) or absence (----) of 150μg of heparin/ml at 20°C in the presence of the four nucleoside triphosphates as detailed in the Experimental section. The values for the amount of the product synthesized *in vitro* are expressed as c.p.m. of [³H]UTP incorporated into RNA per μg of DNA.

rates of rRNA formation are identical with the rates of 45S pre-rRNA synthesis in both normal and regenerating liver (Table 1). Consequently we can conclude that all the synthesized 45S pre-rRNA molecules are processed in the nucleus to 28S and 18S rRNA and are transferred to the cytoplasm. This means that no degradation (wastage) of 28S and 18S rRNA takes place in the nucleus of normal or regenerating rat liver.

Most likely, the reasons for the differences obtained in the rates of formation of 28S and 18S rRNA are: (*a*) some overestimation of the rate of 18S rRNA formation owing to contamination of cytoplasmic 18S rRNA by labelled mRNA, which is proportionally higher at shorter labelling time intervals; (*b*) at shorter labelling periods the radioactivity in 28S RNA is found mainly in the pre-rRNA species. Some degradation of nuclear pre-rRNA during the thermal extraction may also take place, lowering the total radioactivity of cellular 28S rRNA, which could explain the underestimation of the rate of 28S rRNA formation.

Synthesis of pre-rRNA in vitro in normal and regenerating liver

A different approach to compare the rate of pre-rRNA synthesis in normal and regenerating liver is to study the kinetics of RNA synthesis *in vitro* by RNA polymerase A. It has been shown that under optimal conditions rRNA genes are transcribed faithfully in isolated nucleoli (Grummt, 1978; Onishi & Muramatsu, 1978). Experiments with heparin have shown that RNA synthesis *in vitro* in isolated nucleoli represents elongation of RNA chains initiated *in vivo* (Onishi *et al.*, 1977).

Under our experimental conditions isolated nucleoli are capable of incorporating nucleoside triphosphates linearly for 30min (Fig. 4). No differences in the time course of the reaction in the presence or absence of heparin have been detected, showing that (i) the radioactivity is found in polynucleotide chains initiated *in vivo* (Onishi *et al.*, 1977) and (ii) the elongation rate of RNA polymerase A is not affected by heparin (Coupar & Chesterton, 1977). From the slope of the kinetic curves we calculated that the rRNA synthesis *in vitro* is activated 2.68-fold in regenerating liver (in five different experiments the values vary from 2.46 to 2.83). The amount of the product synthesized after 50–60min, when the reaction approaches a plateau, is about 2.5 times higher. If the termination of rRNA transcription *in vitro* is identical in normal

and regenerating liver, than the amount of the product synthesized pending the completion of the reaction should be proportional to the number of bound RNA polymerase A molecules.

Discussion

The results presented here give information about the regulation of the biosynthesis of ribosomes in rat liver under conditions when their production in the cell is activated.

On the basis of labelling kinetics experiments we have determined the absolute rates of synthesis of 45S pre-rRNA and the rates of formation of cellular 28S and 18S rRNA in normal and 12h-regenerating rat liver. Our analyses were made taking into account the alterations in the labelling of the precursor free pyrimidine nucleotides as well as the changes in the pool sizes and in the labelling of pre-rRNA and rRNA.

A critical point in the direct estimation of the rate of synthesis of RNA is the possibility for intracellular compartmentation of the free nucleotides precursor pool (Wiegers *et al.*, 1976; Genchev *et al.*, 1980). However, no compartmentation of the free nucleotides precursor pool in rat liver has been shown to exist (Stärk *et al.*, 1978). On the other hand, the direct estimation of the rate of synthesis of 45S pre-rRNA in normal liver gives values very close to those obtained by an indirect method, which does not require determination of the specific radioactivity of the free nucleotides pool (Dudov *et al.*, 1978). We can conclude that the intracellular compartmentation of UTP in rat liver, if any, is not significant for the reliable assessment of the rate of precursor rRNA synthesis.

Analysing the labelling of pre-rRNA and of cytoplasmic rRNA in regenerating rat liver, some authors have found a considerable enhancement in the appearance of label in cytoplasmic ribosomes and a much smaller increase in the uptake of label into 45S pre-rRNA (Chaudhuri *et al.*, 1967; Chaudhuri & Lieberman, 1968; Rizzo & Webb, 1972). This fact has been explained by suggesting that 45S pre-rRNA is more efficiently utilized in regenerating liver and that the degradation (wastage) of newly synthesized ribosomes is decreased (Chaudhuri & Lieberman, 1968; Rizzo & Webb, 1972). From our experiments it is also evident that, although the label in 45S pre-rRNA increases 2-fold, the radioactivity in cytoplasmic rRNA is 8–10 times higher (see Fig. 1). However, comparing the rate of synthesis of 45S pre-rRNA and the rate of formation of mature rRNA (Tables 1 and 2), it becomes evident that degradation (wastage) of 28S or 18S rRNA sequences in the nucleus does not take place in both normal and regenerating liver. The markedly greater accumulation of label into cytoplasmic rRNA of regenerating liver is a consequence of several factors acting simultaneously: (*a*) the two-fold increase in the specific radioactivity of cellular free UTP and CTP (Fig. 2); (*b*) the 2.7-fold increased synthesis of 45S pre-rRNA and the shortening of its half-life (Table 1); (*c*) the shortening of the time for formation of ribosomes in the nucleus and their transfer to the cytoplasm. This consideration follows from the fact that, whereas rRNA production is stimulated 2.7 times, the amount of nuclear rRNA is increased only 1.6 times (Dabeva & Dudov, 1982). We conclude that the increased production of ribosomes in regenerating liver is determined only at the transcriptional level.

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