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The Ability of C/EBPβ but Not C/EBPα To Synergize with an Sp1 Protein Is Specified by the Leucine Zipper and Activation Domain

YING-HUE LEE,[1] SIMON C. WILLIAMS,[2]† MARK BAER,[2] ESTA STERNECK,[2] FRANK J. GONZALEZ,[1] AND PETER F. JOHNSON[2]*

Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda,[1] and ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick,[2] Maryland

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The rat *CYP2D5* P-450 gene is activated in the liver during postnatal development. We previously showed that liver-specific transcription of the *CYP2D5* gene is dictated by a proximal promoter element, termed 2D5, that is composed of a binding site for Sp1 or a related factor, and an adjacent cryptic C/EBP (CCAAT/enhancer-binding protein) site. Despite the fact that both C/EBPα and C/EBPβ are expressed abundantly in liver, only C/EBPβ is capable of stimulating the 2D5 promoter in HepG2 hepatocarcinoma cells. In addition, activation of the 2D5 promoter by C/EBPβ is completely dependent on the presence of the Sp1 site. Domain switch experiments reveal that C/EBPβ proteins containing either the leucine zipper or the activation domain of C/EBPα are unable to stimulate the 2D5 promoter yet are fully capable of transactivating an artificial promoter bearing a high-affinity C/EBP site. Thus, the leucine zipper and the activation domain of C/EBPβ are absolutely required to support transactivation of the 2D5 promoter. Using *Drosophila* cells that lack endogenous Sp1 activity, we show that the serine/threonine- and glutamine-rich activation domains A and B of Sp1 are required for efficient cooperatively with C/EBPβ. Furthermore, analysis of *c/ebp*β-deficient mice shows that mutant animals are defective in expression of a murine *CYP2D5* homolog in hepatic cells, confirming the selective ability of C/EBPβ to activate this liver-specific P-450 gene in vivo. Our findings illustrate that two members of a transcription factor family can achieve distinct target gene specificities through differential interactions with a cooperating Sp1 protein.

Transcription of most eukaryotic genes is regulated by several activator proteins that bind to *cis*-regulatory sites flanking the gene. Single regulatory elements are rarely sufficient to promote high levels of transcription or to direct proper cell-specific or inducible expression. Usually two or more *cis* elements are required, and frequently these sites function synergistically (superadditively) to activate gene transcription (14). Synergism can occur at the level of transcriptional activation, where two activators may have independent targets in the transcription initiation complex or enhance different rate-limiting steps in the initiation pathway (2, 15, 23). Alternatively, cooperativity may be achieved through facilitated DNA-binding interactions in which binding of one protein increases the affinity of a second activator for its binding site (9, 35, 39). DNA-binding cooperativity involves either protein-protein interactions between two factors or, less likely, a conformational change in the DNA induced by one protein that promotes binding of the second. Although synergism between activators is an important aspect of gene activation in eukaryotic organisms, relatively little is known of the molecular basis for such cooperativity.

We previously reported evidence for cooperative interactions between C/EBPβ (also named NF-IL6, IL-6DBP, LAP, AGP/EBP, CRP2, and NF-M [reviewed in reference 16]) and Sp1, or an Sp1-like protein, in regulating transcription of the *CYP2D5* cytochrome P-450 gene (22). *CYP2D5* is a member of the rat *CYP2D* subfamily of P-450 genes that are developmentally regulated in the liver (10). *CYP2D5* transcripts first appear in liver immediately after birth and attain high levels in 3-week-old prepubertal rats. Mutagenesis experiments identified a region in the *CYP2D5* promoter between nucleotides −80 and −120, termed 2D5, that is essential for the activity of this promoter in transfected HepG2 hepatocarcinoma cells (22). The 2D5 region contains a binding site for a liver nuclear factor that is identical or highly related to the ubiquitous activator protein Sp1. An adjacent *cis*-regulatory element exhibits weak similarity to the consensus C/EBP binding site. Neither C/EBPα nor C/EBPβ, two liver-enriched members of the C/EBP protein family, is capable of binding this site with high affinity. However, in the presence of liver extract or recombinant human Sp1 protein, C/EBPβ was found to bind the 2D5 element as part of a 2D5-Sp1-C/EBPβ ternary complex (22).

Interestingly, C/EBPα and C/EBPβ differ in their capacities to transactivate the 2D5 promoter in HepG2 cells (22). While C/EBPβ stimulated transcription of a 2D5 promoter-chloramphenicol acetyltransferase (CAT) reporter construct approximately fivefold, C/EBPα had no effect on promoter activity. Together with the data from in vitro DNA-binding experiments, these findings suggested that transcriptional activation of the *CYP2D5* gene in hepatocytes involves Sp1-dependent recruitment of C/EBPβ to a cryptic C/EBP site located in the proximal promoter region. In support of this model, C/EBPβ expression in rat liver increases at about 3 weeks after birth, coincident with the elevation of *CYP2D5* transcript levels (22).

In the present study we have further investigated the mechanism underlying cooperative activation of the *CYP2D5* promoter by C/EBPβ and an Sp1-related factor. We show that

* Corresponding author. Mailing address: Eukaryotic Transcriptional Regulation Group, ABL-Basic Research Program, NCI-FCRDC, P.O. Box B, Frederick, MD 21702-1201. Phone: (301) 846-1627. Fax: (301) 846-5991. E-mail: johnsopf@fcrfv1.ncifcrf.gov.

† Present address: Department of Cell Biology and Biochemistry, Texas Tech University Health Science Center, Lubbock, TX 79430.

functional interactions between Sp1 and C/EBPβ are adversely affected by increasing the distance between their binding sites but not by their relative orientation. Cooperativity appears to occur at the level of both DNA binding and transactivation, since dependence on Sp1 was observed even when the cryptic C/EBP binding site was converted to a high-affinity sequence. In *Drosophila* cells, transactivation of the 2D5 promoter by C/EBP proteins requires Sp1, and two of the four Sp1 activation domains are necessary for synergism with C/EBPβ. The C/EBPβ leucine zipper and the activation domain were found to be absolutely required for C/EBPβ-Sp1 cooperativity in HepG2 cells, as hybrid proteins containing the corresponding domains from C/EBPα did not transactivate the 2D5 promoter. Furthermore, analysis of C/EBPβ-deficient mice demonstrates that C/EBPβ is essential for efficient transcriptional activation of a murine *CYP2D5* homolog in hepatocytes.

MATERIALS AND METHODS

Promoter-CAT reporter constructs. The 2D5 promoter mutant-CAT reporters carrying point mutations at the Sp1 or C/EBP site were constructed by PCR amplification of the promoter (nucleotides −120 to +73) using 5′ oligonucleotide primers incorporating the desired base changes. The mutagenic oligonucleotide primers were 2D5, 5′-ACTGAACCTCACTGGCCCCTCCCTACACTTGACCAAACAAAC; 2D5-M1, 5′-ACTGAACCTCACTGGCCCCTCgCTACACTTGACCAAACAAAC; 2D5-M2, 5′-ACTGAACCTCACTGGCCCCTCCCTACCACccGACCAAACAAAC; 2D5-M4, 5′-ACTGAACCTCACTGGCCCCTCCCTACACTTGcgCAAACAAAC; and 2D5-M5, 5′-ACTGAACCTCACTGGCCCCTCgCTACACTTGcgCAAACAAAC. The mutated bases are lowercased, and their effects on the binding of Sp1 or C/EBP to the promoter are diagrammed in the corresponding figures. The 3′ primer was a 20-mer spanning nucleotides +54 to +73. After amplification, the PCR fragments were treated with Klenow polymerase and ligated to the promoterless CAT vector (22).

2D5 promoter hybrids containing the albumin core promoter were constructed by a two-step PCR approach. The 2D5 mutant oligonucleotides (oligos) described above were paired with oligo 1 (see below), and oligo 2 (below) was paired with the 3′ 20-mer; the 2D5 promoter was used as the template. The two amplified fragments were combined and joined by a second round of PCR using the 2D5 mutant oligos and the 3′ 20-mer as primers. The final products were gel purified, blunt ended with Klenow, and ligated to the promoterless CAT vector. The orientation and sequence were confirmed by DNA sequencing. oligo 1 is nucleotides −55 to −36 of 2D5 fused to nucleotides −35 to +3 of the mouse albumin promoter; oligo 2 is nucleotides −17 to +22 of albumin joined to nucleotides +23 to +73 of 2D5.

***Drosophila* cell expression plasmids.** pPacSp1 and a series of human Sp1 expression plasmids containing various deletions were kindly provided by Robert Tjian (4). To construct C/EBPα and C/EBPβ expression plasmids driven by an insect actin gene promoter for use in the transfection studies with *Drosophila* cells, a promoter fragment (−340 to +88) of the *Drosophila* actin 5C gene (3) was PCR amplified from pPacSp1 and inserted into pGEM-3Z (Promega) as an *Eco*RI fragment. This shorter actin 5C region reportedly has promoter strength similar to that of pPacSp1, which contains 2.3 kb of actin 5C 5′-flanking DNA (including upstream enhancers and a negative regulatory element) (3, 4). A 1.1-kb *Sal*I fragment containing the *Drosophila* actin 5C gene polyadenylation signal (pol-A) was then excised from pPacSp1 and inserted into the *Sal*I site of the vector. The resulting expression vector containing the actin 5C gene promoter and pol-A was named pGac. The C/EBPα and C/EBPβ coding sequences were isolated from pMEX-C/EBP and pMEX-CRP2 (37), respectively, as *Bam*HI fragments and inserted into the *Bam*HI site of the polylinker between the actin 5C promoter and pol-A to generate pGac. C/EBPα and pGac.C/EBPβ. The sequence of the PCR-amplified 5C promoter and the insert orientation for each construct were confirmed.

C/EBPα and C/EBPβ expression plasmids. Vectors capable of expressing the wild-type C/EBP proteins have been described previously (36); pMEX-C/EBP is now designated pMEX-C/EBPα, and pMEX-CRP2 is renamed pMEX-C/EBPβ. pMEX is an expression vector containing the murine sarcoma virus long terminal repeat (37). PCR was used to introduce restriction sites into the coding sequences of C/EBPα, C/EBPβ, VP16, and GCN4; the construction of deletions and chimeric genes has been described previously (36). In general, segments located at the N terminus of a gene carry either a naturally occurring or a PCR-derived *Nco*I site at the 5′ end and a *Bam*HI site at the 3′ end. Internal segments were constructed as *Bgl*II-*Bam*HI fragments, and the DNA-binding domain (DBD) fragments were generated as *Bam*HI-*Hin*dIII fragments. Restriction fragments were combined to generate chimeric and deleted genes which are named according to the amino acid residues that they contain. DBD fragments encode amino acids 272 to 358 of C/EBPα and 192 to 276 of C/EBPβ, and the VP16 activation domain consists of amino acids 429 to 456 from the herpes simplex virus (HSV) VP16 protein. Leucine zipper chimeras were constructed by introducing *Xho*I sites into the coding sequences of C/EBPα at codons 314 and 315, C/EBPβ at codons 235 and 236, and GCN4 at codons 250 and 251. The leucine zipper modules were then exchanged as *Xho*I-*Hin*dIII fragments.

Transient transfection and CAT assay. HepG2 cells were transfected essentially as described elsewhere (22), except that the cell density was reduced to 30% confluency and the internal standard, the luciferase reporter pSV232AL.A_5′ (6), was reduced to 0.5 μg per flask. *Drosophila melanogaster* Schneider SL-2 cells were transfected by the calcium phosphate method (12). Briefly, cells were seeded at 2×10^6 per flask (25 cm^2) in 5 ml of M3 medium (Quality Biological, Inc.) containing 10% fetal bovine serum (HyClone) 24 h before transfection. Six micrograms of the DNA was mixed with 200 μl of 0.5 M $CaCl_2$, and 200 μl of 2× HN (50 mM HEPES, 280 mM NaCl, 1.75 mM NaH_2PO_4 [pH 7.09]) was added dropwise. The mixture was added to the cells after 15 min of incubation. The cells were left undisturbed at room temperature for 40 to 48 h before harvesting. Cells were lysed by five rounds of freezing and thawing, and the cell lysates were then used directly for luciferase activity measurements and CAT assays (11). Unless otherwise indicated, CAT assays were performed at 37°C for 1 h.

Each HepG2 cell lysate (10 μl) was analyzed by Western blotting to verify expression of the C/EBP proteins. The extracts were electrophoresed on sodium dodecyl sulfate (SDS)–12% polyacrylamide gels, transferred to nitrocellulose membranes (Schleicher & Schuell), and probed with an antibody (panCRP) that recognizes an epitope in the C/EBP basic region (36). Antigen-antibody complexes were visualized with an Amersham Enhanced Chemiluminescence kit.

Gel mobility shift assay. All probes were end labeled with [γ-^{32}P]ATP. The 2D5 gel shift probe has been described previously (22), and the sequence of the 2D11 gel shift probe is 5′-ACTGAACCTCACTGGGTCCTCCCTATCCCGGGCCAAAAAAAAAC-3′. Gel mobility shift experiments using recombinant human Sp1 (Promega) and C/EBPβ were performed as described elsewhere (22).

Liver RNA extraction and Northern blot analysis. Mouse liver tissue was homogenized in the RNA STAT-60 reagent (TEL-TEST "B," Inc., Friendswood, Tex.), and total RNA was extracted according to the manufacturer's protocol. Twenty micrograms of RNA was heated at 65°C in an RNA sample buffer containing 50% formamide and 6% formaldehyde and loaded onto a 1% agarose gel. Electrophoresis was carried out at 100 V with buffer recirculation. The RNA was transferred to a nylon membrane (Dupont) with 10 mM NaOH. After transfer, the membrane was neutralized with Tris-HCl (pH 7.0) and prehybridized at 50°C in 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–10% dextran sulfate–1% SDS–0.5% BLOTTO for 2 h. A ^{32}P-labeled 2D11 oligonucleotide probe was added at a specific activity of 5×10^7 cpm/ml, and hybridization was continued for 16 h. The membrane was then washed three times with 4× SSPE–1% SDS for 10 min at 50°C. The membrane was scanned with a PhosphorImager detector, and the signals were quantitated. The 2D11 probe is 5′-GCTAAAAATGCTGAAAGAATGTTTCACA; the albumin probe is 5′-CACTACAGCACTTGGTAACATGCTCACTC. The albumin probe was hybridized in 4× SSPE–1% SDS–0.5% BLOTTO at 55°C. The actin cDNA probe was hybridized in 4× SSPE–1% SDS–0.5% BLOTTO at 65°C.

RESULTS

Ternary 2D5 complex formation by Sp1 and C/EBPβ is dependent on spacing but not relative positions of binding sites. In many cases of DNA-binding cooperativity between two activator proteins, spacing and orientation of the binding sites are critical parameters (39). Therefore, we examined the effects of altering the spacing and relative positions of the Sp1 and C/EBPβ sites in the 2D5 region. Poly(dA) spacers of 5, 10, 20, 30, or 40 bp were inserted between the Sp1 and C/EBP binding sites, and the mutant templates were tested for Sp1-C/EBPβ-2D5 ternary complex formation in vitro by using purified recombinant proteins. As shown in Fig. 1A, The efficiency of ternary complex formation decreased as the distance between the binding sites was lengthened. Increasing spacing did not affect Sp1 binding appreciably, whereas cooperative binding of C/EBPβ to templates containing 30-bp or larger spacers was severely diminished. Switching the positions of the Sp1 and C/EBP binding sites within the 2D5 oligonucleotide did not reduce and, indeed, slightly increased the efficiency of ternary-complex formation.

To examine whether ternary complexes can form when the Sp1 and C/EBP sites are on separate DNA fragments, a labeled oligonucleotide containing the 2D5 Sp1 binding site was mixed with a second oligonucleotide containing either the 2D5 C/EBP site or a high-affinity C/EBP site. The various pairs of oligonucleotides were tested for ternary complex formation with purified C/EBPβ and Sp1 proteins in gel shift assays.

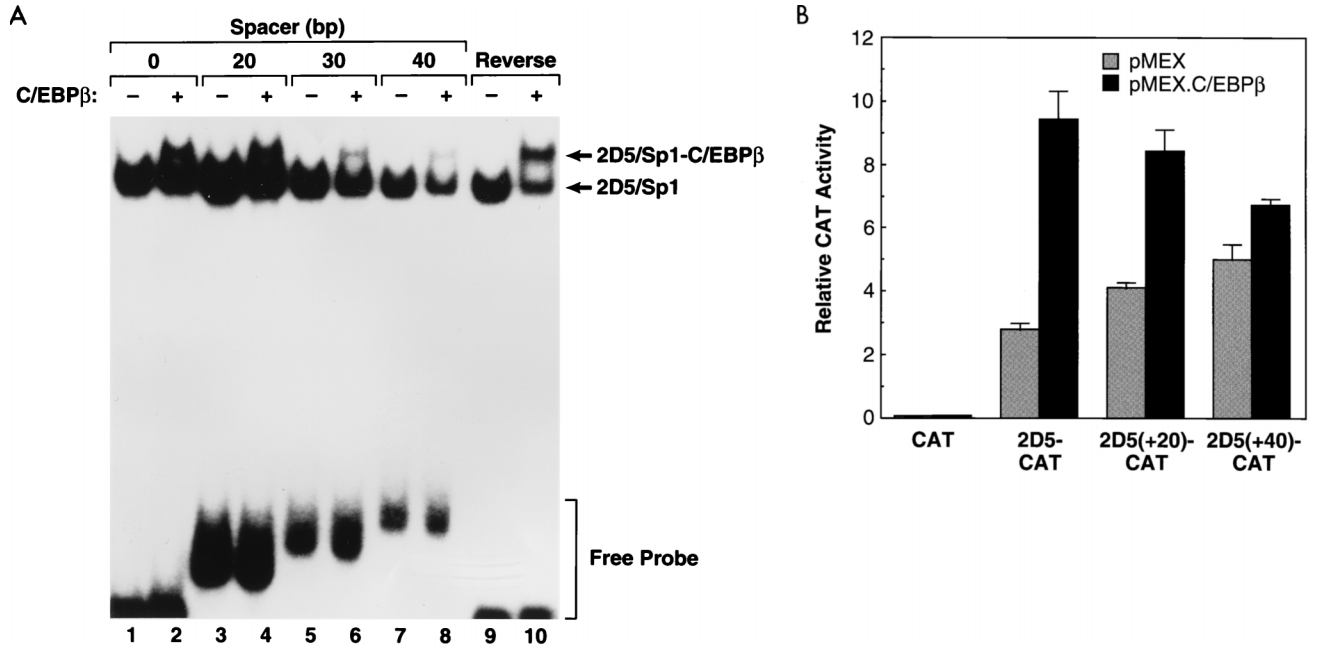

FIG. 1. Effects of altering spacing and orientation of Sp1 and C/EBP binding sites in the 2D5 promoter. (A) 2D5-Sp1-C/EBPβ ternary complex formation is reduced by increasing Sp1-C/EBP spacing. Human Sp1 protein (4 ng) and recombinant C/EBPβ were incubated with 0.1 ng of ^{32}P-labeled oligonucleotide probes containing various lengths of poly(dA) spacer between the 2D5 Sp1 and C/EBP sites and analyzed by gel mobility shift. In lanes 9 and 10 the order of the two sites was reversed. Oligonucleotides with spacer were ACTGAACCTCACTGGCCCTCCCT-$(A)_n$-ACACTTGACCAAACAAAAC; the oligonucleotide with reversed Sp1-C/EBP sites was CCCTACACTTGACCAACCAAACCTCACTGGCCCTCCCTACAC. The Sp1 and C/EBP binding sites are underlined. + and −, presence and absence of C/EBPβ in the binding reactions, respectively. (B) Effects of spacing changes on C/EBPβ transactivation of the 2D5 promoter. The indicated spacing mutations were introduced into the 2D5 proximal promoter (−120 to +73) and inserted upstream of CAT. These reporter genes (5 μg) were transfected into HepG2 cells in the presence and absence of 15 μg of the C/EBPβ expression vector pMEX-C/EBPβ. The control transfections included 15 μg of the pMEX expression vector, and all transfections included a luciferase vector (pSV232AL.A_5′) as an internal standard. Cells were harvested 40 h after transfection, and the CAT and luciferase activities were measured. The CAT activity for each sample was normalized to luciferase to control for transfection efficiency. The data are averages of three independent experiments.

Ternary complex formation was not observed with the separated binding sites (data not shown), indicating that cooperativity requires binding of both proteins to a single DNA molecule. In addition, we could detect no association between Sp1 and C/EBPβ in coimmunoprecipitation experiments (data not shown). These results suggest that interactions between the two proteins occur only upon binding to adjacent sites on DNA.

We also tested the effects of the spacing changes on transactivation by C/EBPβ in HepG2 cells. Figure 1B shows that increasing the distance between the Sp1 and C/EBP sites caused a modest (twofold at most) increase in the basal level of expression from transfected 2D5-CAT reporter genes. However, when the same reporters were cotransfected with a C/EBPβ expression vector, the increase in transcription elicited by C/EBPβ diminished as a function of increasing spacer length. Thus, while wild-type 2D5-CAT was activated fourfold by C/EBPβ, CAT expression from 2D5(+40)-CAT was essentially unaffected by cotransfecting the activator. Taken together, the results in Fig. 1 demonstrate that synergistic interactions between Sp1 and C/EBPβ occur only when their binding sites are closely juxtaposed.

Cooperativity between Sp1 and C/EBPβ involves DNA binding and transcriptional activation. We next examined the requirements for cooperativity between the Sp1 and C/EBPβ binding sites in transfected HepG2 cells. As shown in Fig. 2A, C/EBPβ transactivated the proximal 2D5 promoter (residues −120 to +73) fused to the CAT reporter gene nearly fourfold, whereas C/EBPα had no effect on CAT expression. Mutating the Sp1 site (2D5-M1) reduced basal transcription from the 2D5-CAT construct and eliminated transactivation by C/EBPβ, while altering the cryptic C/EBP site (2D5-M2) did not affect basal transcription but abolished stimulation by C/EBPβ. These data confirm our previously reported results (22).

The observations that human Sp1 recruits C/EBPβ to the cryptic C/EBP site on the 2D5 promoter in vitro and that C/EBPβ fails to transactivate the 2D5 promoter in vivo when the Sp1 site is mutated imply that C/EBPβ-mediated activation of the 2D5 gene requires an Sp1 factor bound to the promoter. These results raise the question of whether the role of Sp1 is simply to recruit C/EBPβ to the DNA template or whether Sp1 and C/EBPβ also cooperate in transcriptional activation. To address this issue, the cryptic 2D5 C/EBP binding site was converted to a high-affinity C/EBP site by site-directed mutagenesis. As shown in Fig. 2A, a 2D5 promoter bearing the high-affinity C/EBP site in combination with a mutated Sp1 site (2D5-M5) was not significantly transactivated by either C/EBPβ or C/EBPα in HepG2 cells. However, when placed immediately upstream of the mouse albumin core promoter (2D5/M5-alb-CAT), the high-affinity C/EBP site was transactivated by both C/EBP proteins but more efficiently by C/EBPα (Fig. 2A, right panel). The level of transactivation was similar to that observed for the C/EBP site from the albumin proximal promoter (DEI-alb-CAT) (36). These results indicate that the sequence context of a C/EBP site, even if it is a high-affinity site, is critical for its ability to mediate transactivation by C/EBP proteins. Moreover, the fact that binding of C/EBPβ to the 2D5/M5 promoter fails to activate transcription suggests

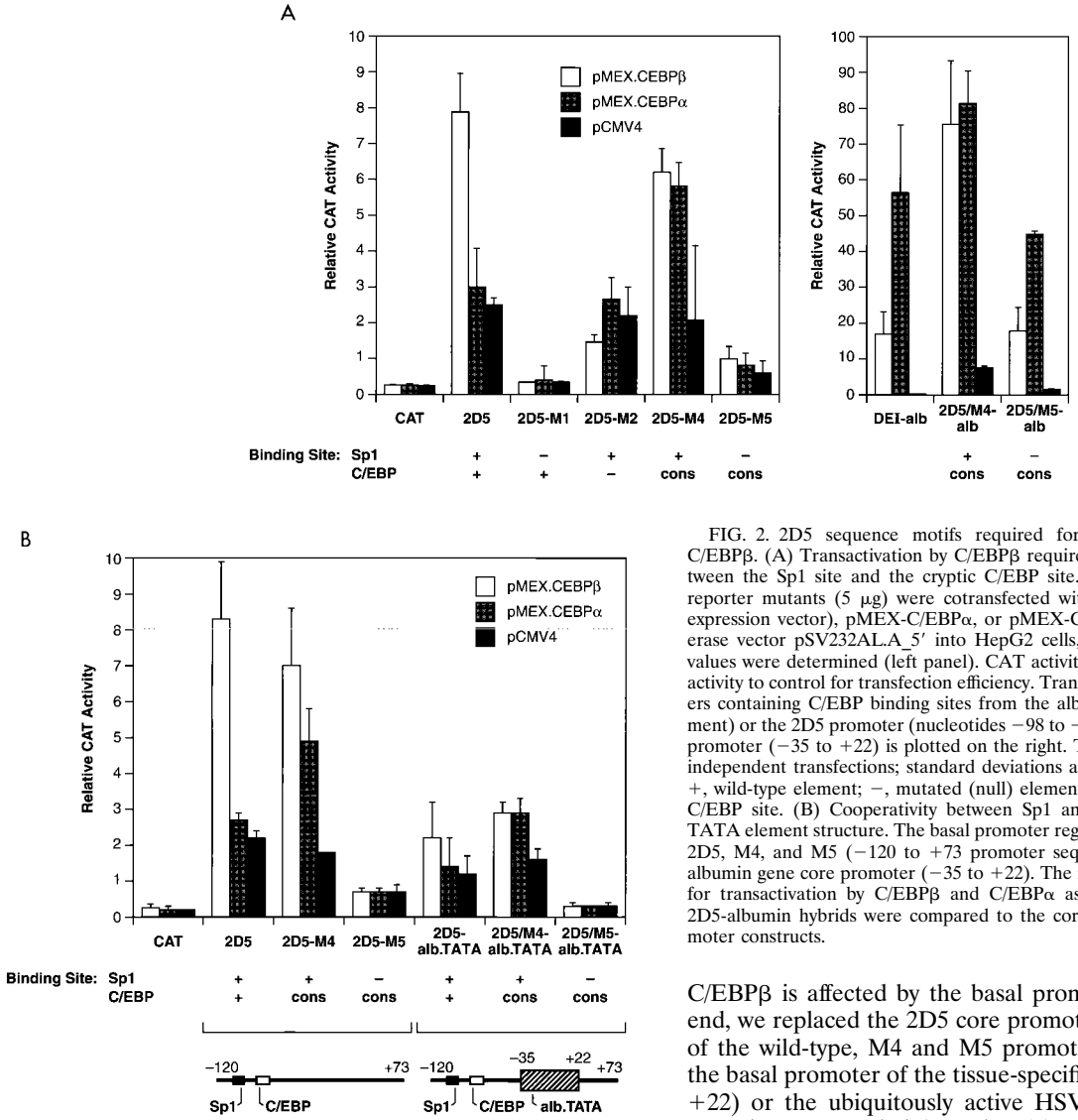

FIG. 2. 2D5 sequence motifs required for selective transactivation by C/EBPβ. (A) Transactivation by C/EBPβ requires cooperative interactions between the Sp1 site and the cryptic C/EBP site. Various 2D5 promoter-CAT reporter mutants (5 μg) were cotransfected with 15 μg pCMV-4 (an empty expression vector), pMEX-C/EBPα, or pMEX-C/EBPβ and 1 μg of the luciferase vector pSV232AL.A_5′ into HepG2 cells, and the CAT and luciferase values were determined (left panel). CAT activity was normalized to luciferase activity to control for transfection efficiency. Transactivation of artificial promoters containing C/EBP binding sites from the albumin promoter (the DEI element) or the 2D5 promoter (nucleotides −98 to −80) fused to the albumin basal promoter (−35 to +22) is plotted on the right. The data are averages of three independent transfections; standard deviations are indicated by the error bars. +, wild-type element; −, mutated (null) element; cons, consensus high-affinity C/EBP site. (B) Cooperativity between Sp1 and C/EBPβ is independent of TATA element structure. The basal promoter regions (−35 to +22) of wild-type 2D5, M4, and M5 (−120 to +73 promoter sequences) were replaced by the albumin gene core promoter (−35 to +22). The resultant reporters were tested for transactivation by C/EBPβ and C/EBPα as described for panel A. The 2D5-albumin hybrids were compared to the corresponding parental 2D5 promoter constructs.

that, in addition to its ability to facilitate binding of C/EBPβ, Sp1 synergizes with C/EBPβ at the transcriptional level.

C/EBPβ activated transcription equivalently from 2D5-CAT and 2D5/M4-CAT, which contains the high-affinity C/EBP site and the wild-type Sp1 site (Fig. 2A). C/EBPα also stimulated transcription from 2D5/M4-CAT, whereas it failed to activate the wild-type 2D5 promoter. C/EBPβ was less active than C/EBPα on the DEI-alb-CAT and 2D5/M5-alb-CAT reporters, in which the Sp1 binding site is either absent or mutated, respectively. However, C/EBPβ and C/EBPα were equally effective in transactivating 2D5/M4-CAT and 2D5/M4-alb-CAT. We conclude from these data that C/EBPα is able to synergize with Sp1 to elicit transcriptional activation provided that a high-affinity C/EBP site is present. However, C/EBPβ cooperates with Sp1 to enhance 2D5 expression even when the promoter contains the normal cryptic C/EBP site, suggesting that an Sp1 factor can recruit C/EBPβ, but not C/EBPα, to this site.

It has been reported that the TATA box and other core promoter elements can contribute to the specificity and efficiency of transcriptional activation (13, 31). We therefore sought to determine whether cooperativity between Sp1 and C/EBPβ is affected by the basal promoter sequence. To this end, we replaced the 2D5 core promoter region (−35 to +22) of the wild-type, M4 and M5 promoter-CAT constructs with the basal promoter of the tissue-specific albumin gene (−35 to +22) or the ubiquitously active HSV thymidine kinase (*tk*) gene (−36 to +51) (Fig. 2B). Although the overall levels of transactivation were reduced for these constructs, neither the albumin nor the *tk* (data not shown) core promoter altered the cooperativity between C/EBPβ and Sp1, demonstrating that the basal promoter sequence is not critical for C/EBPβ-Sp1 synergism.

Cooperativity between Sp1 and C/EBPβ in *Drosophila* cells. To further investigate cooperativity between Sp1 and C/EBPβ, we employed a transfection assay using *Drosophila* SL-2 tissue culture cells. *Drosophila* cells lack endogenous Sp1 activity (4) and therefore can be used to test the requirement for Sp1 in activating 2D5 transcription and to examine the effects of mutations in the Sp1 protein. Human Sp1 was expressed in *Drosophila* cells from the vector pPacSp1 (4), in which Sp1 transcription is driven by the *Drosophila* actin 5C promoter/enhancer. C/EBPβ and C/EBPα were expressed from the related vector pGac.

Potential cooperativity between C/EBPβ and Sp1 was examined in SL-2 cells by cotransfecting the expression vectors for C/EBPβ and Sp1 along with various 2D5 promoter-CAT reporters. As shown in Fig. 3, Sp1 and C/EBPβ transactivated the 2D5-CAT reporter in a synergistic manner. C/EBPβ alone did not transactivate, while Sp1 stimulated 2D5-CAT expression

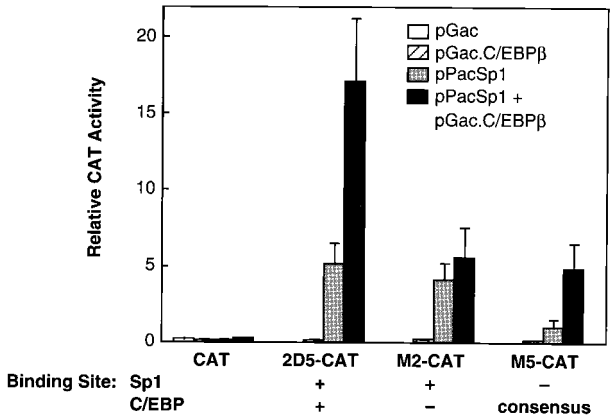

FIG. 3. Synergism between Sp1 and C/EBPβ in *Drosophila* cells. Two micrograms of the indicated 2D5 promoter-CAT reporters was cotransfected into SL-2 cells with pPacSp1 (100 ng), pGac.C/EBPβ (2 μg), or both, and the total amount of DNA was adjusted to 6 μg with pGac. Forty to forty-eight hours after transfection, lysates were prepared and assayed for CAT activity. Relative CAT activity was calculated as percent conversion of [^{14}C]chloramphenicol per milligram of cell lysate protein. The data are averages of three independent transfections. The M2 and M5 mutants are described in the legend to Fig. 2. Expression of the C/EBPβ protein in *Drosophila* cells was confirmed by immunoblotting analysis (data not shown).

approximately 25-fold. However, when cotransfected with the Sp1 vector, C/EBPβ caused a greater than threefold increase in wild-type 2D5-CAT expression compared to Sp1 alone. In cells transfected with 2D5/M2-CAT (mutant C/EBP binding site) and pPacSp1, no significant increase in promoter activity resulted from coexpressing C/EBPβ. The ability of Sp1 to activate 2D5/M5-CAT (mutant Sp1 binding site) was noticeably reduced compared to the wild-type promoter but not completely abolished. This low level of activity was again enhanced nearly fivefold by cotransfecting pGac.C/EBPβ. These results demonstrate that cooperative interactions occur between the two activators in *Drosophila* cells.

Glutamine- and serine/threonine-rich activation domains of Sp1 are required for cooperativity with C/EBPβ. We next tested various Sp1 mutants for their abilities to activate the 2D5 promoter cooperatively with C/EBPβ. As depicted in Fig. 4, Sp1 contains a zinc finger DNA-binding domain and four activation domains, designated A, B, C, and D (4). Sp1 expression plasmids bearing deletions of one or two of these domains (kindly provided by R. Tjian) were cotransfected with 2D5-CAT into SL-2 cells, with or without pGac.C/EBPβ (Fig. 4A). By themselves, all of the activation domain deletion mutants were poorer activators of 2D5 than wild-type Sp1. However, when tested for synergism with C/EBPβ, some of the mutants exhibited equivalent or greater activity than wild-type Sp1. For

A

	Fold Activation		Fold Enhancement
	– C/EBPβ	+ C/EBPβ	
None	1.0	1.1±0.1	1.1
Sp1	18.0±2.2	80.0±9.0	4.7
N636	2.6±0.5	24.0±8.4	9.2
N539	1.1±0.1	1.1±0.0	0.9
Δint162	3.7±0.9	21.1±9.0	5.7
Δint349	4.2±0.6	22.0±9.0	5.2
516C	2.5±0.2	5.7±0.6	2.3

B

	Fold Activation		Fold Enhancement
	– C/EBPβ	+ C/EBPβ	
None	1.0	1.0±0.1	1.0
Sp1	14.3±2.3	59.8±4.1	4.2
Δint349	2.4±0.1	11.7±0.3	4.9
516CΔint112	0.9±0.2	2.1±0.1	2.3
327C	0.8±0.1	1.1±0.2	1.2
168C	1.0±0.2	0.9±0.1	0.9

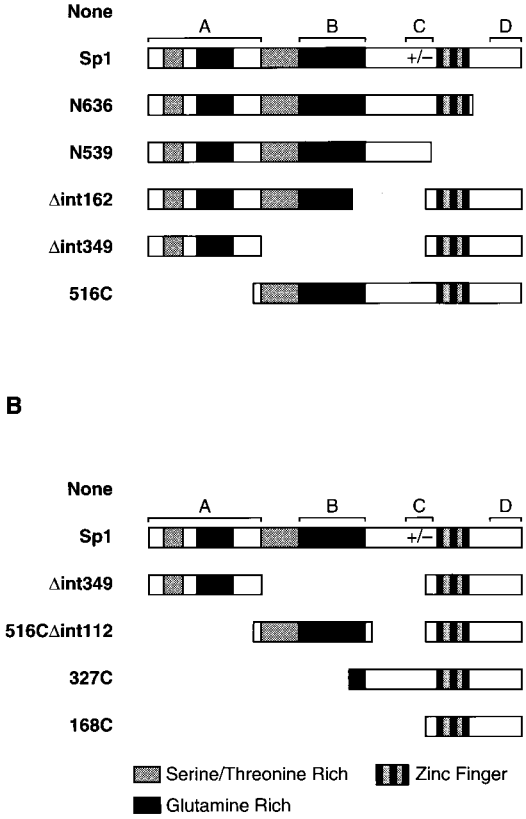

FIG. 4. Two serine/threonine and glutamine-rich activation domains of Sp1 are required for cooperativity with C/EBPβ. Expression plasmids containing Sp1 deletion mutants (300 ng) (4) and the 2D5 promoter-CAT reporters (2 μg) were cotransfected into *Drosophila* SL-2 cells, with or without the C/EBPβ expression vector. The transcriptional activity of the 2D5-CAT reporter alone was assigned a value of 1, and the CAT activities after cotransfection with the Sp1 vectors were normalized accordingly (fold activation). The data are averages of at least four independent transfections ± standard errors. Fold enhancement is the increase in CAT activity resulting from cotransfection of C/EBPβ.

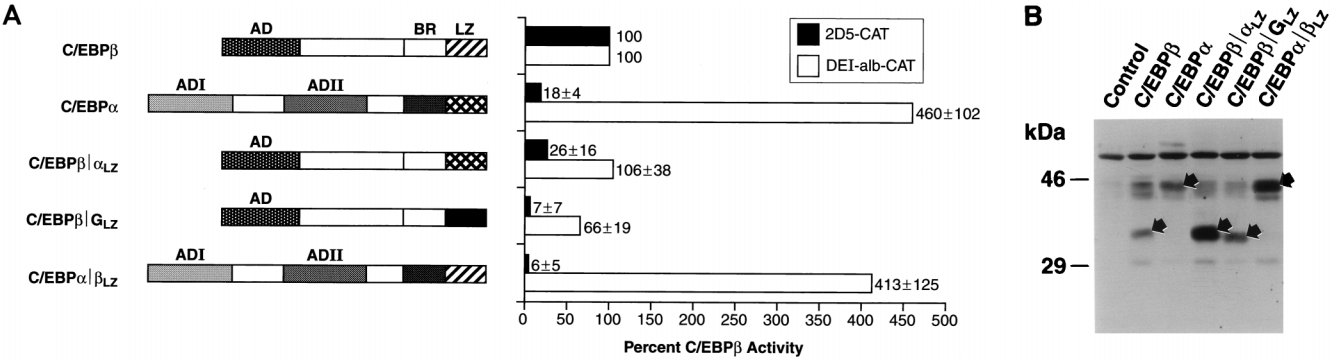

FIG. 5. The C/EBPβ leucine zipper is specifically required for transactivation of the 2D5 promoter. Expression plasmids for C/EBPβ, C/EBPα, or leucine zipper swap mutants (15 μg) were cotransfected with 2D5-CAT or DEI-alb-CAT reporters (5 μg) and 1 μg of pSV232AL.A_5′ into HepG2 cells, and the CAT-luciferase values were determined (A). The activity of wild-type C/EBPβ was assigned a value of 100%, and the activities of the mutants were normalized accordingly. The data are averages of four independent transfections ± standard errors. AD, activation domain; BR, basic region; LZ, leucine zipper; α_{LZ}, C/EBPα LZ; G_{LZ}, GCN4 LZ; β_{LZ}, C/EBPβ LZ. (B) Equivalent amounts of the transfected cell extracts (approximately 10 μg of protein) were analyzed by immunoblotting using the panCRP antibody (36), which recognizes an epitope in the basic region common to all C/EBP proteins.

example, the activity of a D-domain deletion mutant (N636) was more strongly enhanced (9.2-fold) by coexpression of C/EBPβ than wild-type Sp1, which was stimulated 4.7-fold. Mutants lacking the C (Δint162) or B plus C (Δint349) domains showed normal levels of cooperativity with C/EBPβ (5.7- and 5.2-fold, respectively). Removal of the A domain (516C) decreased cooperativity to 50% of that of intact Sp1, suggesting that the A activation domain is involved in transcriptional synergy with C/EBPβ. As expected, removing the zinc finger DNA-binding domain (N539) completely abolished transactivation of the 2D5 promoter as well as any cooperativity with C/EBPβ.

We also tested Sp1 proteins consisting of the zinc finger region fused to single activation domains (or combinations of domains) for cooperativity with C/EBPβ (Fig. 4B). The experiments with Sp1 deletion mutants (Fig. 4A) suggested that the C and D activation domains are not involved in cooperativity with C/EBPβ. In accordance with this result, the D (168C) and C plus D (327C) domains did not mediate synergistic transactivation with C/EBPβ. However, the A domain (Δint349) retained full cooperativity with C/EBPβ, and the B domain (516CΔint112) showed moderate synergism (2.3-fold). The latter protein exhibited activity similar to that of the deletion mutant 516C (Fig. 4A), which includes the B, C, and D domains. Collectively, the data of Fig. 4 indicate that the serine/threonine- and glutamine-rich activation domains A and B (4), and especially domain A, mediate functional synergism with C/EBPβ in *Drosophila* cells.

Identification of C/EBPβ domains required for transactivation of the 2D5 promoter in HepG2 cells. The ability of C/EBPβ but not C/EBPα to transactivate the 2D5 promoter in HepG2 cells presumably arises from differences in specific domains that mediate direct or indirect interactions with an Sp1 factor. To identify the domain(s) of C/EBPβ that confers this capability, we tested several mutant C/EBPβ proteins for their abilities to transactivate 2D5-CAT in HepG2 cells. The mutants included various deletions as well as chimeras containing domains from C/EBPα and other activator proteins. To assess the effects of the mutations on intrinsic (i.e., Sp1-independent) transcriptional activity, we also tested each protein for its ability to transactivate DEI-alb-CAT.

We first asked whether the C/EBPβ leucine zipper domain is involved in synergism with Sp1 by replacing it with either the C/EBPα zipper or the zipper from the yeast bZIP protein GCN4. A C/EBPβ chimera bearing the C/EBPα leucine zipper (C/EBPβ|α_{LZ}) was a strong activator of DEI-alb-CAT (6% greater than wild-type C/EBPβ) but exhibited only 26% of wild-type C/EBPβ activity on 2D5-CAT (Fig. 5). Similarly, replacement of the C/EBPβ zipper with the GCN4 zipper (C/EBPβ|G_{LZ}) decreased its ability to activate 2D5-CAT (7% of the wild-type level), while transactivation of DEI-alb-CAT was largely unaffected (66% of the wild-type level). These results show that the leucine zipper of C/EBPβ is essential for its ability to function cooperatively with Sp1. However, a reciprocal chimera consisting of C/EBPα and the C/EBPβ leucine zipper (C/EBPα|β_{LZ}) was unable to stimulate expression of 2D5-CAT. This protein was otherwise fully functional since, like wild-type C/EBPα, it transactivated DEI-alb-CAT efficiently (fourfold greater activity than C/EBPβ). We conclude that the C/EBPβ zipper is necessary but not sufficient for synergism with Sp1.

To identify additional C/EBPβ sequences that are necessary for cooperativity with Sp1, we analyzed a set of deletion mutations in C/EBPβ (Fig. 6A), as well as C/EBPβ|C/EBPα chimeras in which sequences external to the bZIP domain were exchanged (Fig. 6C). Figure 6A shows that the tripartite activation domain (AD) located at the C/EBPβ N terminus (19, 36) is essential for transactivation of the 2D5 promoter. An N-terminal deletion removing the AD (83 to 276) eliminated transactivation of both 2D5-CAT and DEI-alb-CAT. Constructs containing portions of the AD region [β(43-276) and β(1-47)|DBD] yielded 40% of wild-type activity, while proteins retaining the full AD [β(1-83)|DBD] or AD modules 2 and 3 [β(31-83)|DBD] were nearly fully active, even though the latter protein transactivated DEI-alb-CAT poorly. A chimera in which residues 48 to 83 were replaced by the VP16 AD (β|VP16) exhibited only 27% of wild-type activity on 2D5-CAT. Thus, the AD region, particularly the segment between amino acids 31 and 83 (modules 2 and 3) (36), is necessary for transactivation of the 2D5 promoter.

This conclusion was confirmed and extended by the experiments of Fig. 6C, which demonstrate that the N-terminal AD of C/EBPα (AD-I) is unable to substitute for the C/EBPβ AD in the 2D5 transactivation assay. Although the C/EBPα and C/EBPβ AD regions show significant sequence conservation (19, 26, 36), none of the chimeras containing AD-I from C/EBPα caused appreciable stimulation of the 2D5 reporter. However, most of these hybrid proteins activated DEI-alb-CAT as well as or better than C/EBPβ, showing that they are not generally defective in transactivation. For example, the

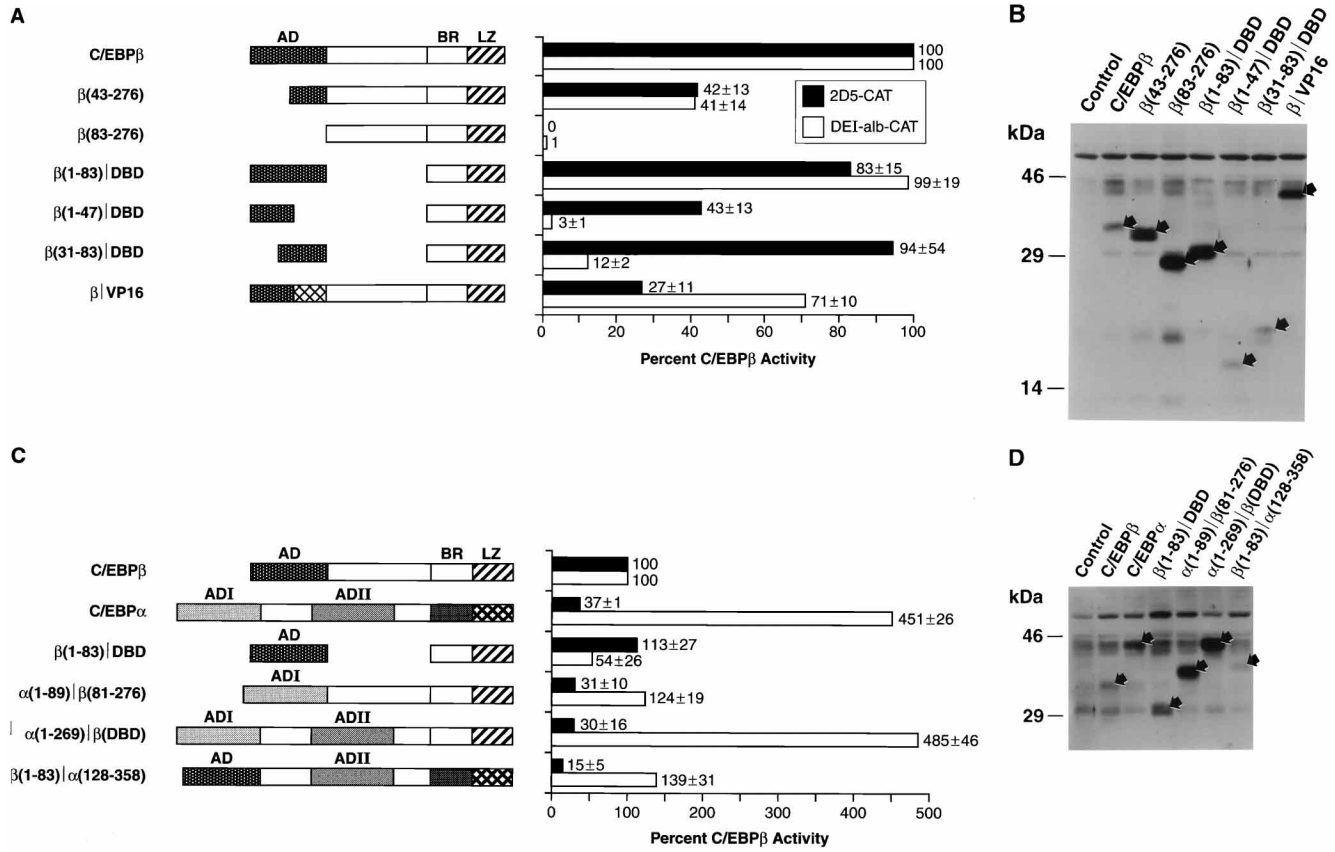

FIG. 6. Analysis of C/EBPβ deletion mutants and C/EBPα-C/EBPβ chimeras. Deletion mutants of C/EBPβ (A) and hybrid proteins composed of C/EBPα and C/EBPβ domains (C) were tested for transactivation of the 2D5-CAT or DEI-alb-CAT reporter genes as described for Fig. 5. β|VP16 is a hybrid protein in which the core activation domain of C/EBPβ (residues 48 to 83) was replaced with the acidic activation domain of HSV VP16. The data are averages of three independent experiments ± standard errors. Expression of the C/EBP proteins was analyzed by Western blotting (B and D) as described for Fig. 5.

chimera α(1-89)|β(81-276) displayed only 31% of C/EBPβ activity on 2D5-CAT yet was 24% more active than C/EBPβ on DEI-alb-CAT. Proteins retaining both AD-I and AD-II of C/EBPα were four- to fivefold more active than C/EBPβ on the DEI-alb-CAT reporter yet failed to stimulate 2D5. Thus, while the presence of AD-II increases the overall transcriptional activity of C/EBPα severalfold, it does not confer the ability to transactivate 2D5. We conclude that the C/EBPβ AD, in addition to the leucine zipper, is absolutely required for enhancement of the 2D5 promoter.

Liver-specific expression of a murine *CYP2D5* homolog is diminished in C/EBPβ-deficient mice. The experiments presented thus far show that the rat *CYP2D5* promoter is selectively activated by C/EBPβ but not C/EBPα in transfected hepatoma cells. To demonstrate that C/EBPβ is required for *CYP2D5* expression in vivo, we analyzed liver-specific transcription of a murine *CYP2D5*-related gene, *CYP2D11* (38), in mice bearing a homozygous null mutation at the *c/ebp*β locus. Construction of *c/ebp*β-deficient mice will be described elsewhere (33a); *c/ebp*β knockout mice have also been reported previously by Tanaka et al. (34) and Screpanti et al. (32).

Figure 7A shows a sequence alignment of the rat *CYP2D5* and murine *2D11* promoters. Although the sequences of the cryptic C/EBP sites in the *2D11* and *CYP2D5* promoters differ somewhat, the overall features of this region, including the presence of an adjacent Sp1 site and the nucleotide sequence extending to the transcription start site, are well conserved. To test whether Sp1 and C/EBPβ bind cooperatively to the *2D11* promoter, we performed an electrophoretic mobility shift assay using recombinant DNA-binding proteins and an oligonucleotide probe encompassing the putative Sp1 and C/EBPβ sites. Figure 7B shows that Sp1 binds to the *2D11* promoter and that recruitment of C/EBPβ to the promoter is dependent on Sp1. Indeed, binding of C/EBPβ to the 2D11 probe was more dependent on Sp1 than for the 2D5 probe; C/EBPβ alone did not bind to 2D11, whereas weak binding to 2D5 was detected (compare lanes 1 and 5). However, in the presence of Sp1, comparable amounts of the C/EBPβ-Sp1-DNA complex were formed with the two probes (compare lanes 4 and 8). These data demonstrate that Sp1 facilitates C/EBPβ binding to the 2D11 probe, indicating that the proximal promoter regions of the *CYP2D5* and *CYP2D11* genes contain functionally analogous Sp1 and C/EBP sites.

We next analyzed the liver expression of *2D11* mRNA in homozygous wild-type, heterozygous, and *c/ebp*$\beta^{-/-}$ mice. *2D11* mRNA was analyzed by Northern blotting using an oligonucleotide probe specific for the *2D11* coding region. *2D11* expression levels differed considerably between *c/ebp*$\beta^{-/-}$ and wild-type or heterozygous animals at 7 weeks after birth (Fig. 7C). The *c/ebp*β-deficient mouse showed nearly undetectable levels of *2D11* mRNA in the liver (lane 2), whereas its normal littermate expressed the gene efficiently (lane 1). Quantitation of the hybridization signals showed a 7.2-fold reduction in *2D11* mRNA in the *c/ebp*$\beta^{-/-}$ animal. A similar difference was observed at 2 months (9.7-fold; lanes 3 and 4); at three months

there was also a significant distinction between normal and mutant animals, although the magnitude of the effect was reduced (4.3-fold; lanes 5 and 6).

For comparison, we also examined the effect of the *c/ebp*β mutation on expression of the liver-specific albumin gene. The albumin promoter and enhancer regions contain C/EBP binding sites (reviewed in reference 16), and the promoter can be transactivated by both C/EBPα and C/EBPβ (5, 8, 37). Figure 7C shows that albumin mRNA levels are unchanged in *c/ebp*$\beta^{-/-}$ mice compared to their normal littermates. C/EBPβ is therefore dispensable for expression of the albumin gene. We conclude from Fig. 7 that efficient liver-specific transcription of the *CYP2D11* P-450 gene requires C/EBPβ. Furthermore, the effect on *CYP2D11* transcription is specific, since the absence of C/EBPβ does not lead to a general decrease in expression of hepatic mRNAs.

DISCUSSION

An important question in the study of transcriptional activators is how members of a family that display similar or identical DNA-binding specificities are able to regulate distinct sets of target genes. Our studies show that C/EBPβ can transactivate the *CYP2D5* gene promoter in HepG2 cells whereas the closely related C/EBPα protein cannot. This target specificity results from the different abilities of these two proteins to cooperate with an Sp1-related protein bound to an adjacent site in the promoter. Our data suggest that the mechanism of cooperativity involves both DNA binding and transcriptional activation. Moreover, both the activating region and the leucine zipper of C/EBPβ are necessary for transactivation of the 2D5 promoter in HepG2 cells. The different abilities of the two C/EBP proteins to activate this class of liver-specific P-450 genes in vivo were confirmed by the observation that *CYP2D11* mRNA levels are greatly diminished in *c/ebp*β-null mice. These results demonstrate that C/EBPα is unable to substitute for C/EBPβ in regulating a specific target gene.

Sp1-C/EBPβ synergism may involve both DNA-binding and transcriptional cooperativities. The idea that Sp1 facilitates C/EBPβ binding to the cryptic C/EBP site is suggested by three observations. First, C/EBPβ binding to the 2D5 oligonucleotide in vitro is greatly enhanced by addition of Sp1 (22) (Fig. 7B). Second, physical and functional cooperativities between C/EBPβ and Sp1 are adversely affected by increasing the distance between the two binding sites (Fig. 1). Third, changing the weak C/EBP site to a consensus C/EBP motif (mutant M4) increases the ability of C/EBPα to transactivate the 2D5 promoter but has no effect on transactivation by C/EBPβ. This result is consistent with the notion that an Sp1-related protein in HepG2 cells promotes C/EBPβ binding to the cryptic C/EBP site but does not facilitate C/EBPα binding.

A mutant 2D5 promoter containing a high-affinity C/EBP site and a defective Sp1 site (M5) is not transactivated by either C/EBP protein (Fig. 2A). Thus, dependence on Sp1 is observed even when C/EBPα and C/EBPβ presumably are able to bind to the 2D5 template autonomously. This result indicates that transcriptional synergy also occurs between Sp1 and C/EBPβ or C/EBPα. It should be noted that C/EBPα is a more potent transcriptional activator than C/EBPβ due to the presence of a second activation domain (AD-II) that is absent in C/EBPβ (Fig. 6). If one takes this intrinsic four- to fivefold difference in activity into account, the fact that C/EBPα and C/EBPβ transactivate the M4 mutant equally (Fig. 2A) may be misleading, and C/EBPβ may indeed be more efficient than C/EBPα in synergizing with Sp1 at the transcriptional level.

Although the properties of wild-type 2D5 and the M4 mutant in transactivation assays suggest that an Sp1 factor facilitates C/EBPβ binding to the promoter but does not promote C/EBPα binding, we have not observed differences between the two C/EBP proteins in forming ternary complexes with recombinant Sp1 in vitro (data not shown), in contrast to our previous observation (22). There are several possible reasons for the apparent inconsistency between the transfection and DNA-binding experiments. First, Sp1 itself may not be the factor that interacts with C/EBPβ in vivo. A family of Sp1-like proteins has been described (18), one of which could be responsible for C/EBPβ-specific transactivation of the 2D5 promoter in HepG2 cells. The possibility that Sp1 per se is not the relevant factor in mammalian cells is supported by the observation that human Sp1 synergizes with both C/EBPα and C/EBPβ in *Drosophila* cells and that an Sp1 antibody supershifts only a portion of 2D5-Sp1 complexes formed by using HepG2 nuclear extracts (22). Second, the specificity of interactions with Sp1 in vivo could be influenced by the assembly of 2D5 DNA into chromatin or by the presence of accessory factors that are absent from reactions using purified components. Third, productive 2D5-Sp1-C/EBPβ complexes might occur only with a heterodimeric C/EBPβ species, and C/EBPα may be unable to associate with this heterodimeric partner. This hypothesis is attractive because it would explain the inability of the C/EBPα or GCN4 leucine zipper to substitute for the C/EBPβ zipper in 2D5 transactivation assays. At present we cannot distinguish among the three models proposed here, nor are the possibilities mutually exclusive.

The activation and leucine zipper domains of C/EBPβ are essential for 2D5 transactivation. Perhaps our most unexpected finding is that C/EBPβ chimeras bearing either the leucine zipper or activation domain I from C/EBPα are completely unable to activate 2D5-CAT expression. These results are especially surprising because these regions exhibit strong sequence similarity between C/EBPα and C/EBPβ (16). Nonetheless, our studies reveal clear functional differences between the C/EBPα and C/EBPβ dimerization and activation domains. As discussed above, the leucine zipper effect could result from the different abilities of the α and β zippers to dimerize with an unknown bZIP partner. C/EBPα-C/EBPβ chimeras fused at different positions in the zipper exhibited intermediate phenotypes (22a), suggesting that there is not a specific segment in the C/EBPβ zipper that mediates the ability of this protein to transactivate 2D5. This result tends to support the heterodimer model. Irrespective of the mechanism, the 2D5 promoter specificity conferred by the C/EBPβ leucine zipper is, to our knowledge, the first example in which a zipper region has been shown to determine differences in target gene selectivity among members of an activator protein family.

How does the C/EBPβ activation domain contribute to 2D5 specificity? It is conceivable that C/EBPα AD-I is unable to substitute for the C/EBPβ AD because these two sequences differ slightly in their targets within the transcriptional initiation complex or associate with different coactivators (40). The nature of these protein-protein contacts would then determine whether transcriptional synergism with Sp1 occurs. It is also possible that the AD region of C/EBPβ physically contacts the Sp1 factor and that this interaction stimulates the ability of one or both proteins to activate transcription. Although we were unable to detect stable interactions between C/EBPβ and Sp1 from rat liver in the absence of DNA (data not shown), this result does not rule out the possibility that C/EBPβ is associated with a different Sp1-related factor in hepatic cells or that binding to DNA is a prerequisite for this interaction.

Synergism between C/EBPβ and Sp1. Transactivation experiments in mammalian and *Drosophila* cells demonstrate that

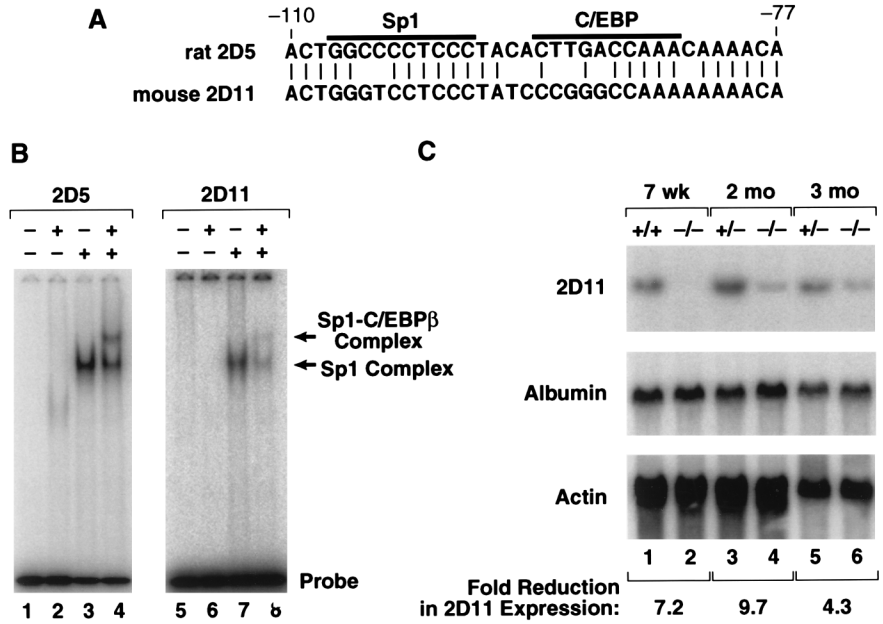

FIG. 7. Liver-specific expression of the murine *CYP2D5* homolog, *CYP2D11*, is diminished in C/EBPβ-deficient mice. (A) Sequence comparison of promoter regions from rat *CYP2D5* and mouse *CYP2D11* (38). Positions of the Sp1 and C/EBP binding sites in the 2D5 sequences are indicated. (B) Cooperative binding of C/EBPβ and Sp1 to the *CYP2D11* promoter. Recombinant human Sp1 (15 ng) and/or rat C/EBPβ (320 ng) was incubated with ^{32}P-labeled, double-stranded oligonucleotide probes containing the Sp1-C/EBP regions of 2D11 (lanes 5 to 8) or 2D5 (lanes 1 to 4). DNA-protein complexes were analyzed by electrophoresis on 4% polyacrylamide gels. (C) *2D11* and albumin expression in normal and *c/ebp*β$^{-/-}$ mice. Total liver RNA was prepared from littermates at the indicated ages after birth. Twenty micrograms of RNA was analyzed by Northern blotting using an oligonucleotide probe specific for the *2D11* gene. Signals were scanned and quantitated with a PhosphorImager. The blot was stripped and reprobed for actin mRNA as an internal control. For each pair of animals, the *2D11*/actin ratio was determined and used to calculate the fold decrease in *2D11* expression in C/EBPβ$^{-/-}$ mice compared to wild-type or heterozygous littermates. The same RNA samples were also analyzed for albumin mRNA levels by using an oligonucleotide probe complementary to a segment of the coding region.

Sp1 is capable of synergizing with C/EBPβ to activate 2D5 transcription. Previous studies indicate that C/EBP proteins can function synergistically with other transcription factors as well, including NF-κB proteins. C/EBP proteins physically interact with the p50 subunit of NF-κB (21, 33), and in the case of C/EBPβ, this association requires the integrity of the leucine zipper domain (21). C/EBP and NF-κB binding sites occur in close proximity within several promoters, particularly those of proinflammatory cytokine genes (1). Efficient induction of the interleukin-6 (IL-6) promoter by lipopolysaccharide requires binding sites for both C/EBP and NF-κB (41), and C/EBPβ (NF-IL6) and NF-κB have been shown to transactivate the IL-6 promoter in a synergistic fashion (24). Thus, certain similarities exist between C/EBPβ–NF-κB interactions and those we have observed for C/EBPβ and Sp1.

Sp1 is also capable of interacting with multiple activator proteins. For example, the erythroid-specific factor GATA-1 synergizes with Sp1 and with the related Krüppel family protein EKLF to activate transcription of target promoters (7, 25). GATA-1 also physically interacts with these proteins in vitro. Furthermore, in *Drosophila* cells Sp1 is capable of recruiting GATA-1 to a promoter lacking GATA binding sites. Perkins et al. (28) observed that efficient activation of transcription from the human immunodeficiency virus type 1 long terminal repeat requires both NF-κB and Sp1 binding sites, which occur in close proximity on the promoter. Synergism between NF-κB p65 and Sp1 involves cooperative DNA binding, and physical association between the two proteins occurs via their respective DNA-binding domains (27, 28). These and other examples illustrate that ubiquitous Sp1 factors can serve as essential coregulators of inducible or developmentally controlled genes by collaborating with cell-specific transcription factors.

C/EBPα and C/EBPβ are not functionally redundant. The different abilities of C/EBPα and C/EBPβ to activate the *CYP2D5* promoter demonstrate that members of a family possessing similar DNA-binding specificities can regulate distinct, or at least partially overlapping, sets of target genes. Although one reason for the existence of multiple *c/ebp* genes could be to increase the complexity of expression of C/EBP proteins, our results demonstrate that the proteins also exhibit functional diversity. In theory, these functional differences serve to expand the potential repertoire of genes activated by C/EBP proteins because differential cooperative interactions with other factors increase the combinatorial possibilities for transcriptional regulation (17, 20).

One principle illuminated by our studies of *CYP2D5* gene regulation is the importance of weak protein-DNA interactions in establishing cooperative relationships between activator proteins. The existence of a cryptic C/EBP site in the 2D5 region appears to be critical for differential transactivation of the promoter by C/EBPα and C/EBPβ, since the differences between the two proteins are much less apparent when the site is converted to a consensus C/EBP motif. Interestingly, most naturally occurring C/EBP sites are not perfect matches to the consensus recognition sequence (reviewed in reference 16). Lower-affinity sites may predominate in promoters because they increase the dependence on adjacent transcription factors and thereby enhance cooperative interactions between activator proteins.

***CYP2D11* gene expression is diminished in C/EBPβ-deficient mice.** It has been proposed that one function of C/EBPβ in liver is to mediate the induction of genes encoding acute-phase plasma proteins such as C-reactive protein and serum amyloid A, which are expressed in response to infection or

trauma (reviewed in reference 29). The finding that targeted disruption of the *c/ebp*β gene greatly reduces expression of the murine *CYP2D5* homolog, *CYP2D11* (Fig. 7), demonstrates that C/EBPβ also controls the transcription of developmentally regulated genes in hepatic cells. It is now of interest to determine whether other cytochrome P-450 genes require C/EBPβ for their expression in hepatocytes. Our data also show that the serum albumin gene is not a C/EBPβ target gene, as its expression was unaffected in *c/ebp*β-null mice. Surprisingly, both C/EBPβ and HNF1, each of which can bind to and transactivate the albumin promoter, have now been found to be dispensable for albumin expression in vivo (30). These results emphasize the importance of confirming information obtained in mammalian cell culture experiments by analyzing the effects of targeted gene disruptions in animal models.

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