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Research Communications

Regulation of albumin synthesis after hepatectomy and in the acute inflammation phase of rat liver

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Hepatic albumin synthesis is down-regulated after both inflammation and hepatectomy. The transcriptional control of albumin synthesis was investigated in models of both conditions to differentiate the underlying mechanisms. Male Donryu rats underwent 70% hepatectomy or turpentine injection. Serum albumin and mRNA levels of albumin and promoter binding proteins (D site binding protein [DBP] CCAAT/enhancer binding protein-α [C/EBP-α], -β, and hepatocyte nuclear factor-1 [HNF-1]) in the liver were measured from 0 to 96 hr. After hepatectomy, the albumin mRNA level decreased to 0.6 at 36 hr and then recovered. After turpentine injection, it decreased to 0.4 at 36 hr and then recovered. The serum level of albumin decreased in a time-dependent manner in both models. The C/EBP-α mRNA level decreased to 0.5 at 6 and 12 hr after hepatectomy and to 0.6 at 24 hr after turpentine injection. The DBP mRNA level decreased to 0.3 at 6 hr, to 0.2 at 24 hr after hepatectomy, and to 0.3 at 30 hr after turpentine injection. The C/EBP-β mRNA level increased to 1.7 at 3 hr after hepatectomy and to 1.5 at 12 hr after turpentine injection. On the other hand, HNF-1 mRNA levels showed no consistent change in either model. The change in mRNA of the nuclear factors (C/EBP-α, C/EBP-β, and DBP) thus precedes that of albumin. In conclusion, transcriptional regulation of albumin synthesis in the regenerating and the acute inflammation phase of the liver can be assessed by monitoring the mRNA levels of nuclear factors. The mechanisms for down-regulation of albumin in both conditions share substantial similarities. (J. Nutr. Biochem. 6:522–527, 1995.)

Keywords: albumin synthesis; C/EBP; DBP; HNF-1; hepatectomy; turpentine

Introduction

The role of albumin in human physiology and pathophysiology has been extensively studied. Its serum level has been utilized as an indispensable factor for evaluation of nutritional status and liver function in various pathological conditions.[1,2] The serum level is not only influenced by the balance between albumin synthesis and degradation but it is also vulnerable to changes in plasma volume and vascular permeability. Therefore, in a pathological condition, such as an inflammatory or postoperative state, where body fluid distribution is altered, the serum albumin level does not correlate with the synthesis of albumin. Several reports have dealt with methods for measuring albumin synthesis by estimating fractional incorporation of radiolabeled amino acids into albumin in plasma.[3]

There is also a large body of literature dealing with albumin synthesis and mRNA levels following hepatectomy.[4,5] Furthermore, the structure and transcriptional regulation of the albumin gene have recently been clarified. It was shown that the synthesis rate of albumin correlates with the mRNA level of albumin,[5,6] which is transcriptionally controlled under pathophysiologic conditions.[7] At least six binding sites for nuclear factors have been identified in the promoter region of the albumin gene (*Figure 1*).[8,9] Many experiments have shown that factors binding to sites B and D act as strong transcriptional regulators.[10,11] Hepatocyte nuclear factor-1 (HNF-1) binds to site B,[12] while the following factors are known to bind to site D: D site binding

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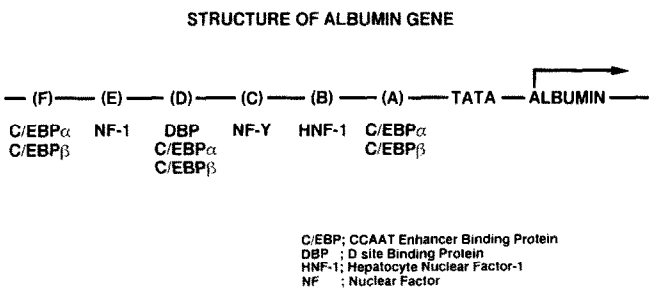

Figure 1 Schematic representation of albumin DNA and its promoter region. Six sites (A–F) have been identified in the promoter region, in which various nuclear factors bind to their specific binding site to activate the transcription of the albumin gene.

protein (DBP),[13] CCAAT/enhancer binding protein (C/EBP-α),[14,15] and C/EBP-β,[15] which has been named a C/EBP-like liver activator protein (LAP),[16] NF-IL6,[17] or CRP2.[18] All of these factors are capable of *trans*-activating the albumin promoter.[13,16,19]

Hepatectomy and turpentine-induced inflammation are apparently different stress conditions, but many similar responses occur in the liver in regard to an increase in the mRNA of acute phase proteins, such as α1 acid glycoprotein, α1 antitrypsin, and haptoglobin and to a decrease in the mRNA of negative acute phase proteins, such as albumin, retinol binding protein, and transthyretin.[20] Because of these similarities, it is of interest to clarify the underlying mechanism of down-regulation of albumin synthesis in the liver in the regenerating and the acute phase in order to determine whether there is a common cascade reaction to induce down-regulation or if the regenerating liver requires down-regulation as a compensation for cell growth in a manner different from that of the stress-induced acute phase liver. To differentiate the mechanisms of down-regulation of albumin synthesis, this study measured the sequential changes in mRNA levels of promoter binding nuclear factors (DBP, C/EBP-α, C/EBP-β, and HNF-1) along with changes in albumin mRNA after hepatectomy and turpentine-induced inflammation. The results indicated that there is a common mechanism at work in the two models and that surgical stress after hepatectomy is responsible for down-regulation of albumin in the regenerating liver.

Methods and materials

Animal experiment

Male Donryu rats (230 to 250 g of body weight), obtained from SLC (Shizuoka, Japan) were housed in cages with a 12 hr light/dark cycle in an air-conditionated environment. They were fed standard laboratory rat chow and water ad libitum for 1 week for acclimatization to the laboratory conditions. All studies were approved by the Committee for Use of Laboratory Animals at Osaka University Medical School. Experimental inflammation was induced under ether anesthesia by a subcutaneous injection of 0.5 mL/100 g of body weight of mineral turpentine (Nacarai Task, Kyoto, Japan) in the bilateral dorsolumbar regions in the turpentine injection model. Partial hepatectomy (70%) for the hepatectomy model was performed under ether anesthesia according to the method of Higgins and Anderson.[21] After partial hepatectomy or turpentine injection, rats were allowed free access to food and water. Since the mRNA level of DBP is known to show a circadian rhythm,[22] a preliminary experiment was carried out to measure mRNA levels of albumin and its nuclear factors in three normal rats at various time points (0400, 1000, 1600, and 2200 hr). The highest mRNA level of DBP was found to occur at 2200 hr. So the rats were sacrificed at this time for the subsequent experiments. Hepatectomy or turpentine injection was therefore carried out at predetermined set intervals before sacrifice (*Figure 2*). Three rats were used at any given time, and the livers were combined to measure mRNA levels. To evaluate the effect from feeding for each of the two models, three normal rats were pair-fed for 36 hr and sacrificed at 2200 hr. For the sham operation model, three rats underwent laparotomy and mobilization of the left and middle lobes of the liver and were sacrificed after 24 and 48 hr.

Analytical procedures

Before sacrification, and under ether anesthesia, blood was taken from the inferior vena cava for measurement of serum albumin, and the liver was removed and rapidly frozen for RNA extraction. Serum albumin levels were measured with the B.C.G. method.[23] Liver materials from three animals were combined at each time point for isolation of RNA. Guanidium isothiocyanate and a previously reported method[24] were used to extract total RNA from the combined liver samples obtained from three rats at each time point. RNA (20 μg/lane) was separated by electrophoresis on agarose gel and then transferred to a nylon membrane (Hybond N^+, Amersham, Buckinghamshire, U.K.). Albumin cDNA was a generous gift from Dr. K. Sugiyama, National Cancer Center Research Institute East, Chiba, Japan. cDNA probes of C/EBP isoforms, HNF-1, and DBP were kindly donated by Dr. Gonzalez, Laboratory of Molecular Carcinogenesis, National Cancer Institute, Maryland, USA.

The probes, digested from cloned cDNAs, were radiolabeled with the random primer synthesis method using an Amersham Kit (Megaprime DNA labeling system) with α ^{32}P dCTP (Amersham Japan, Tokyo, Japan) in accordance with the manufacturers instructions. The nylon membrane filters were hybridized with the respective probes using the method of Church,[25] and after washing they were exposed to autoradiography on a Kodak XAR-5 film (Eastman Kodak, Rochester, NY USA). Relative amounts of mRNA for albumin and nuclear factors were determined by densitometric analysis of the film, and signals of the 18s rRNA were used to confirm that the amounts of RNA loaded were the same.

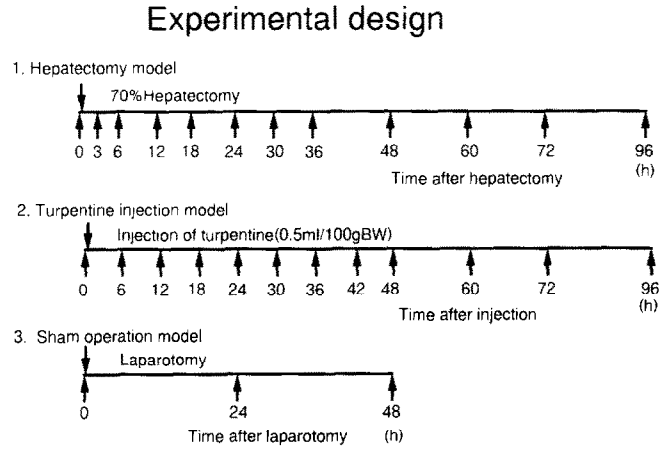

Figure 2 Diagram of experimental design and sampling. Three rats were sacrificed at each sampling point indicated by an arrow (↑). All rats were sacrificed at 10:00 p.m.

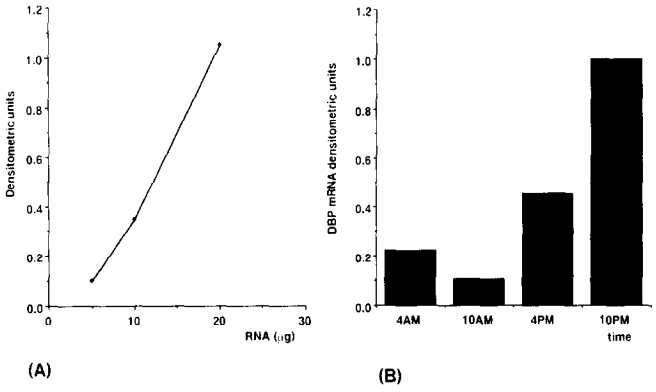

Figure 3 (A) The relation between densitometric units and quantities of applied RNA. Twenty, 10, and 5 μg of total RNA from normal rat liver were applied and hybridized with the albumin cDNA to examine the abundance of albumin mRNA. A good correlation was obtained at the range shown here. (B) The circadian change of DBP mRNA levels in normal rats. Three normal rats were used at each point. The solid bar indicate the amounts of DBP mRNA. The density on the film obtained at 10:00 p.m. was arbitrarily defined as a densitometric unit of 1.0.

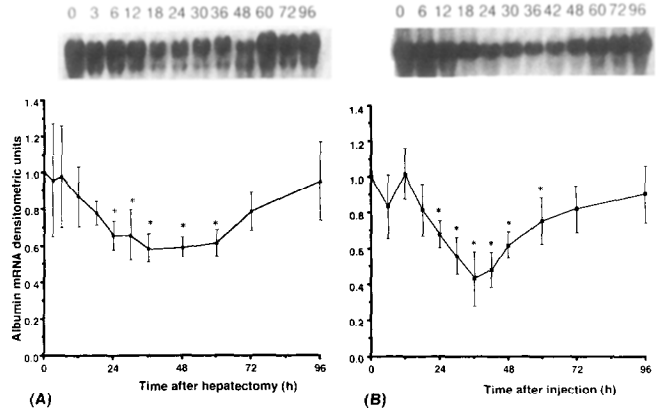

Figure 5 Changes in albumin mRNA levels after hepatectomy (A) and turpentine injection (B). (Upper section) Northern blot analysis results at the time point indicated above each result. (Lower section) The relative amount of albumin mRNA in the control (0 hr) was arbitrarily defined as a densitometric unit of 1.0. Data are expressed as the mean value ± SD for three experiments. ANOVA and the Fisher test were performed. $^*P < 0.05$ vs. 0 hr.

RNA extraction from the combined liver samples was repeated three times for the Northern blot analysis. Linearity of the densitometric analysis was confirmed by measuring various quantities of RNA run on the gel (*Figure 3A*). Since the efficiency of hybridization and film exposure conditions were somewhat different at different times of the Northern blot analysis, the density of the controls on the film was arbitrarily taken as a densitometry unit of 1.0. Data were expressed as means ± SD of the three Northern blot analysis measurements. ANOVA analysis and the Fisher test were employed to perform statistical analysis for serum albumin levels and mRNA levels. A P value of less than 0.05 was considered significant.

Results

As shown in *Figure 3B*, the mRNA level of DBP showed a clear circadian rhythm. It was highest at 2200 hr and lowest at 1000 hr. With the mRNA level of DBP at 2200 hr defined as 1.0, the levels at 0400, 1000, 1600 hr were 0.22, 0.11, and 0.46, respectively. On the other hand, the mRNA levels of albumin and other nuclear factors did not show such a circadian rhythm (data not shown).

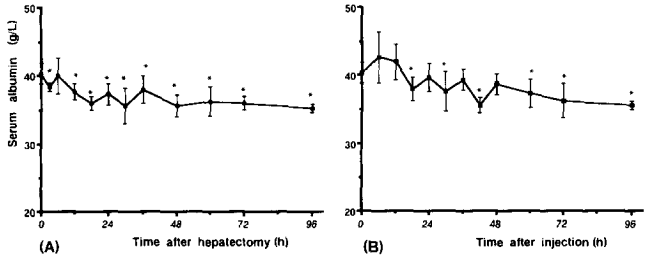

Figure 4 Sequential changes in serum albumin levels in rats after hepatectomy (A) and subcutaneous injection of turpentine (B). Data are expressed as mean ± SD for three rats. Evaluation with ANOVA and Fischer tests showed that the levels of serum albumin in both models decreased significantly after the insults. $^*P < 0.05$ vs. 0 hr.

The serum level of albumin significantly decreased from 0 to 96 h after hepatectomy or turpentine injection (*Figure 4*). The mRNA level of albumin decreased to 0.6 at 36 hr after hepatectomy, and thereafter returned to the preoperative level. Likewise, in turpentine-induced inflammation, the mRNA level of albumin had dropped at 36 hr to 0.4 and then began to increase before returning to the control level at 96 hr (*Figure 5*). C/EBP-α mRNA levels temporarily decreased to 0.5 at 6 and 12 hr after hepatectomy and to 0.6 at 24 hr after turpentine injection. They then returned to the control level before the albumin mRNA level had recovered (*Figure 6*). The C/EBP-β mRNA level rapidly increased to 1.7 at 3 hr after hepatectomy and to 1.5 at 12 hr after turpentine injection and then returned to their respective control levels (*Figure 7*). DBP mRNA levels showed the

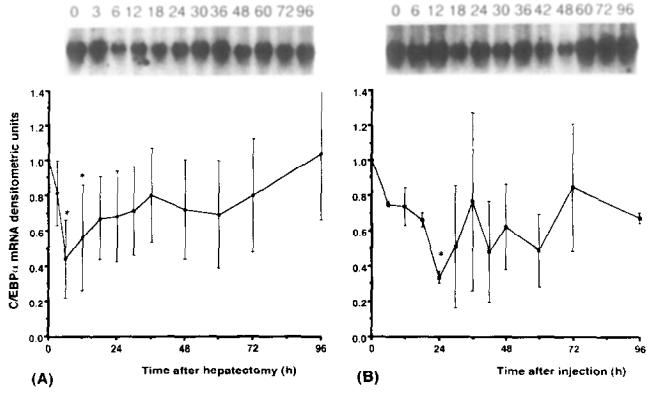

Figure 6 Changes in C/EBP-α mRNA levels after hepatectomy (A) and turpentine injection (B). (Upper section) Northern blot analysis results at the time point indicated above each result. (Lower section) The relative amount of C/EBP-α mRNA in the control (0 hr) was arbitrarily defined as a densitometric unit of 1.0. Data are expressed as the mean value ± SD for three experiments. ANOVA and Fisher test were performed. $^*P < 0.05$ vs. 0 hr.

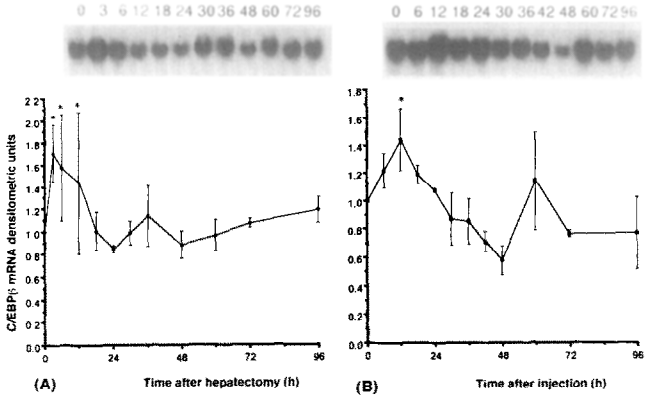

Figure 7 Changes in C/EBP-β mRNA levels after hepatectomy (A) and turpentine injection (B). (Upper section) Northern blot analysis results at the time point indicated above each result. (Lower section) The relative amount of C/EBP-β mRNA in the control (0 hr) was arbitrarily defined as a densitometric unit of 1.0. Data are expressed as the mean value ± SD for three experiments. ANOVA and Fisher test were performed. *$P < 0.05$ vs. 0 hr.

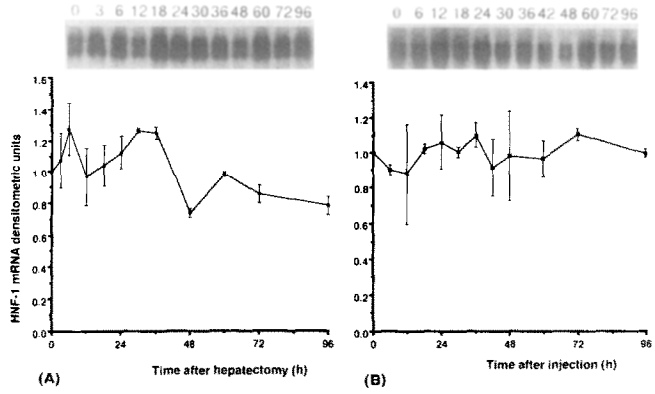

Figure 9 Changes in HNF-1 mRNA levels after hepatectomy (A) and turpentine injection (B). (Upper section) Northern blot analysis results at the time point indicated above each result. (Lower section) The relative amount of HNF-1 mRNA in the control (0 hr) was arbitrarily defined as a densitometric unit of 1.0. Data are expressed as the mean value ± SD for three experiments. ANOVA and the Fisher test were performed; no significant changes were found.

greatest change after insult. After hepatectomy, they decreased to 0.3 at 6 hr and, after a short recovery period, further decreased to 0.2 at 24 hr and then returned to the preoperative value at 96 hr. After turpentine injection, they also decreased to 0.3 at 30 hr and then recovered (*Figure 8*). Because of the different polyadenylation sites, Northern blot analysis of HNF-1 mRNA showed two bands at 3.2 and 3.6 kb.[26] Neither of the HNF-1 mRNA levels in either model showed any significant changes during the observation period (*Figure 9*). In the pair-fed control for each model, the mRNA levels of albumin and its nuclear factors examined at 36 hr showed no changes compared with the respective control levels. In the sham-operated group, albumin and DBP mRNA levels showed slight decreases at 48 and 24 hr respectively, but these changes did not reach significance (*Figure 10*).

Discussion

In hepatectomy and turpentine-induced inflammation models, production of albumin mRNA in the liver was down-regulated and sequential changes in the levels albumin mRNA and its nuclear factors were similar. The albumin mRNA level began to decrease 24 hr after induction of the stress stimuli and then increased after 72 hr. As expected, the serum albumin level did not reflect the changes in the albumin mRNA level, which is a good indicator of albumin synthesis.[4,6] The transcription rate of the albumin gene is regulated by the interaction of a variety of promoter binding proteins, such as C/EBP-α, C/EBP-β, DBP, and HNF-1.

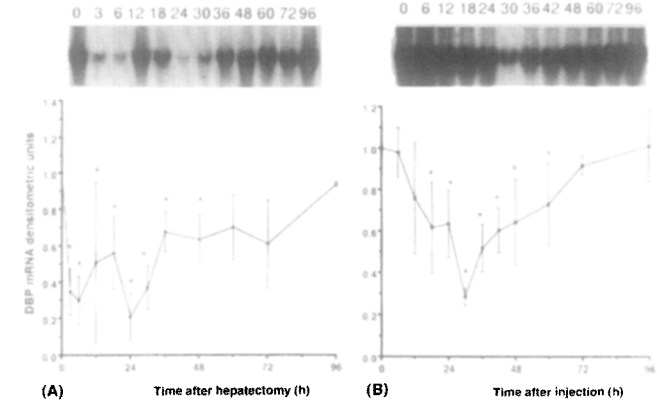

Figure 8 Changes in DBP mRNA levels after hepatectomy (A) and turpentine injection (B). (Upper section) Northern blot analysis results at the time point indicated above each result. (Lower section) The relative amount of DBP mRNA in the control (0 hr) was arbitrarily defined as a densitometric unit of 1.0. Data are expressed as the mean value ± SD for three experiments. ANOVA and the Fisher test were performed. *$P < 0.05$ vs. 0 hr.

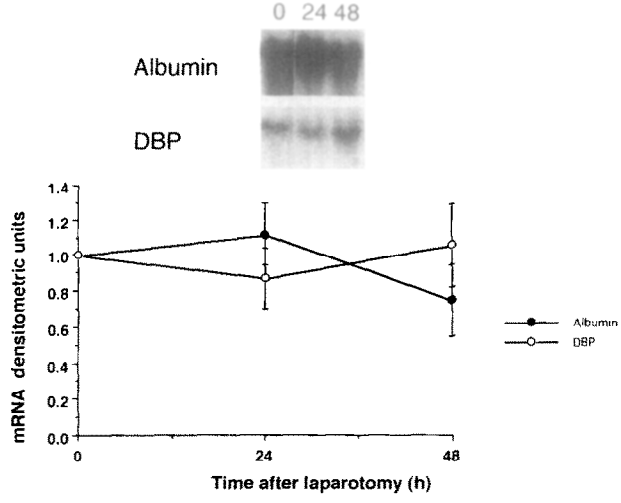

Figure 10 Changes in albumin and DBP mRNA levels after sham operation. (Upper section) Northern blot analysis results at the time point indicated above each result. (Lower section) The relative amount of albumin (●) and DBP (○) mRNA in the controls (0 hr) was arbitrarily defined as a densitometric unit of 1.0. Data are expressed as the mean value ± SD for three experiments. ANOVA and the Fisher test were performed; no significant changes were found.

By monitoring the dynamic changes of these proteins, it was thought feasible to elucidate the transcriptional regulation for the down-regulation of albumin. After both hepatectomy and inflammation, C/EBP-α mRNA dropped at 24 hr, C/EBP-β mRNA peaked at 12 hr, and DBP mRNA dropped within 30 hr. The changes in all these mRNA levels preceded those in the albumin mRNA level. Finally, the mRNA levels of these proteins had returned to their respective control levels before the albumin mRNA level had increased to its control level.

The liver is rich in C/EBP-α, which may play a role in the transcription of several liver-specific genes and can activate the promoter of the albumin gene.[19] This protein is a prototypical leucine zipper transcription factor, forming dimers via a leucine-rich region located in the C-terminal part of the protein.[14] C/EBP-β is capable of *trans*-activating the albumin promoter[16] and is responsible for the expression of acute phase proteins such as the C reactive protein, hemopexin, haptoglobin, and α1-acid glycoprotein.[17,27] It is induced by IL-6.[27] It was recently reported that C/EBP-β mRNA is not directly related to albumin gene regulation, but that C/EBP-α, C/EBP-β, and other nuclear factors compose a homodimer or heterodimer with each other to bind to the promoter responsive element,[16,19] although details of this mechanism remain unclear. Changes in DBP mRNA levels were the most prominent and may play an important role in transcriptional regulation of albumin after hepatectomy and inflammation. The DBP promoter contains a (glucocorticoid response element) (GRE),[28] which may be responsible for the circadian rhythm of DBP expression. Furthermore, DBP gene expression after hepatectomy and inflammation may be connected to the glucocorticoid mediating process. C/EBP-α, C/EBP-β, and DBP are D site binding proteins, suggesting that a decrease in D site binding nuclear factors may be related to down-regulation of the albumin gene. However, HNF-1 mRNA levels did not show any change in either model. Since HNF-1 is a B site binding protein,[11] the B site may not be involved in down-regulation in our models, but one cannot rule out the possibility that HNF-1 may be regulated translationally rather than transcriptionally.

IL-6 is one candidate for the common mediator involved in the down-regulation of albumin. Scotte et al.[29] measured the blood level of IL-6 after hepatectomy and turpentine injection. They found that the blood level of IL-6 reaches a maximum at 4 to 6 hr and thereafter declines and is barely detectable at 72 hr after 30% and 80% hepatectomy, turpentine injection, and laparotomy. The change in the plasma IL-6 level remained identical regardless of stress stimuli. Similar to the change in the blood IL-6 level, down-regulation of the albumin gene seems to be caused by a common process induced by stress response. Krieg et al.[4] also reported a decrease in albumin mRNA levels not only after hepatectomy but also after sham operation. In the sham-operated rats in our present study, however, down-regulation of albumin synthesis was not evident since the DBP and albumin mRNA levels showed little change. In addition to hepatic regeneration after hepatectomy, surgical stress may therefore contribute, but only minimally, to down-regulation of albumin synthesis.

In conclusion, transcriptional regulation of albumin synthesis in the regenerating and the acute inflammatory phase of the liver can be assessed by monitoring the mRNA levels of nuclear factors. The mechanisms for down-regulation of albumin in both conditions share substantial similarities.

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