Transcriptional and Posttranscriptional Regulation of Apolipoprotein E, A-I, and A-II Gene Expression in Normal Rat Liver and during Several Pathophysiologic States†

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ABSTRACT: Assessment of the relative transcription rates and mRNA steady-state levels for apolipoprotein genes E, A-I, and A-II has been performed in normal rat liver, during liver regeneration and following induction of cirrhosis, as well as in rats with inherited analbuminemia associated with hyperlipidemia. Apo E exhibits primarily transcriptional control with an additional component of posttranscriptional control, whereas Apo A-I is controlled primarily at the posttranscriptional level, thus indicating that these genes are regulated independently. The level of control for Apo A-II has not been determined, because of difficulty experienced in measuring the transcription rate of this gene. During liver regeneration, cirrhosis, and analbuminemia, there is a marked increase in the ratio of Apo A-I to Apo E mRNA, resulting from an increase in the Apo A-I mRNA steady-state level and a decrease in Apo E mRNA. These changes are similar in the three pathophysiologic states and seem to occur through a combination of transcriptional and posttranscriptional mechanisms.

The level of high-density lipoproteins (HDL) in the plasma has been shown to be inversely correlated with development of coronary heart disease (Miller & Miller, 1975; Heiss et al., 1980). Apolipoprotein (Apo) A-I, a major component of HDL, appears to be an even better marker of coronary heart disease than HDL (Maciejko et al., 1983). Variations in the plasma concentration of Apo A-I also correlate directly with the degree of liver injury in alcoholic patients (Haskell et al., 1984; Poynard et al., 1986), and this protein is elevated in the serum in several animal models of liver disease (Stern et al., 1965; Narayan, 1967; Felker et al., 1982). However, it is not known whether such alterations in plasma apolipoprotein concentrations are controlled at the transcriptional or posttranscriptional level.

In the rat, the major protein components of HDL are Apo A-I, Apo A-IV, and Apo E (Swaney et al., 1977). These apolipoproteins are synthesized principally by the liver and small intestine. However, Apo E mRNA is present at high levels in the liver but is barely detectable in the intestine (McLean et al., 1983; Elshourbagy et al., 1985b). In contrast, Apo A-I and Apo A-IV mRNAs are more abundant in intestine than in liver (Elshourbagy et al., 1985a). As released from the liver, HDL contains considerably more Apo E than Apo A-I (Marsh & Sparks, 1979; Felker et al., 1977, Lusk et al., 1979). However, in rats with experimental nephrotic syndrome, Apo A-I secretion from the liver increases 8.4-fold, whereas Apo E secretion increases only 1.8-fold (Marsh & Sparks, 1979). The ratio of Apo A-I to Apo E in serum lipoprotein particles is thus reversed, so that Apo A-I becomes the most abundant apolipoprotein. In addition, it has been shown that the increase in expression of Apo A-I in rat experimental nephrosis correlates with an increase in the level of Apo A-I mRNA (Tarugi et al., 1986).

In previous studies, we observed that during development of the rat liver, the levels of Apo A-I and Apo E mRNA are regulated independently (Panduro et al., 1987). Both mRNAs show a strong element of posttranscriptional control in regulating the mRNA steady-state level, but this regulation occurs in opposite directions; i.e., as rats mature, Apo E mRNA becomes more stable, whereas Apo A-I mRNA becomes less stable (Panduro et al., 1987).

The present study analyzes the steady-state levels of Apo A-I, Apo A-II, and Apo E mRNAs in the mature liver and compares these levels with the relative transcription rate of each specific apolipoprotein gene during liver regeneration after toxic injury with CCl_4, in mutant rats with analbuminemia, and in cirrhotic rats. In all these pathologic states, there is an increase in the ratio of Apo A-I/Apo E mRNA resulting from a posttranscriptional increase in the Apo A-I mRNA steady-state level. Under most circumstances, Apo E mRNA appears to exhibit significant down-regulation through a combination of transcriptional and posttranscriptional control. Apo A-II mRNA steady-state levels are also reduced, but the primary level of control of Apo A-II mRNA, i.e., transcriptional or posttranscriptional, has not been determined. However, Apo A-II does exhibit some aspects of posttranscriptional control, especially during cirrhosis. These results indicate that apolipoprotein genes are regulated independently and that changes in expression of these genes during pathophysiologic states occur by a combination of transcriptional and posttranscriptional mechanisms consistent with our previous findings during different stages in normal rat liver development.

Experimental Procedures

Animals and Models for Induction of Liver Regeneration and Cirrhosis. Male Sprague-Dawley rats (Marland Farms)

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were maintained in day–night cycles of 12 h with a diet "ad libitum". In all studies, rats were sacrificed between 8 and 9 am. Two different methods were utilized to induce liver regeneration: partial hepatectomy or administration of a single dose of CCl_4. Partial hepatectomy was performed by resecting 68% of the liver, according to the method of Higgins and Anderson (1931). The use of CCl_4 to induce acute liver injury followed by regeneration was as previously reported (Panduro et al., 1986). Rats of 200–250 g were used for these experiments. A single dose of a mixture of CCl_4/corn oil, 1:1 (v/v), was administered intragastrically, and animals were sacrificed at the times indicated.

Liver fibrosis was induced in young rats (50–60 g initial weight) by intraperitoneal injection of 0.2 mL of a mixture of CCl_4 with mineral oil 3 times per week for 7 weeks, as previously reported (Panduro et al., 1988). The percentages of CCl_4 in mineral oil (v/v) were as follows: week 1, 13%; week 2, 16%; week 3, 20%; and weeks 4–7, 25%. At the times and under conditions indicated in the figures, the rats were killed by vertebral dislocation, and the liver was removed quickly and perfused with a solution of 0.25 M sucrose and 1 mM $MgCl_2$.

RNA Transcription and Isolation of Labeled RNA. Liver nuclei were isolated as previously described (Lamers et al., 1982; Panduro et al., 1986). For transcription assays, incubations were performed in a 250-μL reaction volume and contained (0.25–4.0) $\times$ 10^7 nuclei, 25% glycerol, 75 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 200 mM KCl, 4 mM dithiothreitol, 0.5 mM cytosine triphosphate, 0.5 mM guanosine triphosphate, 1.0 mM adenosine triphosphate, and 50 μCi of uridine [^{32}P]triphosphate (specific activity 410 Ci/mmol; Amersham/Searle Corp.). Reactions were incubated for 20 min at room temperature and were terminated by addition of deoxyribonuclease I (DNase I) to a final concentration of 20 Mg/mL and further incubation for 5 min. Labeled RNA was isolated by the method of Groudine et al. (1981).

Assay for Specific Gene Transcription Products. For quantitative determination of transcriptional activity for individual genes, Apo E, Apo A-I, and Apo A-II cDNAs were bound to nitrocellulose filter disks as previously reported (Panduro et al., 1986). 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 $\times$ Denhardt's solution, 4 $\times$ SET buffer (1 $\times$ 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 $\times$ Denhardt's solution, 5 $\times$ SET buffer, and 0.1% SDS; 400 μg/mL wheat germ RNA was added and the filter prehybridized for 4–6 h at 42 °C. The solution was removed and replaced by fresh hybridization solution containign 1.0 $\times$ 10^7 cpm of the radioactivity 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 hybridization mixture at 42 °C for 20 min each wash, washed with 2 $\times$ SCC (0.30 M NaCl–0.03 M sodium citrate) at room temperature, digested with RNase, and treated with 200 μg/mL proteinase K, as described previously (Panduro et al., 1986). After RNase and proteinase K treatment, filters were washed and dried, and radioactivity remaining on the filters was determined by liquid scintillation spectroscopy.

Isolation of Total Cellular RNA. Total cellular RNA was isolated by using a minor modification 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, using a 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 at −20 °C, resuspended in 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA, quantitated by A_{260} spectrophotometry, and used for molecular hybridization.

Northern Blot Analysis of Total RNA. Ten micrograms of total RNA was denatured for 15 min at 60 °C in buffer containing 50% deionized formamide, 6% formaldehyde, and 1 $\times$ MOPS buffer [20 mM 3-(*N*-morpholino)propanesulfonic acid, 5 mM sodium acetate, and 1 mM Na_2EDTA], placed in separate lanes of a 1% agarose gel prepared in 1 $\times$ MOPS buffer with 6% formaldehyde, and electrophoresed for 4–5 h at 70 mA, essentially according to the method of Thomas (1980). After electrophoresis, the RNA was transferred to a Gene Screen filter sheet (New England Nuclear Corp., Boston, MA), as described by the manufacturer, and hybridized with a ^{32}P-labeled 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 Dupont Lightening Plus intensifier screens.

Results

Regulation of Apo E, A-I, and A-II mRNA Levels during Rat Liver Regeneration. Twenty-four hours after normal rats were treated with a single dose of CCl_4, the steady-state levels of Apo E and A-II mRNAs decreased (Figure 1). The level of Apo A-I mRNA showed little or no change at 24 h and then increased, becoming 3 times normal at 72 h. Apo E mRNA levels began to recover at 48 h and reached normal levels at 72 h and 7 days (Figure 1). Apo-II mRNA responded similarly to Apo E mRNA but still exhibited lower levels than the control on the seventh day posttreatment, indicating a slower return to normal.

The relative transcription rates for Apo-E, Apo-I, and Apo A-II genes were determined simultaneously with the steady-state level of each mRNA (Figure 1). Twenty-four hours after CCl_4 administration, the relative transcription rate of Apo E decreased by 55–60% and returned to normal by 72 h. Since the steady-state levels of Apo E mRNA followed the same pattern, this suggested trancriptional control in regulating Apo E expression following CCl_4 administration. However, the reduction in Apo E mRNA steady-state level at 24 (80–85%) was proportionately greater than the decrease in the relative transcription rate (~55–60%), suggesting a posttranscriptional component in regulating expression of this gene.

After acute CCl_4 administration, the relative transcription rate for the Apo A-I gene was slightly higher than normal at 24 h, but this difference was not statistically significant (Figure 1). Subsequently, Apo A-I transcription rates were normal for the 7 days. Since the steady-state level of Apo A-I mRNA increased 3–4-fold at 3–7 days following acute CCl_4 administration (Figure 1), this indicated substantial posttranscriptional control in Apo A-I mRNA regulation. We were not able to detect specific transcription of Apo A-II above the background obtained with control plasmid pBR322 (Figure 1), suggesting that the relative transcription rate of this gene

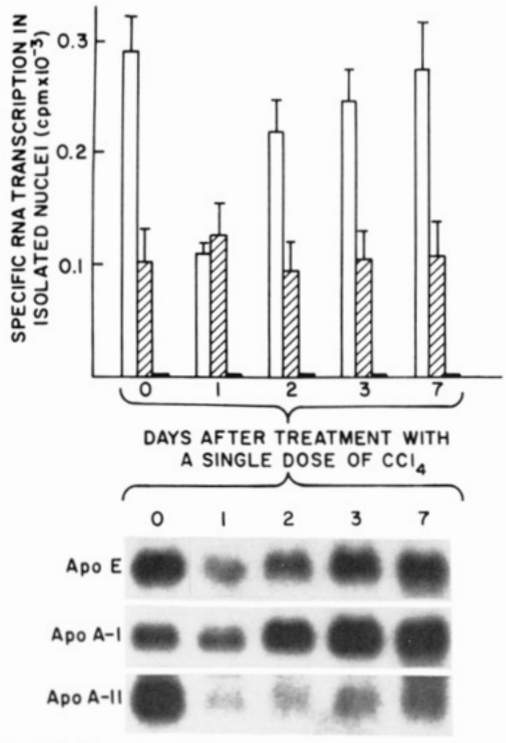

FIGURE 1: Relative transcription rates and mRNA steady-state levels for Apo E, Apo A-I, and Apo A-II genes during CCl_4-induced liver regeneration. For transcription studies (top of figure), nascent RNA molecules in the process of transcription were labeled in isolated nuclei with [^{32}P]UTP for 25 min. 10×10^6 cpm of labeled RNA was then hybridized to cloned specific cDNA sequences fixed in large excess to individual nitrocellulose filter disks. Hybridized labeled RNA was determined for each disk. Results represent mean values with standard deviations for three separate experiments. The relative transcription rates are illustrated for Apo E (open bars), Apo A-I (hatched bars), and Apo A-II (filled bars). Under the conditions used, the hybridization signal for Apo A-II was not significantly above the background obtained with nonrecombinant plasmid pBR322. Thus, the relative transcription rate for Apo A-II was not measurable. For mRNA steady-state levels (Northern blots on bottom of figure), total RNA was extracted from a separate portion of the liver used for in vitro nuclear RNA labeling, and 10 μg of RNA was applied to each lane of a 1% agarose gel under denaturing conditions and electrophoresed in the usual fashion. Hybridization was performed with ^{32}P-labeled cDNA probes of specific activity $(2–6) \times 10^8$ cpm/μg of DNA. For this and subsequent figures, Northern blots shown are representative examples from three independent sets of experiments.

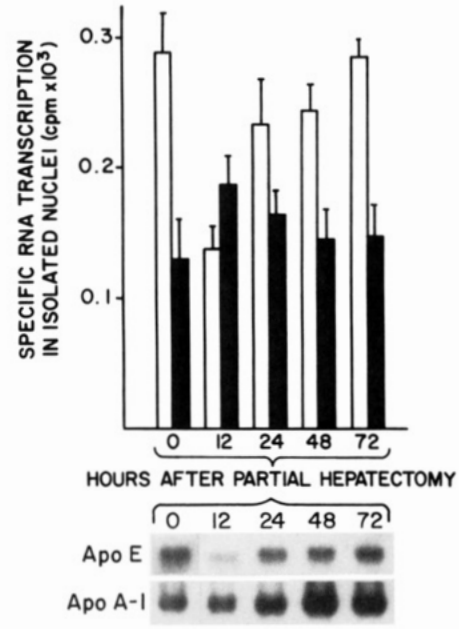

FIGURE 2: Relative transcription rates and mRNA steady-state levels for Apo E and Apo A-I genes after partial hepatectomy. Technical aspects are as described under Experimental Procedures and in the legend of Figure 1. For relative transcription rates (upper portion), data are presented for Apo E as open bars and for Apo A-I as filled bars. For the Northern blots (lower portion), 0, 1, 2, 3, and 7 represent days after partial hepatectomy. No changes were detected in Apo E or Apo A-I transcription rates or mRNA steady-state levels in sham-operated rats.

is low. In conjunction with a relatively high mRNA steady-state level, this suggests that Apo A-II mRNA has a long half-life and may be regulated posttranscriptionally. The rapid decrease in Apo A-II mRNA levels following CCl_4 treatment suggests further that administration of this agent reduced the half-life or stability of this mRNA. However, since the Apo A-II transcription rate is not measurable by our current methods, we cannot be sure whether or not transcription is also affected.

In the CCl_4 model of liver regeneration, maximum incorporation of [^{3}H]thymidine into DNA occurs 48 h after administration of this agent (Panduro et al., 1986). Since the decrease in Apo E mRNA levels occurred at 24 h, i.e., preceding the peak of [^{3}H]thymidine incorporation, and the maximum increase in the Apo A-1 mRNA levels occurred at 72 h after CCl_4 administration (following the peak of [^{3}H]-thymidine incorporation), it appears that changes in the steady-state levels of these mRNAs reflect changes in specific gene expression related to the liver cell growth state. We, therefore, investigated whether the same changes could be observed during liver regeneration following partial hepatectomy. In the partial hepatectomy model, [^{3}H]thymidine incorporation is maximal at ~24 h after surgery (Bucher & Malt, 1971). As shown in Figure 2, the greatest decrease in the steady-state level of Apo E mRNA occurred 12 h after partial hepatectomy (again before maximal [^{3}H]thymidine incorporation). Apo E mRNA returned to near normal levels by 48–72 h after surgery. In contrast, Apo A-I mRNA increased following partial hepatectomy, with maximum levels 3–4 times greater than control at 72 h following surgery. Furthermore, when gene transcription studies were performed after partial hepatectomy, the same pattern was observed as in the CCl_4 model of liver regeneration (Figure 2). Apo E transcription was reduced by ~50% at 12 h, whereas Apo A-I transcription was essentially unchanged. This indicates that similar mechanisms for controlling the levels of Apo E mRNA (transcriptional and posttranscriptional) and Apo A-I mRNA (posttranscriptional) are utilized in partial hepatectomy and after acute CCl_4 administration (cf. Figures 1 and 2).

Regulation of Apo E and Apo A-I mRNAs in Analbuminemic Rats. A mutant strain of rats with inherited analbuminemia (NAR) was developed in Japan (Nagase et al, 1979). In this rat, there is aberrant nuclear processing of the primary albumin RNA transcript secondary to a 7 bp deletion in the albumin H-I intron near the 5′ splice junction (Esumi et al., 1983). This leads to skipping of the H-exon and markedly reduced levels of albumin mRNA in the cytoplasm (Shalaby & Shafritz, 1990). In addition to analbuminemia, these animals show hyperlipidemia (Nagase et al., 1979). Consistent with their hyperlipidemia, analbuminemic rats showed a significant increase in Apo A-I mRNA (Figure 3). However, once again transcription of the Apo A-I gene was not changed in analbuminemic compared to normal rats, indicating a posttranscriptional mechanism for increasing the Apo A-I mRNA steady-state level (Figure 3). Albumin mRNA was nearly undetectable in analbuminemic rat liver RNA, but transcription of the albumin gene in isolated nuclei showed

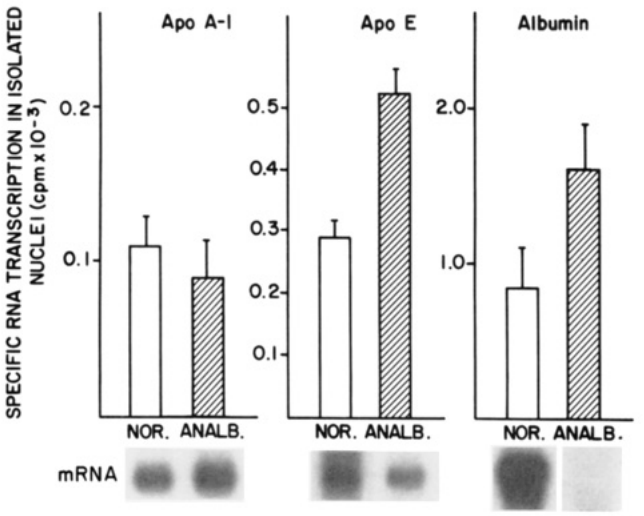

FIGURE 3: Relative transcription rates and mRNA steady-state levels for Apo A-I, Apo E, and albumin genes in normal versus analbuminemic rats.

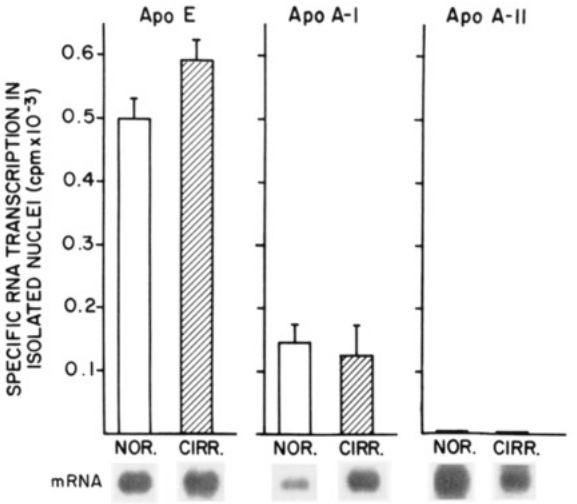

FIGURE 4: Relative transcription rates and mRNA steady-state levels for Apo E, Apo A-I, and Apo A-II genes in normal versus cirrhotic rats. To induce fibrosis, 0.20 mL of CCl_4/mineral oil was administered intraperitoneally 3 times per week during a period of 8 weeks. The amount of CCl_4 administered the first 2 weeks has 0.03 mL, the third week, 0.04 mL, and from the fourth to the eighth week, 0.05 mL.

twice normal levels of activity (Figure 3). These animals also showed increased transcriptional activity of the Apo E gene; however, the Apo E mRNA steady-state level was again decreased. This decrease in Apo E mRNA steady-state levels in face of an increased relative transcription rate of the Apo E gene suggests a strong influence of posttranscriptional control in regulating the steady-state level of Apo E mRNA in analbuminemia.

Regulation of Apo E and Apo A-I mRNAs in Cirrohtic Rats. At the end of chronic CCl_4 treatment, rats show a moderate degree of hepatic fibrosis (Panduro et al., 1988). The steady-state level of Apo E mRNA was unchanged in cirrhotic rats compared to controls, as was the relative transcription rate of the Apo E gene (Figure 4). The steady-state level of hepatic Apo A-I mRNA in cirrhotic rats was increased to 4 times higher than in normal rats (Figure 4). However, no change was observed in Apo A-I gene transcription as compared to controls. This again indicated a posttranscriptional mechanism controlling the level of Apo A-I mRNA in cirrhotic rat liver. Transcription of Apo A-II was undetectable in normal or cirrhotic rat liver; however, in three different experiments, the level of Apo A-II mRNA was consistently lower in cirrhotic compared to control rats (Figure 4).

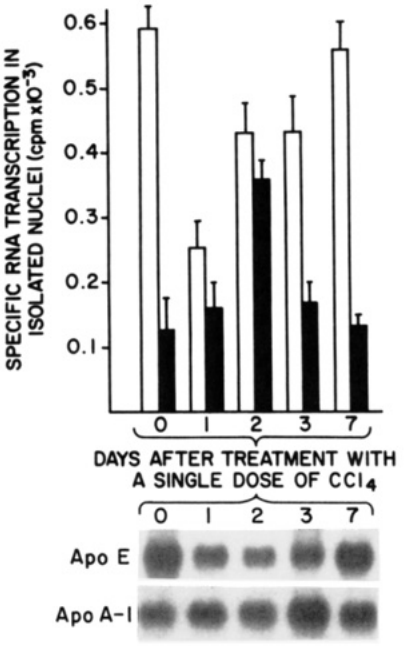

FIGURE 5: Relative transcription rates and mRNA steady-state levels for Apo E and Apo A-I genes during CCl_4-induced liver regeneration in cirrhotic rats. To induce liver regeneration, a single dose of CCL_4 (0.5 mL/100-g body weight) was administered intragastrically. Both the relative transcription rates of Apo E (open bars) and Apo A-I (filled bars) (upper portion of figure) and specific mRNA steady-state levels by Northern blots (lower portion of figure), were determined at the times indicated.

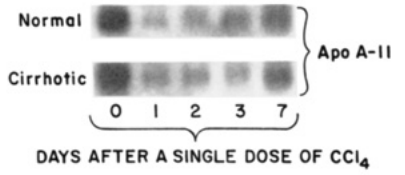

FIGURE 6: Steady-state levels of Apo A-II mRNA at various times during liver regeneration in normal versus cirrhotic rats. Both cirrhosis and liver regeneration were induced as noted under Experimental Procedures and in the legend to Figure 5.

Liver Regeneration in Cirrhotic Rats. After a single dose of CCl_4 was administered to cirrhotic rats, the steady-state levels of Apo E mRNA decreased steadily until 48 h after treatment (Figure 5). Thereafter, it increased and returned to normal by 7 days. The transcription rate of Apo E decreased by ~60% 24 h after CCl_4 treatment, returning to normal by 7 days (Figure 5). As observed in normal rats, the level of Apo A-I mRNA in cirrhotic rat liver began to increase at 24 h after a single dose of CCl_4, peaked at 72 h, and returned to normal by 7 days. However, in contrast to normal rats, in cirrhotic rats, transcription of Apo A-I increased after a single dose of CCl_4, reached a peak at 48 h, and then returned to normal. This was the only circumstance in which there was a significant increase in Apo A-I gene transcription. A decrease in the steady-state level of Apo A-II mRNA was also observed after CCl_4 treatment of cirrhotic rats and persisted for the full 7 days (Figure 6).

Discussion

Previous studies have reported that acute administration of CCl_4 to rats causes disaggregation of membrane-bound polyribosomes and reduction in protein synthesis in the liver (Smuckler & Benditt, 1965). More recently, we showed that this model can also be used to study regulation of specific gene

expression during liver regeneration (Panduro et al., 1986), which occurs in a synchronous fashion following administration of a single dose of CCl_4. Since apolipoprotein genes constitute a multigene family of major physiologic significance (Luo et al., 1986) and these genes are expressed in liver in a regulated fashion during development (Panduro et al., 1987; Staels et al., 1989; Manseney et al., 1989), we studied regulation of certain members of this family, i.e., apolipoproteins A-I, A-II, and E, during liver regeneration, as well as during several pathophysiologic states, such as cirrhosis and analbuminemia, in which apolipoprotein metabolism is known to be altered (Poynard et al., 1986; Nagase et al., 1979).

Two methods were utilized to induce liver regeneration: acute CCl_4 administration and partial hepatectomy. Since the same changes in apolipoprotein gene transcription and/or mRNA steady-state levels were observed in both models, it would appear that these changes reflect fundamental changes in apolipoprotein gene regulation during liver regeneration, rather than idiosyncratic responses to either treatment protocol. In both models, there was a reduction in Apo E mRNA preceding cell division and an increase in Apo A-I mRNA following cell division and during the period of rapid hepatocyte growth. The Apo E response had both transcriptional and posttranscriptional components, whereas the Apo A-I response appeared to be principally posttranscriptional. These changes are similar to those we observed earlier during normal development of the rat liver (Panduro et al., 1987).

In the developing rat liver (late gestational and perinatal periods), Apo A-I mRNA levels are relatively high, and Apo E mRNA levels are low (Panduro et al., 1987). However, as rats mature, the relative levels of Apo A-I to Apo E mRNA in the liver become reversed, and these genes show opposing mechanisms of posttranscriptional control; i.e., Apo A-I mRNA becomes less stable, whereas Apo E mRNA becomes more stable (Panduro et al., 1987). In other studies, it has been found that very little Apo A-I mRNA is present in fetal intestine and that Apo A-I mRNA begins to accumulate in rat jejunum at approximately 1 week of age (Elshourbagy et al., 1985a). Since the level of Apo A-I mRNA in the liver at days 17 and 19 in utero is much higher than in the adult liver (Panduro et al., 1987), the fetal or rapidly growing hepatocyte may represent an important source for Apo A-I synthesis during this stage of development. The reduction of Apo A-I mRNA in mature hepatocytes also correlates with increased intestinal synthesis of this protein (Elshourbagy et al., 1985a).

Our findings with Apo E and Apo A-I genes during liver regeneration are consistent with the notion that these specific responses reflect reactivation or recapitulation of the normal liver differentiation program. Both Apo A-I and Apo E genes code for secretory proteins, which are geneally considered nonessential for cell survival. In this sense, down-regulation of Apo E expression during liver regeneration, which is similar to the transcriptional shut-off of the albumin gene (Panduro et al., 1986), is consistent with the need for hepatocytes to synthesize cellular constituents rather than secreted proteins during periods of rapid cellular growth. However, there is no obvious reason for increased liver Apo A-I expression during this period. Since we observed a simlar phenomenon during late gestation, when liver mass is expanding rapidly (Panduro et al., 1987), increased Apo A-I expression during liver regeneration may represnt a manifestation of hepatocyte growth or proliferation.

Studies performed many years ago (Narayan et al., 1968) showed a drop in total serum proteins (20%) and lipids (15%) during the first 24 h following partial hepatectomy. There was a greater drop in serum phospholipids and total cholesterol (40–60%), and these effects were most noted in the HDL and IDL fractions. Acute CCl_4 administration likewise produces a decrease in plasma lipids and cholesterol within the first few hours (Stern et al., 1965; Lombardi & Ugazio, 1965). This effect has been thought to be related to decreased apolipoprotein synthesis and secretion and is associated with intracellular lipid accumulation (i.e., fatty liver). The increase observed in the present study in the relative ratio of Apo A-I/Apo E mRNA during liver regeneration induced by either partial hepatectomy or CCl_4 administration is consistent with these findings and the notion that these changes represent a regulted response of the liver.

A similar mechanism could explain increased serum lipoproteins and the altered Apo A-I/Apo E ratio in the rat model of the nephrotic syndrome (Marsh & Sparks, 1979). In this model, there is hyperlipemia, hypoalbuminemia, and proteinuria, associated with increased production of Apo A-I by the liver and a reversal of the Apo A-I/Apo E ratio in serum lipoprotein particles (Marsh & Sparks, 1979). From these studies, it was concluded that independent mechanisms are responsible for regulating hepatic synthesis and secretion of various apolipoproteins. More recently, an increase in functional Apo A-I mRNA within the liver was also found in this model (Tarugi et al., 1986), but the mechanism for increased Apo A-I mRNA was not determined. The present study establishes not only that the increase in Apo A-I mRNA is by a posttranscriptional mechanism but also that a similar mechanism is used in other pathophysiologic states, including liver regeneration, cirrhosis, and analbuminemia. We therefore speculate that these changes may result from a common mechanism used to maintain or increase serum lipoproteins in a variety of pathophysiologic states associated with hypoproteinemia.

In the rat model of CCl_4-induced cirrhosis, we also found an increase in Apo A-I mRNA, but no reduction in Apo E mRNA. In addition, the cirrhotic rat was also the only model in which an increase in Apo A-I gene transcription was found during the regenerative response. Since Apo A-I mRNA is already induced in cirrhosis by posttranscriptional stabilization, it is possible that the only mechanism available for further augmentation is increased transcription. Other factors which might influence the level of Apo A-I mRNA in cirrhosis include a possible effect of estrogenic steroid hormones, which are known to be elevated in cirrhosis and cause increased Apo A-I expression (Staels et al., 1989; Weinstein et al., 1986; Archer et al., 1986).

From variety of studies, it is clear that regulation of apolipoprotein gene expression is multifactorial, involving noncoding 5′ gene regulatory signals (Chao et al., 1988; Das et al., 1988 Sastry et al., 1988), hormones (Staels et al., 1989; Pollinger et al., 1989; Dashti et al., 1989), and intracellular mediators (Wyne et al., 1989; Ruiz-Opazo & Zannis, 1989). Most of these studies have focused on questions concerning transcriptional regulation of specific gene expression. The present work illustrates that posttranscriptional control also plays a significant role in tissue or cell-specific regulation f apolipoprotein gene expression, primarily for Apo A-I. Other apolipoprotein genes, such as Apo E and possibly Apo A-II, also appear to be regulated at least in part by posttranscriptional mechanisms. This is reflected by the decreases in Apo A-II mRNA steady-state level during liver regeneration and reduced Apo E mRNA in analbuminemic rats. Precisely how these changes in apparent apolipoprotein mRNA stability are

mediated will require further study.

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