Autoregulated Induction of the Acute-Phase Response Transcription Factor Gene, *agp/ebp*

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ABSTRACT

AGP/EBP (C/EBP β) is a key transcription factor responsible for transcriptional induction of many acute-phase protein genes. To characterize the regulation of this gene, we have isolated the mouse genomic DNA and sequenced the 5'-regulatory region. Using nuclear extracts from lipopolysaccharide (LPS)-stimulated or unstimulated mouse liver, three protein factor-binding motifs, UF1(–376 to –352), UF2(–254 to –223), and UF3(–220 to –190), as well as two Sp1 motifs (–309 to –277, and –264 to –241) were identified by DNase I footprinting assays. Biochemical analysis has shown that the AGP/EBP protein can bind to UF1 and UF2 sites, whereas an ubiquitous factor of unknown identity can bind to the UF3 site. Functional characterizations indicate that all of these factors play a major role in AGP/EBP induction. Thus, the *agp/ebp* gene is autoregulated during the acute-phase response.

INTRODUCTION

DURING THE LAST FEW YEARS, the 5'-flanking regions of many acute-phase protein genes have been studied extensively. The goal most of these studies was to identify regulatory elements required for cytokine-mediated induction of these genes. One type of cytokine response element has been found in the promoters of several acute-phase protein genes, representing binding sites for multiple members of the C/EBP family of transcription factors (Akira and Kishimoto, 1992). We have previously cloned and identified a key nuclear transcription factor, AGP/EBP, which is responsible for induction of the α -1 acid glycoprotein gene (Chang *et al.*, 1990; Lee *et al.*, 1993) and other acute-phase protein genes (Isshiki *et al.*, 1991; Alam *et al.*, 1992) during acute inflammation. AGP/EBP is a mouse liver-enriched transcription factor that belongs to the C/EBP transcription factor family. Several closely related or homologous factors from other species have been described, such as LAP (Descombes *et al.*, 1990), IL-6DBP (Poli *et al.*, 1990), C/EBP β (Cao *et al.*, 1991), and CRP2 (S.C. Williams *et al.*, 1991) from rat and NF-IL6 (Akira *et al.*, 1990) from human. C/EBP β is the common name for these factors isolated from different sources.

The regulation of AGP/EBP expression is very complex. In mouse liver and several other tissues, AGP/EBP expres-

sion is highly induced at both mRNA and protein levels during acute inflammation. Expression can also be induced by treating the animals with lipopolysaccharide (LPS) or recombinant cytokines, *e.g.*, interleukin-6 (IL-6), and IL-1 (Akira and Kishimoto, 1992). The *agp/ebp* gene has been shown to encode three polypeptides through a novel translational mechanism employing three in-frame translation initiation codons (Descombes and Schibler, 1991). Among these three proteins, the larger two are activators, whereas the smaller one functions as a repressor. The activator-to-repressor ratio varies during liver differentiation.

This may result in the dynamic regulation of the activity of AGP/EBP and its closely related members. Furthermore, IL-6 treatment can result in post-translational modifications of IL-6DBP (Poli *et al.*, 1990). AGP/EBP has been shown to be modified by phosphorylation under different physiological conditions (Wegner *et al.*, 1992; Nakajima *et al.*, 1993; Trautwein *et al.*, 1993). Another example of the regulation of NF-IL6 activity involves the interaction of NF-IL6 and the glucocorticoid receptor (P. Williams *et al.*, 1991; Nishio *et al.*, 1993). This interaction between proteins may be responsible for the induction of certain genes (*i.e.*, α -1 acid glycoprotein) by glucocorticoids.

AGP/EBP is a key molecule required for the LPS induction of various cellular genes, and recent data have indicated that members of the AGP/EBP family play important roles in

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the consolidation of long-term facilitation in *Aplysia* (Alberini *et al.*, 1994) and in the induction of the *c-fos* proto-oncogene (Metz and Ziff, 1991). It is extremely important to have a better understanding of how the *agp/ebp* gene is regulated transcriptionally. In this report, we present data on factors involved in the regulation of *agp/ebp* gene transcription, especially its autoregulation during acute inflammation.

MATERIALS AND METHODS

Isolation of genomic DNA for AGP/EBP

Mouse genomic DNA was digested with *Eco* RI. The restricted fragments were separated by agarose gel. Fragments of 4–8 kb in size were recovered from the gel and used for construction of a λ gt10 library. A recombinant λ clone containing the AGP/EBP gene was obtained by probing with an AGP/EBP cDNA probe. This genomic DNA fragment was characterized by restriction mapping and by partial DNA sequencing. Subsequently, a 5-kb *Eco* RI fragment containing the entire coding region of AGP/EBP and a 3-kb upstream regulatory sequence were subcloned into pGEM4 vector (Promega).

Plasmids and constructs

The plasmids pCMV-AGP/EBP and AGP-CAT were described in our earlier work (Lee *et al.*, 1993). An *Eco* RI–*Sph* I fragment spanning from –3 kb to +82 was isolated from genomic AGP/EBP DNA and cloned into the pGEM4 vector (Promega). This fragment was used for generating a series of 5' deletion fragments using *Bal*-31 digestion. The following CAT constructs were obtained: –685/+82-CAT, –390/+82-CAT, –343/+82-CAT, –250/+82-CAT, –156/+82-CAT, –76/+82-CAT, and –44/+82-CAT. pCAT-basic and pCAT-promoter vectors were purchased from Promega.

–390mtUF1/+82-CAT and –390mtUF2/+82-CAT were generated by site-directed mutagenesis using the following primers: 5'-GATCTGAGTCCCAAG-3' and 5'-GCTGGCGTTCCTCCCGTCCGT-3'. pAcSp1 (a *Drosophila* cell Sp1 expression vector) was obtained from Dr. R. Tjian's laboratory.

Cell cultures and transfections

HepG2 cells were grown in Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum. HepG2 cells were plated on 6-cm petri dishes at about 50% confluence when used for transfections. Transfections were performed using the calcium phosphate precipitation method. Twenty-four hours after transfection, plates were rinsed with DMEM and DMEM/10%FCS was added. The cells were harvested 48 hr post-transfection and 40 μ g of protein from the cellular extracts was used for chloramphenicol acetyltransferase (CAT) assays. The Schneider cell line SL.2 was cultured in Schneider's *Drosophila* medium (GIBCO).

Nuclear extracts and DNase I footprinting assays

Nuclear extracts from normal or LPS-treated mouse/rat liver were prepared as described (Lee *et al.*, 1993). For

DNase I footprinting assays, nuclear extracts from liver were used. Recombinant AGP/EBP was used as a control (Lee *et al.*, 1993). The end-labeled probe was derived from the –390 to +82 fragment of the AGP/EBP 5' upstream regulatory region. Preliminary experiments indicated that sequences between –685 and –390 do not have detectable *trans*-acting factor binding. Therefore, fragment containing –390 to +82 was chosen for footprinting studies. Purified Sp1 from HeLa cells was obtained from Promega.

Bandshift assays

For bandshift assays, nuclear extracts from unstimulated and LPS-stimulated mouse livers were incubated in a 20- μ l reaction mixture containing 20 mM HEPES pH 7.5, 50 mM NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, 1 μ g of poly(dI-dC · dI-dC), and 0.5–1.0 ng (10,000–20,000 cpm) of labeled synthetic oligodeoxynucleotides. The sequences of these double-stranded oligodeoxynucleotides were (sense strand is shown): UF1, 5'-GATCAGGAACGATCTGTTTCCCAAGAGTT-3'; UF2, 5'-GATC-GAAGGGGGCGGGCTGGCGT CACCCGCGTCCGT-3'. For the supershift assays, monoclonal or polyclonal antibodies to AGP/EBP were added to the reaction mixture 10 min before terminating the reaction.

Nuclear run-on assays

Nuclear fractions were prepared as described (Lee *et al.*, 1993). Nuclear pellets were resuspended in storage buffer (40% glycerol, 40 mM Tris-HCl pH 7.9, 10 mM MgCl₂, 280 mM KCl, 4 mM DTT, 2 mM MnCl₂) at 10⁷ nuclei/50 μ l. The total nuclear run-on experiment was performed in a reaction mixture of 100 μ l containing 100 μ Ci of [α -³²P]UTP (800 Ci/mmol). The ³²P-labeled RNA probe was hybridized with nitrocellulose strips carrying slot-blotted DNA (2 μ g per slot) for 3 days.

RESULTS

Mouse *agp/ebp* gene

To study the transcriptional regulation of the *agp/ebp* gene, nuclear run-on assays using nuclear preparations from mouse liver with or without LPS treatment were performed. The transcription rate of AGP was dramatically induced when the mouse was treated with LPS. AGP/EBP was also stimulated albeit to a lesser degree than AGP (Fig. 1). These results indicate that both *agp* and *agp/ebp* gene expression are transcriptionally regulated when the animal is treated with LPS. To identify regulatory elements involved in transcriptional regulation of the *agp/ebp* gene, we cloned a genomic DNA containing the entire *agp/ebp* gene (5 kb) including 3 kb of its 5' upstream sequence. We mapped its restriction sites and determined the sequence containing the –685 upstream region. The sequence from –685 to +82 is shown in Fig. 2A. To identify the regions that are important for the AGP/EBP promoter activity, we prepared nested deletions of the 5'-flanking sequence using *Bal*-31 and linked

FIG. 1. Nuclear run-on analysis of mouse liver with or without LPS treatment. Liver nuclear fractions were prepared from normal (–) and LPS-stimulated (+) livers. LPS (100 μ g/10–15 grams of BALB/c mouse) treatment was performed by i.p. injection for 4 hr. Specific DNAs bound to the nitrocellulose membrane were hybridized with [α - 32 P]UTP-labeled run-on probes.

them to the CAT reporter gene. Plasmids derived from these constructs were used for transfection assays. The results suggest that the region between –685 and –390 contains a negative element(s) while that between –390 and –44 contains positive elements (Fig. 2B).

Trans-acting factors involved in the *agp/ebp* gene regulation

To demonstrate that the *agp/ebp* gene transcription is regulated by different *trans*-acting factors, we performed DNase I footprinting analysis using fragment derived from the –390 to +82 5' regulatory region of the AGP/EBP gene as a probe. Nuclear extracts prepared from normal and LPS-treated mouse livers were prepared and used for footprinting assays. We detected two prominent protection regions [from –376 to –352 (UF1), and –220 to –190 (UF3)], together with two weak protection regions (–309 to –277, and –264 to –241) within this sequence (Fig. 3A, lanes 2 and 3). Nuclear extract from LPS-treated livers was used for footprinting assays; a prominent protection region spanning from –254 to –223 (UF2) was also seen in addition to the UF1 and UF3 sites (Fig. 3A, lanes 4 and 5).

The nucleotide sequence of the two weak protection regions (–309 to –277 and –264 to –241) suggests that they are potential binding sites for transcription factor Sp1. Therefore, purified Sp1 was shown to bind to these sequences by a footprinting assay (Fig. 3B, lanes 1–4). One of the two regions (–264 to –241) overlaps with UF2. Because the sequence of UF1 is very similar to the AGP/EBP DNA-binding consensus motif, we tested the footprinting pattern of recombinant AGP/EBP. The results demonstrate that low amounts of recombinant AGP/EBP (10 ng) protect the UF1 region, whereas high amounts additionally protect the UF2 region (Fig. 3B, lanes 5–8). These results are consistent with the results obtained from the nuclear extract,

i.e., the UF2 motif can be recognized by induced nuclear extract in which AGP/EBP (or other factors) activity or level is highly increased.

AGP/EBP protein binds to the UF1 and UF2 regions and activates *agp/ebp* gene expression

To characterize further the protein factors that bind to UF1 and UF2 motifs, we synthesized oligonucleotides corresponding to these regions and used them for both competition and bandshift assays. Nuclear extracts from LPS-treated liver cells was used for footprinting analysis. Factors that bind to both UF1 and UF2 could be independently competed by oligonucleotides from UF1, UF2, and the AGP/EBP binding motif (C, D, and E motifs from the AGP promoter; Lee *et al.*, 1993). An oligonucleotide recognized by Sp1 was used as a negative control (Fig. 4A, lanes 4–9). Furthermore, nuclear extracts from both normal and LPS-treated liver, together with monoclonal and polyclonal antibodies against AGP/EBP, were tested with a gel mobility-shift assay. The protein–DNA complex formed by the UF1 oligonucleotide and protein(s) from both normal and LPS-stimulated nuclear extracts could be supershifted by AGP/EBP antibodies (Fig. 4B). In contrast, only the complex formed by the UF2 oligonucleotide and protein(s) from LPS-stimulated nuclear extract, but not normal nuclear extract, were supershifted (Fig. 4C). Consequently, these results indicate that UF1- and UF2-binding protein complexes share the common AGP/EBP factor present in the LPS-stimulated liver nuclear extracts.

To determine whether the partial palindromic UF1 (5'-GTTTCCCAAG-3') and UF2 (5'-CTGGCGTCAC-3') motifs are indeed recognized by AGP/EBP, we synthesized these oligonucleotides and used them as probes for bandshift assays. Complexes formed by these short oligonucleotides and nuclear extracts were also recognized by AGP/EBP antibodies (data not shown).

To dissect further the factor that was induced by LPS, we performed a footprinting analysis using probes containing the UF1- or UF2-mutated sequence (in which the critical nucleotides of the AGP/EBP binding motif was destroyed; see Fig. 5, upper panel) and LPS-treated liver nuclear extracts. When mutated UF1 or UF2 probe was used, the footprinting protection of UF1 or UF2 was abolished (Fig. 5). These results suggest that the factors binding to these two motifs independently, and the binding of AGP/EBP is essential for UF1 and UF2 footprints.

Therefore, we tested the possibility that AGP/EBP is involved in the induction of its own gene. We cotransfected HepG2 cells with various deletion constructs of AGP/EBP-CAT and pCMV-AGP/EBP or pCMV-C/EBP α . Both AGP/EBP and C/EBP α can *trans*-activate the AGP/EBP promoter, but cannot *trans*-activate the pCAT-promoter (Fig. 6A). When the UF1 site was deleted (–250-CAT), only slight decrease in AGP/EBP activation was observed. But when both UF1 and UF2 sites were deleted (–156-CAT), the activation effect of AGP/EBP was affected dramatically. In addition to the above-mentioned AGP/EBP-binding sites, the sequence in the TATA box that can be recognized by recombinant AGP/EBP (data not shown; also see Lee *et al.*,

FIG. 2. Sequence and functional analysis of *agp/ebp* gene 5' regulatory regions. A. Nucleotide sequence of the 5'-flanking region of *agp/ebp* gene. The transcription initiation site is indicated as +1. The TATA box is underlined. The first translation initiation codon is in boldfaced letters. The footprinting-protected regions of UF1, UF2, and UF3 are indicated by overlining, and Sp1 binding sites are indicated by dashed underlines. B. Transfection analysis of serially deleted AGP/EBP promoter linked to CAT reporter gene. HepG2 cells were seeded in 60-mm petri dishes and transfected with 5 μ g of AGP/EBP-CAT plasmids. Reporter activity is shown as percent conversion relative to 100% activity obtained with (-390)AGP/EBP-CAT. Data are representatives of at least three independent experiments.

FIG. 3. DNase I footprinting analysis of the 5'-flanking sequence of *agp/ebp* with nuclear extracts from mouse liver, recombinant AGP/EBP, and purified Sp1. A. Probe containing -390 to +82 was incubated with 10 and 20 μ g of normal (lanes 2 and 3) and LPS-treated lanes 4 and 5) liver nuclear extracts. Lane 1 is a BSA control. The left brackets indicate two weak protection regions (-309 to -277, -264 to -241) and right brackets represent the UF1 (-376 to -352), UF2 (-254 to -223), and UF3 (-220 to -190) protection sites. B. Purified Sp1 and recombinant AGP/EBP were used for the footprinting experiment. Lanes 1 and 5, BSA controls; lanes 2-4, increasing amounts of Sp1 (6, 12, and 24 ng); lanes 6-8, recombinant AGP/EBP (10, 20, and 40 ng). The probe is the same as in A.

1993) may be responsible for the observed low activation of -156-CAT and -44-CAT by pCMV-AGP/EBP or pCMV-C/EBP α (Fig. 6A). The stimulatory effect of pCMV-AGP/EBP on wild-type-, mutant UF1-, or mutant UF2-AGP/EBP-CAT was further investigated by cotransfection of these plasmids into HepG2 cells. The results indicate that both mutant UF1- and UF2-AGP/EBP-CAT respond to AGP/EBP less well than the wild-type-AGP/EBP-CAT (Fig. 6B). These data suggest that AGP/EBP is involved in the transcriptional activation of *agp/ebp* gene during acute inflammation.

The role of Sp1 in agp/ebp gene expression

There are two motifs in the *agp/ebp* 5'-flanking region recognized by Sp1. We tested the involvement of Sp1 in activation of the *agp/ebp* gene. The SL.2 Schneider cell line was used for cotransfection assays with various constructs, including an Sp1 expression vector, pAcSp1, and AGP/EBP-CAT. Sp1 can activate the *agp/ebp* gene (Fig. 7A), but cannot

trans-activate the *agp* gene (which was used as a control). The promoter region of AGP/EBP is GC-rich, which may be responsible for the activation of a minimal promoter sequence such as -44-CAT. One of the Sp1 motifs (-264 to -241, overlapping with UF2) is undetectable in the presence of nuclear extracts from LPS-treated liver but detectable when nuclear extract from normal liver was used (Fig. 3). Detailed footprinting analysis was performed using fixed amounts of purified Sp1 and increasing amounts of nuclear extracts from normal or LPS-treated liver cells (the level of AGP/EBP in these two nuclear extracts has been adjusted to the same amount by Western blot assay). This experiment clearly established that factors derived from the LPS-treated liver could abolish the Sp1 binding to the motif overlapped with UF2, but normal liver nuclear extract could not (Fig. 7B). This suggested that, in addition to AGP/EBP, the UF2 protein complex contained other LPS-stimulated factors. This possibility will be further discussed.

DISCUSSION

AGP/EBP is involved in transcriptional regulation of the agp/ebp gene

Two lines of evidence suggest that AGP/EBP mediates the initiation of transcription of the *agp/ebp* gene: (i) biochemical characterization of *trans*-acting factors from normal and LPS-treated liver nuclear extracts indicate (a) that AGP/EBP is involved in the binding to AGP/EBP upstream regulatory sequence(s) and (b) that the recombinant AGP/EBP can recognize both UF1 and UF2 motifs. (ii) differences between the footprinting patterns of normal and LPS-liver nuclear extracts demonstrate that the UF2-binding factor exists only in the LPS-treated but not in the normal liver nuclear extracts. The UF2-binding factor can be recognized by antibodies to AGP/EBP as demonstrated by gel mobility-shift assay. Thus, the UF2-binding factor is an acute inflammation-induced nuclear factor containing AGP/EBP. In contrast to the up-regulation of the *agp/ebp* gene during the acute-phase response, the *c/ebp α* gene is down-regulated (Isshiki *et al.*, 1991). However, *c/ebp α* gene expression is increased during differentiation of 3T3-L1 preadipocytes into adipocytes (Christy *et al.*, 1991). Thus, there are some similarities between *agp/ebp* gene regulation during the acute-phase response and *c/ebp α* gene regulation during 3T3-L1 differentiation. The *c/ebp α* gene is autoregulated by its own product during 3T3-L1 differentiation (Christy *et al.*, 1991; Legraverend *et al.*, 1993). The sequence located at -198 to -176, TCAGTGGGCGTTGCGCCAC, which is recognized by C/EBP α , is strikingly similar to the UF2 sequence in the *agp/ebp* promoter GGGGGCGGGCTGGCGTCAC-CCGC. The sequence in the UF2 region must play an important role in LPS-induced *agp/ebp* gene expression that is much like the importance of the -198 to -176 sequence in the *c/ebp α* promoter for C/EBP α production during 3T3-L1 differentiation. The nucleotide sequence of -256 to -234 in the 5' regulatory region of C/EBP α is CUP/Sp1-like GT box element (Vasseur-Cognet, and Lane, 1993). During differentiation of 3T3-L1 preadipocytes into adipocytes, CUP activity decreases as expression of C/EBP α increases. There may be a parallelism between the decrease of CUP/Sp1 activity upon the induction of C/EBP α during adipocyte differentiation and the disappearance

FIG. 4. Biochemical and serological characterizations of UF1- and UF2-binding factors. A. Footprinting competition analysis of binding specificity of UF1 and UF2 elements. The probe is same as in Fig. 3; nuclear extracts are from LPS-treated mouse liver. Lane 1, BSA control; lane 2, 10 µg of nuclear extract; lanes 3–9, 20 µg of nuclear extracts. Lanes 4–9 are in the presence of 40 ng of oligonucleotide competitors of UF1, UF2, C, D, E elements (AGP/EBP binding sites of AGP gene; Lee *et al.*, 1993), and Sp1. B. Gel retardation of antibodies supershift assay. A 1-ng amount of UF1 oligonucleotide probe was incubated with nuclear extracts from normal (lanes 1–5) or LPS-stimulated (lanes 6–10) mouse liver. Lanes 1 and 6, Controls (without antibody); lanes 2 and 7, nonspecific antibody controls; lanes 3 and 8, in the presence of AGP/EBP monoclonal antibody; lanes 4 and 9, rabbit preimmune serum controls; lanes 5 and 10, AGP/EBP polyclonal antibody. C. A 1-ng amount of UF2 oligonucleotide probe was used instead of UF1, and the experimental conditions were the same as B.

or diminishing of Sp1 footprinting activity upon the induction of AGP/EBP during the acute-phase response.

A number of homeotic genes, proto-oncogenes, and transcriptional factor genes have been shown to be autoregulated by their own products (Serfling, 1989). For example, the *jun* proto-oncogene is positively autoregulated by its product, Jun/AP1 (Angel *et al.*, 1988). Fos can repress its own transcription (Sassone-Corsi *et al.*, 1988; Konig *et al.*, 1989; Schonthal *et al.*, 1989). Also the *c-myc* gene is negatively autoregulated by its own product (Penn *et al.*, 1990) and Pit-1/GHF-1, MyoD, and NF-κB are involved in their own regulation (Thayer *et al.*, 1989; Chen *et al.*, 1990; Ten *et al.*, 1992). These examples further demonstrate that it is not unusual that the *agp/ebp* gene is autoactivated by its own product or by closely related C/EBP family members.

Inducible versus constitutive expression of the agp/ebp gene

AGP/EBP is a liver-enriched transcription factor. Several *trans*-acting factors, such as C/EBPα, AGP/EBP, Sp1, and

UF3-binding factor, have been shown to be involved in AGP/EBP transcriptional regulation. The UF1-bound C/EBPα (antibody supershift assay, data not shown) and AGP/EBP, in conjunction with Sp1 and the UF3-binding factor, may constitute the predominant factors that are necessary for the constitutive expression of the *agp/ebp* gene. This is supported by two lines of evidence: (i) the level of Sp1 and the UF3-binding factor remains relatively constant before and after LPS treatment (data not shown) and (ii) C/EBPα is very abundant in liver cells under normal physiological conditions, suggesting that these factors are crucial in maintaining the constitutive expression of hepatic *agp/ebp*. AGP/EBP itself may form heterodimers with C/EBPα or members of the C/EBP family, thus exerting its regulatory function for its own gene.

When UF2 factors are induced by LPS treatment, one of the Sp1 footprinting patterns (–264 to –241) disappears. In contrast to this, two regions (–309 to –277 and –264 to –241) can clearly be recognized by the purified Sp1 (Fig. 3B). The UF2-binding factors may inhibit the Sp1 binding by steric hindrance or competition. The mere presence of the

FIG. 5. DNase I footprinting analysis of mutant AGP/EBP promoter probe generated by site-directed mutagenesis. Sequences of the mutated AGP/EBP binding motif of mutant UF1 and mutant UF2 are shown in the upper panel. Both probes were derived from -390 to $+82$ fragments. Footprinting patterns are shown in the lower panel. Mutant UF1 (lanes 1–3) or mutant UF2 (lanes 4–6) probe was incubated with mouse liver nuclear extract prepared from LPS-stimulated animal ($10 \mu\text{g}$ for lanes 2 and 5; $20 \mu\text{g}$ for lanes 3 and 6). Lanes 1 and 4 are controls.

Sp1 motif does not constitute obligatory binding by Sp1 in the nuclear extract. We have shown that AGP/EBP is involved in UF2 binding. The amount of AGP/EBP protein increases during LPS stimulation (data not shown). Under physiological conditions, C/EBP α or its closely related member is bound to UF1 and may function in regulating the basal transcriptional activity of the *agp/ebp* gene. During LPS-stimulated conditions, increasing amounts of AGP/EBP and its heterodimers with other factors may bind to the UF2 region and may be responsible for *agp/ebp* gene induction. Recently, we have obtained some evidence indicating that

FIG. 6. Activation of AGP/EBP promoter by AGP/EBP or C/EBP α expression vector. **A.** HepG2 cells were transiently transfected with $2 \mu\text{g}$ of AGP/EBP-CAT or pCAT-promoter (control) reporter plasmids in the presence of $0.2 \mu\text{g}$ of pCMV-AGP/EBP or pCMV-C/EBP α expression vector, or pCMV control. Values are percentages of the CAT conversion. **B.** Cotransfection experiments using plasmids ($2 \mu\text{g}$) derived from wild-type AGP/EBP promoter (-390 to $+82$), mtUF1-, or mtUF2-CAT reporter and increasing amounts (0.25 , 0.5 , and $1.0 \mu\text{g}$) of pCMV-AGP/EBP expression vector. Data are representatives of three independent experiments.

the transcription factors of CREB/ATF family may be involved in UF2-mediated function (unpublished data).

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FIG. 7. Sp1 activates the AGP/EBP promoter. **A.** Schneider cell line SL.2 was cotransfected with 3 μ g of CAT reporter linked to a series of AGP/EBP deletion promoter (AGP/EBP-CAT) and 0.25 μ g of pAcSp1 expression vector. AGP-CAT was used as a control. **B.** DNase I footprinting experiments of AGP/EBP probe (–390 to +82) and nuclear extract prepared from normal or LPS-stimulated mouse liver and purified Sp1. Lane 1, BSA control; lanes 2–6, 15 ng of purified Sp1; lanes 3–4, 25 and 50 μ g of normal liver nuclear extract; lanes 5–6, 10 and 20 μ g of LPS-liver nuclear extract.

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