Transcriptional Switch from Albumin to α-Fetoprotein and Changes in Transcription of Other Genes during Carbon Tetrachloride Induced Liver Regeneration†

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ABSTRACT: During liver regeneration induced by CCl_4 administration to rats, changes in the relative transcription rates of albumin and α-fetoprotein genes have been measured in conjunction with other liver-specific and general cellular function genes. Within 24 h following CCl_4 administration, albumin gene transcription decreases by 85%, whereas α-fetoprotein transcription increases from undetectable levels to 50% of that observed for albumin. These changes precede maximal [^{3}H]thymidine incorporation into DNA which peaks at 48 h. Other genes related to liver-specific functions, such as ligandin, α_1-antitrypsin, and cytochrome P-450's, as well as general cellular genes pro α_1- and pro α_2-collagen, β-actin, and α-tubulin, respond in kinetic patterns often distinct from each other and from albumin and α-fetoprotein. Changes in the steady-state levels of albumin and α-fetoprotein mRNA correlate with changes in transcription, but there is a lag in α-fetoprotein mRNA accumulation, which peaks at 72 h following CCl_4 administration. These studies indicate that reciprocal changes in albumin and α-fetoprotein gene transcription occur during CCl_4-induced liver regeneration, leading to changes in the level of these specific mRNAs. These changes precede DNA synthesis and would appear to represent an alteration in differentiated function of hepatocytes in conjunction with the liver regenerative process.

The mammalian liver represents an excellent system to study regulation of eukaryotic gene expression as it relates to cellular differentiation and growth control. Although the liver contains many cell types, hepatocytes comprise approximately 60% of the cells and more than 90% of the total mass (Greengard et al., 1972; Stocker et al., 1972). Since hepatocytes synthesize a number of unique proteins, such as albumin and α-fetoprotein, changes in expression of these proteins can be studied as markers of specialized or differentiated behavior both in normal development and in pathophysiologic circumstances which may alter liver function.

One such circumstance, which has been the focus of considerable attention, is liver regeneration. Following surgical removal of approximately two-thirds of the liver (partial hepatectomy), this organ regenerates to normal mass and cell number within 1-2 weeks [for a review, see Bucher & Malt (1971)]. All mammalian species, including man, are capable of liver regeneration, and during this process, most hepatocytes have been shown to undergo at least one round of cell division (Grisham, 1962; Bucher & Malt, 1971). Hepatocyte proliferation ceases precisely when liver cell mass is restored, and in order to gain a basic understanding of regulatory factors in growth control, recent studies have evaluated various aspects of gene expression during hepatocyte regeneration.

Initial studies focused on general changes in liver mRNA amount, average size, polyadenylation, subcellular distribution, sequence complexity, and abundancy classes [for a review, see Fausto (1984)]. Aside from an increase in the amount of cytoplasmic and polysomal poly(A+) mRNA (Glazer, 1976; Walker & Whitfield, 1981; Atryzek & Fausto, 1979), there was little discernible difference in the general characteristics of liver mRNAs during regeneration. These studies were limited, however, in that they could not detect changes in specific mRNAs, especially those present in relatively low abundance.

With advances in recombinant DNA technology, a number of genes expressed in and/or unique to liver have been cloned (Derman et al., 1981) and used as probes to study regulation of specific mRNAs in normal and regenerating liver (Friedman et al., 1984). The developmental switch from α-fetoprotein to albumin occurring just prior to birth has also been utilized as a specific marker of changing or differentiated hepatocyte function (Tilghman & Belayew, 1982; Selten et al., 1982; Andrews et al., 1982; Powell et al., 1984). It has been postulated (Uriel, 1979; Petropoulos et al., 1983) that if hepatocytes dedifferentiate prior to cell division as part of the proliferative response, then the increase in α-fetoprotein gene expression during liver regeneration should precede DNA synthesis and albumin transcription should be turned off. Although various studies have shown an increase in α-fetoprotein mRNA during liver regeneration (Chiu et al., 1981; Belayew & Tilghman, 1982; Princen et al., 1982; Petropoulos et al., 1983), a reciprocal and specific decrease in albumin mRNA has not been reported (Krieg et al., 1980; Princen et al., 1982; Petropoulos et al., 1983). In a most recent study in mice, simultaneously comparing transcription of albumin and α-fetoprotein genes to mRNA steady-state levels (Friedman et al., 1984), there were no significant changes in the transcription or steady-state level of either albumin or α-fetoprotein mRNA following partial hepatectomy.

Since serum α-fetoprotein and liver α-fetoprotein mRNA increase more dramatically following CCl_4 administration than after partial hepatectomy (Watanabe et al., 1976; Petropoulos et al., 1983), the liver regenerative response may be greater in the former than in the latter. It has also been shown that different mouse strains vary genetically in their ability to

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increase α-fetoprotein expression, a process which is under control of genes Rif and Raf, distinct from the α-fetoprotein structural gene (Belayew & Tilghman, 1982). Therefore, we decided to reexamine expression of the albumin/α-fetoprotein gene locus, as well as other genes of liver-specific vs. general cellular function, under optimal conditions of CCl_4-induced liver injury in the rat. Our results indicate not only that there are reciprocal changes in albumin and α-fetoprotein gene transcription during liver regeneration but also that these and other changes in specific gene transcription may reflect a change in the state of liver cell differentiated function during CCl_4-induced regeneration.

Experimental Procedures

Preparation of Animals. Male Sprague-Dawley rats, 250 g (Holzmann Farms), were used for all studies. To induce liver regeneration, rats were given a single intragastric dose of CCl_4 [0.5 mL of a 1:1 (v/v) mixture of CCl_4 in mineral oil per 100 g body weight]. For induction of liver cell hyperplasia, nafenopin, 30 mg/100 g body weight, was administered as a single intragastric dose. At various times after treatment, the rats were killed by cervical dislocation. The livers were removed quickly, weighed, and perfused at 4 °C with 50–100 mL of a solution containing 0.25 M sucrose, 1 mM $MgCl_2$, and 10 units/mL heparin.

Histology and [^3H]Thymidine Incorporation. Thin (2–3 mm thick) slices of liver were cut immediately after sacrifice and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The material was embedded in paraffin, cut in sections of 3-μm thickness, and stained with hematoxylin and eosin. [^{3}H]Thymidine incorporation into DNA was determined by a 1-h pulse-labelling of intact animals by intraperitoneal injection of 1 μCi/g body weight of methyl[^{3}H]-thymidine (specific activity 77.8 Ci/mmol, New England Nuclear Corp.) and determination of radioactivity in homogenized liver by trichloroacetic acid precipitation according to the method described by Munro (1966).

Isolation of Nuclei. Nuclei were isolated essentially as described by Lamers et al. (1982). The liver was minced and homogenized at 4 °C in 5–10 volumes of 0.3 M sucrose, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, and 0.1% Triton X-405. The homogenate was filtered through four layers of cheesecloth and the crude nuclear fraction isolated by centrifugation at 800*g* for 5 min at 4 °C. The nuclei were resuspended in the same buffer, but lacking Triton X-405, and sucrose was added to a final concentration of 1.65 M. The mixture was centrifuged in a Sorvall centrifuge at 25000*g* in the HB-4 rotor for 60 min at 4 °C through 1.0 mL of 2 M sucrose, 10 mM Tris-HCl, pH 7.5, and 2 mM $MgCl_2$. The pellet was resuspended in 50% glycerol, 50 mM *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (Hepes), pH 7.5, 5 mM $MgCl_2$, and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The nuclei were counted and used directly or stored frozen at −86 °C. After storage for 1–2 months, frozen nuclei retained 80% of the initial transcription activity, and there was no significant change in the percent transcription of specific gene products as determined below.

RNA Transcription and Isolation of Labeled RNA. Incubations were performed in a 250-μL reaction volume and contained (0.25–4.0) × 10^7 nuclei, 25% glycerol, 75 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 100 mM KCl, 4 mM dithiothreitol, 0.5 mM guanosine triphosphate, 0.5 mM cytosine triphosphate, 1.0 mM adenosine triphosphate, and 50 μCi of uridine [α-^{32}P]triphosphate (specific activity 410 Ci/mmol, Amersham/Searle Corp). Reactions were incubated for 20–30 min at room temperature and were terminated by addition of deoxyribonuclease I (DNase I) to a final concentration of 20 μg/mL and further incubation for 5 min. Labeled RNA was isolated by the method of Groudine et al. (1981).

Binding of Cloned cDNAs to Nitrocellulose Filters. For quantitative determination of transcriptional activity for individual genes, cloned specific cDNAs were bound to nitrocellulose filter disks essentially as described by Gillespie and Spiegelman (1965) and Melli et al. (1975). The following cDNA clones, kindly provided by the individuals noted, were used: rat albumin (M. Zern and D. Shafritz), mouse α-fetoprotein (S. Tilghman), rat α-fetoprotein (T. Sargent), rat pro α_1- and pro α_2-collagen (D. Rowe), human α_1-antitrypsin (S. Woo), rat ligandin and α_1-acid glycoprotein (J. Taylor), rat cytochrome P-450's, R-17, and TF-1 (M. Adesnik), mouse β-globin (A. Skoultchi), and rat β-actin, α-tubulin, and mouse 28S rDNA (L. Reid and D. Clayton). Cytochrome P-450 R-17 is derived from one of two major phenobarbital-inducible cytochrome P-450 B isoenzymes (Kumar et al., 1983). Cytochrome P-450 TF-1 is one of two clones obtained by immunoscreening of a pBR322 library of rat cDNA, using an antibody directed against cytochrome P-450 PB1 (M. Adesnik et al., unpublished results). This enzyme is constituitive in rat liver but is increased 2-fold in microsomes by phenobarbital. Clone TF-1 has 50% sequence homology with R-17. For simultaneous but only qualitative comparison of transcriptional activity of multiple gene products in a given RNA extract, a series of cloned probes (5 μg each) was applied to a nitrocellulose filter sheet according to the method of Clayton and Darnell (1983).

Assay for Specific Gene Transcription Products. Determination of the percent transcription of specific gene products was performed by filter hybridization as described by Kafatos et al. (1979). The filters were soaked for at least 1 h in 10 × Denhardt's solution and 4 × SET buffer (1 × SET buffer is 30 mM Tris-HCl, 150 mM NaCl, pH 8.0, and 1 mM EDTA) and then transferred to a sterile siliconized scintillation vial containing the hybridization mixture consisting of 50% deionized formamide, 2 × Denhardt's solution, 5 × SET buffer, and 0.1% sodium dodecyl sulfate (SDS). Wheat germ RNA (400 μg/mL) was added and the filter prehybridized for 4–6 h at 42 °C. The solution was removed and replaced by fresh hybridization solution containing the radioactively labeled RNA and 200 μg/mL carrier wheat germ RNA in a total volume of 2 mL and hybridized for 36 h at 42 °C.

After hybridization, filters were washed 3 times with the hybridization mixture at 42 °C for 20 min each wash. The filters were then washed with 2 × SCC at room temperature for 10 min, digested with RNase A at a concentration of 20 μg/mL for 45 min at 37 °C, and washed again with 2 × SSC (SSC is a solution containing 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). Proteinase K was added to a concentration of 200 μg/mL in a buffer solution containing 50 mM Tris-HCl, pH 7.5, 150 mM EDTA, and 1% SDS and incubated at 37 °C for 30 min. After RNase A and proteinase K treatment, filters were washed and dried, and radioactivity was determined by liquid scintillation spectroscopy for individual filter disks or by autoradiography for nitrocellulose filter sheets. Separate filters or spots containing nonrecombinant plasmid pBR322 were employed as negative controls.

Isolation of Total Cellular RNA and Poly(A+) RNA. RNA was isolated by using minor modifications of the procedure of Chirgwin et al. (1979). A portion of the liver was dropped into liquid N_2, pulverized, and then homogenized in 3.5 mL of 4 M guanidine thiocyanate solution by using a

Table I: Characteristics of in Vitro Transcription System with Isolated Rat Liver Nuclei[a]

	[^{32}P]UTP incorporation (cpm)
complete system	28×10^6
−nuclei	0.14×10^6
$-MgCl_2$	17.6×10^6
−KCl	10.5×10^6
−ATP, CTP, GTP	4.0×10^6
+α-amanitin (1 μg/mL)	11.7×10^6
albumin cDNA specific transcripts	6825
+α-amanitin (1 μg/mL)	792

[a] For this experiment, 20×10^6 nuclei were used in a 250-μL reaction mixture, and incubation was performed at 23 °C for 30 min. For determination of cpm incorporated into albumin RNA transcripts, total RNA was isolated, and 10^7 cpm were then hybridized with a 25-mm diameter nitrocellulose filter containing 100 μg of rat pAlb 87. The final result was corrected for the length of the cDNA clone compared to the full-length albumin coding sequence and for the proportion of total cpm utilized for hybridization. See Experimental Procedures for additional information.

Polytron homogenizer. The homogenate was cleared of cellular debris by centrifugation at 5000 rpm for 10 min at 10 °C in an HB-4 rotor and the RNA pelleted through a CsCl gradient. The resultant RNA was redissolved in 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA, adjusted to 0.1 M sodium acetate, pH 5.5, precipitated with 2.5 volumes of absolute ethanol, quantitated by A_{260} spectrophotometry, and used for molecular hybridization.

Hybridization Analysis of Total RNA Transferred to a Membrane Filter ("Northern" Transfer). Ten micrograms of total RNA was denatured for 15 min at 60 °C in buffer containing 50% deionized formamide, 6% formaldehyde, and 1 × MOPS buffer [20 μM 3-(*N*-morpholino)propanesulfonic acid (MOPS), 5 mM sodium acetate, and 1 mM Na_2EDTA], placed in separate lanes of a 1% agarose gel prepared in 1 × MOPS buffer with 6% formaldehyde, and electrophoresed for 4–5 h at 100 mA essentially according to the method of Thomas (1980). After electrophoresis, the RNA was transferred to a Gene Screen filter sheet (New England Nuclear) as described by the manufacturer and hybridized with a radiolabeled probe. The cDNA clones complementary to specific messenger RNAs were radioactively labeled by primer extension as described by Summers (1975), using [^{32}P]dCTP (specific activity 3000 Ci/mmol) to obtain a specific activity of $(2–6) \times 10^8$ cpm/μg of DNA. After hybridization, filters were washed and exposed to autoradiography at −86 °C on Kodak XAR-5 film using Du Pont Lightening Plus intensifier screens. The autoradiographs were then evaluated by densitometry scanning.

Results

Characteristics of the in Vitro Nuclear Transcription System. To ensure that labeling of nascent RNA chains in isolated rat liver nuclei was occurring under uniform and reproducible conditions, a series of preliminary experiments was performed (Table I). There was increasing incorporation of [^{32}P]UTP into RNA with increasing numbers of nuclei up to 50×10^6 nuclei/250-μL reaction. This reaction was dependent on added ribonucleotide substrates and partially dependent on $MgCl_2$ and KCl and showed increasing incorporation of [^{32}P]UTP with time for 30–45 min. Total RNA transcription was inhibited ~60% by 1 μg of α-amanitin. When individual gene products (such as albumin) were assayed, the relative proportion of labeled transcripts was constant at 10, 20, or 30 min. Labeled albumin coding sequences

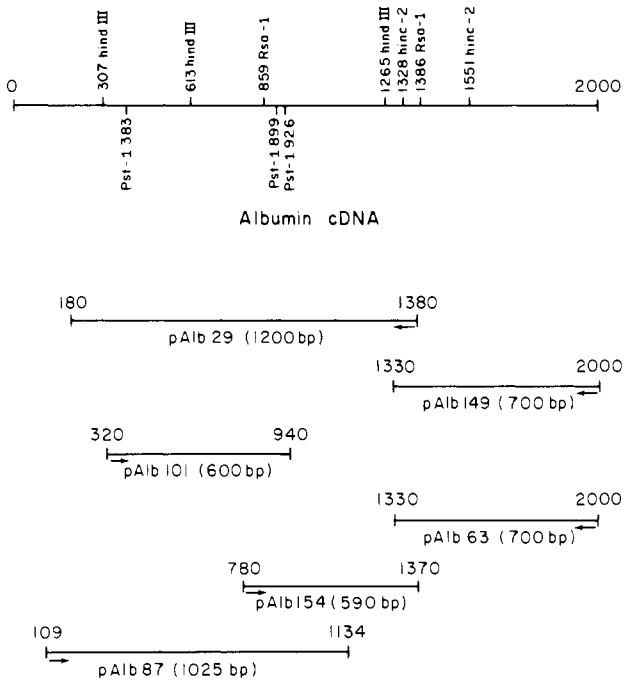

FIGURE 1: Restriction map of rat albumin cDNA and characteristics of rat albumin cDNA subclones used in the present studies.

represented ~0.024% of total transcripts. These results are similar to those obtained in other systems for highly abundant eukaryotic gene products (Lowenhaupt et al., 1978; McKnight & Palmiter, 1979; Derman et al., 1981; Groudine et al., 1981). Transcription of albumin was inhibited 89% by 1 μg/mL α-amanitin. Therefore, at least 50% of the total [^{32}P]UTP incorporated was into RNA polymerase II directed gene products and represented transcription of specific nuclear mRNA precursors.

To further characterize this system, we compared labeling of transcripts representing the 5′, middle, and 3′ regions of the albumin gene. Previously, we isolated six clones of rat albumin cDNA from cytoplasmic poly(A+) RNA (Zern et al., 1983). These clones have been mapped in comparison to those reported previously by Sargent et al. (1981). As shown in Figure 1, clones were identified representing the 5′ (pAlb 29, 87, and 101), middle (pAlb 154), and 3′ (pAlb 63 and 149) regions of the albumin coding sequence. In subsequent experiments, clones pAlb 87, pAlb 154, and pAlb 149 were used to detect labeling of nascent RNA transcripts in different stages of elongation. The 5′ clone detected the greatest number of cpm, but after correction for the length of the probes, this represented only 20% greater incorporation than either the middle or the 3′ clones, which were virtually identical. No correction was made for transcription of intron sequences which were not detected in our assay.

Influence of CCl_4 Treatment on Specific Gene Transcription. Within 24 h following CCl_4 administration, there was cytoplasmic vacuolization, fat accumulation, and a moderate degree of hepatocyte necrosis in the central lobular area, associated with hemorrhage and an inflammatory infiltrate. Aside from fatty metamorphosis, the periportal area was spared. Mitotic activity associated with liver regeneration was first seen in the periportal area and peaked at 48–72 h. The hepatic lobular architecture returned to normal within 1 week. Figure 2 shows [^{3}H]thymidine incorporation into DNA over time as an index of the liver regenerative response following CCl_4 administration. [^{3}H]Thymidine incorporation began to increase at 24 h and became maximal at 48 h. At the peak, [^{3}H]thymidine incorporation was approximately 12 times that

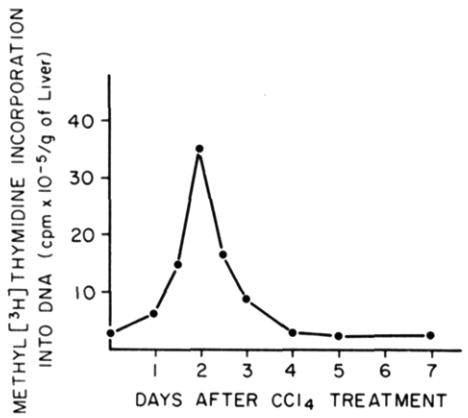

FIGURE 2: [^{3}H]Thymidine incorporation into rat liver DNA following administration of CCl_4. CCl_4 was administered to 250-g male rats as a single intragastric dose. After the times noted, methyl[^{3}H]-thymidine was given as a 1-h pulse, and counts incorporated into trichloroacetic acid insoluble material in liver were determined (see Experimental Procedures for additional details).

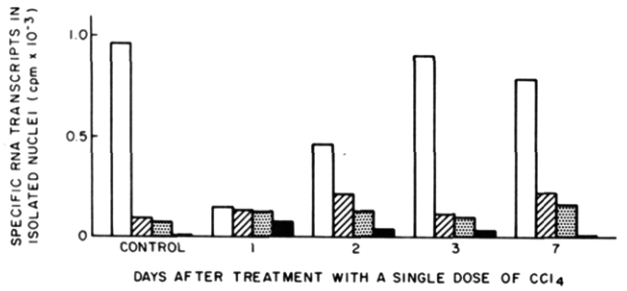

FIGURE 3: Relative transcription of albumin and α-fetoprotein during rat liver regeneration following CCl_4 administration. Rats were given a single intragastric dose of CCl_4 and were then sacrificed at the times noted. Nuclei were isolated from the liver, and in vitro transcriptional elongation was performed as noted under Experimental Procedures. [^{32}P]UTP-labeled RNA was then isolated, and 10^7 cpm was used for hybridization with one-fourth portions of 25-mm-diameter nitrocellulose filter disks to which specific albumin and α-fetoprotein cDNA clones had been attached. See Experimental Procedures for additional details. Experiments were repeated at least 3 times and for α-fetoprotein cpm transcription were control [7.25 ± 7.25 (SE)], 1 day following CCl_4 administration [69.8 ± 3.38 (SE) ($p < 0.01$)], and 2 days following CCl_4 administration [33.3 ± 4.4 (SE) ($p < 0.05$)]. Open bars, albumin; hatched bars, pro α_1-collagen; stippled bars, pro α_2-collagen; closed bars, α-fetoprotein.

occurring in untreated animals, returning to normal after 3–7 days. This brisk response was quite reproducible and was more marked than that usually observed following 68% partial hepatectomy, in which [^{3}H]thymidine incorporation becomes maximal at 24 h (Bucher & Malt, 1971; Goyette et al., 1983; Petropoulos et al., 1983).

We then compared total transcription and the relative transcription rates for the albumin and α-fetoprotein gene following administration of CCl_4. Both the total [^{32}P]UTP incorporation and the specific activity of labeled RNA transcripts were unchanged in CCl_4 vs. control liver nuclei. This indicated that treatment with a single dose of CCl_4 had no effect on general transcriptional activity. Following CCl_4 administration, albumin transcription was abruptly inhibited (85%) (Figure 3). α-Fetoprotein transcripts, which were not detected initially, became evident within 24 h, gradually returning to normal after 3 days. These experiments were repeated 3 times, and in each instance, the increase in α-fetoprotein transcription was maximal on the first day (data statistically significant at $P < 0.01$). Transcription of pro α_1- and pro α_2-collagen mRNAs was also increased but remained elevated for the entire week (Figure 3).

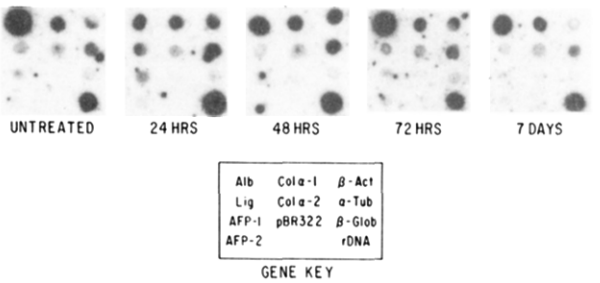

FIGURE 4: Transcriptional response of liver-specific vs. general cellular genes during CCl_4-induced liver regeneration. Nuclei and ^{32}P-labeled RNA were prepared from untreated and CCl_4-treated rats at the times indicated after administration of a single dose of CCl_4. The various cDNA clones (5 μg each) were fixed to a nitrocellulose sheet as illustrated in the gene key and hybridized with ^{32}P-labeled RNA as noted under Experimental Procedures. Nonrecombinant plasmid pBR322 and a mouse β-globin recombinant were used as negative controls, and 28S rDNA at 0.5 μg/spot was used as a positive control.

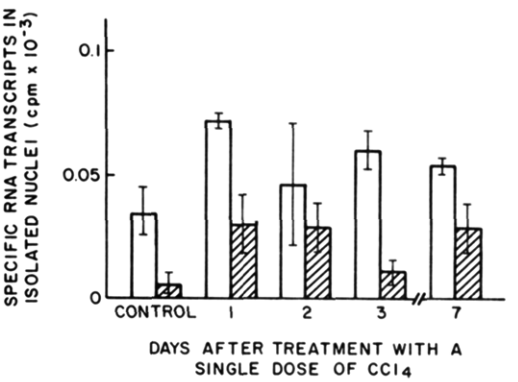

FIGURE 5: Transcriptional response of α_1-antitrypsin and ligandin genes to CCl_4-induced liver regeneration. 10^7 cpm of ^{32}P-labeled RNA was used for each time point (see Figure 4 and Experimental Procedures for additional details). Open bars, α_1-antitrypsin; hatched bars, ligandin. Error bars indicate the range of results in two separate experiments.

To determine whether changes occurred in other specific gene products following CCl_4 administration, the qualitative dot blot assay of Clayton and Darnell (1983) was utilized. As shown in Figure 4, simultaneous with the decrease in albumin transcription, there were modest increases in ligandin, α-fetoprotein (AFP-1), pro α_1- and pro α_2-collagen, β-actin, and α-tubulin. While the latter four genes are transcribed by a wide variety of cell types, albumin, α-fetoprotein, and ligandin are liver specific or highly liver enriched. We have also tested other genes normally associated with liver function and have found an increase in α_1-antitrypsin (α_1-AT) but no increase in α_1-acid glycoprotein transcription (data not shown).

Although the dot blot assay has the advantage of rapidly detecting changes for a large number of gene products in one analysis, the results needed independent confirmation. The increases in ligandin and α_1-AT transcription following CCl_4 administration using quantitative analysis with individual filters are shown in Figure 5. With both α_1-AT and ligandin, there was a 2–3-fold increase in transcription within 24 h following CCl_4 administration, and this transcription remained elevated for up to 1 week. Other genes important in liver function showed differential responses, as, for example, with two different cytochrome P-450 genes (Figure 6). In one case (R-17), there was a modest initial decrease reaching a low point on the second day, followed by a return to normal or slightly increased transcription after 1 week. In the other case (TF-I), there was a marked inhibition of transcription at 24 h, a return to normal by 48 h, and a continued increased to 3 times normal after 1 week.

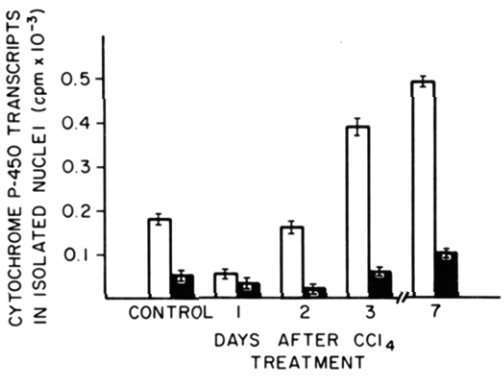

FIGURE 6: Differential response in transcription of two cytochrome P-450 genes during CCl_4-induced liver regeneration. Experiments were performed as noted in Figure 4 and under Experimental Procedures. Open bars, cytochrome P-450 TF-1; closed bars, cytochrome P-450 R-17. Error bars indicate the range of results in two separate experiments.

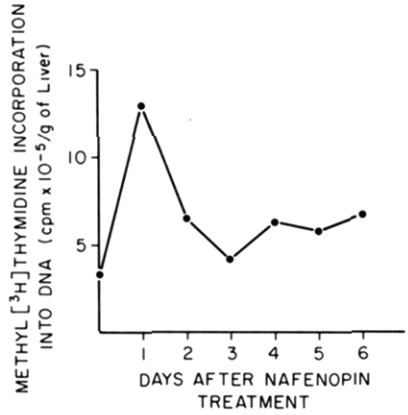

FIGURE 7: [^{3}H]Thymidine incorporation into liver DNA of rats treated with nafenopin. Nafenopin was administered as a single intragastric dose. At the times indicated, methyl[^{3}H]thymidine incorporation into DNA was determined as noted under Experimental Procedures.

To be certain that the reduction in albumin gene transcription compared to other genes during liver regeneration was due to a change in the functional state of hepatocytes, rather than a toxic or nonspecific effect of CCl_4, a second method was utilized to induce liver cell division. This was accomplished by intragastric administration of nafenopin, a hypolipidemic agent which causes liver cell hyperplasia (Moody et al., 1977). Following a single dose of nafenopin, there was no evidence histologically of liver cell necrosis or inflammation, but [^{3}H]thymidine incorporation into DNA increased 4–5-fold within 24 h and returned to normal by day 3 (Figure 7). The maximal response was one-third to half that observed with CCl_4. Changes in relative transcriptional rates for a series of specific gene products 24 h after nafenopin administration are given in Table II. Albumin gene transcription was reduced 60%. No detectable transcription above background was observed with α-fetoprotein, perhaps because the proliferative response with nafenopin, as judged by [^{3}H]thymidine incorporation, was much less than that with CCl_4. Following nafenopin administration, transcription of β-actin was increased by 50%, but α-tubulin was decreased by 44%; 28S rDNA transcription remained unchanged (Table II).

Liver-Specific mRNA Levels following CCl_4 Administration. In order to assess the contribution of transcriptional vs. posttranscriptional factors in specific gene functions of the liver and their potential alteration during liver regeneration, we measured the steady-state level of five specific mRNAs: albumin, α-fetoprotein, pro α_2-collagen, β-actin, and α-tubulin.

Table II: Changes in Transcription Rates of Specific Genes in Rat Liver following Nafenopin Administration[a]

specific gene	control (cpm)	nafenopin (cpm)	% change
albumin	1080	435	−60
α-fetoprotein	0	0	0
β-actin	90	135	+50
α-tubulin	140	78	−44
28S rDNA	51000	51500	0

[a] Changes in the relative transcription of these specific genes 1 day following nafenopin administration were determined by measuring specific transcripts per 10^7 cpm total transcription in isolated liver nuclei from treated vs. untreated rats.

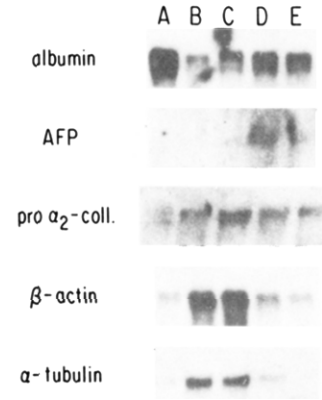

FIGURE 8: Changes in the steady-state level of specific mRNAs in rat liver following CCl_4 administration. Total cellular RNA (10 μg) isolated from the liver of control rats and from animals at various times after CCl_4 treatment was denatured and applied to separate lanes of a 1% agarose gel under denaturing conditions and electrophoresed for 4 h at 100 V. The RNA was then transferred to a Gene Screen membrane filter and hybridized with the specific cDNA probes noted on the left (see Experimental Procedures for additional details). (A) Untreated rat; (B) 1 day; (C) 2 days; (D) 3 days; (E) 7 days after CCl_4 administration.

These results were obtained by Northern gel hybridization of constant amounts of total cellular RNA in control vs. CCl_4-treated animals. Representative examples of these gels are shown in Figure 8. At all time points, there was a distinct band of albumin RNA sequences at 19–20 S indicating the presence of normal, full-length albumin mRNA. On the day following CCl_4 administration, the intensity of the albumin mRNA signal was reduced by more than 50%, returning to near normal after 3–7 days. α-Fetoprotein mRNA was clearly identified on day 3 following CCl_4 administration, the time at which previous investigators have reported the α-fetoprotein mRNA steady-state level to be maximally increased (Petropoulos eet al., 1983). There was a 2-fold increase in pro α_2-collagen mRNA, returning to near-normal levels by day 7, and large increases in β-actin and α-tubulin mRNAs, both peaking on day 2 and returning to normal by day 7. Table III quantitates these data by densitometry scanning from several experiments. Results are reported as relative increases or decreases in the steady-state level of the various mRNAs using RNA extracts from control liver as reference standards. Although the changes in albumin, α-fetoprotein, collagen, and β-actin mRNA steady-state levels are consistent with decreased or increased transcription of these genes following CCl_4 administration, the increase in α-tubulin mRNA is proportionately greater than that expected from the very slight increase in transcription.

Discussion

Several recent studies have evaluated changes in the steady-state level of albumin and α-fetoprotein mRNA during

Table III: Steady-State Level of Specific mRNAs in Rat Liver following Acute CCl_4 Administration[a]

mRNA	control	days following CCl_4 administration			
		1	2	3	7
albumin	1.0	0.4	0.6	0.69	0.76
α-fetoprotein	−	−	−	+	−
pro α_2-collagen	1.0	1.7	1.9	1.9	1.3
β-actin	1.0	9.3	12.1	2.6	1.3
α-tubulin	1.0	7.7	8.7	1.6	0.92

[a] Densitometry tracings of the mRNAs illustrated in Figure 8 were obtained at several levels of autoradiographic exposure. The area under the curve for mRNAs from untreated animals was then set at 1.0 for comparison with densitometry tracings of the mRNAs at various days following CCl_4 administration. The data represent the average of two experiments.

liver regeneration (Krieg et al., 1980; Princen et al., 1982; Petropoulos et al., 1983). The major observation following 68% partial hepatectomy (Krieg et al., 1980; Princen et al., 1982; Petropoulos et al., 1983) or CCl_4-induced hepatic necrosis (Chiu et al., 1981; Belayew & Tilghman, 1982; Petropoulos et al., 1983) has been that α-fetoprotein mRNA increases, but there is no reciprocal change in the albumin mRNA steady-state level. These findings have been taken as evidence for a lack of coordinate or reciprocal regulation of α-fetoprotein and albumin genes once these genes have been induced (Belayew & Tilghman, 1982; Petropoulos et al., 1983; Fausto, 1984). Since the increase in α-fetoprotein mRNA follows the peak of DNA synthesis after CCl_4 administration (Petropoulos et al., 1983), it has been concluded that increases synthesis of α-fetoprotein mRNA does not reflect "dedifferentiation" or "retrodifferentiation" of hepatocytes. However, in this study, changes in the rate of α-fetoprotein transcription during liver regeneration were not assessed, and evidence against a change in the albumin mRNA steady-state level was also limited in that only one time point, the third day following CCl_4 administration, was determined.

In the present study, we observed that following a single large dose of CCl_4 to rats, albumin transcription decreased 85% in 24 h and α-fetoprotein transcription increased from unmeasurable levels to 50% of that observed for albumin. Albumin gene transcription then increased sharply, and α-fetoprotein transcription returned to normal after 3 days. There was an associated decrease (~60%) in cytoplasmic albumin mRNA also within the first 24 h following acute CCl_4 treatment, returning to normal within 1 week. Total RNA transcription was normal, and the changes in albumin and α-fetoprotein expression appeared to be associated with the liver regenerative response. This was documented by histologic analysis and [^{3}H]thymidine incorporation into liver DNA.

By comparison of the changes in albumin and α-fetoprotein gene transcription with the kinetics of [^{3}H]thymidine incorporation, the transcriptional changes clearly preceded the increase in DNA synthesis. This suggested that changes in the cell program preceded cell division and were perhaps coordinated. However, the present studies could not distinguish whether hepatocytes previously synthesizing albumin were those in which the α-fetoprotein gene became induced or whether differentiated hepatocytes previously synthesizing albumin were preferentially eliminated by CCl_4 administration and a preexisting, less differentiated subpopulation of hepatocytes was induced to transcribe α-fetoprotein. It is also possible that the rates of both albumin and α-fetoprotein transcription vary in different subpopulations of hepatocytes within the hepatic lobule and that changes in expression of these genes in these various subpopulations may vary considerably during liver regeneration, as observed previously following injury with chemical toxins (Sell, 1980).

In developing mouse liver, Tilghman and Belayew (1982) have observed that transcriptional regulation is the major factor governing the level of α-fetoprotein and albumin mRNA. In utero, both genes turn on in parallel at approximately the 15th day of gestation (α-fetoprotein slightly before albumin), and then α-fetoprotein transcription decreases selectively at birth. However, during liver regeneration, Friedman et al. (1984) were unable to detect significant changes in the relative transcription rate or mRNA steady-state level for either α-fetoprotein or albumin. The major conclusion in these and other studies with cultured primary hepatocytes (Clayton & Darnell, 1983; Jefferson et al., 1984; Reid et al., 1985) is that both transcriptional and posttranscriptional factors (i.e., changes in mRNA half-life) play a role in regulating specific gene function and that the contribution of transcriptional and posttranscriptional factors varies with the individual mRNA, as well as with different physiologic, developmental, or cell culture conditions.

The marked reduction of the albumin mRNA steady-state level at 24 h in the present study was unexpected and rather surprising, since this mRNA has been thought to have a relatively long half-life. One possibility was that activation of nucleases during hepatic necrosis led to degradation of albumin mRNA. However, there was no evidence for increases nuclease activity in cytoplasmic extracts from CCl_4-treated rats (our unpublished results); albumin mRNA did not show increased degradation on Northern blots, and other mRNAs, such as β-actin and α-tubulin, were increased dramatically as observed by Friedman et al. (1984). Since the $T_{1/2}$ for albumin mRNA is currently unknown, we still cannot provide a precise explanation for the rapid decrease in albumin mRNA following acute CCl_4 treatment. However, our results suggest that the albumin mRNA $T_{1/2}$ may be shorter than previously suspected.

Following both CCl_4 and nafenopin administration, albumin gene transcription decreases, suggesting that these changes were associated with responses related to cell growth and proliferation. The subsequent turnoff of the α-fetoprotein gene during the latter stages of liver regeneration associated with increased albumin transcription also mirrors changes in the expression of these genes in late fetal development. Tilghman and Belayew (1982) have demonstrated that transcription of α-fetoprotein decreases relative to albumin at birth and that the relative fall in α-fetoprotein transcription precedes a parallel fall in the α-fetoprotein/albumin mRNA steady-state ratio. These findings are consistent with our present results in which the increase in α-fetoprotein transcription in regenerating liver precedes the increase in α-fetoprotein mRNA by several days.

In studies to be reported elsewhere (A. Panduro et al., unpublished results), we have also observed differential transcriptional responses for a large series of liver-specific genes in normal rat development. In several instances, these changes are sequential and similar to those occurring during liver regeneration. Therefore, it would appear that changes in albumin and α-fetoprotein transcription (and possibly other liver-specific genes) during the regenerative response reflect a return to an earlier state of liver cell differentiation. These changes are transient and are followed rapidly by reappearance of normal function as newly proliferated hepatocytes mature.

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