**p300 Coactivates the Adipogenic Transcription Factor CCAAT/Enhancer-binding Protein α**

Robin L. Erickson, Nahid Hemati, Sarah E. Ross, and Ormond A. MacDougald

*From the Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0622*

Despite the knowledge that CCAAT/enhancer-binding protein α (C/EBPα) plays an important role in preadipocyte differentiation, our understanding of how C/EBPα interacts with nuclear proteins to regulate transcription is limited. Based on the hypothesis that evolutionarily conserved regions are functionally important and likely to interact with coactivators, we compared the amino acid sequence of C/EBPα from different species (frog to human) and identified four highly conserved regions (CR1–CR4) within the transactivation domain. A series of amino-terminal truncations and internal deletion constructs were made creating forms of C/EBPα which lack single or multiple conserved regions. To determine which regions of the C/EBPα transactivation domain are important in its ability to induce spontaneous differentiation of 3T3-L1 preadipocytes, we infected preadipocytes with expression vectors encoding the C/EBPα conserved region mutants and observed their ability to induce differentiation. We found that CR1 fused to the DNA binding domain of p53 was able to induce spontaneous differentiation independent of the other conserved regions. However, CR2 was not necessary for the adipogenic action of C/EBPα because a combination of CR1 and CR3 can also induce adipogenesis. Because the transcriptional coactivator p300 participates in the signaling of many transcription factors to the basal transcriptional apparatus, we examined whether functional interaction exists between C/EBPα and p300. Cotransfection of p300 with p42C/EBPα results in a synergistic increase in leptin promoter activity, indicating that p300 acts as a transcriptional coactivator of C/EBPα. Analyses using C/EBPα conserved region mutants suggest that multiple regions (CR2 and CR3) of the C/EBPα transactivation domain functionally interact with p300.

Adipose tissue plays multiple roles in the mechanisms controlling homeostasis. Adipocytes serve not only as an energy reservoir by storing excess calories as triacylglycerol, but they also have endocrine and immune functions (1–4). The importance of understanding adipocyte biology is emphasized further by the complications that arise from either too much or too little adipose tissue. Obesity and its associated disorders, such as type 2 diabetes and cardiovascular disease, are an epidemic in the developed world today (5). Conversely, lipatrophy, the lack of adipose tissue, is also associated with diabetes and a number of other metabolic abnormalities (6). Understanding the factors that govern the transcriptional control of adipogenesis will aid in our understanding of how these disorders arise.

A number of the key factors controlling the adipocyte differentiation cascade have been identified (for review, see Refs. 3 and 7–9), including the transcription factors CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ). Although the structure, function, and regulation of PPARγ have been studied extensively (10–13), similar analyses have yet to be performed upon C/EBPα.

Consistent with C/EBPα being a key component in the adipogenic cascade, mice with the cebpα gene deleted have a deficiency in lipid accumulation in both white and brown adipose tissue (14). Likewise, 3T3-L1 preadipocytes (15–17) are unable to differentiate after hormonal induction if C/EBPα antisense RNA is expressed in the cells (18). Moreover, the enforced expression of C/EBPα in 3T3-L1 fibroblasts is sufficient to induce spontaneous adipogenesis (19, 20) and overcome the effects of repressors of adipogenesis such as Wnt-1 (21). C/EBPα is a prototypical basic region/leucine zipper (bZIP) transcription factor consisting of a well characterized carboxyl-terminal leucine zipper that confers dimerization ability and a neighboring DNA binding domain and nuclear localization signal, which are rich in basic residues (22–24). The remaining amino-terminal 273 amino acids, termed the transactivation domain, contains insulin-responsive sites of phosphorylation (25–27) and regions that affect the transcriptional activity of C/EBPα (28–30). Previous studies have arbitrarily divided the transactivation domain and identified regulatory regions within it by examining the ability of these C/EBPα constructs to transactivate reporter genes under the control of the liver serum albumin promoter (29, 30) or an artificial Gal4-responsive promoter (28). In contrast, the current study approaches the functional analyses of the C/EBPα transactivation domain by targeting regions of C/EBPα which are highly conserved across several species (31). We examined the role of these conserved regions (CR) in the ability of C/EBPα to activate the genes necessary to induce spontaneous preadipocyte differentiation. Furthermore, we demonstrate that the nuclear coactivator p300 is able to potentiate C/EBPα-mediated transcription of the leptin (ob) promoter through multiple conserved regions within the C/EBPα transactivation domain.

* This work was supported by Natural Sciences and Engineering Research Council of Canada predoctoral fellowships (to R. L. E. and S. E. R.) and by a grant from the American Diabetes Association and National Institutes of Health Grant DK51563 (to O. A. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Physiology, 7744 Medical Science Bldg. II, 1301 E. Catherine, Ann Arbor, MI 48109-0622. Tel.: 734-647-7721; Fax: 734-936-8813; E-mail: macdoug@umich.edu.

1 The abbreviations used are: C/EBP, CCAAT/enhancer binding protein; PPAR, peroxisome proliferator-activated receptor; bZIP, basic region/leucine zipper; CR, conserved region(s); CMV, cytomegalovirus; βGal, β-galactosidase; pRb, retinoblastoma protein; GFP, green fluorescent protein; CREB, cAMP response element-binding protein; CBP, CREB-binding protein; TBP, TATA-binding protein.
Human embryonic kidney 293T cells were cultured in high glucose Dulbecco's modified Eagle's medium (Life Technologies, Inc.) containing 10% calf serum (Life Technologies, Inc.), 1 mM sodium pyruvate (Life Technologies, Inc.), 105 μg/ml penicillin G (Sigma PEN-K), 65 μg/ml streptomycin sulfate (Sigma), and 8.4 μg/ml biotin (Sigma). Cells were incubated in a 10% CO2 water-jacketed incubator. 3T3-L1 fibroblasts were cultured as described (26).

Cloning of C/EBPα Constructs

To facilitate cloning of C/EBPα deletion mutants, a 5′-nucleotide to +2,111-nucleotide clone of mouse C/EBPα in pcDNA3.1 (+Stratagene) containing an ideal Kozak translational start site (32) and silent mutations was used. These silent mutations, as described previously (27), introduce unique KpnI (+534 nucleotides), SphI (+823 nucleotides), and XhoI (+1,072 nucleotides) restriction sites. BamHI to KpnI (pSER13) and KpnI to XhoI (pSER23) fragments of C/EBPα were each subcloned into pBluescript KS+ (Stratagene) and ligated in a 3′ overhang orientation.

Cloning of CR1 (5′-3′/5′-3′ Vector)—pSER13 was digested with BamHI and AatII (+307 nucleotides), and a BamHI-AatII oligonucleotide (Table I) was inserted (pSER13A/CRI). The BamHI-KpnI fragment of pSER13A/CRI was excised and subcloned into pCDNA3.1 (+Invitrogen) containing full-length p42CRE (pSER28) and resulted in a C/EBPα clone lacking CR1 (pSER28A/CRI).

Cloning of CR1 (3′-5′ Vector)—pSER13 was digested with AatII and KpnI and religated with an AatII-KpnI oligonucleotide (Table I). The resulting vector (pSER13A/CRI) was then digested with BamHI plus KpnI, and the excised fragment was subcloned into pSER28. The resulting clone lacks CR2 (pSER28A/CRI2).

Cloning of CR1/2 Vector—C/EBPα containing CR1 and CR2 was created by digesting pSER23 with KpnI and SphI and ligating in a KpnI-SphI oligonucleotide (Table I; pSER23A/CRI2). The KpnI-XhoI fragment of pSER23A/CRI2 was then excised and inserted into the KpnI-XhoI site of pSER28, generating CR1/2 (pSER28A/CRI3). Constructs

Cloning of CR1 and CR2 Vectors—To create expression vectors containing CR1 or CR2 alone fused to the bZIP, the BamHI-KpnI fragment from pSER13A/CRI2 or pSER13A/CRI1, respectively, was subcloned into the BamHI-KpnI site of pSER23A/CRI4.

Cloning of CR4 (3′-5′ Vector)—A C/EBPα fragment lacking CR3 was synthesized by polymerase chain reaction using CR3 primers (Table I) with pSER28 as a template. TOPO TA cloning (Invitrogen) was used to subclone the product into pCR2.1-TOPO (Invitrogen). A KpnI-XhoI fragment was then excised and subcloned into pSER28A/CRI or pSER28A/CRI2, yielding CR2/4 or CR1/4, respectively.

Cloning of CR4 Vector—The MluI-EcoRV fragment (+698 to +2,111 nucleotides) from the C/EBPα gene (33) was cloned into pBHEB1s (Invitrogen), as described previously (His-p18/EBPα (27)). To create a non-His-tagged form of CR4, His-p18/EBPα was linearized with HindIII, and a fill-in reaction was performed to blunt end the vector. The linearized vector was then digested with BglII, and the resulting fragment was subcloned into the BamHI-EcoRV site of pcDNA3.1 (+) (Invitrogen) containing an ideal Kozak consensus sequence oligonucleotide (AGCTTGGC GCCGCACCATGGG) 5′ to the BamHI site. The expression vectors encoding p30C/EBPα (CR3/4) and p12C/EBPα (bZIP) were described previously (27).

To create retroviral expression vectors, pLXSN (34) was linearized with HindIII, blunt ends were created using DNA polymerase I, large (Klenow) fragment (New England Biolabs) and ligated with T4 DNA ligase (New England Biolabs). The resulting plasmid was digested with HpaI, and a HindIII linker (dpCaAGCTTG), New England Biolabs) was ligated to form pNH2. The full-length, p30, CR1, and CR3/34 isoforms of C/EBPα were each excised from pcDNA3.1 (+) with EcoRI and HindIII and subcloned into the EcoRI-HindIII sites of pcNH2. CR2/3/4 and CR2 in pcDNA3.1 (+) were first digested with BamHI. The resulting fragment was filled in with DNA polymerase I, large (Klenow) fragment and ligated with an EcoRI linker (dpCGGAAATTCGGC, New England Biolabs). This vector was subsequently digested with EcoRI and HindIII, and the resulting insert was placed in pNEH21.

An expression vector encoding a chimeric protein consisting of the Gal4 DNA binding domain and the C/EBPα transactivation domain was constructed by subcloning a KpnI-Sacl fragment of pSER23 into pSG424 (35). This construct was subsequently digested with BamHI and KpnI, and the BamHI-KpnI fragment from pSER28 was inserted.

Luciferase Reporter Gene Assays

Reporter gene assays were performed using leptomin promoter-luciferase reporter genes, pObLuc-760 and pObLuc-m760 (from Dr. Daniel Lane, Johns Hopkins University (36)), a murine PPARγ promoter-luciferase reporter gene (from Dr. Jeffrey Gimble, Artecel Sciences, Inc. (37)), and a (Gal4)-SV40-luciferase reporter gene (from Dr. Mitchell A. Lazar, University of Pennsylvania). The human p300 expression vector, pVR1012-p300, was donated by Dr. Gary Nabel (National Institutes of Health). pmCV-125 E1A-WT and pCMV-125 E1A-R2G were created by subcloning the HindIII-Notl fragment of pRc/RSV-12S E1A and pRc/RSV-12S E1A R2G (from Dr. Roland Kwok, University of Michigan, 38) into the HindIII-Notl site of pcDNA3.1 (+).

Human embryonic kidney 293T cells (100-mm plates) were transiently transfected with 20 μg of total DNA by calcium phosphate coprecipitation, including 1 μg of luciferase reporter gene, 500 ng of CMV-β-galactosidase (pGal), and 10 μg of sheared herring sperm DNA. Additional plasmid DNA amounts varied based upon experimental conditions and are documented in the figure legends. A constant amount of CMV promoter was maintained in all conditions to control for the potential squelching of the transcriptional machinery. After precipitation for 4–5 h, cells were shocked with 12.5% glycerol in phosphate-buffered saline (157 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 5.5 mM Na2HPO4, pH 7.3) for 3 min. Cells were incubated for 24–48 h in 10% calf serum and Dulbecco's modified Eagle's medium before they were lysed in 1 × reporter lysis buffer (100 mM KH2PO4, 0.2% (w/v) Triton X-100, 1 mM dithiothreitol). Samples were vortexed and subsequently centrifuged in a microcentrifuge for 30 s at 16,000 × g. To assay the samples for luciferase activity, 100 μl of the supernatant was mixed with 350 μl of luciferase buffer 2 (25 mM glycylglycine (Fisher Bieche), pH 7.8, 30 mM MgSO4, 4 mM EGTA, pH 8.0, 0.0027% (w/v) Triton X-100, 15 mM KH2PO4, 2 mM ATP, 1 mM dithiothreitol) in glass test tubes. The tubes were then placed in an Optocomp II luminometer (MGM Instruments, Hamden, CT) and injected with 100 μl of luciferase buffer 2 (25 mM glycylglycine, 30 mM MgSO4, 4 mM EGTA, pH 8.0, 200 μl luciferin (Promega), 2 mM dithiothreitol), and the relative light units emitted were measured and normalized against the relative light units obtained from a chemiluminescent β-galactosidase assay. β-galactosidase activity was measured by taking 1 μl of cell lysate and incoating 100 μl of β-galactosidase reaction buffer (100 μM KH2PO4, 1 mM MgCl2, 1% (w/v) Galacton (Tropix)) and incubating for 45 min to 1 h at room temperature. Chemiluminescence was measured after the injection of 900 μl of light emission accelerator (10% (w/v) Emerald enhancer (Tropix) in 0.2% NaOH (39)).

Retroviral Infection and Oil Red-O Staining of 3T3-L1 Preadipocytes

293T cells were transfected as described above with the retroviral C/EBPα expression vectors and the viral packaging vectors SV-E-MLV-env and SV-F-E-MLV (7.5 μg of each) (21, 34). Virus-containing medium was collected at each 12-h intervals post-transfection and at each harvest and passed through a 0.45-μm filter. 5 μg/ml filter-sterilized polybrene (hexadimethrine bromide; Sigma) was added to the virus-loaded medium. This medium was then applied to preconfluent (40%) 3T3-L1 preadipocytes. The infection protocol was repeated two additional times. After the third round of infection, 3T3-L1 preadipocytes were trypsin treated and replated on multiple plates, in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum and 10% fetal bovine serum.
400 μg/ml Geneticin (Life Technologies, Inc.). Cells were then allowed to proliferate to confluence, at which point the cells were fed with Dulbecco's modified Eagle's medium containing only 10% calf serum. For 14 days postconfluence the cells were observed for evidence of spontaneous differentiation. To detect cytoplasmic lipid accumulation, retrovirus-infected 3T3-L1 cells were stained by Oil Red-O, essentially as outlined previously (40).

**Immunoblot Analysis**

Protein expression levels in samples used for reporter gene assays were determined by immunoblot analysis. For detection of p300, supernatant from cells lysed in 1 × reporter lysis buffer was combined with 4 × SDS loading buffer (4% (w/v) SDS, 350 mM β-mercaptoethanol, 240 mM Tris, pH 6.8, 40% (v/v) glycerol, 0.01% (w/v) bromphenol blue) and electrophoresed on an SDS-polyacrylamide gel (5%). Proteins were transferred to a polyvinylidene difluoride (Osmonics) membrane, and immunoblotting was performed with mouse monoclonal p300 antibody (BD PharMingen). To determine the expression of C/EBPα, pellets from reporter gene samples were transferred to new tubes, resuspended in Western lysis buffer (1% SDS, 60 mM Tris, pH 6.8), and sonicated. Lysate was mixed with 4 × SDS loading buffer and separated on an SDS-polyacrylamide gel (11.5%), transferred to polyvinylidene difluoride, and immunoblotted with an affinity-purified polyclonal C/EBPα antibody generated against a synthetic polypeptide corresponding to amino acids 253–265 (32). Immunoblot analyses of proteins from infected 3T3-L1 preadipocytes and adipocytes were performed as described previously (18, 26). The adipocyte marker 422/aP2 was detected using a polyclonal antibody received from Dr. David Bernlohr (University of Minnesota).

**RESULTS**

**Multiple Conserved Regions Contribute to the Adipogenic Action of C/EBPα—Conserved regions of the C/EBPα transactivation domain were identified by aligning the primary amino acid sequences of human, bovine, mouse, chicken, and frog C/EBPα (31). Analysis of the alignment revealed four regions within the transactivation domain with a high degree of sequence homology (Fig. 1A). Homology among CR1, 2, 3, and 4 from the mouse and chicken isoforms of C/EBPα is 66, 87, 87, and 94%, respectively.

To determine the region within the C/EBPα transactivation domain capable of inducing differentiation, retroviral expression vectors encoding the C/EBPα isoforms depicted in Fig. 1A were infected into 3T3-L1 preadipocytes. The adipogenic activity of the various isoforms was measured by the expression of the adipocyte marker 422/aP2 (Fig. 1B) and Oil Red-O staining of cytoplasmic lipid accumulation (Fig. 1C). Consistent with the findings of other investigators (19, 20), p42C/EBPα induces spontaneous differentiation, but the p30C/EBPα isoform that contains only CR3 and CR4 does not (Fig. 1). Based upon these results and our previous results that a C/EBPα deletion mutant containing only CR1 and CR2 is able to induce differentiation at a level comparable to p42C/EBPα (31), we examined the contribution of CR1 and CR2 to the adipogenic action of C/EBPα. The C/EBPα construct CR2/3/4, which lacks CR1, was able to induce 422/aP2 expression (Fig. 1B) and lipid accumulation (data not shown) comparable to p42C/EBPα, suggesting that CR1 is not required for C/EBPα-induced adipogenesis and that CR2 is the adipogenic region. CR1 alone was not sufficient to induce 422/aP2 expression (Fig. 1B) nor promote cytoplasmic lipid accumulation (Fig. 1C), whereas CR2 alone was able to induce spontaneous differentiation. This was consistent with CR2 being sufficient to mediate C/EBPα-induced adipogenesis. However, despite lacking CR2, CR1/3/4 is also able to induce adipogenesis (Fig. 1B). Thus it appears that multiple conserved
regions within C/EBPα are involved in the adipogenic effect. CR2 is able to contribute independently of the other regions, whereas CR1 and CR3 work in combination.

*p300 Coactivates C/EBPα-mediated Transcription of the Leptin Promoter—*p300 is a nuclear coactivator that has been shown to interact with transcription factors that are important for a number of differentiation paradigms (for review, see Ref. 41). We performed a series of experiments to investigate whether p300 coactivates C/EBPα through the adipogenic domains. To determine if p300 is a limiting component and func-
tions as a coactivator of C/EBPα transcription from an adipocyte-specific promoter, reporter gene assays were performed using a C/EBPα-responsive, leptin-luciferase reporter gene (ob-luc). 293T cells, which do not contain endogenous C/EBPα, were transfected with a constant amount of expression vector encoding C/EBPα and an increasing amount of p300 expression vector. The ob-luc activity showed a p300-dependent increase (Fig. 2, top) in the presence of a constant level of C/EBPα, as seen by immunoblotting (Fig. 2, bottom). p300 had no effect on ob-luc activity in the absence of C/EBPα. Similar results were seen in assays using the PPARγ promoter (data not shown). To ensure that the coactivational effects observed were the result of C/EBPα binding the ob promoter and not nonspecific or non-DNA binding events, we performed the reporter gene assay with an ob-luciferase reporter gene with a mutation in the C/EBPα binding site (mob-luc). This mutation prevents C/EBPα from binding to the promoter (36) and abrogates C/EBPα-dependent transactivation. Consistent with p300 coactivation being mediated through the C/EBPα binding site, coactivation was disrupted in reporter assays utilizing mob-luc (Fig. 3, solid bars). The ability of p300 alone to activate both ob-luc and mob-luc is the result of utilizing high amounts of p300 expression vector (5 μg) in this experiment and is not seen at lower plasmid concentrations (Figs. 2, 4, and 6). These data strongly suggest that p300 is a rate-limiting component in the C/EBPα transcriptional machinery.

**Functional Interaction between C/EBPα and p300 Is Inhibited by Adenovirus E1A**—p300 was first cloned as an adenovirus E1A-associated protein (42). It was subsequently shown that E1A prevents the association of p300 with a number of transcription factors (38, 43, 44) and inhibits the interaction required for coactivation. We examined whether E1A could inhibit coactivation of C/EBPα transcription by p300. Cotransfection of an expression vector encoding wild-type 12S E1A repressed ob-luc activity by 36-fold. This result was confirmed by immunoblotting. These results confirm that p300 acts as a coactivator of C/EBPα. Furthermore, the inhibition of basal C/EBPα activity by E1A is consistent with endogenous p300 coactivating C/EBPα transcription.

**Multiple Regions of the C/EBPα Transactivation Domain Functionally Interact with p300**—Having firmly established that p300 functions as a coactivator of C/EBPα transcription, we then investigated the region of the C/EBPα transactivation domain which interacts with p300. To determine if coactivation of C/EBPα by p300 is independent of the bZIP domain we created a chimeric protein consisting of the Gal4 DNA binding domain (Gal4DBD) and the C/EBPα transactivation domain (Gal4-C/EBPα). We then performed a luciferase reporter gene assay using a Gal4-responsive reporter construct and tested the ability of p300 to coactivate the chimeric protein (Fig. 5). Cotransfected p300 with the reporter gene alone or with the Gal4 DNA binding domain generated minimal reporter gene activity. Co-transfection of Gal4-C/EBPα resulted in a robust induction in reporter gene activity, whereas cotransfection with p300 potentiates the basal Gal4-C/EBPα transactivation by ~36-fold. This response confirmed our hypothesis that p300 functionally in-
interacts with the transactivation domain of C/EBPα.

To determine which conserved regions of the C/EBPα transactivation domain mediate the interaction with p300, we created C/EBPα transactivation domain truncations (Fig. 6A, top) and conserved region deletion mutants (Fig. 6B, top). We then tested the ability of p300 to coactivate transcription of ob-luc by the deletion mutants. Immunoblot analysis was used to ensure that expression levels of the C/EBPα mutants were similar (data not shown). Truncation of the transactivation domain revealed that the removal of CR1 did not have an effect on basal transcription or on coactivation by p300 (Fig. 6A, CR2/3/4). Further truncation of the amino terminus showed that upon the loss of CR2 there was a substantial decrease in the basal activation, but there was no change in the potentiation by p300 (~2.6-fold in both CR2/3/4 and p30). Removal of CR3 resulted in a further decrease in basal transcription and the loss of coactivation by p300 (Fig. 6A, p30 versus CR4). These results suggest that CR3 is a region within the C/EBPα transactivation domain which interacts with p300. However, when a construct containing only CR1 and CR2 (Fig. 6B, CR1/2) was assayed, p300 cotransfection potentiated the transcriptional activity, indicating that CR3 is able to interact functionally with p300 but is not required for p300 interaction with C/EBPα. Based upon the observation that the deletion of CR1 had a minimal effect on coactivation by p300 (Fig. 6A, CR2/3/4), we suspected that CR2 was also able to interact functionally with p300. Consistent with this hypothesis, a C/EBPα construct lacking both CR2 and CR3 (Fig. 6B, CR1/4) was not coactivated by p300. Furthermore, deletion of all of the conserved regions in the C/EBPα transactivation domain except CR2 (Fig. 6B, CR2) revealed that like CR3 (data not shown), CR2 alone can be coactivated by p300. Thus it appears that multiple regions of the C/EBPα transactivation domain interact functionally with p300.

DISCUSSION

In this study we find that multiple conserved regions of the C/EBPα transactivation domain are able to stimulate adipogenesis and interact functionally with p300. C/EBPα, β, and δ are related transcription factors in the C/EBP family of genes which play a role in adipogenesis (for review, see Ref. 7). These proteins display a high degree of homology between their bZIP domains (93% homology between C/EBPα and C/EBPδ) and recognize the same consensus DNA binding site (46). However, their sequences diverge markedly within the transactivation domain. Work by Elberg et al. (47) demonstrated the important role of the transactivation domain by showing that despite the similarities in the DNA binding domains, C/EBPβ homodimers are unable to bind to and activate the PPARγ2 promoter. C/EBPα is able both to bind and transactivate the PPARγ2 promoter. These investigators found that fusing the C/EBPα transactivation domain to the C/EBPβ bZIP allows transactivation, whereas fusing the C/EBPβ transactivation domain to the C/EBPα bZIP blocks transactivation (47). Furthermore,
because of the use of alternative translation start sites (32, 48). C/EBPα (p42/p30) and C/EBPβ (LAP/LIP) have isoforms that vary in the composition of their transactivation domain. The ratio of expression between these isoforms is critical for normal 3T3-L1 differentiation (49), again implicating the C/EBPα transactivation domain as a key component in understanding the control of adipogenesis. In our study, we have identified highly conserved regions of the C/EBPα transactivation domain which mediate C/EBPα-induced differentiation of 3T3-L1 preadipocytes. Of the four conserved regions in the transactivation domain, CR2 is the only region capable of inducing spontaneous differentiation alone when fused to the C/EBPα bZIP (Fig. 1). Although CR1 and CR3 are incapable of inducing spontaneous differentiation when placed individually next to the bZIP, a construct containing both regions but lacking CR2 (CR1/3/4) promotes cytoplasmic lipid accumulation and the expression of adipocyte markers.

The ability of CR2 to act as a strong activation domain and induce differentiation independent of the other conserved regions may be the result of its ability to interact with multiple coactivators and components of the basal transcription apparatus. The retinoblastoma protein (pRb) has been shown to coactivate C/EBPα expression (50) and is thought to interact with a site within CR2 (51). Furthermore, TBP and transcription factor II B (TFIIB) have been shown to interact within the CR2 region of C/EBPα (52) to promote C/EBPα-mediated transactivation. The coactivator p300 is also able to potentiate C/EBPα transactivation (Fig. 2 and Ref. 43); and based upon our results, this is mediated, in part, by functional interaction with CR2 (Fig. 6D). Spontaneous adipogenesis induced by CR1/3/4 (Fig. 1), which lacks the putative pRb interaction site (51), suggests that C/EBPα can induce differentiation in 3T3-L1 preadipocytes independent of pRb. This is in contrast with the observation that C/EBPα fails to induce differentiation of pRb−/− 3T3 fibroblasts (50). The conflicting observations may be the result of differences in the cell models or the presence of a previously unpredicted pRb-interacting region within the C/EBPα transactivation domain.

We show that p300, in addition to interacting functionally with C/EBPα transactivation (Fig. 2 and Ref. 43), and both of these proteins was demonstrated (50) to promote C/EBPα-mediated tran-

Acknowledgments—We thank Drs. G. Hammer and J. Schwartz for a critical review of this manuscript and the MacDougall laboratory for comments. We also acknowledge Drs. F. Schaufele and M. Lazar for advice on protein-protein interaction studies.

REFERENCES

1. Lin, Y., Lee, H., Berg, A. H., Lissanti, M. P., Shapiro, L., and Scherer, P. E. (2000) J. Biol. Chem. 275, 24255–24263
2. MacDougall, O. A., Hwang, C.-S., Fan, H., and Lane, M. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9035–9037
3. Morrison, R. F., and Farmer, S. R. (1999) J. Cell Biochem. 32-33, 59–67
4. Pond, C. M., and Mattacks, C. A. (1998) Immunol. Lett. 63, 159–167
5. Wickelgren, I. (1998) Science 271, 1364–1367
6. Reitmans, L. M., Arioglu, E., Gavrilova, O., and Taylor, S. I. (2000) Trends Endocrinol. Metab. 11, 410–416
7. Darlington, G. J., Ross, S. E., and MacDougall, O. A. (1990) J. Biol. Chem. 265, 371–385
8. Rangwala, S. M., and Lazar, M. A. (2000) Annu. Rev. Nutr. 20, 535–559
9. Rosen, E. D., Walley, C. J., Puigserver, P., and Spiegelman, B. M. (2000) Genes Dev. 14, 1293–1307
10. Hu, E., Kim, J. B., Surra, P., and Spiegelman, B. M. (1996) Science 274, 2100–2103
11. Reginato, M. J., Krakow, S. L., Bailey, S. T., and Lazar, M. A. (1998) J. Biol. Chem. 273, 1855–1864
12. Shao, D., Rangwala, S. M., Bailey, S. T., Krakow, S. L., Reginato, M. J., and Lazar, M. A. (1998) Nature 396, 377–380
13. Zhang, B., Berger, J., Zhou, G., Elbrecht, A., Biewa, S., White-Carrington, S., Sittka, E., and de Laat, W. (1996) J. Biol. Chem. 271, 31711–31714
14. Wang, N. D., Finegold, M. J., Bradley, A., Ou, C. N., Abdel-sayed, S. V., Wilde, M. D., Taylor, L. R., Wilson, D. R., and Darlington, G. J. (1995) Science 269, 1108–1112
15. Green, H., and Kehinde, O. (1979) J. Cell Physiol. 101, 169–172
16. Green, H., and Kehinde, O. (1975) Cell 19, 29–27
17. Green, H., and Meuth, M. (1974) Cell 3, 127–133
18. Lin, F.-T., and Lane, M. D. (1995) Genes Dev. 9, 533–544
19. Freytag, S. O., Paielli, D. L., and Gilbert, J. D. (1994) Genes Dev. 8, 1654–1663
20. Lin, F.-T., and Lane, M. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8757–8761
21. Rocheleau, S. E., Hemati, N., Longo, K. A., Bennett, C. N., Lam, P. C., Erickson, R. L., and MacDougall, O. A. (2000) Science 289, 950–953
22. Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1989) Science 243, 1681–1688
23. Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1988) Science 240, 1759–1764
24. Williams, S. C., Angerer, N. D., and Johnson, P. F. (1997) Gene Expr. 6, 217–385
25. MacDougall, O. A., Cornelius, P., Liu, R., and Lane, M. D. (1995) J. Biol. Chem. 270, 647–654
26. Hemati, N., Ross, S. E., Erickson, R. L., Groblewski, G. E., and MacDougall, O. A. (1997) J. Biol. Chem. 272, 25933–25939
27. Ross, S. E., Erickson, R. L., Hemati, N., and MacDougall, O. A. (1999) Mol. Cell. Biol. 19, 8433–8441
28. Pei, D., and Shih, C. (1991) Mol. Cell. Biol. 11, 1480–1487
29. Friedman, A. D., and McElhaney, E. H. (1995) Genes Dev. 9, 1416–1426
30. Neman, C., and Ziff, E. B. (1994) Genes Dev. 8, 350–362
31. Erickson, R. L., Longo, K. A., Ross, S. E., Hemati, N., and MacDougall, O. A. (1999) in Adipocyte Biology and Hormone Signaling (Niimi, J., ed) pp. 79–90, IOS Press, Amsterdam
32. Lin, F.-T., MacDougall, O. A., Diehl, A. M., and Lane, M. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8636–8640
33. Christy, R. J., Kaestner, K. H., Geiman, D. E., and Lane, M. D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2593–2597
34. Miller, A. D., and Rosman, G. (1989) BioTechniques 7, 890–990
35. Szalkowski, D., and Moller, D. E. (1996) J. Biol. Chem. 271, 13711–13714
36. Hwang, C.-S., Fan, H., and Lane, M. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 93, 873–877
37. Clarke, S. L., Robinson, C. E., and Gimble, J. M. (1997) Biochem. Biophys. Res. Commun. 240, 989–993
38. Lundblad, J. R., Kwok, R. P. S., Laurance, M. E., Harter, M. L., and Goodman, R. H. (1995) Nature 374, 85–88
39. Brasher, A. R., and Fortin, J. J. (1995) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. J., Seidman, J. G., Smith, J. A., and Struhl, K., eds) Vol. 1, pp. 9.7.12–9.7.21, John Wiley & Sons, New York
40. Preece, A. (1972) A Manual for Histogenic Technicians, Little Brown, Boston
Functional Interactions between p300 and C/EBPα

16355

41. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577
42. Eckner, R., Ewen, M. E., Newsome, D., Gerdes, M., DeCaprio, J. A., Lawrence, J. B., and Livingston, D. M. (1994) Genes Dev. 8, 869–884
43. Mink, S., Haenig, B., and Klempnauer, K.-H. (1997) Mol. Cell. Biol. 17, 6609–6617
44. Puri, P. L., Avantaggiati, M. L., Balsamo, C., Sang, N., Graessmann, A., Giordano, A., and Levrero, M. (1997) EMBO J. 16, 369–383
45. Wang, H. G., Rikitake, Y., Carter, M. C., Yaciuk, P., Abraham, S. E., Zerler, B., and Moran, E. (1993) J. Virol. 67, 476–488
46. Osada, S., Yamamoto, H., Nishihara, T., and Imagawa, M. (1996) J. Biol. Chem. 271, 3891–3896
47. Elberg, G., Gimble, J. M., and Tsai, S. Y. (2000) J. Biol. Chem. 275, 27815–27822
48. Descombes, P., and Schibler, U. (1991) Cell 87, 569–579
49. Calkhoven, C. F., Muller, C., and Leutz, A. (2000) Genes Dev. 14, 1920–1932
50. Classon, M., Kennedy, B. K., Mulloy, R., and Harlow, E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10826–10831
51. Chen, P.-L., Riley, D. J., Chen, Y., and Lee, W.-H. (1996) Genes Dev. 10, 2784–2804
52. Nerlov, C., and Ziff, E. B. (1995) EMBO J. 14, 4318–4328
53. Zhang, J. J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C. M., and Darnell, J. E., Jr. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15092–15096
54. Zhong, H., Voll, R. E., and Ghosh, S. (1998) Mol. Cell 1, 661–671
55. Tang, Q. Q., and Lane, M. D. (1999) Genes Dev. 13, 2231–2241
p300 Coactivates the Adipogenic Transcription Factor CCAAT/Enhancer-binding Protein α
Robin L. Erickson, Nahid Hemati, Sarah E. Ross and Ormond A. MacDougald

J. Biol. Chem. 2001, 276:16348-16355.
doi: 10.1074/jbc.M100128200 originally published online February 8, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100128200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 36 of which can be accessed free at http://www.jbc.org/content/276/19/16348.full.html#ref-list-1