MOLECULAR AND CELLULAR BIOLOGY, Mar. 1995, p. 1364–1376 Vol. 15, No. 3
0270-7306/95/$04.00+0

Decreased Expression of Hepatocyte Nuclear Factor 3α during the Acute-Phase Response Influences Transthyretin Gene Transcription

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Received 11 July 1994/Returned for modification 26 September 1994/Accepted 19 December 1994

Three distinct hepatocyte nuclear factor 3 (HNF-3) proteins (α, β, and γ) are known to regulate the transcription of numerous liver-specific genes. The HNF-3 proteins bind to DNA as monomers through a winged-helix motif, which is also utilized by a number of developmental regulators, including the *Drosophila* homeotic *fork head* (*fkh*) protein. We have previously characterized a strong-affinity HNF-3S site in the transthyretin (TTR) promoter region which is essential for expression in human hepatoma (HepG2) cells. In the current study, we identify an activating protein 1 (AP-1) site which partially overlaps the HNF-3S sequence in the TTR promoter. We show that in HepG2 cells the AP-1 sequence confers 12-*O*-tetradecanoylphorbol-13-acetate inducibility to the TTR promoter and contributes to normal TTR transcriptional activity. We also demonstrate that the HNF-3 proteins and AP-1 bind independently to the TTR AP-1–HNF-3 site, and cotransfection experiments suggest that they do not cooperate to activate an AP-1–HNF-3 reporter construct. In addition, 12-*O*-tetradecanoylphorbol-13-acetate exposure of HepG2 cells results in a reciprocal decrease in HNF-3α and -3γ expression which may facilitate interaction of AP-1 with the TTR AP-1–HNF-3 site. In order to explore the role of HNF-3 in the liver, we have examined expression patterns of TTR and HNF-3 during the acute-phase response and liver regeneration. Partial hepatectomy produced minimal fluctuation in HNF-3 and TTR expression, suggesting that HNF-3 expression is not influenced by proliferative signals induced during liver regeneration. In acute-phase livers, we observed a dramatic reduction in HNF-3α expression which correlates with a decrease in the expression of its target gene, the TTR gene. Furthermore, consistent with previous studies, the acute-phase livers are induced for c-*jun* but not c-*fos* expression. We propose that the reduction in TTR gene expression during the acute phase is likely due to lower HNF-3α expression levels and that the induction of primarily c-*jun* homodimers, which are poor transcriptional activators, is insufficient to maintain normal TTR expression levels. We also discuss the role of reduced HNF-3α expression in mediating decreased transcription of HNF-3 target genes which respond negatively to cytokine signalling.

Cellular differentiation results in transcriptional induction of distinct sets of tissue-specific genes whose expression is critical for organ function. Deciphering transcriptional control mechanisms is thus critical to understanding cellular differentiation and development. We have utilized the transthyretin (TTR) DNA regulatory regions as a model in seeking to understand hepatocyte-specific gene transcription. TTR is expressed in hepatocytes and secreted into the serum, where it functions as a carrier protein for thyroxine and vitamin A (27). Functional analysis of the TTR 5′ flanking sequences in human hepatoma (HepG2) cell transfection assays defined a TTR proximal promoter and a distal enhancer region which provided a 10-fold increase in promoter activity (18, 19). Furthermore, this minimal TTR promoter and enhancer region is sufficient for normal hepatic expression in transgenic mice (71). Studies of the TTR regulatory region suggest that hepatocyte-specific expression relies on combinatorial interaction of multiple DNA binding sites by several distinct families of liver-enriched transcription factors (17). One of these regulatory families consists of the hepatocyte nuclear factor 3 (HNF-3) α, β, and γ (43, 44) proteins, whose recognition is essential for TTR expression in HepG2 cells (18). In collaboration with other liver-enriched factors HNF-1 (9, 26), HNF-4 (67), and C/EBP (1, 11, 12, 21, 40, 45, 56, 60, 70), the HNF-3 proteins are involved in regulating expression of numerous hepatocyte-specific genes (reviewed by Costa [16] and Zaret [73]) via recognition of the consensus DNA binding site A(A/T)TRTT(G/T)RYTY (57).

The HNF-3s not only are associated with adult liver- and lung-specific gene expression (14, 44, 64) but also participate in cellular differentiation and embryogenic pattern formation. In situ hybridization studies of stage-specific embryos demonstrate that the HNF-3 genes are expressed during endoderm, node, and notochordal mesoderm determination as well as during the formation of the neural tube, as evident from expression in the floor plate (4, 55, 62, 63). The HNF-3 genes are also induced during retinoic acid-mediated differentiation of embryonic carcinoma F9 cells, further supporting a role in visceral yolk sac differentiation (37). Zaret and colleagues further demonstrate that HNF-3α is the critical factor in maintaining the differentiative potential of H2.35 cells (4, 23). HNF-3 also participates with HNF-4 in the hierarchical transcriptional activation of HNF-1 in the hepatocyte, verifying its role in establishing a cellular environment sufficient for combinatorial control of transcription (42). Furthermore, mammalian HNF-3 (43, 44) and the *Drosophila* homeotic gene *fork head* (*fkh*) (69) are prototypes of a family of transcription factors defined by homology within a 100-amino-acid winged-

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helix DNA binding domain, which directs monomeric DNA recognition (13). To date, the mammalian HNF-3/Fkh family consists of more than 30 members which are expressed in different cell lineages and possess distinct DNA binding specificities (4, 7, 14–16, 33, 38, 46–48, 57, 63, 68).

Hepatocytes are specialized secretory epithelial cells that respond to a variety of nutritional and inflammatory signals by inducing the coordinate expression of genes which play a common biological role. For example, trauma or infection results in the release of immune cytokines (e.g., interleukin-6, interleukin-1, and tumor necrosis factor) which bind to hepatocyte receptors and lead to transcriptional induction or repression of specific sets of acute-phase genes (8, 35). Changes in expression of acute-phase proteins alter the serum protein composition to allow recovery from the insult or stress. The activation of acute-phase gene expression is mediated by induction of combinations of transcription factors which recognize distinct target sequences in acute-phase-responsive promoter regions. Among these induced transcription factors are the NF-κB family (52) and the STAT (signal transducers and activators of transcription) family members acute-phase-responsive factor (2) and stat1α/β (20). In acute-phase livers, expression of both C/EBPβ and C/EBPδ is also increased, while C/EBPα expression is reciprocally decreased (1, 3, 36, 40). Furthermore, decreased expression of another group of serum proteins is observed during the acute-phase response (65). These proteins include a number of HNF-3 target proteins such as albumin, TTR, transferrin, ornithine transcarbamoylase, and retinol binding protein (6, 18, 50, 57). Although HNF-3 is required for the transcription of these genes, the effect of acute-phase cytokines on HNF-3 expression has not been investigated.

The liver also possesses the ability to regenerate completely when damage is inflicted by either partial hepatectomy or chemical agents via proliferation of the remaining hepatocytes (30). This compensatory hepatocyte proliferation activates many of the immediate-early genes (e.g., c-*jun*, c-*fos*, and c-*myc*) as well as the expression of specific transcription factors and their cognate target genes (25, 53, 54). Decreased expression of the liver-enriched transcription factors DBP and C/EBPα is observed during liver regeneration, while C/EBPβ, C/EBPδ, and HNF-1α expression is stimulated and HNF-4 does not fluctuate (25, 53, 56). The effect of hepatocyte proliferation on HNF-3 gene expression remains unknown.

In this study we identify an activating protein 1 (AP-1) site which partially overlaps a previously characterized HNF-3S site in the TTR promoter. We show that the AP-1 sequence confers 12-*O*-tetradecanoylphorbol-13-acetate (TPA) inducibility in HepG2 cells and that it is required for normal TTR promoter activity. Consistent with independent recognition of the AP-1–HNF-3 sequence, HNF-3 expression is diminished with TPA exposure, which may facilitate AP-1 interaction with the TTR recognition sequence. In order to correlate these HepG2 studies to in vivo responses, we evaluated expression levels of HNF-3 and TTR during the acute-phase response and liver regeneration. We found that neither HNF-3 nor TTR expression was influenced by signalling induced in proliferating hepatocytes. In contrast, acute-phase livers exhibited a dramatic reduction of HNF-3α expression which coincided with diminished TTR mRNA levels. We describe a model suggesting that decreased HNF-3α expression during the acute-phase response is involved in the reduction of TTR gene expression. We also discuss the implications of our findings with respect to the decreases in other negative acute-phase genes that are dependent on HNF-3 function.

MATERIALS AND METHODS

Cell culture, transfections of TTR AP-1–HNF-3 reporter constructs, and chloramphenicol acetyltransferase (CAT) assays. Human hepatoma HepG2 cells (41) were grown in Ham's F12 medium (GIBCO) supplemented with 7% heat-inactivated fetal calf serum (GIBCO), 100 U of penicillin-streptomycin per ml, 0.5× minimum essential medium amino acids, and 0.5 U of recombinant human insulin (Eli Lilly) per ml. Where indicated, HepG2 cells were treated with 160 nM of TPA (dissolved in dimethyl sulfoxide) in Dulbecco's modified Eagle's medium containing 0.5% fetal calf serum and actinomycin D was applied to cells at 5 μg/ml in ethanol. Calcium phosphate DNA transfections were carried out in Dulbecco's modified Eagle's medium containing 7% heat-inactivated fetal calf serum, 100 U of penicillin-streptomycin per ml, 1× nonessential amino acids, and 0.5 U of human insulin per ml as described previously (58, 59).

The oligonucleotides containing wild-type TTR AP-1 and HNF-3 sites with and without mutations in either the HNF-3S or AP-1 sequence (see Fig. 1A) were ligated in a head-to-tail orientation next to the TATA box-driven CAT (28) reporter plasmid as described previously (18, 58). The AP-1 mutation was introduced into the −202 TTR promoter sequence by PCR-mediated site-directed mutagenesis (32) using the following mutagenesis primers and the appropriate 3′ and 5′ flanking primers: 5′-TTATTCTCCTTTTGTctgCTAAGTCAATAAT CA-3′ (sense) and 5′-TGATTATTGACTTAGcagACAAAAGGAGAATAA-3′ (antisense). The AP-1 mutation was verified by dideoxy DNA sequencing using the enzyme Sequenase (United States Biochemicals).

HNF-3, c-*fos*, and c-*jun* expression plasmids were cotransfected with the indicated reporter constructs into HepG2 cells, and cytoplasmic extracts were prepared 48 h later as described previously (58, 59). Cytoplasmic extracts were used to determine CAT enzyme levels in the presence of [^{14}C]chloramphenicol (ICN) and *n*-butyryl coenzyme A (Pharmacia); this was followed by xylene extraction of *n*-butyryl chloramphenicol and determination of product formation via liquid scintillation counting (61a). The cytomegalovirus (CMV)-driven β-galactosidase plasmid was included in each transfection to normalize extracts for differences in transfection efficiency as described previously (58, 59). In the transfection study, 40 μg of wild-type or mutant TTR AP-1–HNF-3 site reporter plasmids was used in the transfection assays. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 0.5% fetal calf serum for 12 to 24 h before and during TPA treatment. In the cotransfection assay, we used 5 μg each of HNF-3α, HNF-3β, c-*jun*, and c-*fos* expression plasmid; 40 μg of TTR AP-1–HNF-3 reporter construct; and 1 μg of CMV β-galactosidase reporter plasmid. In other cotransfection studies, 10 μl of Lipofectin reagent (Bethesda Research Laboratories) was used to transfect 35-mm-diameter plates of HepG2 cells with 50 to 150 ng of expression plasmid, 1 μg of TTR promoter CAT construct, and 100 ng of CMV β-galactosidase reporter plasmid.

LPS-induced acute-phase response in mice and partial hepatectomy of rat livers. CEN/HeN responder mice (5 to 6 weeks old) were treated with 600 μg of lipopolysaccharide (LPS; serotype O11B4; Sigma) per kg of body weight by intraperitoneal injection and 10 μg (in 10 μl) of LPS by nasal aspiration to induce the acute-phase response in the liver and lung (39). Total RNA was prepared from liver and lung tissue at 1, 4, 8, and 24 h after LPS administration by using RNAzol as described by the manufacturer (Tel-Test Inc.). Control mice were injected with the same volume of phosphate-buffered saline and sacrificed at 4 h postinjection. In the liver regeneration study, male Fisher rats (200 g) were anesthetized with ether and subjected to midventral laparotomy with approximately 70% liver resection (left lateral and median lobes) essentially as described by Higgins and Anderson (30). Careful asepsis was maintained throughout the procedure. Regeneration was terminated at 1, 2, 4, 24, 48, and 72 h after the operation, and liver RNA was isolated with RNAzol. Livers from untreated animals were used as controls. To serve as reference points, sham operations were performed by subjecting rats to midventral laparotomy and closure, followed by removal of livers for RNA extraction at the designated postoperative time points.

Gel shift and antibody supershift assays. Nuclear protein extracts were prepared from HepG2 cells and liver tissue as described previously (18, 59). Protein-DNA complexes were formed at room temperature for 30 min in 20 μl of reaction mix containing 1 ng of ^{32}P-end-labeled double-stranded oligonucleotide, 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.9), 4% Ficoll, 2 mM $MgCl_2$, 40 mM KCl, 0.1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.5 mM dithiothreitol, 4 μg of poly(dI-dC) · poly(dI-dC) (Pharmacia) per ml, 4 μg of salmon testis DNA (Sigma) per ml, and 5 μg of nuclear extract (57). A 100- to 300-fold molar excess of unlabeled oligonucleotide was included in the reaction when competition experiments were performed. In the antibody supershift assay, labeled DNA probe was incubated with nuclear extract for 30 min and then affinity-purified HNF-3 antibodies were added and incubated for an additional 30 min prior to polyacrylamide gel electrophoresis. The generation of antisera specific to rodent HNF-3α (synthesized against amino acids 7 to 103) and HNF-3β (synthesized against amino acids 7 to 86) is described by Jacob et al. (37). We also used c-*jun* and c-*fos* cDNA templates (a gift from Lester Lau, Department of Genetics, University of Illinois at Chicago) to synthesize RNA which was used to program a reticulocyte lysate system (Promega) to generate in vitro-translated protein. The in vitro-translated c-*jun* and c-*fos* proteins were used for complex formation

with TTR AP-1–HNF-3 oligonucleotide to demonstrate that this sequence is recognized by authentic c-*jun*–c-*fos* heterodimers.

Synthesis of antisense RNA probes, isolation of total RNA, and RNase protection assays. Antisense RNA probes were generated by in vitro transcription of linearized templates by using either SP6 or T7 RNA polymerase in the presence of [^{32}P]UTP. Antisense glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA probe was synthesized with T7 RNA polymerase (New England Biolabs) from a *Sal*I-linearized pGEM5 template containing a *Pst*I and *Nco*I subclone from the GAPDH cDNA (15). Antisense C/EBPβ RNA probe was synthesized from an *Eco*RI-linearized pGEM1 template containing an *Sst*I-*Sal*I fragment derived from the 3′ region of the C/EBPβ cDNA. A *Pst*I fragment of rat HNF-3α genomic DNA containing part of the first intron and 193 bp of the second exon of HNF-3α was subcloned into pGEM1 vector (Promega). The resulting construct was digested with *Hin*dIII and used to generate an HNF-3α-specific antisense probe with T7 RNA polymerase. For the HNF-3β antisense probe, a plasmid subclone of a *Bgl*II fragment of HNF-3β genomic DNA containing part of intron II and 480 bp of the third exon of rat HNF-3β was digested with *Stu*I and in vitro transcribed with SP6 RNA polymerase. To generate HNF-3γ antisense riboprobe, a *Sma*I-*Pst*I fragment containing the DNA sequences encoding rat HNF-3γ amino acids 67 to 216 was subcloned to pGEM1 vector. This construct was then digested with *Hin*dIII and in vitro transcribed with SP6 RNA polymerase. A mouse c-*fos* cDNA pGEM1 subclone containing the *Eco*RI-*Stu*I fragment was digested with *Bgl*II and transcribed by SP6 RNA polymerase (gift from Pradip Raychaudhuri, Department of Biochemistry, University of Illinois at Chicago). A cDNA clone containing mouse c-*jun* (kindly provided by Lester Lau) and an *Ava*I-*Hin*cII fragment was subcloned into pGEM1 vector. The resulting construct was digested with *Eco*RI and transcribed by SP6 RNA polymerase to generate antisense probe. The TTR probe was prepared as described previously (18).

RNAzol (Tel-Test, Inc.) was used to isolate total RNA from tissue culture cells, mouse liver or lung, and rat liver. The integrity of RNA samples was demonstrated by RNase protection assay using a rat GAPDH RNA probe. To detect HNF-3, c-*fos*, c-*jun*, C/EBPβ, and TTR transcripts in rat liver or HNF-3α in mouse liver, antisense RNA probes were hybridized with 40 μg of total RNA. Hybridization was performed in 30 μl of hybridization buffer [80% formamide, 40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.4), 400 mM sodium acetate, and 1 mM EDTA] overnight at 60°C. Three hundred microliters of RNase I buffer containing 10 U of RNase One (Promega) was then added to the hybridization mix. After digestion for 1 h at 37°C, the reaction was stopped by addition of 5 μl of 10% sodium dodecyl sulfate and 4 μg of yeast tRNA per μl. Nucleic acids were precipitated with ethanol, resuspended in formamide dye, denatured at 100°C for 3 min, and then loaded onto an 8% denaturing sequencing gel. Protected fragments were visualized by autoradiography. For detection of HNF-3β and HNF-3γ in mouse liver RNA using antisense probes generated from rat DNA, T_2 RNase (Bethesda Research Laboratories) was substituted for RNase One. For detection of HNF-3 transcripts in HepG2 cells, a similar procedure was performed with the exceptions of use of 20 μg of total RNA and 60 U of T_2 RNase per ml for digestion.

RESULTS

AP-1 and HNF-3 proteins bind independently to the adjacent sites in the TTR promoter. The TTR proximal promoter region contains both strong- and weak-affinity HNF-3 binding sites as well as weak-affinity sites for the HNF-1 and HNF-4 proteins (Fig. 1A). Site-directed mutagenesis had previously demonstrated that the TTR HNF-3S binding sequence was absolutely required to attain expression in HepG2 cells (17). These studies also revealed that disruption of the weak-affinity HNF-1, HNF-4, and HNF-3 binding sites resulted in a 30 to 50% reduction in TTR transcriptional activity, suggesting that multiple binding factors are required for normal expression levels. In this current study, we noticed a 2-nucleotide overlap between an AP-1 binding site and the TTR HNF-3S site (57) (Fig. 1A). Because the AP-1 binding site confers stimulation to TPA via c-*jun*–c-*fos* heterodimer recognition (5), we wanted to determine whether the TTR AP-1 site would function in the same manner.

Double-stranded oligonucleotides containing either wild-type or mutated TTR AP-1–HNF-3 (bp −111 to −88) sequences were synthesized and used to examine AP-1 complex formation with nuclear extracts prepared from TPA-stimulated HepG2 cells (Fig. 1). The simian virus 40 (SV40) AP-1 site served as a positive control showing that TPA-treated HepG2 nuclear extracts were induced for AP-1 binding activity

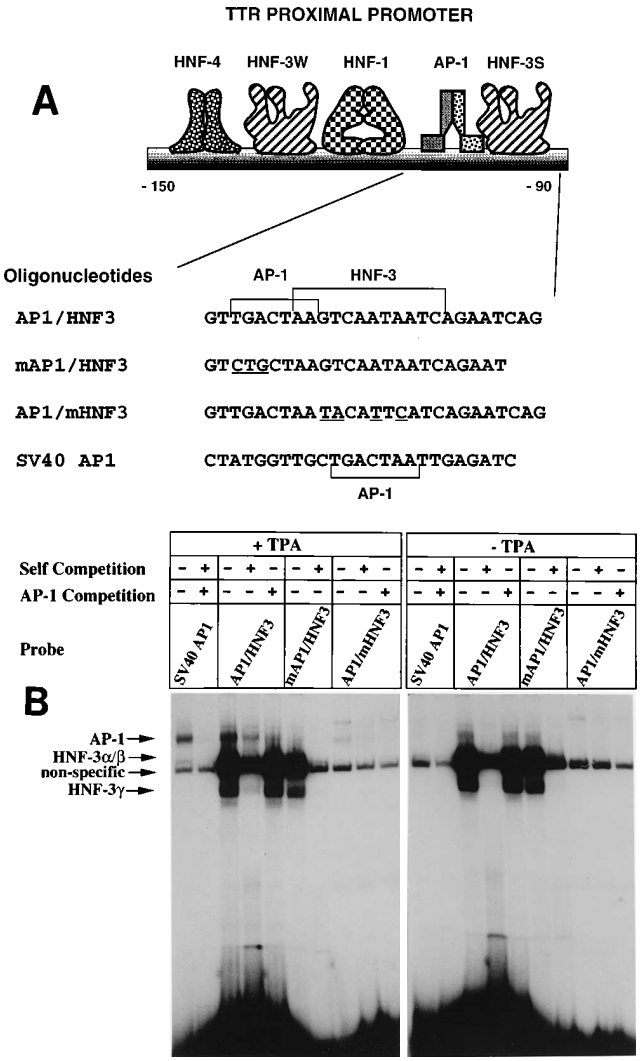


FIG. 1. The strong-affinity HNF-3 binding sequence in the TTR promoter overlaps with an AP-1 site. (A) Schematically shown are the TTR proximal promoter region and the locations of the strong-affinity HNF-3S site, a partially overlapping AP-1 site, and several weak-affinity sites for liver-enriched transcription factors HNF-1, HNF-4, and HNF-3 (17, 18). Shown below are the oligonucleotide sequences of wild-type AP-1–HNF-3 and those containing mutations in the AP-1 (mAP1/HNF3) and HNF-3 (AP1/mHNF3) recognition sequences. Also shown is the AP-1 recognition sequence derived from the SV40 enhancer region. (B) Gel shift assay with the oligonucleotides described above and nuclear extracts prepared from human hepatoma (HepG2) cells that were either stimulated with TPA for 4 h (+TPA) or left untreated (−TPA). Included are lanes containing a 100-fold molar excess of oligonucleotide consisting of itself or the SV40 AP-1 binding site. Indicated are the positions of the AP-1, HNF-3α/β, and HNF-3γ protein–DNA complexes as well as a nonspecific band that is not inhibited by competitor DNA.

(Fig. 1B, SV40 AP1, compare +TPA and −TPA). As anticipated, TPA-treated HepG2 extracts formed an AP-1 complex with the TTR promoter site which migrated at the same position as the SV40 AP-1 complex and more slowly on the gel than did the HNF-3 bands (Fig. 1B). This AP-1–DNA complex was inhibited more efficiently by the SV40 AP-1 site than by itself, suggesting that the TTR sequences possessed lower binding affinity (Fig. 1B, AP1/HNF3, +TPA). A nonspecific band which is not diminished with homologous competition migrates near the HNF-3α and HNF-3β protein-DNA complexes (Fig. 1B, AP1/HNF3 probe). We also used in vitro-

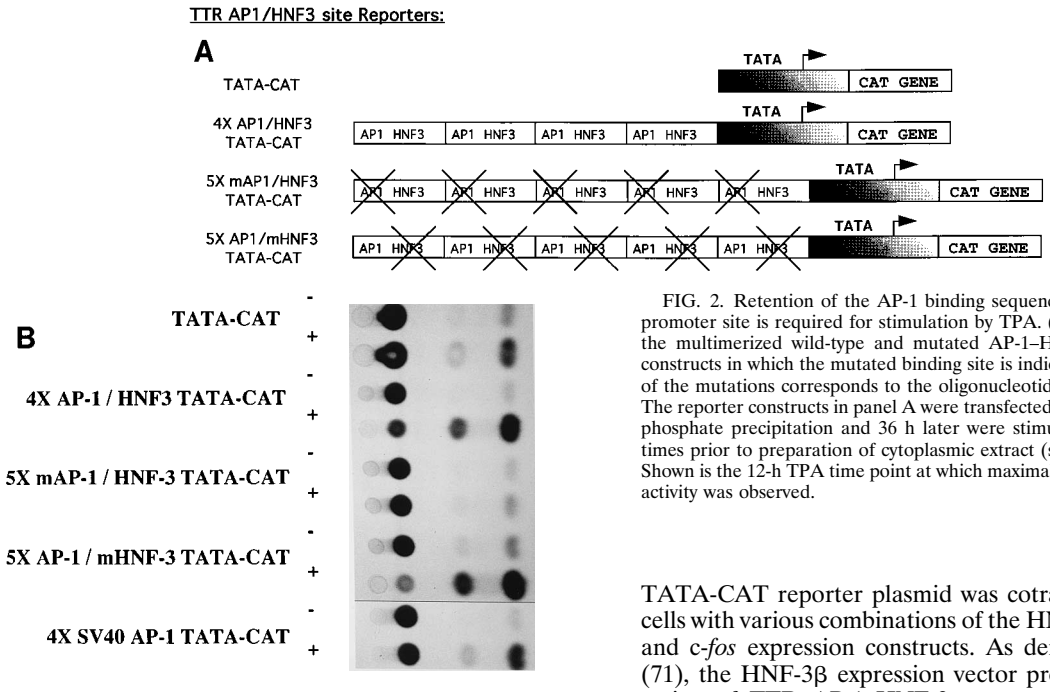


FIG. 2. Retention of the AP-1 binding sequence in the AP-1–HNF-3 TTR promoter site is required for stimulation by TPA. (A) Schematically shown are the multimerized wild-type and mutated AP-1–HNF-3 TATA-CAT reporter constructs in which the mutated binding site is indicated by an X. The sequence of the mutations corresponds to the oligonucleotides presented in Fig. 1A. (B) The reporter constructs in panel A were transfected into HepG2 cells by calcium phosphate precipitation and 36 h later were stimulated with TPA for various times prior to preparation of cytoplasmic extract (see Materials and Methods). Shown is the 12-h TPA time point at which maximal stimulation of CAT enzyme activity was observed.

translated c-*jun*–c-*fos* proteins to confirm that authentic c-*jun*–c-*fos* proteins recognize the TTR AP-1–HNF-3 oligonucleotide (data not shown; a gift from Lester Lau).

Mutations in the AP-1 sequence of the TTR promoter site abrogated AP-1–DNA complex formation without influencing HNF-3 protein recognition (Fig. 1B, mAP1/HNF-3). Likewise, alteration of the HNF-3 site did not diminish AP-1–DNA complex formation but prevented HNF-3 protein recognition (Fig. 1B, AP1/mHNF3). The AP-1 complex with the AP-1–mHNF-3 sequence appeared to be somewhat reduced, but this was due to the probe's lower specific activity. These experiments demonstrate that the TTR AP-1–HNF-3 site can be independently recognized by the AP-1 and HNF-3 proteins.

The TTR AP-1 sequence is sufficient to confer transcriptional induction by phorbol esters. We used TATA box-driven CAT reporter plasmids containing tandem copies of the wild-type and mutant TTR AP-1–HNF-3 oligonucleotides to examine whether this AP-1 site confers TPA responsiveness (Fig. 2A). The reporter constructs were transfected into HepG2 cells, treated for various periods of time with TPA, and then analyzed for CAT enzyme activity. We found that the 4× AP-1–HNF-3 TATA-CAT construct was maximally induced after 12 h of TPA treatment and the resulting 10-fold stimulation was similar to that obtained with the 4× SV40 AP-1 TATA-CAT plasmid (Fig. 2B). TTR promoter sites that were mutated in the AP-1 binding sequence elicited poor activation in response to TPA (Fig. 2B, 5× mAP1/HNF-3 TATA-CAT). Conversely, nucleotide substitution that disrupted HNF-3 binding did not reduce stimulation by TPA (Fig. 2B, 5× AP1/mHNF3 TATA-CAT). Taken together, these results suggest that the AP-1 site confers TPA stimulation and that retention of the HNF-3 site is not required for this inducible response.

Noncooperative activation of the TTR AP-1–HNF-3 reporters in cotransfection assays by the HNF-3 and c-*jun*–c-*fos* expression plasmids. We next sought to determine whether the TTR promoter site reporters interacted with the HNF-3 and c-*jun*–c-*fos* proteins in a cooperative or independent manner. In order to examine this question, the 4× AP-1–HNF-3 TATA-CAT reporter plasmid was cotransfected into HepG2 cells with various combinations of the HNF-3β, HNF-3α, c-*jun*, and c-*fos* expression constructs. As demonstrated previously (71), the HNF-3β expression vector produced a 20-fold activation of TTR AP-1–HNF-3 reporter expression compared with that by transfections of the CMV expression plasmid containing no cDNA insert (Fig. 3A, compare HNF-3β and None). Cotransfection of c-*jun* and c-*fos* expression plasmids together allowed for the formation of heterodimers and elicited greater transactivation than did HNF-3β alone (Fig. 3A). Introduction of c-*jun* and c-*fos* expression vectors separately provided lower and no activation, respectively (Fig. 3A). These results are consistent with published observations of lower binding affinity of c-*jun* homodimers and the inability of c-*fos* to form homodimers (5). Transactivation levels comparable to those obtained with HNF-3β or HNF-3α alone were observed when equal amounts of c-*jun*, c-*fos*, and HNF-3 expression vectors were cotransfected with the 4× AP-1–HNF-3 reporter construct (Fig. 3A and data not shown). TTR promoter activity was also stimulated when HNF-3 or c-*jun*–c-*fos* expression vectors were introduced separately, but they failed to cooperate when combined (Fig. 3B). On the basis of these results we suggest that HNF-3 and AP-1 do not synergize to activate TTR promoter expression.

TPA elicits decreased HNF-3α DNA binding activity and mRNA levels in HepG2 cells. Because the previous set of experiments suggested that the HNF-3 and c-*jun*–c-*fos* proteins did not cooperate to activate the overlapping AP-1 and HNF-3 site reporter construct, we wanted to determine whether HNF-3 expression is influenced by TPA. We treated HepG2 cells with TPA at various times, then harvested the cells, and prepared nuclear extract to examine both HNF-3 and AP-1 complex formation by gel shift assay. Unlike the transient response elicited by growth factor stimulation (54), TPA induced a prolonged stimulation of AP-1 activity in HepG2 cells (Fig. 4A). Abundant AP-1 complex is visible within 1 h of TPA treatment, reaches a maximum at 6 h, and persists for at least 24 h. These extracts displayed little fluctuation in complex formation with the UF1-H3β binding site derived from the HNF-3β promoter (bp −128 to −98) (70) (Fig. 4A). In contrast, HNF-3α DNA binding activity was significantly reduced in response to TPA, whereas minimal fluctuations in HNF-3β and HNF-3γ complex formation were observed (Fig. 4A). This decrease is better visualized when affinity-purified HNF-3β or

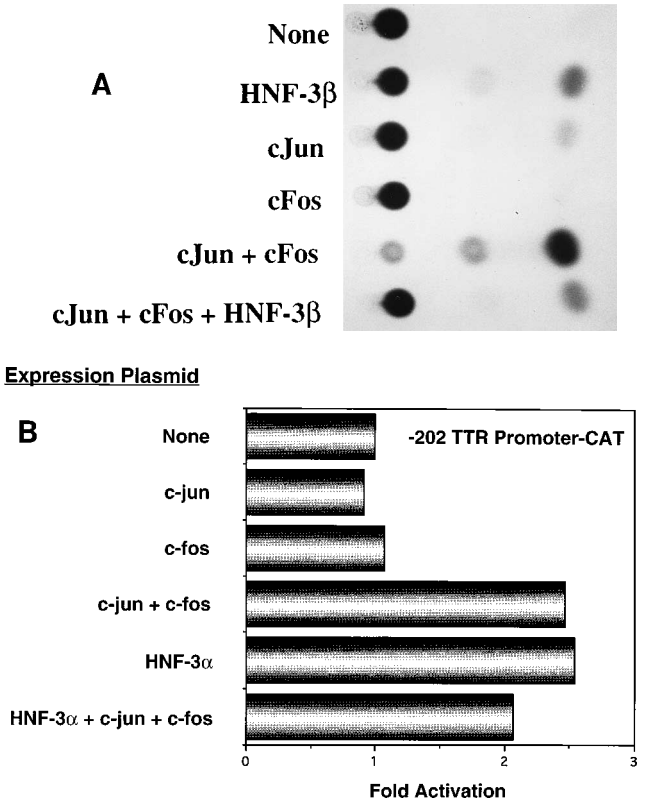


FIG. 3. Noncooperative activation of TTR reporter expression by the HNF-3 and c-*jun*–c-*fos* proteins. (A) The AP-1 and HNF-3 proteins do not cooperate to activate TTR AP-1–HNF-3 reporter expression. The 4× AP-1–HNF-3 TATA-CAT reporter construct was cotransfected in HepG2 cells with the HNF-3α, HNF-3β, c-*jun*, and c-*fos* expression constructs alone or in various indicated combinations, and then CAT enzyme levels were determined. Transfection of the CMV expression vector lacking a cDNA insert with the TTR reporter construct served as our control (None). (B) The TTR promoter construct is not activated in a cooperative manner. The −202 TTR promoter-CAT construct was cotransfected in HepG2 cells with the indicated combinations of expression vectors, and then CAT enzyme levels were determined. The results of the cotransfection experiment are presented as the fold activation of nontransfected TTR expression levels.

HNF-3α antibodies are included in the gel shift reaction (37). Removal of the HNF-3β protein–DNA complex by antibody supershift showed that the remaining HNF-3α complex exhibited a fourfold decrease within the first hour of TPA treatment and did not recover during the course of the experiment (Fig. 4B). Conversely, supershift of the HNF-3α band allowed visualization of a less than twofold increase in HNF-3β protein–DNA complex at the later time intervals (Fig. 4B).

To determine whether TPA influences HNF-3α message levels, we isolated cytoplasmic RNA from TPA-treated HepG2 cells and analyzed it for HNF-3 mRNA abundance via T_2 RNase protection assays (Fig. 4C). TPA treatment resulted in a 10-fold decrease in HNF-3α mRNA expression after 4 h, and expression levels remained depressed throughout the experiment (Fig. 4C). Consistent with an increase in DNA binding activity, TPA elicited less than twofold induction in HNF-3β mRNA levels (compare Fig. 4A and C, 2 and 4 h) and caused a small reduction in HNF-3γ message after 6 h of treatment (Fig. 4C). To verify that reduced HNF-3α message levels were not due to a TPA-induced decrease in mRNA stability, we compared HNF-3α message levels from HepG2 cells that were treated with TPA for 2 h or mock treated prior to inhibition of

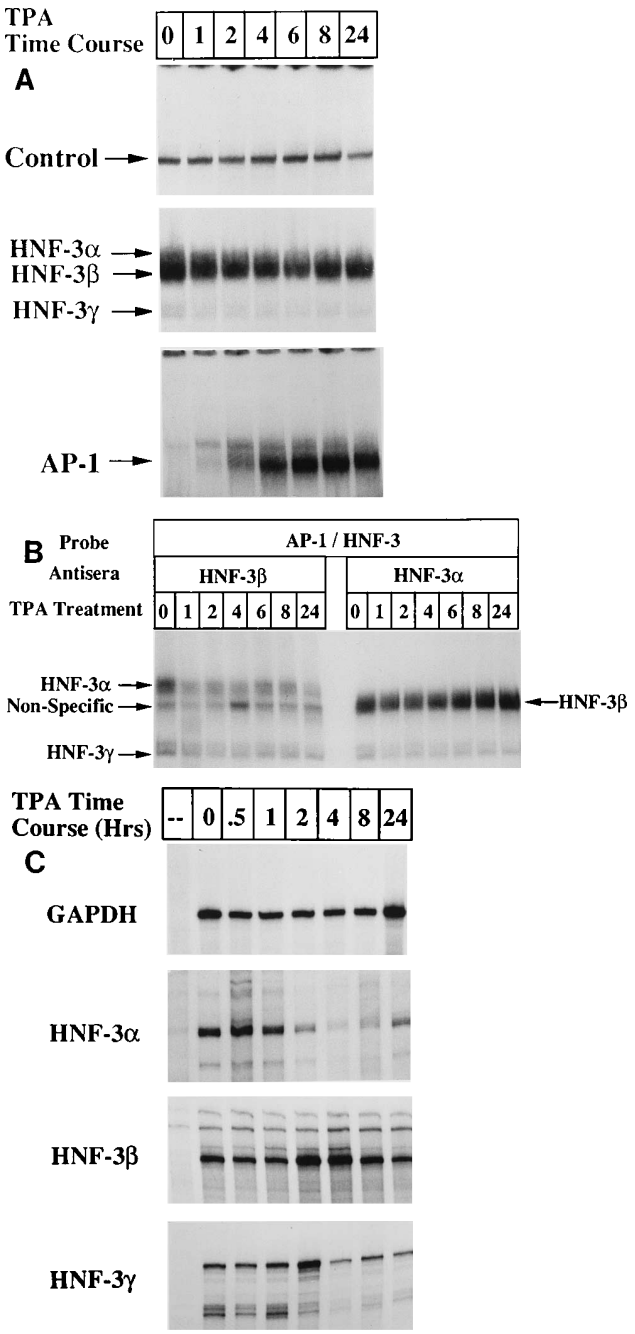


FIG. 4. TPA treatment of HepG2 cells reduces HNF-3α expression. (A) Gel shift with the AP-1–HNF-3 TTR promoter site and HepG2 nuclear extracts prepared after indicated periods of TPA treatment (numbers are in hours). The positions of the HNF-3 protein–DNA complexes are determined from HNF-3-specific antibody supershift experiments (see panel B). Included as controls are the AP-1 binding site derived from the SV40 enhancer and the UF1-H3β binding site obtained from the HNF-3β promoter (−128 to −98) (59). (B) Nuclear extracts from a TPA time course were used for gel shift assay in the presence of either HNF-3β or HNF-3α antisera (supershift; see Materials and Methods). Removal of the HNF-3β band by supershift with its cognate antisera allows visualization of the remaining HNF-3α protein–DNA complex, and HNF-3α supershift allows visualization of the HNF-3β complex (37). (C) Decrease in HNF-3α mRNA levels in response to TPA stimulation. Cytoplasmic RNA was isolated from HepG2 cells after the indicated stimulation with TPA and analyzed for HNF-3 expression by T_2 RNase protection assay as described previously (18). Included are the GAPDH RNA probe as a control for the amount of RNA and tRNA control to identify nonspecific bands (--). Note that heterogeneity between the rat RNA probes and human HepG2 RNA causes internal cleavage sites for T_2 RNase, sometimes resulting in additional bands.

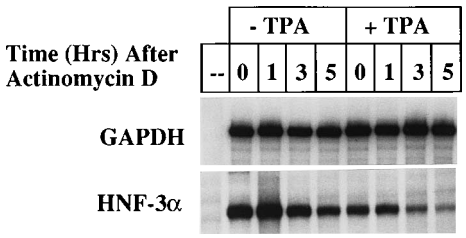


FIG. 5. TPA does not influence HNF-3α mRNA stability. HepG2 cells were either treated with TPA for 2 h prior to the addition of actinomycin D or not treated, and RNA was isolated at the indicated times thereafter. HNF-3α mRNA levels were assessed by T_2 RNase protection assay and compared with GAPDH.

RNA synthesis with actinomycin D. The experiments show that the HNF-3α mRNA decay rates for TPA-stimulated cells were comparable to those of untreated cells (Fig. 5). Taken together, these data suggest that TPA-dependent reduction of HNF-3α mRNA levels does not involve a change in HNF-3α message stability and is likely due to decreased promoter utilization.

The AP-1 site contributes to both TPA-stimulated and uninduced TTR promoter activity. In order to determine the role of the AP-1 site in TTR gene regulation, we introduced within the TTR promoter sequence the identical AP-1 mutation which disrupted c-*jun*–c-*fos* recognition (see Materials and Methods; Fig. 1 and 2). The transcriptional activity of this TTR promoter mutation was also assessed in the context of the TTR distal enhancer region. HepG2 cell transient expression assays were performed with these TTR reporters and are presented as a ratio of wild-type TTR promoter activity (Fig. 6B). The AP-1 mutation elicited an 87% reduction in TTR promoter activity in the enhancerless construct and a 70% decrease in CAT expression levels when the enhancer was present (Fig. 6B). This result suggests that retention of the AP-1 site is important for maintenance of normal TTR expression levels and is consistent with previous analyses of TTR promoter mutations within the other weak-affinity sites (17). In contrast, similar mutations in the HNF-3S site eliminate TTR expression, suggesting that the HNF-3S site provides a greater contribution to promoter activity than does the AP-1 binding sequence (17).

We next determined whether the AP-1 site conferred TPA responsiveness to the TTR promoter region. We transfected HepG2 cells with mutant and wild-type promoter constructs containing or lacking the upstream enhancer and assayed for CAT activity after 4 h of TPA stimulation. TPA elicited a consistent 1.5-fold stimulation of the TTR promoter (Fig. 6C), although induction levels were substantially less than that of the 4× AP-1–HNF-3 TATA-CAT reporter (Fig. 2). As expected, TPA stimulation of the TTR promoter was dependent on the retention of the AP-1 recognition sequence (Fig. 6C).

Dramatic reduction of HNF-3α expression during the acute-phase response may influence TTR transcription. Previous studies examining TTR expression during the hepatic acute-phase response demonstrated a 60% reduction in TTR expression levels (27), accompanied by induction of c-*jun* and JunD but not c-*fos* expression (29). In order to determine whether this established down regulation in TTR expression is the result of diminished HNF-3α mRNA expression, we induced an acute-phase response in the liver and lung via administration of LPS by intraperitoneal injection and nasal aspiration to LPS responder mice (see Materials and Methods). Total RNA was isolated from liver and lung (see below) tissue at various time points thereafter in order to evaluate for HNF-3, c-*jun*, c-*fos*, GAPDH, C/EBPβ, and TTR mRNA levels by T_2 RNase protection assay as indicated. Transient stimulation of both C/EBPβ and c-*jun* expression (1 to 8 h) but not c-*fos* expression was indicative of the acute-phase response in the liver (Fig. 7A and data not shown). Four hours following LPS administration, we noted a 95% decrease in HNF-3α expression in the liver coincident with reduction in expression of its target gene, the TTR gene (Fig. 7B and C). Interestingly, the expression profiles of TTR and HNF-3α genes are parallel throughout the acute-phase response (Fig. 7C). HNF-3β mRNA levels were also diminished by 20% in the acute-phase livers, while the HNF-3γ mRNA level exhibited no change (Fig. 7B and C).

Consistent with the RNA expression levels, diminished HNF-3α protein-DNA complexes were also observed in liver nuclear extracts prepared either 4 or 8 h after LPS administration, as visualized by antibody supershifting of the HNF-3β complexes (Fig. 7D, + α-HNF-3β). The position of the HNF-3α protein-DNA complexes was verified in supershift assays using both HNF-3β- and HNF-3α-specific antibodies (Fig. 7D). Taken together, these results consistently suggest a role for HNF-3α protein in mediating TTR down regulation during the acute-phase response and that this isoform plays an essential role in TTR gene expression.

The acute-phase response does not influence HNF-3 expression in the lung. We next determined HNF-3 message levels in lung tissue isolated from the same LPS-treated mice in order to examine whether the acute-phase response influenced HNF-3 expression in that tissue. A transient increase of C/EBPβ expression in the lung indicated that the acute-phase response was successfully induced by LPS in this tissue (Fig. 7E). In contrast to the observed decreases of HNF-3α and HNF-3β mRNA levels in the liver, HNF-3 expression in the lung was not influenced by immune cytokines secreted during the acute-phase response. The fact that HNF-3 expression is not modulated by cytokines in the lung suggests that the HNF-3 genes utilize distinct *cis*- or *trans*-acting control elements to direct their expression in these different tissues.

Slight fluctuations in HNF-3 and TTR expression levels are observed during liver regeneration and in sham-operated rats. We sought to examine whether hepatocyte proliferation elicited decreases in HNF-3α message in a manner similar to that observed in experiments involving TPA stimulation of HepG2 cells (Fig. 4). Rat liver RNA was prepared at various times after partial hepatectomy or sham operation and evaluated for c-*jun*, c-*fos*, HNF-3, and TTR expression levels (Fig. 8). Again, the GAPDH probe demonstrated little fluctuation in expression levels throughout the experiment (Fig. 8A). Consistent with published results (54), the c-*jun* and c-*fos* genes were transiently induced during liver regeneration, while sham-operated rats displayed a slight induction of primarily c-*jun* at the 1-h time point (Fig. 8A). Surprisingly, only minor fluctuations in HNF-3 and TTR mRNA levels were observed in partially hepatectomized livers, and these were paralleled in the sham-operated control livers. These studies showed biphasic expression profiles in both sham-operated and partially hepatectomized liver samples for the HNF-3α and HNF-3β genes, whereas the HNF-3γ isoform showed smaller fluctuations throughout the experiment (Fig. 8B to D). TTR expression remained constant but exhibited a slight decrease at 48 h post-hepatectomy (Fig. 8B to D). Because similar expression patterns were observed for both partially hepatectomized and sham-operated livers, it appears that the observed changes are caused by the operation rather than hepatocyte proliferation. These in vivo studies suggest that the dramatic reduction in HNF-3α expression during TPA stimulation of HepG2 cells is the result of signalling pathways resembling those stimulated

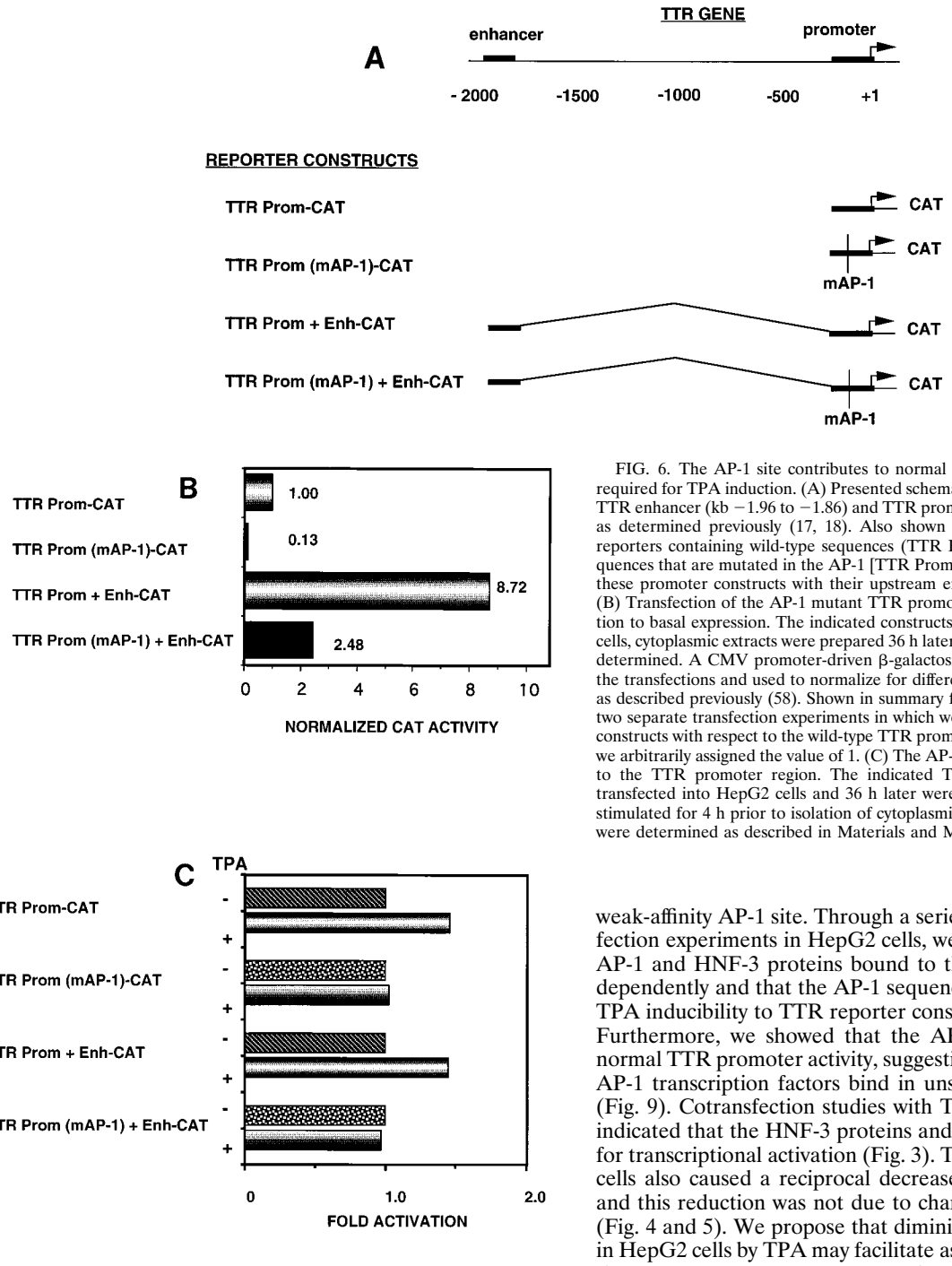


FIG. 6. The AP-1 site contributes to normal TTR promoter activity and is required for TPA induction. (A) Presented schematically are the locations of the TTR enhancer (kb −1.96 to −1.86) and TTR promoter region (bp −202 to +20) as determined previously (17, 18). Also shown are the TTR promoter CAT reporters containing wild-type sequences (TTR Prom-CAT) and promoter sequences that are mutated in the AP-1 [TTR Prom (mAP-1)-CAT] site as well as these promoter constructs with their upstream enhancer region (+Enh-CAT). (B) Transfection of the AP-1 mutant TTR promoter demonstrates its contribution to basal expression. The indicated constructs were transfected into HepG2 cells, cytoplasmic extracts were prepared 36 h later, and CAT enzyme levels were determined. A CMV promoter-driven β-galactosidase plasmid was included in the transfections and used to normalize for differences in transfection efficiency as described previously (58). Shown in summary form are the results of at least two separate transfection experiments in which we compare the activities of the constructs with respect to the wild-type TTR promoter expression level, to which we arbitrarily assigned the value of 1. (C) The AP-1 site confers TPA inducibility to the TTR promoter region. The indicated TTR reporter constructs were transfected into HepG2 cells and 36 h later were either mock treated or TPA stimulated for 4 h prior to isolation of cytoplasmic extracts. CAT enzyme levels were determined as described in Materials and Methods.

by cytokines instead of proliferative signals induced during liver regeneration.

DISCUSSION

TPA stimulation of the TTR AP-1–HNF-3 promoter site in HepG2 cells includes AP-1 recognition as well as decreased HNF-3 levels. Previous analysis of TTR promoter mutations demonstrated that the HNF-3S site is essential for expression and that the other weak-affinity HNF sites also contributed to promoter activity (17, 18) (Fig. 1A). In the present study, we found that the TTR HNF-3S site partially overlaps with a weak-affinity AP-1 site. Through a series of gel shift and transfection experiments in HepG2 cells, we demonstrated that the AP-1 and HNF-3 proteins bound to their respective sites independently and that the AP-1 sequence is sufficient to confer TPA inducibility to TTR reporter constructs (Fig. 1, 2, and 6). Furthermore, we showed that the AP-1 site is essential for normal TTR promoter activity, suggesting that low levels of the AP-1 transcription factors bind in unstimulated HepG2 cells (Fig. 9). Cotransfection studies with TTR reporter constructs indicated that the HNF-3 proteins and AP-1 did not synergize for transcriptional activation (Fig. 3). TPA exposure of HepG2 cells also caused a reciprocal decrease in HNF-3 expression, and this reduction was not due to changes in mRNA stability (Fig. 4 and 5). We propose that diminished HNF-3 expression in HepG2 cells by TPA may facilitate association of AP-1s with the TTR AP-1–HNF-3 promoter site (Fig. 9).

The existence of AP-1 binding sites in close proximity to HNF-3 sites may be a general feature of liver promoters, including α1-antitrypsin and HNF-1 (18, 42). In the case of the HNF-1 promoter, the adjacent AP-1 site was shown to confer regulation by protein kinase C and may operate in a fashion similar to that of the TTR promoter site. Recent studies using the kb −10 albumin enhancer have also identified an AP-1 site in close proximity to the HNF-3 eG site (34), a sequence essential for albumin enhancer activity in transfected hepatocytes (50). In vivo footprinting studies of this albumin enhancer region have demonstrated that the HNF-3 proteins are involved in organizing the nucleosome pattern of the albumin

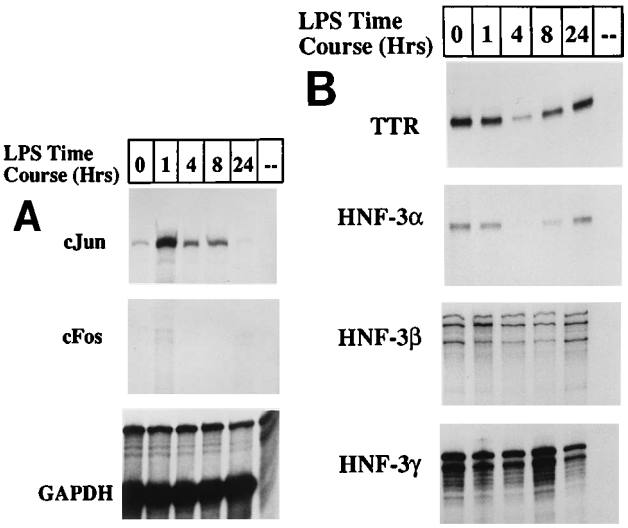
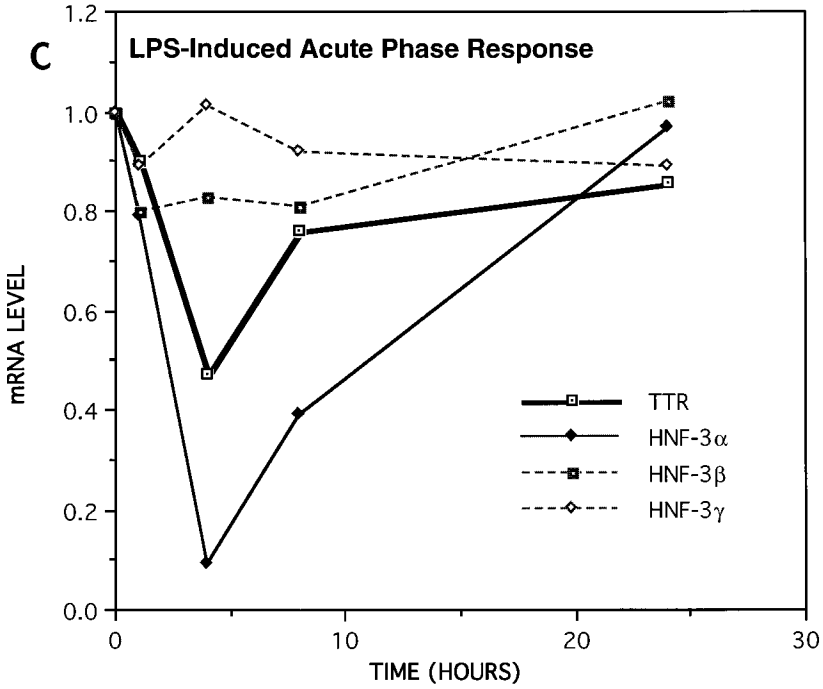
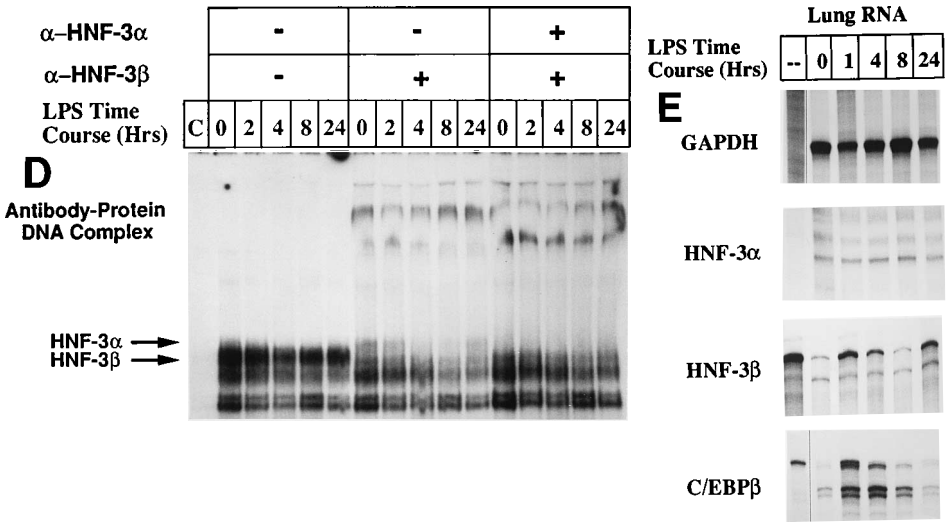


FIG. 7. Decrease in TTR mRNA levels during LPS-induced acute phase coincides with reductions in HNF-3α and HNF-3β expression. LPSs from gram-negative bacteria were administered at a dose of 600 μg/kg by intraperitoneal injection to LPS responder mice, and 10 μl of 1-mg/ml LPS solution was delivered to the lung via nasal aspiration (39). Liver and lung RNA was isolated at various hours thereafter (indicated by numbers) and evaluated for TTR, GAPDH, c-*jun*, c-*fos*, C/EBPβ, and HNF-3 expression by T_2 RNase or RNase I protection assay as described in Materials and Methods. Hybridization with yeast tRNA was included as a control to detect nonspecific protected fragments (--). (A) RNase protection assays with acute-phase liver RNA and probes specific for c-*jun*, c-*fos*, and GAPDH. (B) RNase protection assays with acute-phase liver RNA and probes specific for TTR and HNF-3 isoforms. Note that the specific HNF-3β band migrates below the doublet bands on the gel. (C) Graph summarizing TTR and HNF-3 mRNA levels during hepatic acute-phase response. Densitometer scans were used to determine mRNA levels, and these are expressed as a percentage of untreated levels. (D) Gel shift assays with liver nuclear extracts prepared from LPS-treated mice. The TTR AP-1–HNF-3 site was incubated with nuclear extracts prepared from acute-phase livers (time after LPS administration is indicated) and electrophoresed on a 7% polyacrylamide gel. Included is a homologous competition (lane C). HNF-3β affinity-purified antiserum (α-HNF-3β lanes) was included in the binding reaction to alter the migration of the HNF-3β protein-DNA (supershift) and facilitate visualization of the HNF-3α complex (37). The HNF-3β and HNF-3α antisera (α-HNF-3α) were combined to identify the locations of the HNF-3α complexes which decrease at the 4- and 8-h time points. (E) Lung RNA isolated from the same LPS-treated mice was evaluated for C/EBPβ, HNF-3α, and HNF-3β expression levels by RNase protection assay.





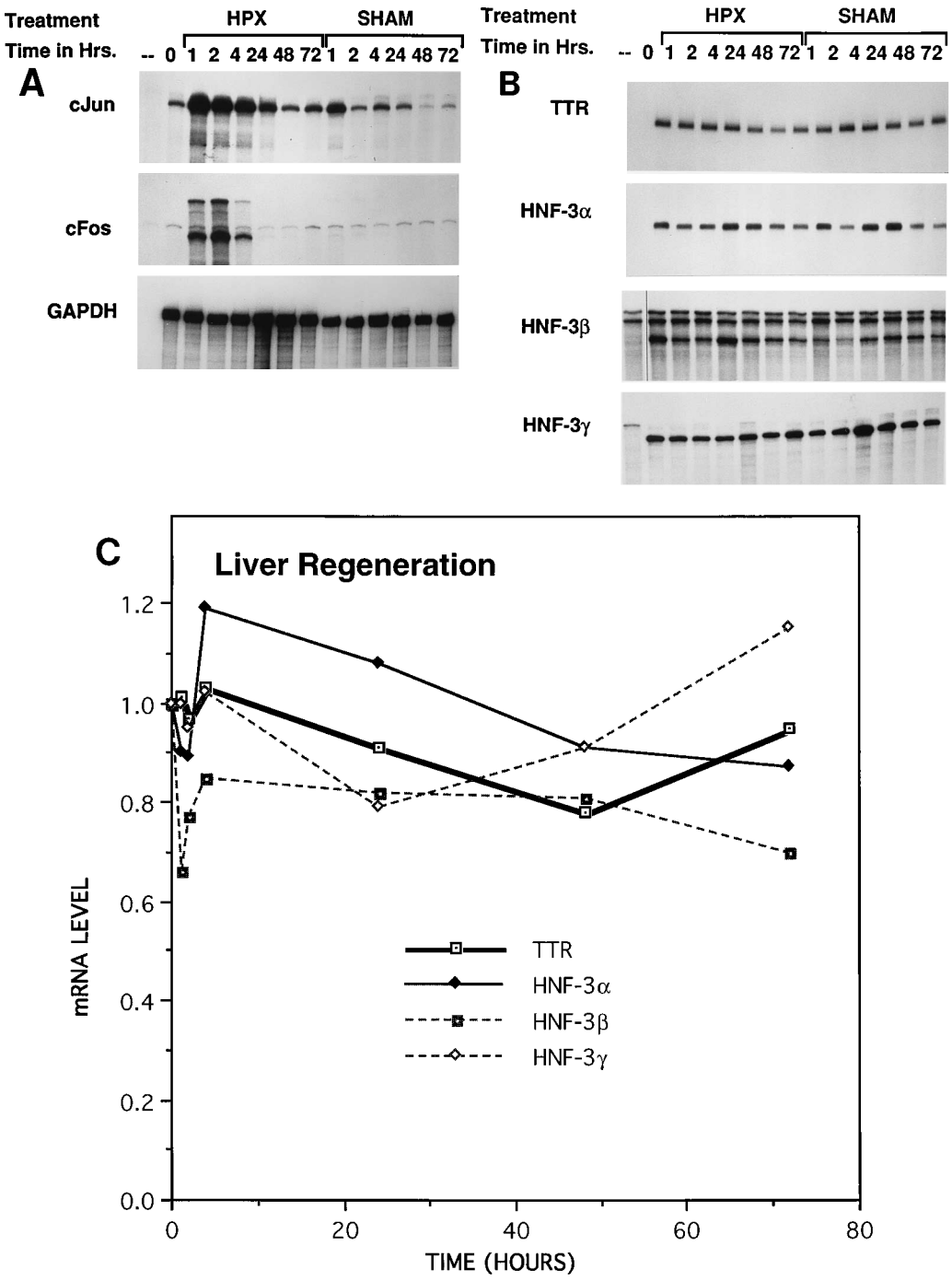
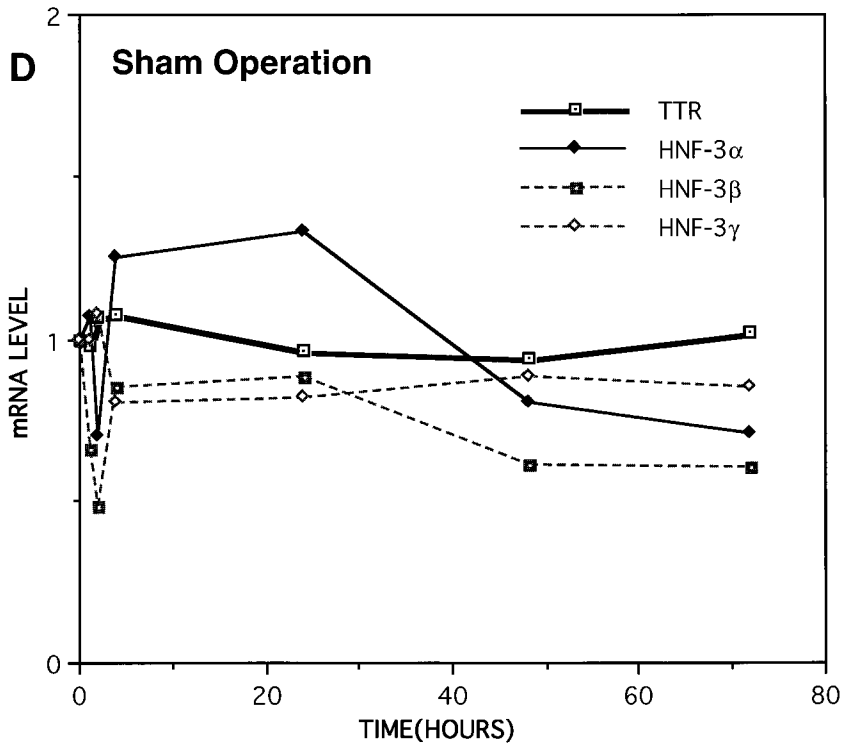


FIG. 8. Liver regeneration elicits only slight fluctuations in HNF-3 and TTR gene expression. Total rat liver RNA was isolated from either partially hepatectomized (HPX) or sham-operated animals at the indicated hours thereafter and analyzed for expression levels by T_2 RNase or RNase I protection assays as described in Materials and Methods. Hybridization with yeast tRNA was included as a control to detect nonspecific protected fragments (--). (A) RNase protection assays with liver RNA prepared from HPX or sham-operated rats and probes specific for c-*jun*, c-*fos*, and GAPDH; (B) RNase protection assays with liver RNA isolated from HPX or sham-operated rats and probes specific for TTR and HNF-3 isoforms; (C) graph summarizing TTR and HNF-3 mRNA levels during liver regeneration and expressed as a percentage of control animals; (D) graph summarizing TTR and HNF-3 mRNA levels in livers isolated from sham-operated control animals.

enhancer exclusively in hepatocytes (51). Although these AP-1 sites confer repression of albumin enhancer region in *ras*-transformed hepatocytes, their function in differentiated hepatocytes may be similar to that found with the TTR promoter region. Moreover, impaired hepatogenesis observed in homozygous c-*jun* mutant mice underscores the importance of c-*jun* expression for normal hepatocyte differentiation (31).

Is the cytokine-induced decrease in HNF-3α expression responsible for reduced expression of negative acute-phase genes? Previous studies demonstrated that TTR gene expression is diminished during the acute-phase response but not in partially hepatectomized livers (27). During liver regeneration both c-*jun* and c-*fos* expression was induced, while the acute-phase response stimulates primarily c-*jun* expression (29, 54).



We hypothesized that the difference in the AP-1 composition in regenerating and acute-phase livers may influence TTR gene expression. To test this model, we first examined the expression of TTR, HNF-3, and c-*fos*–c-*jun* in livers isolated from LPS-induced mice. We found that reduction of TTR expression in acute-phase livers coincided with a large reduction in HNF-3α expression and moderate induction of c-*jun* but not c-*fos* expression. Because of the critical importance of HNF-3 in TTR expression (17, 18), our model proposes that the reduction in TTR gene expression during the acute phase is likely due to lower HNF-3α expression levels (Fig. 9). We also propose that induction of primarily c-*jun* protein may not be sufficient to restore TTR expression because c-Jun homodimers are poor activators of TTR AP-1 site reporters (Fig. 3).

One plausible mechanism for the acute-phase repression involves the requirement of a critical amount of HNF-3 protein for transcriptional activation of particular target genes. Because HNF-3 binding activity is composed of three distinct proteins, α, β, and γ, the reductions in HNF-3α (95% decrease) and HNF-3β (20% decrease) expression may be sufficient to bring the HNF-3 protein below a given threshold, thus diminishing transcription of these target genes. Support for this model comes from studies with H2.35 hepatocyte cells (4, 23, 50). Undifferentiated H2.35 cells contain low levels of HNF-3α protein, yet none of the liver-specific markers are transcribed. However, differentiation of H2.35 cells elicits a large activation in HNF-3α expression, and its increase causes induction of several HNF-3 target genes. Another interpretation of our acute-phase response studies suggests that HNF-3α is the primary isoform which binds to the TTR HNF-3S site in vivo. HNF-3α specificity could be dictated by protein-protein interactions with other transcription factors binding in the vicinity of HNF-3 in the TTR promoter region as well as influences from chromosomal protein structure. This model suggests that each HNF-3 isoform possesses a set of target genes which are dictated by the configuration of the factors binding to the promoter region. In support of this hypothesis, expression of only a subset of HNF-3 target genes is down regulated during the acute-phase response. These genes include those for albumin, TTR, ornithine transcarbamoylase, transferrin, and retinol binding protein (6, 18, 50, 57, 65).

Are different DNA regulatory sequences required for HNF-3 expression in the lung and liver? Recent in situ hybridization studies in the lung demonstrate that HNF-3α expression is confined to bronchiolar epithelial cells and HNF-3β transcripts are found in pulmonary smooth muscle surrounding the arterioles and bronchioles (14). In the current study, we found that the acute-phase response in the lung did not significantly influence either HNF-3α or HNF-3β mRNA levels (Fig. 7D). We determined that the acute phase was induced in lung tissue, because we observed a transient increase in C/EBPβ expression. We show that bronchiolar epithelial (Clara) cells do respond to cytokines because the HFH-4 gene is stimulated by LPS in this cell type (61). Because identical HNF-3 genes are expressed in hepatocytes as in Clara cells and pulmonary smooth muscle, our results further suggest that the immune cytokines are functioning at the level of promoter regulation and not by altering mRNA stability. Taken together, these results suggest that different *cis*- or *trans*-acting control elements are used to direct HNF-3α and HNF-3β expression to hepatocytes, pulmonary bronchiolar epithelium (Clara cells), and smooth muscle, respectively. Moreover, recent studies identified two HNF-3 binding sites required for Clara cell 10-kDa protein promoter activity in NCI-H441 cells, one of which overlaps with both AP-1 and Oct-1 (64). Since Clara cell expression of HNF-3α is not influenced during LPS-induced acute-phase response, it is likely that the AP-1–HNF-3 site in the Clara cell 10-kDa protein promoter functions in a manner that differs from that of the TTR promoter.

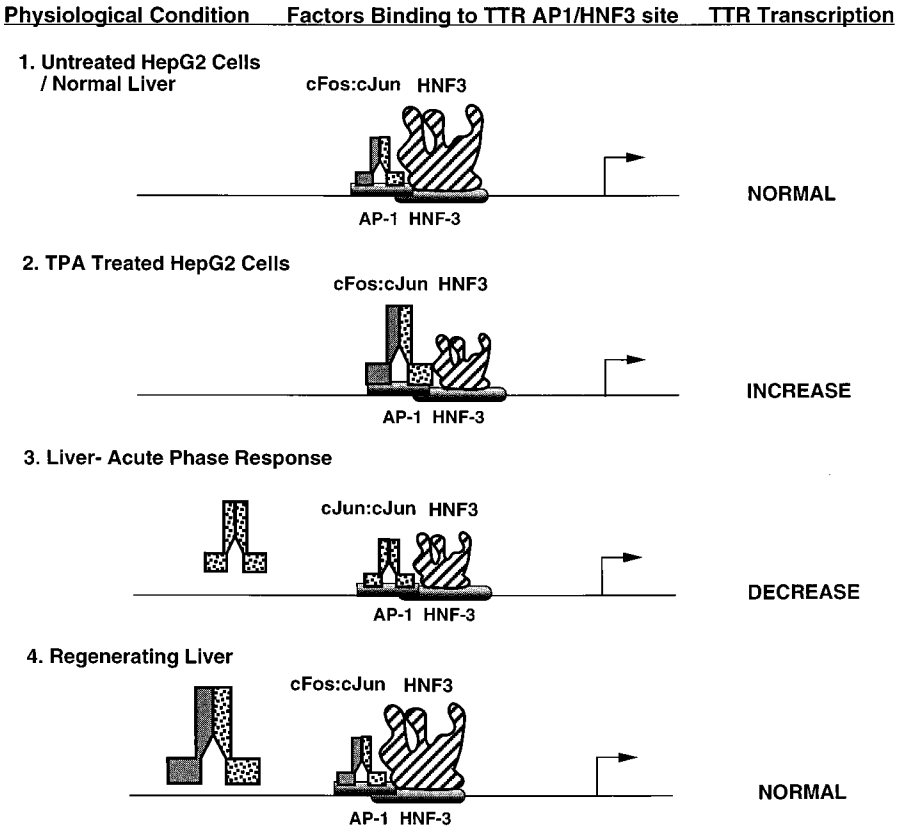


FIG. 9. Proposed model depicting binding of HNF-3 protein and AP-1 to the TTR promoter site under different conditions. Schematically shown are the TTR AP-1–HNF-3 site and the cognate transcription factors HNF-3, c-*jun* homodimers, and c-*jun*–c-*fos* heterodimers. The sizes of the transcription factors represent their relative abundance or their frequency of DNA occupancy. (Line 1) In untreated HepG2 cells the TTR AP-1–HNF-3 site is occupied by both HNF-3 protein and AP-1 (c-*jun*–c-*fos*), allowing for contribution to normal TTR promoter activity. We propose that HNF-3 is the dominant factor recognizing its high-affinity sequence in the TTR composite site. (Line 2) In TPA-treated HepG2 cells, we propose that diminished HNF-3α expression facilitates interaction of the induced AP-1 transcription factors to elicit activation of the TTR promoter. (Line 3) In acute-phase livers, we propose that TTR expression decreases because of the reduction in HNF-3α expression and induction of primarily c-*jun* homodimers which are poor binders and activators of the TTR composite site. (Line 4) In regenerating liver, we observed that HNF-3 levels do not fluctuate and therefore caused minimal fluctuation in TTR expression. The induced c-*jun*–c-*fos* complex appears not to activate the TTR promoter above normal levels in proliferating hepatocytes.

What are possible mechanisms of HNF-3 reduction in the acute-phase response versus maintenance during liver regeneration? Our HepG2 studies showed that TPA elicited a negative influence on HNF-3α expression while stimulating AP-1 activity. Because TPA is thought to mimic growth factor stimulation, we were surprised by the fact that HNF-3α mRNA was only slightly reduced in regenerating liver during a period when c-*fos* and c-*jun* were highly activated. Moreover, the sham-operated animals exhibited an HNF-3α expression profile similar to that of partially hepatectomized rats, suggesting that fluctuations in expression are due to the stress of surgery or induction of an acute-phase response but not cell proliferation. TTR expression in regenerating liver changed only slightly in spite of the dramatic activation of the AP-1 transcription factors. The lack of significant decreases in HNF-3α expression in regenerating liver suggests that HNF-3 is the dominant factor binding to the AP-1–HNF-3 TTR promoter site (Fig. 9). These observations are in strong contrast to expression of the C/EBPα, C/EBPβ, C/EBPδ, DBP, and HNF-1 liver factors, which change in response to hepatocyte proliferation (25, 53, 54).

The expression pattern of HNF-3α during liver regeneration and the acute-phase response in vivo suggests that the down regulation of HNF-3α expression in TPA-treated HepG2 cells is mediated by a subset of effectors that are involved in the acute-phase response. This hypothesis is supported by the fact that TPA induces the expression of a subset of acute-phase genes in several different hepatoma cell lines (24). One possible mechanism for the down regulation of HNF-3α expression is that the exclusive induction of c-*jun* during the acute-phase response provides an inhibitory complex not present in regenerating liver, which expresses both c-*jun* and c-*fos*. The c-Jun protein has been shown to interact with helix-loop-helix proteins and glucocorticoid receptors to inhibit activation by members of these families of transcription factors (10, 22, 49, 66, 72). Further analysis of the HNF-3α promoter may allow identification of regulatory sequences mediating reduced HNF-3α expression during the acute-phase response.

ACKNOWLEDGMENTS

We thank Robert Storti, Pradip Raychaudhuri, and members of our laboratory Derek Clevidence, David Overdier, and Richard Peterson for critically reviewing the manuscript and for helpful discussions. We also thank Jim Artwohl and Carolyn Bruzdzinski for their assistance during the liver regeneration studies.

This work was supported by Public Health Service grant R01 GM43241 from the National Institute of General Medical Sciences. U.S. was supported in part by the Department of Ophthalmology and

Visual Sciences, UIC. R.H.C. is an Established Investigator of the American Heart Association/Bristol-Myers Squibb.

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