PART III

TRANSCRIPTIONAL CHANGES IN THE REGENERATING RAT LIVER

INTRODUCTION

Studies described in the previous part, as well as those reported in the literature, have shown that transcription apparatus can be activated in mammalian system in response to several stress conditions (1-6). Subtle stimulatory effects in transcription can also be achieved by administration of suitable doses of specific hormones (3,4,6,7). Several studies aimed at dissecting the underlying biochemical mechanisms have revealed that the events leading to transcriptional changes could be different under different stimuli. These results have no doubt provided many insights into regulatory aspects of eukaryotic transcription.

Considerable upheaval in RNA synthetic pattern is however not a phenomenon that can be elicited only by resorting to changes in external environmental conditions. It has been documented that during cell-cycle, a number of transcriptional events take place in the G_1 phase presumably to prepare the cell for DNA synthesis and subsequent cell division (8-12). To study such alterations in higher organisms, the regenerating liver provides an excellent system since the biochemical events occurring after partial hepatectomy could be expected to be associated with normal cell proliferation and rapid growth. Quiescent cells of the mammalian liver can be triggered to proliferate by removal of two-thirds of the organ. In the hepatocytes of the rats, a wave of DNA synthesis occurs in the nucleus about 18 hr after partial hepatectomy and mitosis follows 6 to 8 hr

later (13). Among the earliest detectable changes which precede DNA synthesis, are the increase in the rate of RNA synthesis (12).

The studies to be reported in this part have attempted to elucidate events associated with stimulation in RNA synthesis in the liver following partial hepatectomy. The results indicate that a number of biochemical changes take place during liver regeneration, which include altered turnover of nonhistone chromosomal proteins, activation of RNA polymerases (Type I and II), increase in chromatin template efficiency and increase in number of RNA chain initiation sites on chromatin.

MATERIALS AND METHODS

Animal experiments

Male Wistar strain rats each weighing between 120-130 g and fed on a laboratory stock diet were used. For liver regeneration experiments, the animals were 67% hepatectomized under ether anesthesia by excision of the left lateral and median lobes (14). Sham-operated rats served as controls. In all the experiments, rats were fasted for 18-20 hr prior to killing.

Measurement of the rate of DNA synthesis in the liver (incorporation of ^{3}H-thymidine into DNA)

Rats were given intraperitoneal injection of ^{3}H-thymidine (80 μCi/100 g body wt) and sacrificed 2 hr later. Radioactivity incorporated into DNA was determined by isolating DNA by the method of Munro and Fleck (15) and measuring counts due to DNA in aliquots of KOH-neutralised TCA-extracts.

Measurement of the rate of RNA synthesis in the liver (incorporation of ^{32}P-orthophosphate into RNA)

The procedure was as described in Part II-Section I.

Sucrose density gradient centrifugation analysis of ^{32}P-labelled liver RNA

As described in Part II-Section I.

Assays of RNA polymerisation by liver nuclei, liver RNA polymerase, template activities of liver chromatin and DNA

As described in Part II-Section I.

Chromatographic fractionation of liver RNA polymerase on DEAE-cellulose

The liver RNA polymerase was isolated and purified up to fraction IV of Roeder and Rutter and fractionation was carried on a DEAE-cellulose column according to the method of Torres (16). Briefly the procedure was as follows. The column (1 x 12 cm) was equilibrated with TGMED buffer pH 8.0 containing 0.025 M KCl. Ten mg of the RNA polymerase preparation in TGMED buffer pH 8.0 (~ 3 ml) was applied onto the column. Ten ml of the same buffer was first passed through the column. Thereafter, the enzyme preparation was eluted with a linear gradient of 0.025 M to 0.5 M KCl in TGMED buffer pH 8.0 with a total volume of 50 ml. One ml fractions were collected and assayed for RNA polymerase activity with calf-thymus DNA and ^{14}C-ATP as described in Part II-Section I.

Analysis of labelling patterns of chromosomal nonhistone proteins

The procedures used were essentially those detailed in Part II-Section II. Sham-operated and partially hepatectomized rats (at specified

times post-operation) were injected with 83 μCi of ^{14}C-DL-leucine and 600 μCi of ^{3}H-DL-leucine respectively and sacrificed 1 hr later. The excised livers from the two groups were homogenised together and nonhistone chromosomal proteins were isolated. These were electrophoretically fractionated on the polyacrylamide-SDS gels, slices of 2 mm thickness were transversely cut along the gel length and counted for ^{14}C- and ^{3}H-radioactivities. The ratios of the two activities reflected rates of labelling of the individual proteins in the liver of partially hepatectomized rats in relation to the corresponding rates of labelling in the liver of sham-operated (control) rats.

A reverse experiment in which sham-operated rats received ^{3}H-DL-leucine and partially hepatectomized animals ^{14}C-DL-leucine, was also carried out using the same procedure as described above.

Determination of molecular weights of electrophoretically separated proteins was as described in Part II-Section II.

Measurement of radioactivity

As described in Part II-Section I.

Chemical analysis

DNA, RNA and protein were estimated as described in Part II-Section I.

Materials

^{3}H-Thymidine (sp.act. 6.2 Ci/mmole), ^{32}P-orthophosphate (carrier free), ^{14}C-DL-leucine (sp. act. 0.25 mCi/mmole), ^{3}H-DL-leucine (sp. act. 5 Ci/mmole), ^{3}H-UTP (Sp. act. 18.2 Ci/mmole) (Isotope Division of this Research Centre), GTP, CTP, UTP

(Sigma Chemical Co., U.S.A.), ^{14}C-ATP (sp. act. 27.2 mCi/mmole, Schwartz Biochemicals, U.S.A.) were used.

RESULTS

Restorative process after partial hepatectomy

As evident from Table 1, the restoration of liver weight that follows the removal of median and left lateral lobes of the liver tissue is quite rapid. This phenomenon can be appropriately termed as a truly hyperplastic process, the proliferation of new cells occurring at a rate exceeding even that of liver tumors (17). During the two week period, the liver weight is increased almost to the pre-operation value.

The increase in liver weight is preceded by acceleration in the rate of DNA synthesis as seen from the data shown in Table 2. The stimulation is discernible at about 12 hr following partial hepatectomy and reaches a maximum at 28 hr post-operation. The results clearly depict that the activity in cells are preparatory to mitosis.

RNA synthesis during liver regeneration

Significant modulations at the level of transcription are expected in quiescent cells given a stimulus for proliferation. Initially, studies were undertaken to examine the nature of these changes during liver regeneration.

As is apparent from the results presented in Table 3, the rate of RNA synthesis in rat liver is enhanced progressively from as

Table 1

Restoration of rat liver weight after partial hepatectomy

Time after partial hepatectomy (hr)	Per cent increase over control in liver weight
10	12.5
20	43.8
40	80.0
60	130.0
300	184.3

Median and left lateral lobes were excised from rats (120 g body wt) by operation. The wet weight of the liver was 7.8 ± 0.3 g out of which the portion excised was 5.2 ± 0.4 g (average of 4 rats). The remaining portion of the liver was taken as '0' hr control (100%). The values shown are averages of two rats.

Table 2

^{3}H-thymidine labelling of rat liver DNA :

effect of partial hepatectomy

	Specific activity (cpm/mg DNA)
Sham-operated	8070 ± 119
Partially hepatectomised (hr after operation)	
8	7125 ± 192
12	11500 ± 270
18	15400 ± 215
21	26580 ± 320
24	44540 ± 295
28	50750 ± 355
36	48915 ± 275

Rats were injected with ^{3}H-thymidine (80/uCi/100 g body wt), 2 hr prior to sacrifice. Each value represents average of three independent experiments ± SEM.

Table 3

RNA synthetic rates in rat liver : effect of partial hepatectomy

	Relative specific activity (*)
Sham-operated	1260 ± 56
Partially hepatectomised (hr after operation)	
2	1302 ± 97
6	1450 ± 105
12	1635 ± 102
18	1885 ± 72
24	1726 ± 113
48	1320 ± 130

* $\frac{\text{cpm/mg RNA}}{\text{cpm/pmole of orthophosphate in liver homogenate}}$

Each rat received ^{32}P-orthophosphate (2 mCi/100 g body wt) 1 hr prior to sacrifice. Other details are as in the text. Each value represents average of three independent experiments ± SEM.

early as 2 hr after partial hepatectomy. At 18 hr post-operation, the increase ~~being~~ is about 50% over control.

Sedimentation profiles of pulse-labelled RNA:

During normal and rapid cell proliferation, the syntheses of all types of RNAs are expected to be stepped up. This has been borne out by the results of experiment in which RNA isolated from regenerating rat liver was resolved by centrifugation on sucrose density gradient (see Fig.1). This observation is similar to the results reported by Maramatsu and Busch (18) for RNA synthesised during liver regeneration.

RNA polymerisation by liver nuclei:

Subsequent experiments were directed to elucidate the mechanism responsible for the early triggering of RNA synthetic machinery. The capacities of liver nuclei to catalyse Mg^{++}-dependent RNA polymerisation and $Mn^{++}/(NH_4)_2SO_4$-dependent RNA polymerisation were assessed first. The nature of these reactions in terms of RNA polymerases involved and RNA products formed are already explained in Part II. As is apparent from Table 4, nuclei from the regenerating liver exhibit greater ability to catalyse either of the RNA polymerisation reactions, as compared to those from the liver of sham-operated rat. The maximum effect is discernible at 18 hr post-operation being about 160% over sham-operated control for Mg^{++}-activated reaction and about 35% over sham-operated control for $Mn^{++}/(NH_4)_2SO_4$-activated reaction.

Activities of liver RNA polymerases:

In further experiments, the activity of RNA polymerase isolated free of DNA from regenerating rat liver was determined and compared with

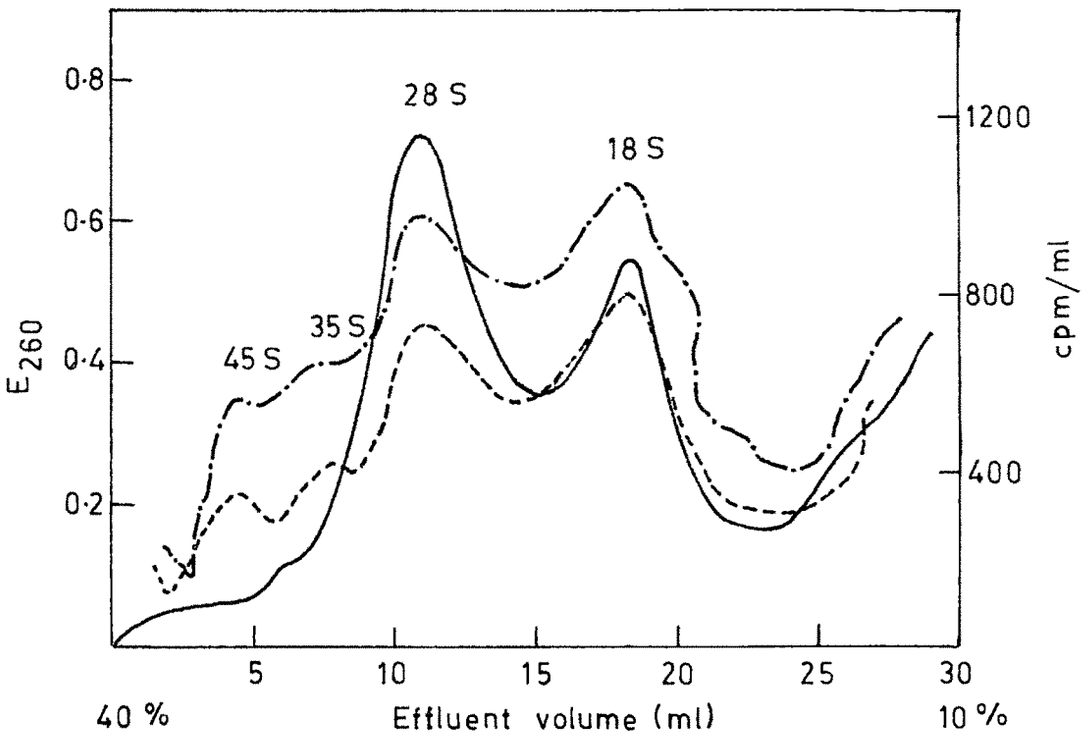


Fig. 1. Sedimentation profiles of ^{32}P-orthophosphate pulse-labelled RNA in the regererating rat liver.

Sham-operated and partially hepatectomized (18 hr after operation) received ^{32}P-orthophosphate (carrier free, 2 mCi/ 100 g body wt) 30 min before sacrifice. An aliquot of RNA solution in acetate buffer pH 5.1 (equivalent to 1 mg, E_{260} units 25) was layered on a 28 ml linear sucrose density gradient (10-40%), centrifuged at 63,000 x g for 15 hr and 1 ml fractions were collected for E_{260} and radioactivity determinations.

———— optical density; - - - - radioactivity, Sham-operated;

-·-·-·-·- radioactivity, partially hepatectomized.

Table 4

RNA polymerisation capacities of nuclei isolated from regenerating rat liver

	Amount of RNA synthesized	
	Mg^{++} activated (*)	$Mn^{++}/(NH_4)_2SO_4$-activated (**)
Sham-operated	230	1125
Time after partial hepatectomy (hr)		
6	298	1209
12	422	1327
18	598	1525

* pmoles of AMP incorporated into RNA/15 min/mg DNA.

** pmoles of AMP incorporated into RNA/45 min/mg DNA.

Both, Mg^{++}-activated and $Mn^{++}/(NH_4)_2SO_4$-activated assay systems contained in a final volume of 0.5 ml: 0.02 µmole of ^{14}C-ATP and nuclei equivalent to 0.3 mg DNA. Other details are as in the text. Each value is average of two independent experiments.

that isolated from sham-operated rat liver. As seen from Table 5, the activity of RNA polymerase, expressed both as units per mg DNA of nuclear suspension from which it was isolated and as units per mg protein of the enzyme preparations, were increased after partial hepatectomy. Since the activity expressed both as total activity and as specific activity is enhanced, it may be presumed that the observed increase is not an artifact arising from differential solubilization of RNA polymerases from the two groups or from differential inactivation of the enzymes during purification (see also Part II-Section I - Results).

These investigations were then extended to ascertain the extent to which the two principal RNA polymerases - Type I and Type II-are activated during liver regeneration. Fractionation of the total RNA polymerase preparation was achieved on a DEAE-cellulose column with a linear gradient of 0.025 M to 0.5 M KCl. The elution profiles of RNA polymerase preparations from sham-operated and partially hepatectomized rats (18 hr post-operation) are illustrated in Fig.2. The enzyme preparation is resolved into two fractions: RNA polymerase I which is eluted in the range of 0.15 M to 0.17 M KCl, and RNA polymerase II which appears in the elution range of 0.34 M to 0.36 M KCl. As discussed earlier, polymerase I is presumably a nucleolar enzyme responsible for ribosomal RNA synthesis, and polymerase II is located in the extranucleolar region catalysing synthesis of DNA-like RNA products. The activity of RNA polymerase I is stimulated to a much greater extent as compared to that of RNA polymerase II during liver regeneration, as can be seen from Fig.2.

Table 5

Changes in RNA polymerase activity during liver regeneration

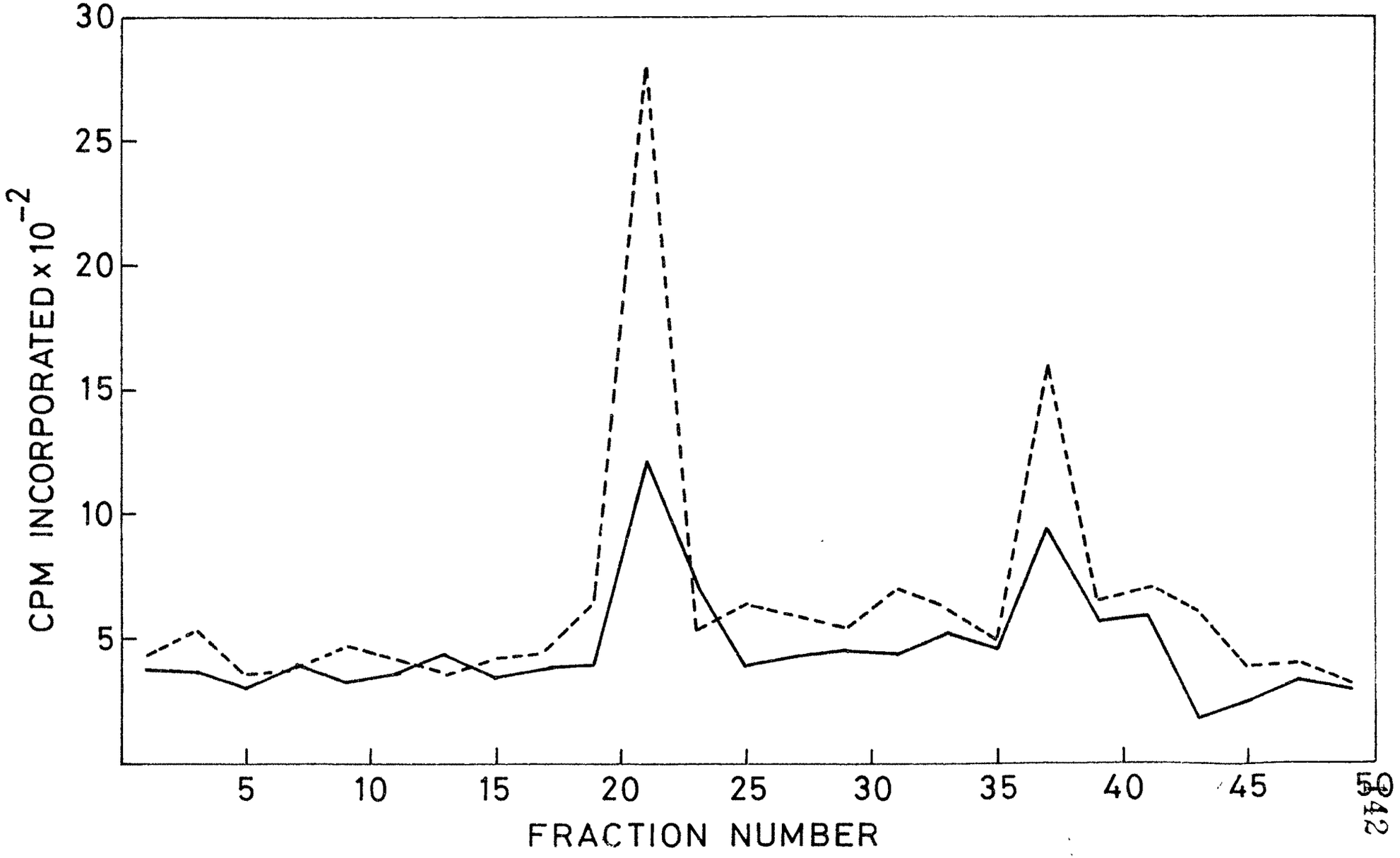
	Total activity (units/mg nuclear DNA)	Specific activity (units/mg protein)
Sham-operated	1672	1465
Time after partial hepatectomy (hr)		
8	1927	1680
18	3475	3137

RNA polymerase was isolated from nuclear suspension of pooled livers (four rats per group) up to fraction IV of the method as described in the Materials and Methods.

The assay mixture contained (in 0.5 ml): 20 µg rat liver DNA, liver RNA polymerase preparation equivalent to 100 µg protein, 0.2 µmole of ^{14}C-ATP. Other constituents are as described under Materials and Methods. One unit of activity is defined as the amount which catalyses the incorporation of 1 pmole of AMP into RNA per 10 min. Each value is average of three independent experiments.

Fig.2. Chromatography of liver RNA polymerase on DEAE-cellulose column. The column (1 x 12 cm) was equilibrated with TGMED buffer containing 0.025 M KCl. Ten mg of the enzyme preparation was applied and eluted with a linear gradient of 0.025 to 0.5 M-KCl in TGMED buffer. One ml fractions were collected and RNA polymerase activity in each fraction was assayed as described in the text.

- - - - - Partial hepatectomised; ——— Sham-operated.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Experiments on liver nuclear RNA polymerisation based on specific inhibition by α-amanitin of RNA polymerase II (i.e. the enzyme catalysing $Mn^{++}/(NH_4)_2SO_4$-reaction; also see Part II-Section I - Results) have also confirmed the conclusion that the activity of RNA polymerase I is stimulated to a greater extent than that of RNA polymerase II in the regenerating rat liver (Table 6).

Transcriptive functions of liver chromatin:

Studies were directed to see whether chromatin transcriptive functions are also subject to alterations in response to the mitotic stimulus. It is apparent from Table 7, that the template activity of chromatin, as assayed using exogenous liver polymerase, is progressively increased up to 18 hr post-operation whence it is about 42% above that seen in sham-operated control rats. At these time intervals, the template activity of DNA however remains unchanged.

In Table 8 are shown the data on number of RNA chain initiation sites (RNA polymerase binding sites) on the liver chromatin after partial hepatectomy. The determination was done as described in Part II-Section I using exogenous rat liver RNA polymerase and ^{3}H-UTP under the conditions which first allow only RNA chain initiation and then allow only RNA chain elongation. At 8 hr after partial hepatectomy, a small increase is discernible in the number of RNA chain initiation sites on chromatin but at 18 hr post-operation a considerable increase in RNA chain initiation sites is evident.

Table 6

Activity (in presence and absence of α-amanitin) of liver RNA polymerase at various times post-partial hepatectomy

	pmoles of AMP incorporated into RNA/10 min	
	without α-amanitin	with α-amanitin
Sham-operated	1390 ± 39.5	834 ± 21.9
Partially hepatectomized (hr after operation)		
4	1638 ± 50.6	981 ± 46.6
12	2085 ± 40.0	1235 ± 38.1
18	2909 ± 60.1	1838 ± 49.2

The assay mixture contained (0.5 ml) 0.2 μmole of ^{14}C-ATP, 20 μg rat liver DNA, with or without 2 μg of α-amanitin and 100 μg of RNA polymerase enzyme protein. Other details are as described in the text. Each value represents average of three independent experiments ± SEM.

Table 7

Template activity of rat liver DNA and chromatin : effect of partial hepatectomy

	Template activity (*)	
	DNA	Chromatin
Sham-operated	131.5	28.0
Time after partial hepatectomy (hr)		
8	130.2	32.1
12	132.0	35.3
18	129.7	39.8
20	132.8	36.4

* pmoles of ^{14}C-AMP incorporated into RNA/10 min.

The assay system for template activity contained 0.2/umole of ^{14}C-ATP, 20/ug of DNA either in the form of chromatin or purified as such and 100/ug RNA polymerase enzyme protein in 0.5 ml final volume. Other details are as in the text. Each value is an average of three independent experiments.

Table 8

Number of RNA chain initiation sites available on rat liver chromatin: effect of partial hepatectomy

Source of liver chromatin	Number of RNA chain initiation sites per 10^6 nucleotide pairs
Sham-operated rats	11.5
Partially hepatectomized rats (hr after operation)	
8	13.8
18	15.0

The number of RNA chain initiation sites were determined as described in Part II-Section I, under the low-salt reaction conditions that allow only RNA chain initiation followed by high-salt reaction conditions which allow only RNA chain elongation, in the presence of ^{3}H-UTP to label growing RNA chains. The calculations were done as described in Part II-Section I. Each value is average of two independent experiments.

Turnover of nonhistone chromosomal proteins

Results presented above indicate that the response of transcription functions of liver chromatin to the mitotic stimulus could be ascribed to non-DNA components of the chromatin. In Part II, it was shown that stimulation in template activity of liver chromatin brought about by whole-body radiation exposure is related to specific alterations in the turnovers of chromosomal nonhistone proteins. Similar examination was made in respect of nonhistone chromosomal protein turnovers in regenerating rat liver. At various times after partial hepatectomy, rats were given injections of ^{3}H-leucine and sacrificed 1 hr later. The liver from partially hepatectomized rats were pooled with those from control (sham-operated) animals which had received injections of ^{14}C-leucine 1 hr prior to sacrifice. Chromosomal nonhistone proteins, isolated from the mixed homogenates, were then subjected to electrophoresis on 10% polyacrylamide-SDS gels. The relative labelling patterns (^{3}H/^{14}C ratio of separated proteins *versus* gel slice no.) of nonhistone chromosomal proteins of the livers at 8 and 18 hr after operation are illustrated in Fig.3. It is clear from the plots that among the proteins separated on polyacrylamide-SDS gel, only one protein (or a group of two or three proteins) migrated to the gel region corresponding to slice No.12 exhibit increased relative rate of labelling. The molecular weight of this component(s) can be calculated by the method described in Part II-Section II and is approximately 55,000 daltons. This is suggestive of a selective increase in the synthesis (or turnover) of the protein(s) in regenerating liver.

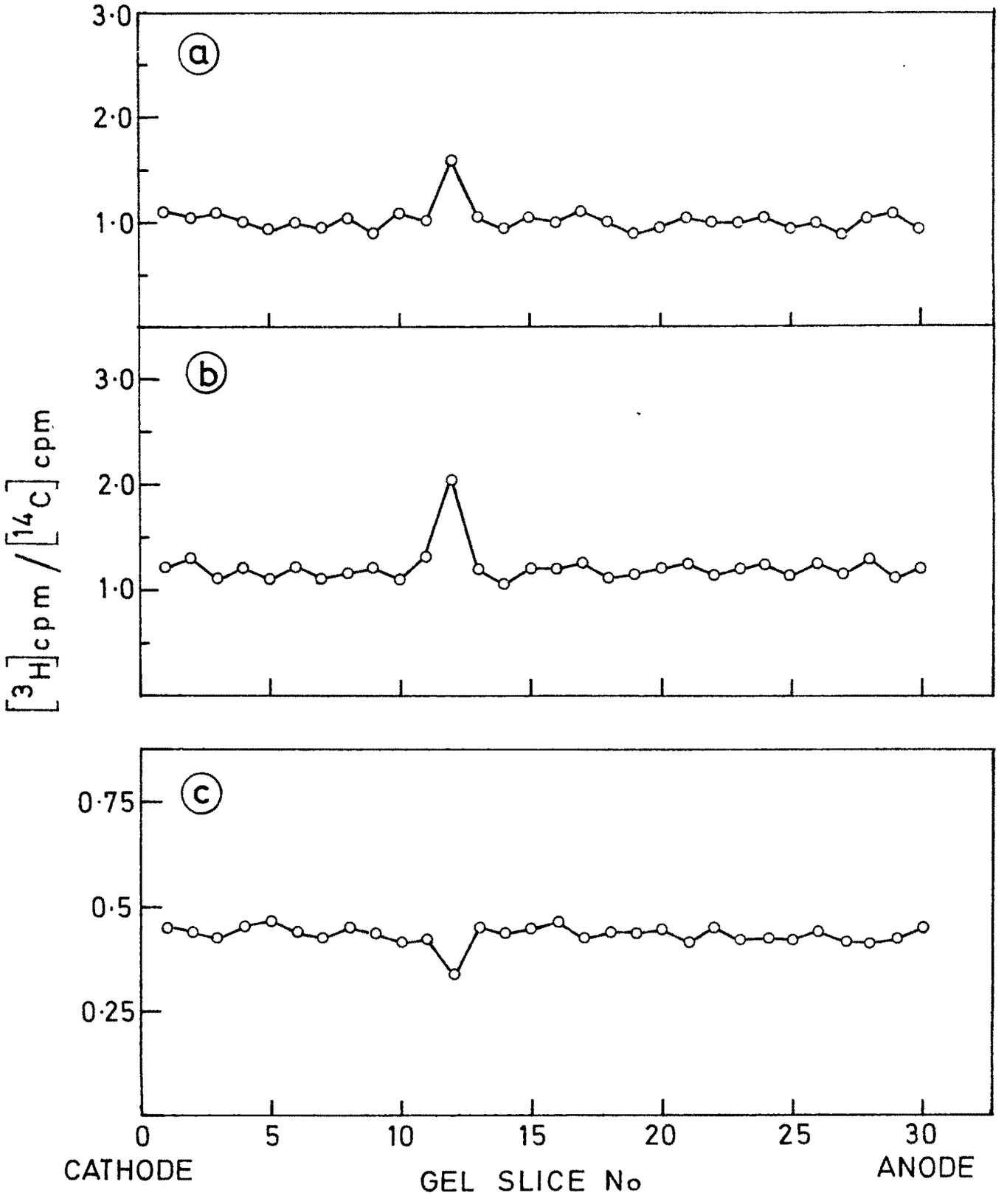
Fig.3. Relative rates of labelling of electrophoretically separated nonhistone chromosomal proteins in regenerating rat liver. Partially hepatectomised rats received (^{3}H)-leucine and sham-operated rats (^{14}C)-leucine. Radioactivity of protein components (absolute counts) are as follows:

(a) Eight hr after partial hepatectomy. Total counts placed on the gel : 4200 cpm of ^{3}H and 3120 cpm of ^{14}C; slice no.12 : 290 cpm of ^{3}H and 180 cpm of ^{14}C.

(b) Eighteen hr after partial hepatectomy. Total counts placed on the gel: 5260 cpm of ^{3}H and 4725 cpm of ^{14}C; slice no.12 : 390 cpm of ^{3}H and 195 cpm of ^{14}C.

(c) Reverse labelling experiment. Partially hepatectomised rats (18 hr post-operation) received (^{14}C)-leucine and sham-operated rats (^{3}H)-leucine.

Total counts placed on the gel : 5910 cpm of ^{14}C and 7505 cpm of ^{3}H; slice no.12 : 112 cpm of ^{14}C and 335 cpm of ^{3}H.

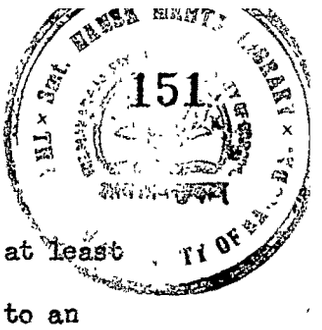

3·0
2·0
1·0
a
3·0
2·0
1·0
b
0·75
0·5
0·25
c
[3H]cpm / [14C] cpm
0
5
10
15
20
25
30
CATHODE
GEL SLICE No
ANODE

A reverse experiment in which sham-operated rats received ^{3}H-leucine and partially hepatectomized animals (18 hr post-operation) ^{14}C-leucine was also carried. As seen in Fig.3c, only the protein(s) moved to slice No.12 display a dip in the relative rate of labelling (^{3}H/^{14}C) again indicating a selective stimulation in its synthesis (or turnover) during liver regeneration. This experiment rules out ~~only~~ any artefact arising from counting anomalies.

DISCUSSION

The results presented here confirm earlier reports that the rate of RNA synthesis in the regenerating liver is considerably higher than that in the sham-operated liver (9,19,20). The enhancement in RNA synthesis occurs much earlier than the onset of cell division indicating that the activation at the transcriptional level is brought about in the remaining hepatocytes and not in the newly formed cells. The liver regeneration thus constitutes a useful system to elucidate the factors responsible for heightened rate of RNA synthesis. The data on RNA polymerisation by liver nuclei support the general belief that the capacity of animal cells to synthesise RNA *in vivo* is a direct reflection of the capacity of their nuclei to synthesise RNA *in vitro* (21,22,23).

Studies reported so far have presented conflicting data on the mechanism underlying accelerated rate of RNA synthesis. In some reports, the activation of RNA synthesis in eukaryotic cells has been ascribed mainly to an effect on chromatin template (3), whereas in others, levels of RNA polymerase have been shown to determine rates of RNA synthesis (24).



Studies presented in the previous part, seem to suggest that at least in some conditions, chromatin template activation could lead to an increase in the rate of RNA synthesis. The activation of transcription machinery during liver regeneration is paralleled by enhancement of both RNA polymerase activities and template activity of chromatin.

To assess the relative importance of the activation of various components of transcription machinery in the regulation of RNA synthetic rate, it would be appropriate here to discuss the molecular mechanisms of eukaryotic transcription as envisaged on the basis of current knowledge. Transcription may be considered as a three-step process comprising: (1) initiation in which RNA polymerase interacts with a promoter region in the DNA template and a purine triphosphate to form the initiation complex (25); (2) propogation in which the enzyme moves along the DNA template with the formation of a growing complementary RNA chain (25,26); and (3) termination in which the complex of enzyme-DNA-RNA chain is dissociated when it reaches a terminating signal on the DNA template (25,26).

This scheme based on studies with prokaryotic system would suggest that rate of RNA synthesis could be governed by all the three steps mentioned above. Let us consider the manner in which DNA template could govern RNA synthetic rate. From a variety of considerations, it can be surmised that the rates of movement of the enzyme molecule along different DNA templates may be nearly same in identical conditions of reactions (25). The rates of termination, i.e. the dissociation of RNA polymerase-DNA-RNA chain complex, may vary by virtue of different nature of terminating signals but it would seem that this may not be a step that could greatly determine RNA synthetic rate. Conceivably,

therefore, the most crucial step in the determination of RNA synthetic rate may be the initiation step. The rate of initiation may govern to a large extent the number of RNA polymerase molecules that may transcribe a cistron at a time. Some specific factors (mostly protein in nature) presumably could govern initiation at certain specific cistrons. At the enzyme level, the regulation should be determined by the number of active RNA polymerase molecules available.

The eukaryotic transcription rate could also be regulated similarly. However, since non-DNA components have a role in restricting the template ~~propensity~~ efficacy of DNA, these may have an important role in RNA synthetic rate regulation. In the present study, a substantial increase in the number of available initiation sites on chromatin is observed in the regenerating rat liver. This can very well account for the increased rate of RNA synthesis. It may be mentioned here however that the assay for available initiation sites on chromatin (as also the chromatin template activity assay) is based on rather artificial conditions since the assay is done in the presence of saturating amounts of RNA polymerase - a condition which may not exist *in vivo*. Nevertheless, the results should give a fairly good idea of the degree of increase that might have been brought about in the number of initiation sites on chromatin during liver regeneration. One more factor that should also be taken into consideration is: whether the increase in the number of available initiation sites would necessarily mean de-repression of that many cistrons on the genome. The magnitude of increase, which is 30% over control, could be considered as related entirely to the synthesis of new species of RNAs. Such extensive gene activation may perhaps be necessitated for the synthesis of proteins needed for chromosomal replication and subsequent cell division. On the other hand, it is

quite possible that there may not be such increase in the induction of new species of RNA. The genes which have been de-repressed could simply be repressed copies of genes, only one or a few may be active in normal liver (Also see Discussion in Part II - Section I).

The results presented here also reveal a considerable enhancement in the activities of both RNA polymerases I and II in the regenerating rat liver. That this observation is not an artefact can be surmised from the findings that the nuclei from regenerating rat liver exhibit greater stimulation in their capacity to catalyse Mg^{++}-activated RNA polymerisation reaction than $Mn^{++}/(NH_4)_2SO_4$-activated RNA polymerisation reaction. These reactions are believed to yield ribosomal RNA-like and DNA-like RNA products respectively. The question that should now be considered is whether under normal conditions, RNA polymerase molecules are present in amounts which could regulate rate of transcription. Chambon (27) has shown that the number of RNA polymerase II molecules in liver cells are of the order of 3.9×10^4 per haploid genome. This number appears more than adequate for normal active transcription of an estimated 40000-50000 RNA initiation sites per haploid genome. However, this number may still be insufficient to saturate the genome if the genes become highly active, i.e., to pack themselves to the extent of about 100 nucleotide base pairs per polymerase molecule. As far as RNA polymerase I is concerned, Roeder et al.(28) give the estimate of about 2×10^4 molecules per mouse liver cell. These authors have calculated that for saturating genes of ribosomal RNA, 0.5 to 1.8×10^5 RNA polymerase I molecules are required. If it is presumed that the number of RNA polymerase I molecules are nearly equal to these of RNA polymerase II molecules (viz. of the order of 3.9×10^4 per haploid genome), the normal level of RNA polymerase I may also fall short of Roeder et al.'s estimate for the saturating level. These estimates would indicate that at least part of the increase in the rate of RNA synthesis during liver regeneration

may have been the consequence of increased activities of both RNA polymerases I and II. It may be noted that increase in RNA polymerase I activity is greater than in RNA polymerase II activity. The profile of pulse-labelled RNAs indicates a high proportion of ribosomal RNA precursor in regenerating liver as in sham-operated liver. To cope with increased rate of ribosomal RNA precursor, it is expected that the activity of RNA polymerase I would be stimulated to a greater extent than RNA polymerase II. This is further confirmed by the experiments involving RNA polymerisation by liver nuclei (RNA polymerase) in the presence of α-amanitin, an agent which specifically inhibits RNA polymerase II. It may be noted, however, that the activities of RNA polymerisation by liver nuclei could be the combined effect of both increase in RNA polymerase activities and increase in template activities of chromatin. The increased activities of the enzymes can be either due to activation of already existing enzyme molecules or, more likely, the consequence of increased production of more number of molecules.

Similar to the studies presented in Part II-Section II, it appears that the activation of transcription machinery in the liver regenerating system is also associated with the changes in non-DNA components of the chromatin. As in radiation-provoked gene activation, nonhistone chromosomal protein turnover has been found to be selectively altered during liver regeneration. The alterations are progressively magnified with time during the first 18 hr liver regeneration and closely correspond to the extent of activation at the template level. Selective changes in nonhistone chromosomal protein labelling have

likewise been reported in the other mammalian systems where quiescent cells were made to proliferate in response to appropriate stimuli. These include the stimulation of rat uterus by estrogen (29), mouse salivary gland by isopreterenol (30), rat mammary glands by explantation (31), human diploid fibroblast by serum (32) and guinea pig lymphocyte by phytohemagglutinin (33). The proteins-syntheses of which are selectively stimulated- are however different under different cell proliferation triggering conditions. The molecular weight of the nonhistone protein(s) whose synthesis is enhanced during liver regeneration has been estimated as 55000 daltons. The protein may not be any of the RNA polymerases since the molecular weights of the sub-units range from 16000 daltons to 190000 daltons (34). However, the possibility that the nonhistone protein could be a single RNA polymerase sub-unit having molecular weight close to 55000 daltons exists; such a sub-unit may have an important regulatory role. The protein in question may also be conceived as a factor involved in control of transcription.

It may be pointed out that the nonhistone protein is different from the two nonhistone proteins whose syntheses are stimulated in response to whole-body radiation exposure (see also the Discussion in Part II-Section II).

It appears from the reports in literature that modifications in the synthesis (11), acetylation (10) and phosphorylation (11) of histones are also brought about in the liver following partial hepatectomy. This is indicative of the occurrence of extensive modifications in structure and distribution of non-DNA components of chromatin during liver regeneration.

As has already been pointed out in the beginning of this 'Discussion', the activation of transcription following whole-body radiation stress and that following partial hepatectomy are two distinct phenomena and this has been amply borne out from the differences in the activation of the components of transcription machinery in respect of the two conditions. Although partial hepatectomy has many more advantages over the other conditions of gene activation where the cells are not made to proliferate, there are also certain limitations. This is especially so in the case of the modifications and turnover of non-DNA chromosomal components, since at least some changes occurring in ~~non-DNA~~ these components could be related to either DNA synthesis or cell-division rather than to the changes at the transcriptional level. Further studies could be able to resolve these complexities.

SUMMARY

Studies were conducted to elucidate events responsible for stimulation in RNA synthetic machinery in the rat liver following partial hepatectomy. The rate of RNA synthesis was stepped up as early as 2 hr after partial hepatectomy and was maximum at 18 hr post-operation being about 50% over the control. Sedimentation profiles of ^{32}P-pulse-labelled RNA indicated that the syntheses of all classes of RNAs might have been enhanced during liver regeneration. Nuclei from theregenerating liver exhibited greater ability to

catalyse Mg^{++}-dependent and $Mn^{++}/(NH_4)_2SO_4$-dependent RNA polymerisation reactions. The activation of transcription machinery during liver regeneration seemed to be associated with enhancement in both chromatin template activity and RNA polymerase activities - unlike radiation-induced activation of transcription which appeared to be the reflection of an increase in template activity of chromatin alone (Part II). Like the template activity, the number of RNA chain initiation sites of the chromatin also exhibited progressive elevation in regenerating liver. Similar changes could not however be seen in the transcription functions of liver DNA. Fractionation of RNA polymerase preparation from the regenerating liver by DEAE-cellulose column chromatography revealed that RNA polymerase I activity was stimulated during liver regeneration to a greater extent than RNA polymerase II activity. Concomitant with the changes at transcriptional level, selective acceleration in the synthesis of a nonhistone chromosomal protein with a molecular weight of 55000 daltons was also noticeable. This protein is different from the two nonhistone chromosomal proteins whose syntheses were found to be selectively stimulated in the liver following whole-body x-irradiation (in parallel with the activation of liver transcription machinery). These results serve to show that the mechanisms underlying activation of transcription during liver regeneration may be somewhat different from those underlying activation of transcription brought about by other stimuli.

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Parts of the work reported in the thesis have appeared in the following publications :

1. Template activity of liver chromatin in rats following whole-body exposure to x-irradiation
M.N.S. Rao, M.S. Netrawali, D.S. Pradhan and A. Sreenivasan
Indian Journal of Biochemistry & Biophysics, 8, 257, 1971 (1971).

2. Changes at transcriptional level in the rat liver following whole-body x-irradiation
M.N.S. Rao, M.S. Netrawali, D.S. Pradhan, and A. Sreenivasan
FEBS Letters, 48, 160, 1974.

3. Alterations at transcriptional level in the rat liver following whole-body x-irradiation
D.S. Pradhan, M.N.S. Rao, M.S. Netrawali and A. Sreenivasan
Abstracts of the papers presented at 5th International Congress of Radiation Research, Seattle, Washington, U.S.A., July 14-20, 1974, p.120.

4. Regulation of transcription in eukaryotes
D.S. Pradhan, M.N.S. Rao, A. Narayani, M.S. Netrawali and A. Sreenivasan
Ind. Natl. Acad. Sci. Symp. on 'Regulation of growth and differentiated function in eukaryotic cells', New Delhi, October 14-17, 1974, p.129.

5. Changes at transcriptional level during liver regeneration in the rat
M.N.S. Rao, M.S. Netrawali, D.S. Pradhan and A. Sreenivasan
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