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THE ELEVATION OF α-FETOPROTEIN MESSENGER RNA IN REGENERATING RAT LIVER

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SUMMARY

The level of serum AFP increased in the rats treated with CCl_4. The increase of serum AFP concentration can be suppressed by administration of dexamethasone. The molecular basis of these increases in serum level of AFP was investigated by examining AFP mRNA concentration in livers of normal, CCl_4-, and hormone-treated animals. The increase in AFP mRNA content in rat liver may be the primary factor responsible for the alteration of AFP serum levels as determined by translation and hybridization assay.

INTRODUCTION

Synthesis of α-fetoprotein (AFP) by hepatocytes differs depending on the stage of development and physiological conditions. Liver cells synthesize AFP at a high level during fetal life (1). The level of AFP in serum dramatically decreases after birth. There is very little AFP synthesis in the liver of adult animals. The decrease of AFP synthesis in the adult liver is not due to an alteration in translation (2). The low level of AFP in the liver of an adult rat results from a lack of AFP mRNA (3-8).

Liver cells of adult rats rarely divide, but under the appropriate stimuli, such as the administration of CCl_4 or partial hepatectomy, hepatocytes reacquire the ability to proliferate. It has been generally assumed that genomic derepression occurs during the early stages of liver regeneration. Most of these assumptions are based on reports indicating the presence of short-lived RNA species in regenerating liver but not in normal livers (9,10). However, the methods used in the original reports measured only the hybridization of repetitive DNA sequences. Presently, there is no information substantiating that a specific

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gene is derepressed during liver regeneration. The production of AFP in the adult liver has been associated with the proliferation of hepatocytes. This has been demonstrated in experiments following hepatectomy and chemically induced liver necrosis, where increases in the level of serum AFP have been observed (11). We studied the regulation of AFP gene expression during the regeneration of the liver, in order to determine if a specific gene is derepressed during liver regeneration. Here we report that the AFP gene is turned on during this process and that the level of AFP mRNA increased in polysomal mRNA.

MATERIALS AND METHODS

Animals

Sprague Dawley male rats (125 gm) were used in all experiments. The animals were kept in temperature-controlled rooms under 12-hr alternating light-dark cycles with food and water always available. Five to six rats were sacrificed at each time point. Experimental rats received 0.2 ml/Kg body weight of 50% CCl_4 (CCl_4: corn oil = 1:1), while control rats received the same amount of corn oil. Dexamethasone was I.P. administered at a concentration of 2 μg/gm body weight.

Serum AFP Measurements

The amount of AFP in the serum was measured by a double antibody radioimmunoassay described previously (12).

Cytoplasmic RNA Isolation

Livers were homogenized in 5 volumes of 25 mM Tris-HCl (pH 7.6), 0.25 M sucrose, 5 mM magnesium chloride, 25 mM NaCl, and centrifuged at 10,000 x g for 10 min. The supernatant was then brought to 25 mM sodium acetate, pH 5.0, 10 mM EDTA, 0.5% SDS, and the solution was extracted once with an equal volume of buffer-saturated phenol and 2-3 times with phenol/chloroform/isoamyl alcohol (49:49:2). The RNA in the aqueous phase was precipitated with ethanol overnight at -20°C.

Preparation of AFP cDNA and Molecular Hybridization

The isolation of AFP mRNA and AFP cDNA were prepared as previously described (4). RNA excess hybridizations were carried out in sealed, siliconized capillary tubes in 20 μl volumes. Increasing amounts of RNA from livers were hybridized with 0.1 mg of [^{3}H]AFP cDNA (1,500 cpm) in 50% formamide, 0.5 M NaCl, 20 mM Tris HCl buffer (pH 7.2), 10 mM EDTA, 0.01% SDS. Hybridization was carried out at 41° for 72 hr. The formed bybrids were assayed utilizing nuclease S_1 from Aspergillus Oryzae (13).

Translational Assay of AFP mRNA Activity

Rat liver cytoplasmic RNA was examined for AFP mRNA translational activity in a cell-free protein synthesizing system utilizing mRNA-dependent rabbit reticulocyte lysates prepared according to Pelham and Jackson (14). The translational assay was performed essentially as described for an untreated lysate system (5). Briefly, liver RNA was added to the reaction mixture (final volume

of 100 µl) which contained 25 µCi/ml of [^{3}H]leucine, unlabeled amino acids (except leucine), creatine kinase, creatine phosphate, ATP and GTP. After incubation for 1 hour at 25°, the reaction mixture was adjusted to a final volume of 200 µl to contain: 20 µg of unlabeled AFP carrier, 1% Triton X-100, 10% sodium deoxycholate and 10 mM unlabeled leucine. The final reaction mixture was centrifuged in a Beckman Microfuge B for 5 min.

A 100 µl portion of the above supernatant fluid was added to sufficient anti-AFP to ensure quantitative immunoprecipitation of the added carrier and cell-free translation product. The immunoprecipitates were centrifuged through 150 µl discontinuous sucrose gradients, collected, and assayed for radioactivity in the AFP translation product (15).

RESULTS AND DISCUSSION

The elevation of serum AFP has been observed in both mice and rats in toxic liver injury caused by CCl_4 (11,16). Here we also found the concentration of AFP increased after administration of CCl_4 to rats. The concentration of serum AFP increased from 70 µg/ml in control rats up to over 1,000 µg/ml in rats treated with CCl_4 for 4 days. The increase of serum AFP level was strongly suppressed by administration of 2 µg/gm body weight of dexamethasone daily (Figure 1).

To investigate the molecular basis by which CCl_4 induced liver AFP production, AFP mRNA levels were determined in livers of normal control and CCl_4-treated animals. Total cytoplasmic liver RNA was isolated by phenol/chloroform extraction. The RNA preparations were assayed for AFP mRNA by translation and hybridization assays. AFP mRNA translational activities of total cytoplasmic RNA from normal control and CCl_4-treated rat liver were compared using mRNA-dependent reticulocyte lysate (Figure 2). There was a progressive increase in the amount of translatable AFP mRNA in liver cytoplasmic RNA of animals after administration of CCl_4. The increase of translatable AFP mRNA activity was strongly reduced by the concomitant administration of dexamethasone and CCl_4.

Hybridization assays were performed also on total cytoplasmic RNAs from livers of normal control and CCl_4-treated rats. Total cellular RNA were chosen for these assays rather than poly (A) containing RNA because some AFP mRNA is not completely retained by affinity matrices currently in use and because of the possibility that AFP mRNA may vary in its poly (A) content during various phases

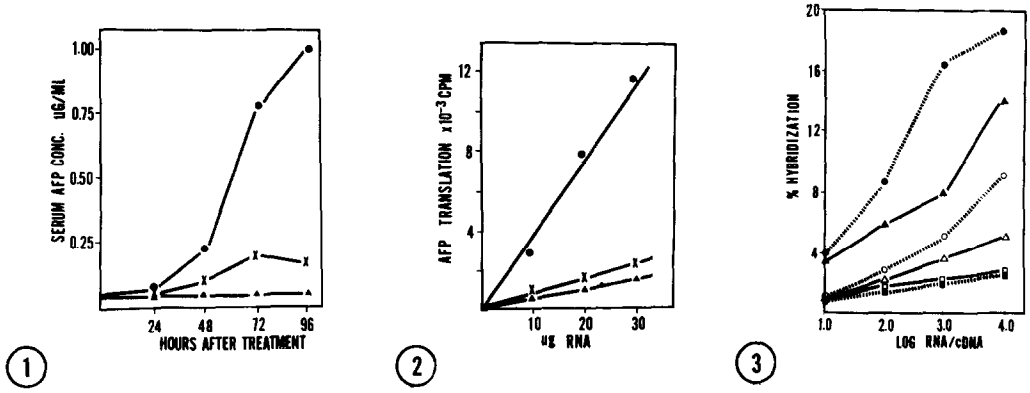

Figure 1. The effect of CCl_4 and dexamethasone on serum AFP concentration. Male Sprague-Dawley rats (125 gm) were administered either CCl_4 intoxication (0.1 ml/100 gm body weight per oral) (●——●), or CCl_4 and dexamethasone (2 µg/gm body weight i.p. injection) concomitantly (x——x). Control rats were given 0.1/ml of corn oil per oral (▲——▲). At each time point, five to six rats were sacrificed and the serum was collected. The amount of AFP in the serum was determined by radioimmunoassay (12).

Figure 2. Translation of AFP mRNA. Total cytoplasmic liver RNA was assayed for its AFP mRNA translational activity by using the mRNA-dependent reticulocyte lysate system as described in the Methods. RNA was from normal control (▲——▲), CCl_4-treated (●——●) and CCl_4 + dexamethasone-treated (x——x) animals.

Figure 3. Hybridization assays of cytoplasmic RNA with AFP cDNA. Total cellular RNA was hybridized up to 10,000-fold weight excess to 0.1 ng of AFP cDNA (1500 cpm). Hybrid formation was assayed by resistance to S_1 nuclease. RNA from the liver of rats treated with corn oil only for 72 hr (□——□) and 96 hr (■----■), or with CCl_4 for 72 hr (▲——▲) and 96 hr (●----●), or with CCl_4 and dexamethasone for 72 hr (△——△) and 96 hr (○-----○) were used.

of CCl_4 effect. The results of these assays are presented in Figure 3. It shows that there is very little, if any, of AFP mRNA present in 72 hr and 96 hr control rat livers. However, after treatment of CCl_4, a significant amount of AFP mRNA sequences appears in regenerating liver. If dexamethasone was given concomitantly with CCl_4, the appearance of AFP mRNA was strongly suppressed. These results suggest that the appearance of AFP in serum and reduction of AFP serum level by dexamethasone is dependent upon availability of AFP mRNA in liver for protein synthesis. These data cited above also indicate that during liver regeneration the AFP synthesis takes place in mature differentiated hepatocytes. This suggests that re-elevation of AFP level in adults liver cells is caused by derepression of the AFP gene. Dexamethasone reduction of the AFP mRNA concentration in CCl_4-treated rat liver is probably due to depression of the transcription of this specific gene in the liver (17).

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