INCREASED LEVELS OF RAT HEPATIC NUCLEAR FREE AND ENGAGED RNA POLYMERASE ACTIVITIES DURING LIVER REGENERATION

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SUMMARY

Rat liver nuclei, seventeen hours after partial hepatectomy, showed a two to three-fold increase in total RNA synthesis *in vitro* over the sham operated controls. When tested with exogenous synthetic template, this was found to be mainly a reflection of increased levels of both the nuclear free and engaged RNA polymerase activities *per se*. It was also observed that there was a greater stimulation of the species of RNA polymerase that are α-amanitin resistant than sensitive (3.2 μg/ml). This observation was further confirmed by DEAE-Sephadex column chromatography of the solubilized nuclear free and engaged RNA polymerases and found RNA polymerase I and IIIa were the major species greatly stimulated during this period of liver regeneration. These data suggest not only that there exists a sensitive equilibrium between the nuclear free and engaged RNA polymerases; they also suggest the possibility that RNA polymerase itself may play a positive role in the regulation of gene expression.

INTRODUCTION

One of the most challenging problems in modern biology is to determine how gene expression is regulated in higher organisms. There are two conceivable possibilities that the cellular information may be regulated at the transcriptional level: 1.) by controlling the availability of the DNA template through various repressor or activator molecules normally found on the chromatin; or 2.) the gene expression may be regulated by the enzyme RNA polymerase which transcribes the information directly from the genome, or both.

Within 24 hours after the partial hepatectomy, the stub that was left at the time of the operation has doubled in size. This is due to hypertrophy and hyperplasia of the liver cells (1). Thus, the regenerating liver system

provides an excellent opportunity to study the biochemical events of gene activation during "normal" cell proliferation and rapid growth. Among the earliest detectable changes after partial hepatectomy are the increase in the rate of RNA synthesis (2-5) with a concomitant increase in both nucleolar size (6) and nucleolar RNA content (7-8) which was later demonstrated from in vivo (9-10) and in vitro (11-13) studies to be a result of increased rate of nucleolar RNA synthesis. Several reports in the literature have attempted to dissect the biochemical mechanism underlying this increased rate of RNA synthesis after partial hepatectomy as to whether it is due to an increased level of RNA polymerase per se, or an increased availability of DNA sites for transcription. Unfortunately, these reports gave conflicting results. Tsukada and Lieberman from a series of studies (9, 12, 14), using actinomycin D, puromycin and p-flurophenylalanine injections to the partial hepatectomized rats at various times, concluded that the increase in RNA polymerase activity after partial hepatectomy was a result of increased enzyme synthesis. On the other hand, Pogo et al. (15) Thaler et al. (16) and Mayfield and Bonner (17), studying in vitro and using isolated nuclei or chromatin with added bacterial RNA polymerase, concluded partial hepatectomy increased the template function. Because the conflicting reports in the literature and the recent findings indicate the use of bacterial RNA polymerase to transcribe mammalian chromatin to be an unreliable method for the measurement of the true template function (18-19), I have re-evaluated this problem with the improved method of quantitative isolation of rat liver RNA polymerase (20). This paper presents direct evidence to indicate that the increased rate of RNA synthesis after partial hepatectomy was mainly a reflection of increased levels of RNA polymerase activity per se and suggests the possibility that RNA polymerase itself may act as a positive regulator in gene expression.

METHODS

Procedures for partial hepatectomy: Male Sprague-Dawley rats of about 200 g body weight were used. Partial hepatectomy, which refers to the removal of

65-75% of the liver, was performed according to Higgins and Anderson (21). Sham operations were identically performed, except that no liver was excised. The rats were maintained on regular Purina Laboratory Chow, 5% glucose and water ad libitum. They were sacrificed, in groups of 3-4 rats, 17 hours after operation.

Isolation of rat liver nuclei: Rat liver nuclei were isolated by the hypertonic sucrose method, as described previously (22).

Separation of the free and engaged RNA polymerases from the isolated nuclei: The nuclear free RNA polymerase could be easily separated from the nuclear engaged enzyme by gentle homogenization of the isolated nuclei in 0.34 M sucrose followed by centrifugation for 5 minutes at 2,000 X g in a refrigerated International centrifuge. The supernatant fluid contained 80% or more of the free enzyme, while the nuclear pellet retained all the engaged enzyme (20).

Solubilization of the nuclear engaged RNA polymerase: The nuclear pellet minus the free enzyme was suspended in 0.01 M Tris-HCl buffer (pH 7.9, 23°), containing 1 M sucrose, 5 mM $MgCl_2$ and 20 mM 2-mercaptoethanol. And the engaged RNA polymerase was solubilized according to the method of Roeder and Rutter (23). A Biosonik IV sonicator (dial 60, microprobe) was used for the sonication.

Assay of RNA polymerase activity: Conditions for RNA polymerase assay were identical to those described previously (24-25).

RESULTS AND DISCUSSION

Table 1 shows that rat liver nuclei, 17 hours after partial hepatectomy, manifested a two to three-fold increase in total RNA synthesis in vitro over the sham-operated controls. This effect was observed when either ^{14}C-UTP or

TABLE 1

Effect of partial hepatectomy (17 hrs) on nuclear RNA polymerase activity measured with endogenous and exogenous templates

	RNA polymerase activity (pmole labeled precursor incorporated/g liver)							
Group	Endo template				poly(dA-dT)		poly(dI-dC)	
	^{14}C-UTP	%	^{14}C-GTP	%	^{14}C-UTP	%	^{14}C-GTP	%
Sham	1120	100	1490	100	1830	100	2890	100
Regenerating liver	2700	241	4170	280	3160	173	6080	210

^{14}C-GTP was used as the labelled RNA precursor. This result is in good agreement with the earlier reports derived from studies in vivo (2-10). In order to determine whether the increased nuclear RNA synthesis after partial hepatectomy was due to endogenous chromatin template activation or increased levels of RNA polymerase activity per se, the technique that I had developed a few years ago was used (22). In these experiments the nuclear endogenous template was effectively blocked by a high dose of actinomycin D (10 μg/ml) (22,24,25), and the RNA polymerase activity was measured independently with the addition of synthetic DNA templates poly(dA-dT) or poly(dI-dC). These templates were capable of directing RNA synthesis because they do not contain deoxyguanosine moiety and hence are not sensitive to actinomycin D inhibition (26). The results of these studies are also shown in Table 1. It is clear from these data that the increased nuclear RNA synthesis after partial hepatectomy was mainly a reflection of increased levels of RNA polymerase activity per se.

It was recently discovered that there are two functionally distinct populations of RNA polymerases in rat liver nuclei. One of these, referred

TABLE II

Effect of partial hepatectomy (17 hrs) on the nuclear free RNA polymerase activity measured with poly(dI-dC) template

Group	α-amanitin (3.2 μg/ml)	RNA polymerase activity	
		pmole of (^{14}C)GMP incorporated/g liver	%
Sham	-	1690	100
Regenerating liver	-	3799	225
Sham	+	647	100
Regenerating liver	+	1946	301

to as the engaged enzyme, is active in transcribing the endogenous chromatin template; the other, called the free enzyme, is inactive (24). It is now known that when the endogenous template is blocked by actinomycin-D (as described in Table I), RNA polymerase activity measured with the exogenous synthetic template represents only the free RNA polymerase population (20). It is also realized that the conventional method of solubilization of RNA polymerase from isolated nuclei yields mainly the engaged enzyme (20).

To isolate quantitatively the total RNA polymerases from nuclei, I have devised an improved method which sequentially extracts the free enzyme and the engaged enzyme (20), and the result from this approach is shown in Tables II and III. In good agreement with the data obtained from Table I, where the free enzyme activity was measured *in situ* in the presence of actinomycin D and synthetic templates in the whole nuclei, here again a two-fold increase in extracted free enzyme activity was observed (Table II) for the regenerating liver group over the control. It should be mentioned here that the extracted free enzyme preparations contained no endogenous chromatin template function because its activity was entirely dependent on the added

TABLE III

Effect of partial hepatectomy (17 hrs) on *solubilized* nuclear engaged RNA polymerase activity measured with poly(dI-dC) template

Group	α-amanitin (3.2 μg/ml)	RNA polymerase activity	
		pmole of (^{14}C)GMP incorporated/g liver	%
Sham	-	3570	100
Regenerating liver	-	5877	165
Sham	+	2287	100
Regenerating liver	+	4693	205

synthetic template (20). Table II also shows that there was comparatively an even greater increase in free enzyme activity in the presence of low concentration of α-amanitin (3.2 μg/ml). These data suggest that there was a rather selective stimulation of species of RNA polymerase that is α-amanitin resistant rather than sensitive. Table III presents the increased levels of the solubilized engaged RNA polymerase activities after partial hepatectomy. The results are quite similar to those observed for the free enzyme in Table II, suggesting that there exists a sensitive equilibrium between the free enzyme and engaged enzyme in the nuclei.

Because of the α-amanitin data from Table II and III, it was decided to analyze further the species of RNA polymerase that was selectively stimulated during liver regeneration. Solubilized free and engaged RNA polymerases derived from equal amounts of livers from each group were put on DEAE-Sephadex columns and chromatographed. Fig. 1 shows a typical elution profile obtained from the free enzymes of the control (A) and the regenerating liver group (B). Four distinct peaks were obtained from both groups. Based on their relative eluting positions in respect to $(NH_4)_2SO_4$ concentration and α-amanitin sensitivity, they are identified* as RNA polymerase I, II, IIIa and IIIb. It was found that RNA polymerase I and IIIa were the major species greatly stimulated during liver regeneration. Changes of RNA polymerase II and IIIb were in general small and variable. Similar results were obtained from the engaged RNA polymerase, as is shown in Fig. 2. Here again a strong stimulation was observed for RNA polymerase I and IIIa, and a weak stimulation for RNA polymerase II and IIIb.

These data agree well with the low-dose α-amanitin experiments (Table II and III). Since indeed the RNA polymerases that were preferentially stimulated in Fig. 1 and 2, i.e., polymerase I and IIIa, are α-amanitin resistant at this dose level (3.2 μg/ml) (27-28), and furthermore, because it is believed that RNA polymerase I is responsible for ribosomal RNA synthesis (23,29-30), these data are also in good agreement with the observations that

*Data submitted for publication elsewhere.

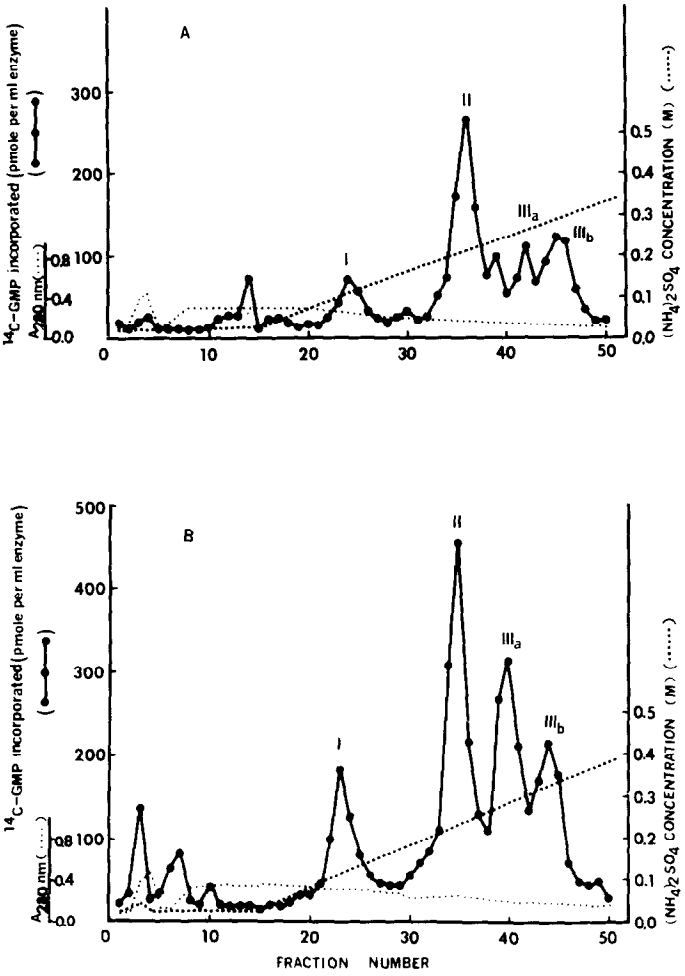

Fig. 1. DEAE-Sephadex (A-25) column chromatography of nuclear free RNA polymerase. The column (15 X 0.3 cm) was equilibrated with TGMEM buffer (0.05 M Tris-HCl, pH 7.9 at 23°, 25% (v/v) glycerol. 5 mM $MgCl_2$, 0.1 mM EDTA, and 20 mM 2-mercaptoethanol) containing 50 mM $(NH_4)_2SO_4$ (A:control) 2.0 ml of the nuclear free RNA polymerase sample (3.3 g liver equivalent) was applied onto the column. After washing with 2 column volumes of the equilibrating buffer, a linear gradient of 50 to 500 mM $(NH_4)_2SO_4$, in total 50 ml, was developed to elute the enzyme. A flow rate of 14-15 ml/hour was maintained. Fractions of 1.2 ml were collected. 0.5 ml aliquots of the eluate were taken and immediately mixed with 50 µl of bovine serum albumin (10 mg/ml). For RNA polymerase assay, 0.1 ml of the enzyme solution was added to 0.2 ml assay medium containing (µmoles): Tris-HCl, pH 7.9 at 23°, 20; $MnCl_2$, 0.4; 2-mercaptoethanol, 5.6; ATP, GTP, CTP and UTP, each 0.04; with 0.1 µCi of ^{14}C-GTP (Schwarz/Mann, sp. act. 40 mCi/µmole), and 1.25 µg Poly(dI-dC) (Boehringer Mannheim). Incubation was carried out at 37° C for 20 minutes. $(NH_4)_2SO_4$ concentration was determined with a conductivity meter. Radioactivity analysis was as previously described (24). (B:Regenerating liver) 2.0 ml of the nuclear free enzyme (3.4 g liver equivalent) was used, and it was column fractionated under identical conditions as the control group.

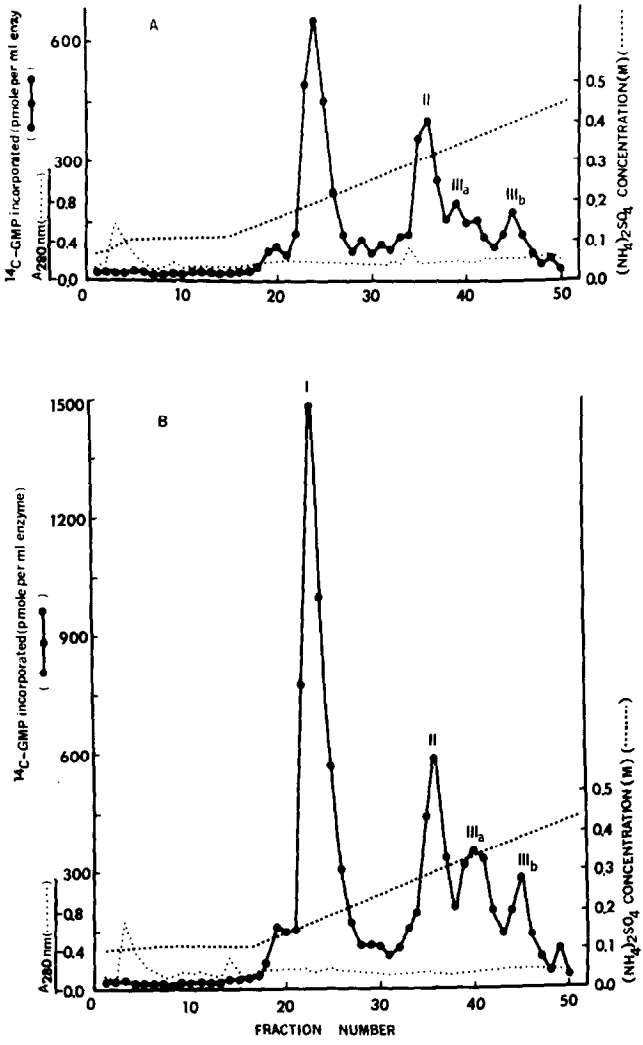

Fig. 2. DEAE-Sephadex (A-25) column chromatography of nuclear engaged RNA polymerase. (A:control) The conditions for enzyme fractionation were identical to those of Fig. 1, except that 1 ml of the engaged enzyme (4.8 g liver equivalent) was used. (B: Regenerating liver) 1 ml of the engaged enzyme (5.2 g liver equivalent) was used.

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increased nucleolar size (6) and predominant stimulation of ribosomal RNA synthesis (9-13) were the main notable features during early liver regeneration. Finally, these data suggest not only that there exists a sensitive equilibrium between the nuclear free and engaged RNA polymerases in support-

ing an earlier proposed model (20), they also suggest the possibility that RNA polymerase itself may act as a positive regulator in gene expression.

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