CCAAT/Enhancer-binding Protein α Is Required for Transcription of the β₃-Adrenergic Receptor Gene during Adipogenesis*

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The β₃-adrenergic receptor (β₃AR) is expressed predominantly in adipocytes, and it plays a major role in regulating lipolysis and adaptive thermogenesis. Its expression in a variety of adipocyte cell models is preceded by the appearance of CCAAT/enhancer-binding protein α (C/EBPα), which has been shown to regulate a number of other adipocyte-specific genes. Importantly, it has been demonstrated that several adipocyte cell lines that fail to express C/EBPα exhibit reduced insulin sensitivity, despite an apparent adipogenic phenotype. Here we show that transcription and function of the β₃AR correlates with C/EBPα expression in these adipocyte models. A 5.15-kilobase pair fragment of the mouse β₃AR promoter was isolated and sequenced. This fragment conferred a 50-fold increase in luciferase reporter gene expression in adipocytes. Two putative C/EBP binding sites exist at −3306 to −3298 and at −1462 to −1454, but only the more distal site is functional. Oligonucleotides corresponding to both the wild-type and mutated −3306 element were inserted upstream of a thymidine kinase luciferase construct. When cotransfected in fibroblasts with a C/EBPα expression vector, reporter gene expression increased 3-fold only in the wild-type constructs. The same mutation, when placed into the intact 5.15-kilobase pair promoter, reduced promoter activity in adipocytes from 50-fold to <10-fold. Electrophoretic mobility shift analysis demonstrated that the site at −3306 generated a specific protein-oligonucleotide complex that was supershifted by C/EBPα antibody, while a probe corresponding to a putative site at −1462 did not. These results define C/EBPα as a key transcriptional regulator of the mouse β₃AR gene during adipogenesis.

The β₃-adrenergic receptor (β₃AR) is a unique member of the βAR family because, unlike the β₁AR and β₂AR, it is expressed predominantly in adipocytes and regulates both lipolysis and nonsnghier thermogenesis (reviewed in Ref. 1). In genetic and dietary models of obesity, progressive accumulation of adipose tissue is associated with defects in the ability of catecholamines to mobilize lipid stores (2–4). We have previously shown that the expression and function of the adipocyte βARs are blunted in most models of obesity (5, 6). Nevertheless, a curious aspect of β₃AR biology is that, despite defects in β₃AR expression and function, selective agonists for this receptor have been shown to prevent or reverse obesity (4, 7–10). The efficacy of these drugs is related to increased brown adipose tissue thermogenesis and a restoration of expression of the β₃AR and β₁AR in white adipose tissue depots (4). For these reasons it is important to understand the tissue-specific and hormonal factors that regulate the expression of this receptor.

Two groups of transcription factors are known to be responsible for initiating and maintaining adipocyte differentiation: the CCAAT/enhancer-binding proteins (C/EBP) (11–17) and PPARs (18–20). From a large body of work in model adipocyte cell lines, such as 3T3-L1, it has been shown that the C/EBPs are expressed in a cascade-like fashion during the early stages of adipocyte differentiation, with C/EBPβ and C/EBPδ preceding the appearance of C/EBPα (15, 21). More recent studies indicate that the expression of the adipogenic transcription factor PPARγ is partially under the control of the C/EBP family of transcription factors and vice versa (22, 23). Additionally, insulin-sensitive glucose uptake has been shown to be impaired in adipogenic cells that lack C/EBPα, due to deficits in insulin receptor and insulin receptor substrate-1 (22, 24). Like other adipocyte-specific genes, the β₃AR is not expressed in preadipocytes, but appears late in the adipogenic program of both white and brown adipocytes (Refs. 25 and 26; this report). Because of the pivotal role of C/EBPs in activating many adipocyte-specific genes, the focus of our studies was to determine the role of C/EBPα in initiating β₃AR gene transcription during adipogenesis.

EXPERIMENTAL PROCEDURES

Materials—Materials were obtained from the following sources: dexmethylasone, 3-isobutyl-1-methylxanthine, insulin, puromycin, benzamidine, poly(dI/dC), and soybean trypsin inhibitor were from Sigma; phenylmethylsulfonyl fluoride was from Life Technologies, Inc.; Dulbecco’s modified Eagle’s medium (DMEM) was from Cellgro; fetal bovine, calf, and cosmic calf sera from Hyclone; [γ-³²P]ATP and [γ-³²P]dCTP from PerkinElmer Life Sciences. CL316,243 was a gift from American Cyanamid Co. (Pearl River, NY). GL237310x (rosiglitazone) and GF211835x (LGD1069) were generous gifts from Drs. Steve Kliewer and Jurgenn Lehmann (GlaxoWellcome).

Isolation and Sequencing of Genomic Clones and Construction of...
TABLE I

Nucleotide sequences of oligonucleotides used in EMSAs

| Oligonucleotides | Nucleotide sequences |
|------------------|----------------------|
| −3306            | 5′-ctagcatctgtaaaGAGGAAATGgacct-3′ |
| −3306            | 5′-ctagcatctgtaaaGACTAGGCgtagctg-3′ |
| −1426            | 5′-ctagcatctgtagtttgtTTCGAGGCAagg-3′ |
| −1426            | 5′-ctagcatctgtagtttagACATTGCGCaggg-3′ |
| C/EBP consensus (CON) | 5′-ctagctgcaatTGGCAGCTctgagg-3′ |
| Sp1              | 5′-attcgcattGGGCGGGGcagac-3′ |
| Geneka C/EBP     | 5′-ctagggtTTGGCGCAATctattcgg-3′ |
| Geneka mut C/EBP | 5′-ctagggtTGGCATTCCCCatattcgg-3′ |

Mutated bases are in bold, and capitalized sequences are (putative) transcription factor binding sites.

βAR Promoter-Luciferase Plasmids—A mouse genomic DNA library constructed in λ-DASH (Stratagene) was screened with a mouse βAR probe comprising the first 108 amino acids of the protein (27). Eight independent positive clones were isolated and analyzed by restriction enzyme mapping and Southern blot hybridization with the 108 amino-terminal probe and a with second probe corresponding to the first 600 nucleotides 5′ to the initiator methionine. A 6641-nucleotide BamHI fragment was subcloned into the pGEM-4Z plasmid, and two isolates containing the insert in opposite orientations were sequenced along both strands at the Duke University Automated DNA Sequencing Facility. The sequence of the 5283-nucleotide BamHI-NarI fragment can be retrieved as GenBank™ accession no. AF303739. This BamHI-NarI fragment was subcloned into the luciferase reporter construct pGL3Basic (Promega) at the BglII/HindIII sites to generate ml3-Luc. A series of deletion mutants was created by digesting the ml3-Luc with the following enzymes to yield the indicated fragments: Nhel and SpeI (3288 base pairs), KpnI (2079 base pairs), EcoRV and MluI (1107 base pairs), or MluI and BglII (557 base pairs). A site-directed mutation of the putative C/EBP response element at −3306 to −3298 was generated using the GeneEditor kit (Promega) such that the sequence TGGAGCAAT was changed to GACTAGCCT. The C/EBP expression vector has been described previously (28). Plasmids for transfections were purified using the Promega Megaprep system.

Cell Culture and Transfections—COS-7 and C3H10T1/2 (T1/2) clone 8 fibroblasts (American Type Culture Collection) were maintained in DMEM 10% fetal bovine serum. 3T3-L1 and NIH-3T3 cells were maintained in DMEM 10% fetal bovine serum. 3T3-L1 cells were cultured in DMEM 10% calf serum. The NIH-C/EBPα, Swiss-PPARγ, and NIH-PPARy cell lines were maintained and differentiated as described (13, 14, 24). T1/2 cells were seeded into six-well dishes and differentiation proceeded as described (29). For most cell lines, 2 μg of DNA were transfected per well using 3 μl of FuGENE 6 as outlined by the supplier (Roche Molecular Biochemicals). For preparation of nuclear extracts enriched in C/EBPα, 20 μg of C/EBPα expression vector were transfected into 10-cm dishes of COS-7 cells by calcium phosphate coprecipitation (30). The T3T-L1 cells were cotransfected with 4 μg of reporter vectors, 4 μg of either the murine sarcoma virus-C/EBPα expression vector or the empty murine sarcoma virus expression vector, and 2 μg of cytomegalovirus-β-galactosidase using calcium phosphate coprecipitation. T1/2 cells were harvested for luciferase activity 72 h after transfection, while all others were harvested after 48 h.

Enzymatic Assays—Luciferase activity was determined in a TD 20/20 luminometer (Promega) using the luciferase assay kit (Promega). β-Galactosidase activity was determined by a colorimetric assay (absorbance at 570 nm) using chlorophenol red-β-D-galactopyranoside as the substrate. Luciferase data were normalized by dividing the light units by β-galactosidase activity.

Oligonucleotides—Oligonucleotides used in this study were synthesized by Life Technologies, Inc. Nucleotide sequences are listed in Table I.

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assays—Nuclear extracts were prepared following the method of Schreiber (31). Protein determinations were made by the Bradford method (32). Double-stranded oligonucleotide probes were annealed and end-labeled with [γ−32P]ATP (1117Bq/mmole) by T4 polynucleotide kinase. Nuclear extracts (10 μg) were incubated with labeled oligonucleotide probes for 30 min on ice before loading on pre-run polyacrylamide gels (Invitrogen) in 0.5× TBE (44.5 m Tris borate, 1 mM EDTA, pH 8.0). For competition studies, unlabeled nucleotides were incubated with nuclear extracts on ice for 15 min prior to addition of the labeled oligonucleotides. Rat liver nuclear extract was obtained from Geneka Biotechnology (Montréal, Québec, Canada) and gel shifts were performed per manufacturer’s instructions, using 2 μg of extract/reaction. Reactions were resolved in 1× TGE (50 mM Tris, 380 mM glycine, and 2 mM EDTA). Where indicated, antibodies used in supershift analysis that are specific to C/EBPα (sc-61x), C/EBPβ (sc-150x), and C/EBPδ (sc-151x) were obtained from Santa Cruz Biotechnology.

Isolation and Analysis of RNA—Total cellular RNA was prepared using TRI reagent according to the manufacturer’s specifications (Molecular Research Center). RNA (40 μg) was denatured by the glyoxal procedure, fractionated through 1.2% agarose gels, and blotted onto Biotrans (ICN) membranes as detailed previously (33). Radiolabeled probes were prepared by random primer synthesis (PrimeIT, Stratagene) of the purified DNA fragments in the presence of [α-32P]deoxy CTP (1117Bq/mmole) to a specific activity > 2 × 108 dpm/μg DNA. The DNA fragments that were used as probes were obtained from the following sources. A fragment specific for mouse βAR was prepared as described previously (5). Mouse PPARγ cDNA was a gift from Jürgen Lehmann. The α2 probe was a gift from Bruce Spiegelman (34). A cDNA fragment for rat C/EBPα was described previously (13). A rat C/EBPα probe for cyclophilin was used as an internal hybridization/quantitation standard as described previously (35). Blots were hybridized and washed as described previously (5, 36).

Adenyl Cyclase Assay—Cells were grown in 12-well plates for cAMP assays according to published methods (37). Briefly, cells were preincubated in serum-free DMEM, 25 mM HEPES, pH 7.5 (SFMM) for 20 min, followed by fresh SFMM containing 0.25 mM isobutylmethylxanthine for 5 min. CL316,243 (5 μM final) was added, and the plates were returned to the incubator for 20 min (determined to be an optimal time in pilot experiments). Treatment was terminated by rapidly aspirating the medium and adding cold 5% trichloroacetic acid (100 μl/well). Two hundred μl of 50 mM KPO4, pH 7.4, was added to each well to partially neutralize the trichloroacetic acid. Cyclic AMP in the cell lysate was determined by radioimmunoassay (38) using a polyclonal antiserum to iodinated cAMP (39).

RESULTS

Differentiation-dependent Expression of the βAR Gene—T1/2 fibroblasts have been shown to differentiate into white adipocytes in response to PPARγ ligands, and these cells can serve as a vehicle for examining the expression of transactivated genes (29), but the expression of βAR in these cells as a function of differentiation was unknown. Fig. 1 presents the pattern and time course of expression of several adipogenic genes, including βAR, in these cells during differentiation. Total RNA was isolated every other day from day 2 after ligand treatment until day 12 and analyzed by Northern blotting (Fig. 1A). A progressive increase in the expression of C/EBPα, PPARγ, and α2 preceded detectable basal expression of βAR at day 3. The internal control transcript, cyclophilin, was unchanged throughout this period. Fig. 1B depicts the concomitant increase in the ability of a selective βAR agonist to stimulate adenyl cyclase activity during differentiation of the T1/2 cells. By day 3 of differentiation, there is a 2-fold increase in βAR-stimulated adenyl cyclase activity. However, in subsequent days, there is a substantial increase in cAMP production such that, by day 10, βAR-stimulated cAMP production is enhanced 25-fold over basal.

C/EBPα Is Necessary for βAR Expression in Adipocytes—Previous studies have shown that ectopic expression of either PPARγ or C/EBPα in nonadipogenic fibroblasts stimulates adipogenesis (11, 20). Coexpression of the two proteins (22) re-
results in a synergism of adipogenesis that is comparable to that seen in the well-studied 3T3-L1 model (20). Fig. 2 compares the expression of β3AR and other relevant adipocyte genes in a collection of cell lines that either possess de novo adipogenic capacity in response to hormonal induction or were engineered to express various combinations of C/EBPs and PPARγ to assess their contribution to the adipocyte phenotype. β3AR cells are NIH-3T3 cells that were designed to express both C/EBPβ and C/EBPα under a tetracycline-responsive promoter to ascertain the role of glucocorticoids and C/EBPβ in the initiation of adipogenesis (13, 14). When these cells are induced to differentiate, they accumulate lipid droplets and they express both PPARγ and aP2 (Fig. 2). However, despite obvious adipocyte morphology, these cells fail to express either C/EBPα or β3AR. In two other adipocyte models, ectopic retroviral expression of PPARγ induces C/EBPα and β3AR expression in the Swiss-3T3 cells (24), but not in NIH-3T3 cells (20), again despite lipid accumulation and expression of aP2. Finally, we examined β3AR expression in another cell line, NIH-α, which ectopically express C/EBPα in the NIH-3T3 fibroblast background (24). As shown in the right-hand side of Fig. 2, when these cells are induced to differentiate they express β3AR mRNA, in addition to PPARγ and aP2, as early as day 6.

In support of these mRNA expression studies, we assessed the functional activity of the β3AR in these various adipocyte cell lines by the ability of the β3AR-specific agonist, CL316,243, to stimulate adenylyl cyclase activity. Table II shows that there is no detectable increase in β3AR-stimulated cyclase activity in undifferentiated versus differentiated NIH-PPARγ cells, whereas there was a 5-fold increase in cAMP production in the NIH-α cells when comparing the differentiated and undifferentiated states. Together, these data strongly suggest that C/EBPα is necessary for β3AR expression and function in adipocytes.

**Structure and Activity of the Mouse β3AR Promoter.**—We sequenced a 5.28-kb BamHI-Nar I fragment containing 150 nucleotides of exon 1 and 5.13 kb of sequence 5' to the transcription start site (40) of the mouse β3AR gene. Sequence analysis with SIGNALSCAN (CCG, University of Wisconsin, Madison, WI) revealed a number of putative transcription factor binding sites within the 5.13-kb promoter. As outlined in Fig. 3, two putative C/EBP protein binding sites between -3306 and -3298 and between -1462 and -1354 were identified. Several other putative regulatory elements, including glucocorticoid and AP-1 binding sites, were also identified (see GenBank® accession no. AF303739).

In experiments designed to evaluate the tissue-specificity of the β3AR promoter, the promoter region was subcloned into the pGL3-Basic luciferase vector (Promega). This construct was then transfected into differentiating T1/2 adipocytes or proliferating NIH-3T3 cells. Relative to the promoter-less parent vector, the 5.13-kb β3AR fragment stimulated luciferase expression 50-fold in the adipocytes (Fig. 4A). By contrast, in NIH-3T3 cells, the same construct induced luciferase expres-
sion <2-fold. These results are consistent with the observation that β3AR mRNA is primarily observed in adipocytes. To determine the importance of the putative C/EBP regions for the activity of the mouse β3AR promoter, we evaluated the activity of several 5′-deletion constructs (Fig. 4B). The activity of the −3138 promoter truncation, which lacks the proposed C/EBP site at −3306, is decreased to less than 50% of the −5.13 kb promoter fragment. The −1929 bp deletion led to a further 30% decrease of luciferase activity, while deletion of the second putative C/EBP site at −1462, shown by the −957 bp truncation, had no further effect. These data support the hypothesis that C/EBPs is necessary for both the expression and function of the β3AR in these adipocyte models.

C/EBPα Binds the β3AR −3306 Element—To establish whether either of the putative C/EBP binding sites was capable of binding C/EBP proteins, we performed a series of gel shift and antibody supershift assays. For these experiments, nuclear extracts were prepared from COS-7 cells that had been transfected with a C/EBPα expression vector, as described previously (41). The oligonucleotides used in these experiments are shown in Table I. Using a consensus C/EBP oligonucleotide as the probe resulted in a major binding species (filled arrow) that was supershifted (open arrow) in the presence of antisera to C/EBPα (Fig. 5A, lanes 2 and 3). Addition of a 100-fold molar excess of the unlabeled β3AR −3306 eliminated this band (lane 4). When the β3AR −3306 element was used as a probe (Fig. 5A, lanes 5–8), several binding species were detected, one major band that comigrated with the major C/EBP band in lane 2. This major band was completely supershifted (open arrow) by anti-C/EBPα antibody (lane 5 versus lane 6), but was eliminated when excess unlabeled oligonucleotide for a consensus C/EBP binding site (42) was included as a competitor (lane 7). In contrast, an oligonucleotide with mutations in the −3306 C/EBP was unable to affect the gel shift pattern of the labeled −3306 oligonucleotide (lane 8). In Fig. 5B, we again used the C/EBP consensus sequence (lanes 1–4) and the −3306 β3AR element (lanes 5–8) as probes to further examine the specificity of binding. The major band (filled arrow) was eliminated in the presence of 100-fold molar excess of the −3306 β3AR C/EBP element, but neither an excess of the −1462 C/EBP element nor of a Sp1 consensus affected binding. In lanes 5–9, the −3306 C/EBP element was used as a probe. A 100-fold molar excess of the C/EBP consensus oligonucleotide inhibited binding of the major band. The addition of the unlabeled C/EBP element at −1462 was able to partially inhibit binding of the −3306 C/EBP element while the addition of unlabeled Sp1 oligonucleotide or the mutant −3306 oligonucleotide had no effect. The relative affinity of C/EBPs for the −3306 C/EBP element versus the consensus sequence was assessed by including 10-, 50-, or 100-fold molar excess of unlabeled oligonucleotides (Fig. 5C). The major band shown in lane 1 (filled arrow) is eliminated in the presence of increasing amounts of the −3306 oligonucleo-

![Fig. 3. Structure of the mouse β3AR promoter. A 5285-nucleotide fragment of the β3AR gene, including 5135 nucleotides of 5′-flanking promoter sequence, was isolated and sequenced as described. The location of restriction endonucleases for creating many of the reporter constructs and the location and sequence of the two putative C/EBP sites are shown.](image)

![Fig. 4. Tissue-specific activity of the mouse β3AR promoter. A, luciferase activity of the 5.13-kb mouse β3AR promoter after transfection into differentiating T1/2 adipocytes and NIH-3T3 cells. Transfections were performed as described. Error bars show the standard error of triplicate wells. The results are representative of several experiments. B, schematic representation of the different reporter constructs. Several 5′-deletion series of mouse β3AR promoter-luciferase constructs were transfected into differentiating T1/2 adipocytes. Error bars show the standard error of triplicate wells.](image)
tide (lanes 2–4), while the mutant −3306 had no effect (lanes 5–7). As anticipated, addition of the unlabeled consensus C/EBP sequence completely blocked the appearance of the major band (lanes 8–10). Finally, as shown in Fig. 5D, the oligonucleotide corresponding to the putative C/EBP site at −1462 produced gel shift bands with nuclear extracts, but these bands do not appear to bind C/EBPα. First, the relative position of these faint bands did not correspond to C/EBP binding as observed for the −3306 sequence and identical to the consensus, nor was the band pattern affected when either the consensus or the −3306 C/EBP were used as competitors. More importantly, the addition of anti-C/EBPα antibody did not affect the abundance or position of these bands.

Finally, we wanted to determine whether binding of the −3306 element was specific for C/EBPα. To address this, we performed gel shifts using rat liver nuclear extract, which contains multiple C/EBP isoforms, and antibodies specific for C/EBPα, C/EBPβ, and C/EBPδ. As shown in Fig. 6, the −3306 C/EBP sequence produced strong gel shift bands when incubated with the rat liver extract (lane 2), while a consensus C/EBP oligonucleotide inhibited the appearance of these bands (lane 3). A C/EBP mutant had no effect (lane 4) (for sequences see Table I). The major band was nearly completely supershifted by antibodies to C/EBPα (lanes 5, 8, and 9). Adding of anti-C/EBPδ supershifted a lower, weaker band (lanes 6, 8, and 10), and anti-C/EBPδ had no effect (lanes 7, 9, and 10). For comparison, the same consensus C/EBP sequence used in Fig. 5 (see Table I) was used as the probe in lanes 11 and 12. The major band was supershifted by the addition of C/EBPα antibody. From these data, we conclude that C/EBPα binds the −3306 C/EBP element within the mouse β3AR promoter.

The β3AR C/EBPα Element Confers Activity to a Heterologous Promoter—Having identified a potential role for C/EBPα in the regulation of the β3AR by correlative expression and gel
shift analysis, we sought to determine whether the putative C/EBP at −3306 was functional. Two approaches were taken. First, wild-type and mutated versions of the −3306 C/EBP element were transfected in the presence or absence of a C/EBPα expression vector. Fig. 7 shows that the insertion of one copy of the putative C/EBPα element results in a 3-fold increase in luciferase activity, while the presence of two tandem C/EBP elements results in a 9-fold enhancement. In contrast, the mutant −3306 C/EBP element abolished transactivation by C/EBPα.

Next we mutated the C/EBP site at −3306 to −3298 from TGGGACCAT to GACTAGCCT within the context of the intact 5.13-kb βAR promoter. This mutation is the same as the one used in the C/EBPα transactivation experiments in Fig. 7, as well as the gel shift assays in Fig. 5. As shown in Fig. 8, when the βAR promoter constructs containing wild-type and mutant C/EBP sites were introduced into differentiating 3T3-L1 cells, luciferase activity from the mutant was reduced by more than half, to a level equivalent to that observed in the −2852 deletion (Fig. 4B). These data demonstrate that this site mediates the effect of C/EBPα on βAR gene expression.

**DISCUSSION**

In this study we have shown that the mouse βAR gene is specifically activated by C/EBPα and that this activation is correlated with the binding of C/EBPα to an element residing between −3306 and −3298 bp upstream of the βAR gene transcription start site. By utilizing several cell lines that contain various combinations of the C/EBPs and PPARγ, we showed that only adipocytes expressing C/EBPα possess βAR transcripts and concomitant functional activity. A 5.13-kb promoter fragment of the mouse βAR gene containing two putative C/EBP binding sites, at −3306 to −3298 at −1454, confers robust expression of a luciferase reporter preferentially in adipocytes. This transcriptional activity is significantly decreased upon deletion of the more distal C/EBP element, while removal of the more proximal element had no further effect. We also showed that this C/EBP element at −3306 conveys transcriptional activity to C/EBPα in vitro. Finally, electrophoretic mobility shift assays provided evidence that C/EBPα interacts specifically with the element at −3306 and not the element at −1462 in the mouse βAR promoter. In every case, mutation of the −3306 C/EBP site eliminates these responses.

Our observation that expression of the βAR gene is positively regulated by C/EBPα is consistent with numerous reports showing the role of this transcription factor in adipogenesis. For example, ectopic expression of C/EBPα is sufficient to induce adipocyte differentiation in a number of cell lines, while the expression of an antisense C/EBPα construct in 3T3-L1 adipocytes blocks differentiation (43). Consistent with these in vitro studies, C/EBPα null mice fail to develop white adipose tissue (44).

In addition to C/EBPα, C/EBPβ and C/EBPδ have also been shown to be critical regulators of adipocyte differentiation. C/EBPβ and C/EBPδ are transiently expressed and precede the appearance of C/EBPα (12). Interestingly, overexpression of C/EBPβ, but not C/EBPδ, in preadipocytes converts them to adipocytes, suggesting that C/EBPβ can substitute for C/EBPα (15). However, the β339 cells, which constitutively express C/EBPβ and C/EBPδ, acquire an adipocyte phenotype, as evidenced by the presence of PPARγ and p2, but fail to express either C/EBPα or βAR. As shown in our gel shift assays, it appears that C/EBPβ is capable of binding the C/EBP element at −3306 in the mouse βAR promoter. Perhaps this is not surprising because C/EBP isoforms have been shown to bind to a common DNA consensus sequence (21, 41). Despite this interaction, it is clear that C/EBPβ or C/EBPδ alone is insufficient to induce βAR.

Although we show a pivotal role for C/EBPα in transactivating the βAR gene during adipogenesis, tissues containing high levels of C/EBPα, such as liver and lung, do not contain appreciable amounts of βAR. In addition, we show that the NIH-α cells do not express βAR in the preadipocyte state, despite the constitutive expression of C/EBPα. Therefore, it is clear that some other transcription factor(s) are important in directing the adipocyte-specific expression of the βAR gene. Another key regulator of adipogenesis is PPARγ. Constitutive expression of PPARγ in fibroblasts can induce the conversion to the adipocyte phenotype (20). PPARγ ligands, such as the thiazolidinediones, which are a class of insulin-sensitizing agents, convert fibroblasts and multipotent stem cells to adipocytes (13, 29). As well, PPARγ(−/−) animals completely lack adipose tissue (45–47). Previous studies showed that, despite expression of PPARγ and development of an adipocyte morphology, a lack of C/EBPα results in decreased insulin-stimulated glucose transport (22, 24). Despite this apparent cooperation between C/EBPα and PPARγ in adipocyte differentiation, our initial
attempts to locate a DR-1 PPAR response element (PPRE) within our mouse \( \beta \)3AR promoter fragment or to demonstrate transactivation of the \( \beta \)3AR by PPARs have been unsuccessful. It is plausible that a PPRE lies outside of the promoter region that we have isolated and studied. However, it is known that PPREs in several PPAR target genes deviate significantly from the consensus DR-1 site (18). For this reason we are currently investigating some regions that weakly resemble a PPRE half-site. To fully understand the role of the \( \beta \)3AR in obesity, it will be important for us to determine not only other critical transcription factor(s) is/are required for the regulation of the \( \beta \)3AR gene, but how their effects on \( \beta \)3AR expression may contribute to obesity.

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