Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2593–2597, March 1991
Developmental Biology

CCAAT/enhancer binding protein gene promoter: Binding of nuclear factors during differentiation of 3T3-L1 preadipocytes

(C/EBP/adipocyte/transcription)

ROBERT J. CHRISTY, KLAUS H. KAESTNER, DEBORAH E. GEIMAN, AND M. DANIEL LANE

Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Contributed by M. Daniel Lane, December 20, 1990

ABSTRACT **Differentiation of 3T3-L1 preadipocytes into adipocytes is accompanied by increased expression of the nuclear protein C/EBP (CCAAT/enhancer binding protein) and by transcriptional activation of a group of adipose-specific genes. We report here the isolation of the murine C/EBP gene and the characterization of its promoter. Consistent with its proposed role in coordinating transcription during preadipocyte differentiation, an increase in the rate of transcription of the C/EBP gene precedes that of several adipose-specific genes whose promoters are transactivated by C/EBP. DNase I cleavage-inhibition patterns (footprinting) of the C/EBP gene promoter by nuclear factors from differentiated and undifferentiated 3T3-L1 cells identified two sites of differential factor binding. One site in the C/EBP gene promoter between nucleotides −252 and −239 binds a nuclear factor(s) present in preadipocytes that is lost or modified upon differentiation. Another site, between nucleotides −203 and −176, exhibits different but overlapping footprints by nuclear factors present in differentiated and undifferentiated cells. Gel retardation analysis with oligonucleotides corresponding to these sites revealed protein–oligonucleotide complexes containing these differentially expressed nuclear factors. The factor present in differentiated cells that binds at this site was identified as C/EBP (possibly in heterodimeric form with a homologous leucine-zipper protein), suggesting that C/EBP may regulate expression of its own gene.**

Differentiation of 3T3-L1 preadipocytes into adipocytes in culture is accompanied by a dramatic rise in the rate of transcription of adipose-specific genes (1, 2). The isolation of a number of these genes (3–5) and characterization of their respective promoters prompted a search for transcription factors that coordinately activate their expression. Recently, it was discovered (6–8) that the CCAAT/enhancer binding protein (C/EBP) exhibits many of the characteristics of such a transcriptional activator.

C/EBP was first isolated from rat liver nuclei as a sequence-specific DNA binding protein with preference for binding to CCAAT boxes and to the enhancer regions of several viral promoters (7). High levels of expression of C/EBP were subsequently found to be limited to terminally differentiated cell types—e.g., hepatocytes and adipocytes—that play a central role in energy metabolism, particularly in the synthesis and mobilization of glycogen and fat (6, 9, 10). Most recently, C/EBP was identified as the differentiation-induced nuclear factor that binds specifically to homologous regulatory elements within the promoters of three genes that are coordinately expressed when 3T3-L1 preadipocytes differentiate into adipocytes (6, 11). It was further demonstrated that C/EBP can function as a trans-activator of the promoters of these genes (6, 11, 12) and that mutation of the C/EBP binding site obliterates trans-activation (6, 12).

Given that C/EBP may play a pleiotropic role in coordinating gene expression in this system, it was of interest to investigate how the C/EBP gene itself is regulated during preadipocyte differentiation. In the present paper, we report the structure of the murine C/EBP gene and characterize its 5′ flanking region.* We show that the increase in the C/EBP message level during preadipocyte differentiation is due to an increase in the rate of transcription of the C/EBP gene. A differentiation-induced nuclear factor, identified as C/EBP, binds to the C/EBP gene promoter.

EXPERIMENTAL PROCEDURES

Isolation and Analysis of a C/EBP Genomic Clone. A partial cDNA of murine C/EBP was obtained by screening a 3T3-L1 adipocyte cDNA library (3) with a 400-base-pair (bp) *Sma* I fragment of the rat C/EBP gene (nucleotides +452 to +867; ref. 7). Two fragments of the cDNA 6a-1 (probes 1 and 2, Fig. 1) were used to screen a NIH 3T3 genomic library, a gift from T. Lanahan and D. Nathans (Johns Hopkins University School of Medicine). Genomic and cDNA fragments were subcloned and sequenced (13).

DNase I Cleavage-Protection (Footprinting) and Gel Retardation Assays. Mouse 3T3-L1 preadipocytes were maintained and differentiated (6), and nuclear extracts were isolated as described (14). A 210-bp *Nar* I–*Nar* I fragment of the C/EBP gene (nucleotides −80 to −291; Fig. 2) was 5′-end-labeled on the noncoding strand and DNase I footprinting was performed (15). Gel retardation assays were carried out as described (6).

Expression of C/EBP in *Escherichia coli*. A 1061-bp *Nco* I fragment of the murine C/EBP gene was inserted into a phage T7 promoter-containing vector pET3d (16). The protein produced starts at the initial methionine of the C/EBP protein but lacks the four carboxyl-terminal amino acids and uses a termination codon from the ϕ10 gene of the bacteriophage T7. Bacterial lysates were prepared as described (17, 18).

Isolation of Nuclei and Run-on Transcription Assays. Nuclei from 3T3-L1 cells were isolated as described (1). Nuclear transcription activity was determined by measuring the incorporation of [α-^{32}P]UTP and using 10^7 nuclei per reaction. Elongated RNA transcripts, hybridized to C/EBP and stearoyl-CoA desaturase 1 cDNAs, were quantified by densitometry of autoradiograms, and the signals were normalized to ^{32}P-labeled transcripts hybridized to 3T3-L1 genomic DNA (19).

RESULTS

Isolation, Sequence, and Structure of the Murine C/EBP Gene. To investigate the mechanism by which expression of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: C/EBP, CCAAT/enhancer binding protein.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M62362).

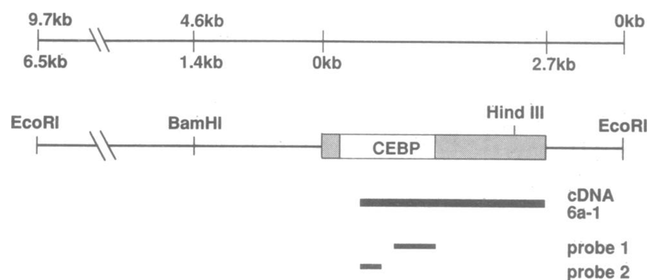

FIG. 1. Map of the murine C/EBP gene. (*Upper*) Scale indicating nucleotide positions in kilobases (kb). (*Lower*) C/EBP 9.7-kb *Eco*RI genomic fragment. The open and shaded boxes indicated 5′ or 3′ untranslated sequences and the coding sequence of the C/EBP gene, respectively. The cDNA clone, 6-a1, is positioned relative to the C/EBP gene, and probes that were used to screen the genomic library are indicated.

C/EBP mRNA is activated during differentiation of 3T3-L1 adipocytes, the mouse C/EBP gene was first isolated. A fragment of the rat C/EBP gene was used to screen a mouse 3T3-L1 adipocyte phage λ ZAP cDNA library (3). The cDNA clone 6a-1 (Fig. 1) extends from nucleotide +490 of the rat C/EBP gene to the poly(A) tail (7). To obtain a murine C/EBP genomic clone, a 400-bp *Sma* I fragment of the 6a-1 cDNA (probe 1 in Fig. 1) was used to screen a mouse NIH 3T3 genomic library. A 9.7-kb *Eco*RI clone was isolated, characterized by restriction enzyme mapping, and partially sequenced. The murine C/EBP gene contains a single exon of 2616 nucleotides and an open-reading frame that encodes a protein of 359 amino acids. The nucleotide and derived amino acid sequences of the mouse and rat C/EBP genes possess 88% sequence identity at the nucleotide level and 99% identity at the amino acid level. The murine C/EBP gene has a single amino acid insertion at position 268 that is not present in the rat C/EBP protein (7).

The start site of transcription was determined by primer extension analysis using total RNA from fully differentiated 3T3-L1 adipocytes (results not shown). An adenine residue, designated nucleotide +1 (Fig. 2), was identified as the primary site of initiation of transcription. This start site was confirmed by S1 nuclease analysis (result not shown) and is four nucleotides 5′ to that reported for the rat C/EBP gene (7). The 5′ flanking region of the mouse C/EBP gene (Fig. 2) contains a sequence with a "TATA" box homology, TATAAA, centered at nucleotide −27 but lacks a "CCAAT" box. However, two putative Sp1 hexanucleotide core sequences, GGCGGG, are located at −115 and, in its reverse complement, at position −151.

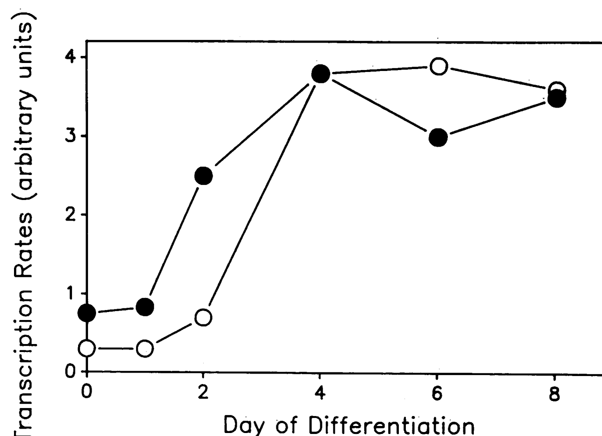

FIG. 3. Transcriptional activation of the C/EBP gene during preadipocyte differentiation. Nuclear run-on transcription activity for the stearoyl-CoA desaturase 1 gene (open circles) and C/EBP gene (closed circles) was determined during differentiation of 3T3-L1 preadipocytes into adipocytes. Day 0 preadipocytes are 2-day post-confluent preadipocytes.

Transcriptional Control of C/EBP mRNA Expression. The differentiation of 3T3-L1 preadipocytes is accompanied by a marked increase in the cellular concentration of C/EBP mRNA (6, 8). [It should be noted that undifferentiated 3T3-L1 preadipocytes express low but detectable levels of C/EBP mRNA and protein (6, 8). After differentiation the levels of C/EBP message and protein rise markedly.] To determine whether this increase in message abundance is due to an increase in transcription rate, nuclei were isolated during the course of differentiation for use in run-on transcription assays. The rate of run-on transcription of the C/EBP gene increased within 48 hr after induction of differentiation and then persisted (Fig. 3), correlating well with the rise in the steady-state level of C/EBP message during differentiation (6). Consistent with its proposed role in coordinating gene transcription during differentiation of 3T3-L1 preadipocytes,

```
-371 CTCCCCTCGC TCGGCCTCTA TATGCTCCCG GGCTCCCTAG TGTTGGCTGG AAGTGGGTGA

-311 CTTAGAGGCT TAAAGGAGGG GCGCCTAACC ACGGACCACG TGTGTGCGGG GGCGACAGCG

-251 CCGCCGGGGT GGGGCTGAGC GCTGCAAGCC GGGTTCGCCT TGCAGCGCAG GAGTCAGTGG

-191 GCGTTGCGCC ACGATCTCTC TCCACTAGCA CTATGCTCCC GCCCCACTCA CCGCCTTGGA
                                              Sp1

-131 AAGTCACAGG AGAAGGCGGG CTCTAAGACC CAGCAGGCAC CATCCTACTG GCGCCTTCGA
                  Sp1

 -71 TCCGAGACCC GTTTGGACAC CAGGGGGCGA TGCCGACCCT CTATAAAAGC GGTCCCCGCG
                                                TATA BOX
                 +1
 -11 CGGGCCTGGC CATTCGCGAC CCGAAGCTGC GCGGGCGCGA GCCAGTTGGG GCACTGGGTG

 +49 GGCGGCGGCG ACAGCGGCGC CACGCGCAGG CTGGAGGCCG CCGAGGCTCG CCATGCCGGG

+109 AGAACTCTAA CTCCCCC ATG
                    Met
```

FIG. 2. Nucleotide sequence of the initial 371 bp of 5′ flanking and 5′ untranslated sequences of the murine C/EBP gene. The transcription initiation site (+1), TATA box (−27), putative Sp1 binding sites (−151 and −115), and the initial methionine residue are indicated. Bold print indicates DNase I protected sequences in 3T3-L1 undifferentiated preadipocyte nuclear extracts. Double underlined sequences indicate DNase I protected sequences detected with both 3T3-L1 undifferentiated preadipocyte and fully differentiated adipocyte nuclear extracts.

the increase in transcription rate of the C/EBP gene precedes that of other adipose-specific genes including the genes for stearoyl-CoA desaturase (EC 1.14.99.5) (Fig. 3), 422(aP2) (1), and GLUT4 (murine-insulin-responsive glucose transporter) (results not shown) whose promoters are trans-activated by C/EBP (6, 11).

DNase I Footprinting of the C/EBP Gene Promoter by Nuclear Extracts from Undifferentiated and Differentiated 3T3-L1 Cells. To determine whether the 5′ flanking region of the C/EBP gene has promoter activity, several chimeric constructs containing segments of the 5′ flanking region, fused upstream of the chloramphenicol acetyltransferase reporter gene, were tested for transcriptional activity. After transient transfection of the chimeric genes into 3T3-L1 cells, it was demonstrated that the segment of the C/EBP gene between nucleotides −350 and +5 is sufficient to drive a high level of expression of the reporter gene (R.J.C. and M.D.L., unpublished results).

To locate potential cis regulatory elements in the promoter of the C/EBP gene and to identify differentiation-specific trans-acting nuclear factors, DNase I footprinting was performed. A 210-bp *Nar* I fragment of 5′flanking sequence in the C/EBP gene (nucleotides −291 to −81, Fig. 2) was subjected to DNase I footprinting with nuclear extracts from undifferentiated 3T3-L1 preadipocytes or fully differentiated 3T3-L1 adipocytes. Three regions were protected from DNase I digestion by nuclear extracts prepared from 3T3-L1 cells (Fig. 4). The 5′-most region footprinted (between nucleotides −275 and −258) by both preadipocyte and adipocyte nuclear extracts contains the sequence CACGTGTGT-GCGGGGGCG. A second region, between nucleotides −252 and −239, is footprinted only by nuclear extracts from preadipocytes and contains the sequence GCCGCCGGGG-TGGG. A third region, from nucleotide −198 to nucleotide −176 (TCAGTGGGCGTTGCGCCACGATC), is protected from DNase I digestion by nuclear extracts from both preadipocytes and adipocytes. However, these footprints are distinctly different, the protected region extending ≈5 bp further in the 5′ direction with nuclear extracts from preadipocytes (compare lanes UNDIFF and DIFF in region −203 to −176 in Fig. 4).

Previous experiments had shown that a heat-stable nuclear factor, which was identified as C/EBP and is present at high levels only in fully differentiated 3T3-L1 adipocytes, binds to and transactivates the promoters of several differentially expressed adipocyte-specific genes (6, 11). To determine whether the differences in DNase I protection (between nucleotides −203 and −176) by nuclear extracts of preadipocytes and adipocytes are due to an interaction of C/EBP with its own promoter, footprinting with recombinant C/EBP protein was investigated, showing the C/EBP gene promoter between nucleotides −198 and −176, with the footprint pattern indistinguishable from that obtained with nuclear extracts from fully differentiated 3T3-L1 adipocytes (compare DIFF lanes in Fig. 4 with lanes in Fig. 5*A*). Like C/EBP, the nuclear factor from 3T3-L1 adipocytes that binds at this site is heat-stable, and heating (70°C for 10 min) does not alter its footprinting pattern (results not shown). Furthermore, the electrophoretic mobility of a synthetic oligonucleotide corresponding to this site (nucleotides −191 to −172) is retarded by recombinant C/EBP protein (Fig. 5*B*). Taken together, these results indicate that the promoter of the C/EBP gene possesses a C/EBP binding site.

Gel-Retardation Analysis of Differentiation-Associated Oligodeoxynucleotide–Protein Complexes. To verify the differences in the DNase I protection patterns detected with nuclear factors from undifferentiated versus differentiated cells (Fig. 4) and to identify specific nuclear factor–DNA complexes, gel retardation assays with synthetic oligonucleotides were performed. An oligonucleotide corresponding to the sequence (nucleotides −191 to −172) in the C/EBP gene promoter that was footprinted both by nuclear factors from differentiated cells and by full-length recombinant C/EBP (Figs. 4 and 5) was tested. Two protein–oligonucleotide complexes (A_1 and A_2) were formed in greater abundance with nuclear extracts from differentiated than from undifferentiated cells (Fig. 6, lanes 1 and 2). That these protein–oligonucleotide complexes contain C/EBP is indicated by their disappearance when the nuclear extract is treated with

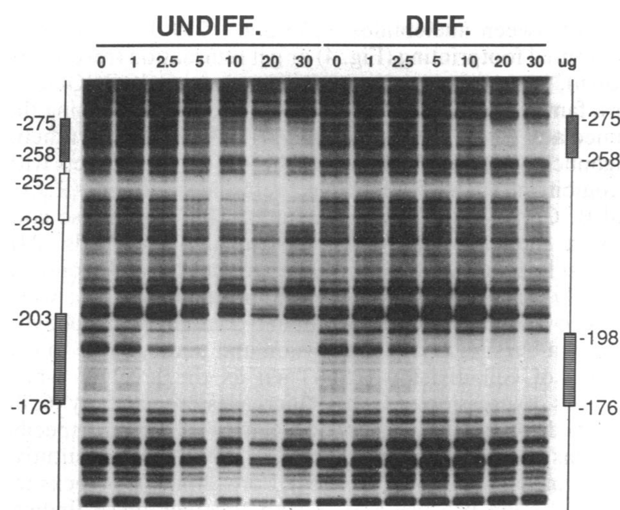

FIG. 4. DNase I footprint analysis of the C/EBP promoter with nuclear extracts of 3T3-L1 cells. 3T3-L1 preadipocytes either were maintained in the undifferentiated state (lanes UNDIFF.) or were induced to differentiate (lanes DIFF.). A 210-bp *Nar* I fragment of the C/EBP promoter was incubated with increasing amounts (1–30 μg) of undifferentiated or differentiated cell nuclear extracts and then was digested with DNase I. The protected regions are indicated by boxes. Lanes 0 contain control footprints with no nuclear extract added.

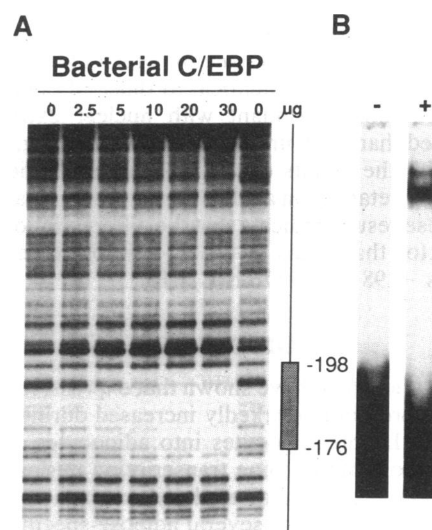

FIG. 5. DNase I footprint analysis with bacterially expressed recombinant C/EBP. (*A*) Increasing amounts of bacterially expressed C/EBP were incubated with the 210-bp *Nar* I fragment and then digested with DNase I. The region protected (nucleotides −198 to −176) is boxed, and the endpoints are indicated. Lane 0 shows a control footprint with no extract added. (*B*) Gel retardation assay using a ^{32}P-labeled oligodeoxynucleotide for the region from nucleotide −191 to nucleotide −172 of the C/EBP gene incubated with control extract (lane −) or bacterially expressed C/EBP (lane +).

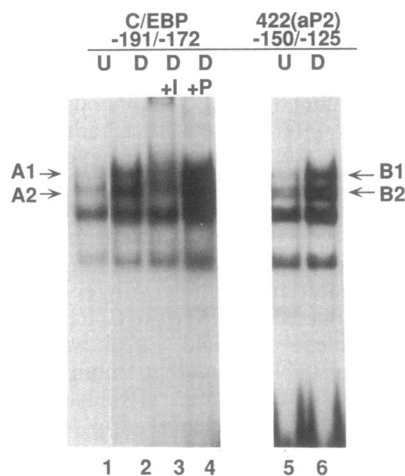

FIG. 6. Gel retardation analysis of the C/EBP binding sites in the C/EBP and 422(aP2) gene promoters. ^{32}P-end-labeled oligonucleotides corresponding to positions −191 to −172 in the C/EBP gene promoter (lanes 1–4) or positions −150 to −125 in the 422(aP2) gene promoter (6) (lanes 5 and 6) were incubated with 4 μg of nuclear extract from 3T3-L1 undifferentiated (lanes U) preadipocytes (lanes 1 and 5) or differentiated (lanes D) adipocytes (lanes 2–4 and 6). In lanes 3 and 4, respectively, anti-C/EBP (lane +I) or preimmune (lane +P) serum was first incubated with nuclear extract from differentiated cells prior to incubation with labeled oligonucleotide. A_1, A_2, B_1, and B_2 indicate protein–oligonucleotide complexes whose formation is increased with nuclear extracts from differentiated cells.

antiserum against C/EBP (Fig. 6, lane 3) but not when treated with preimmune serum (Fig. 6, lane 4).

To ascertain whether the same nuclear factor(s) that binds to the C/EBP promoter between nucleotides −191 and −172 might also bind to the C/EBP binding site of the 422(aP2) gene, the corresponding oligonucleotide (nucleotides −150 to −125) of this gene was subjected to gel retardation analysis with nuclear extracts from 3T3-L1 cells. A comparison of the two oligonucleotides showed that the gel retardation patterns were virtually identical (compare lanes 1 and 2 with lanes 5 and 6 in Fig. 6), suggesting that the same nuclear factors bind to the C/EBP sites of both genes. Moreover, the two complexes (B_1 and B_2 in Fig. 6, lane 6), which have electrophoretic mobilities virtually identical to those of complexes A_1 and A_2, are more abundant with nuclear extracts from differentiated than from undifferentiated cells (Fig. 6). Taken together with the results of the DNase I protection experiments and gel retardation analysis using recombinant C/EBP (Fig. 5), these results indicate that the differentiation-induced nuclear factor that binds to the C/EBP promoter between nucleotides −198 and −176 is C/EBP.

DISCUSSION

Previous studies (6, 8) have shown that expression of C/EBP mRNA and protein is markedly increased during differentiation of 3T3-L1 preadipocytes into adipocytes. A role for C/EBP in coordinating gene transcription during preadipocyte differentiation is indicated by its capacity to transactivate the promoters of several adipose-specific genes (6, 11). We now report that the differentiation-associated increase in the steady-state level of C/EBP is due to an increased rate of transcription of the C/EBP gene (Fig. 3) and that the promoter of the gene contains sequences that interact with nuclear factors (Figs. 4 and 6) likely to be involved in differentiation-induced gene expression. Thus, we show that a nuclear factor whose expression is increased during preadipocyte differentiation and that binds to the C/EBP gene promoter is C/EBP (Figs. 5 and 6) or a closely related gene family member. A scenario can be visualized whereby, after derepression of the C/EBP gene, the continued expression of C/EBP and thus maintenance of the differentiated state would be guaranteed by interaction of the gene product (e.g., C/EBP) with the promoter of its own gene.

Recently, two members of the C/EBP basic region-leucine zipper (C/EBP B-ZIP) family have been sequenced and shown to form heterodimers with C/EBP (18, 20, 21). One of these proteins, LAP (18), interacts with the D region (i.e., the C/EBP binding site) of the albumin promoter and activates transcription of the albumin gene *in vitro*. These and other findings (18, 20, 21) indicate that at least three members of the C/EBP B-ZIP family have indistinguishable binding sites. Preliminary results indicate that LAP is expressed in both 3T3-L1 preadipocytes and adipocytes (P. Cornelius and M.D.L., unpublished results). Gel retardation analysis with C/EBP binding site oligonucleotides and nuclear extracts from 3T3-L1 cells revealed that multiple nuclear factor–oligonucleotide complexes are formed (Fig. 6). Two of these complexes, generated primarily with nuclear extracts from differentiated 3T3-L1 adipocytes (Fig. 6, lanes 2 and 6), contain C/EBP, since anti-C/EBP antibodies disrupt their formation (Fig. 6, lane 3). Preliminary findings suggest that these complexes contain C/EBP homodimer and C/EBP-LAP heterodimer (results not shown).

Evidence was also obtained that a nuclear factor present in preadipocytes and lost upon differentiation binds to another site in the promoter of the C/EBP gene. Thus, the region between nucleotides −252 and −239 is protected from DNase I cleavage by a nuclear factor expressed only in undifferentiated 3T3-L1 cells (Fig. 4). Consistent with these findings, nuclear extracts from preadipocytes also give rise to an oligonucleotide (nucleotides −254 to −236)–protein complex not formed with nuclear extracts from differentiated cells (results not shown). It is conceivable that this site is involved in repressing expression of the C/EBP gene in the undifferentiated state. The sequence of this binding site lacks homology to the consensus binding sites of known transcription regulatory proteins (22). Other regions protected from DNase I cleavage in the C/EBP gene promoter do contain consensus DNA sequences for other nuclear binding proteins. While the region between nucleotides −275 and −259 does not show differential footprinting (Fig. 4) or gel retardation (results not shown), it contains a consensus sequence, GCGGGGGCG, for a family of zinc-finger proteins first defined by using the immediate early gene product Zif 268 (23). A synthetic oligonucleotide corresponding to this sequence interacts with recombinant Zif 268 protein in gel retardation studies (R.J.C. and B. Christy, unpublished results). This region also contains a sequence CACGTG, to which c-myc binds (24). Several lines of evidence suggest that c-myc may function at an early stage of preadipocyte differentiation. Thus, when induced to differentiate, confluent 3T3-L1 preadipocytes exhibit a burst of c-myc expression and undergo one to two rounds of cell division (25). Then as the level of c-myc declines, expression of C/EBP increases dramatically (6, 8), followed by the expression of a family of adipose-specific proteins (1, 2, 4, 5). It should also be noted that constitutive overexpression of c-myc in 3T3-L1 preadipocytes blocks the differentiation process (25). Taken together these findings suggest that c-myc might repress expression of the C/EBP gene and thereby prevent the activation of transcription of adipose-specific genes in the preadipocyte.

It is evident that the 5′ flanking region of the C/EBP gene contains an array of cis elements that interact with differentially expressed nuclear factors in 3T3-L1 cells. To understand how transcription of the C/EBP gene is regulated, it will be necessary to characterize and determine the roles of each of these nuclear trans-acting factors.

We thank Ms. Natalie Tumminia for expert help in preparing this manuscript. This work was supported by a research grant (NIDDK-38418) from the National Institutes of Health. R.J.C was supported by a National Research Service Award from the National Institutes of Health.

1. Bernlohr, D. A., Bolanowski, M. A., Kelly, T. J. & Lane, M. D. (1985) *J. Biol. Chem.* **260**, 5563–5567.
2. Cook, K. S., Hunt, C. R. & Spiegelman, B. M. (1985) *J.Cell. Biol.* **100**, 514–520.
3. Ntambi, J. M., Buhrow, S. A., Kaestner, K. H., Christy, R. J., Sibley, E., Kelly, T. J. & Lane, M. D. (1988) *J. Biol. Chem.* **263**, 17291–17300.
4. Bernlohr, D. A., Angus, C. W., Lane, M. D., Bolanowski, M. A. & Kelly, T. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5468–5472.
5. Kaestner, K. H., Ntambi, J. M., Kelly, T. J. & Lane, M. D. (1989) *J. Biol. Chem.* **264**, 14755–14761.
6. Christy, R. J., Yang, V. W., Ntambi, J. M., Geiman, D. E., Landschulz, W. H., Friedman, A. D., Nakabeppu, Y., Kelly, T. J. & Lane, M. D. (1989) *Genes Dev.* **3**, 1323–1335.
7. Landschulz, W. H., Johnson, P. F., Adashi, E. Y., Graves, B. J. & McKnight, S. L. (1988) *Genes Dev.* **2**, 786–800.
8. Birkenmeier, E. H., Gwynn, B., Howard, S., Jerry, J., Gordon, J. I., Landschulz, W. H. & McKnight, S. L. (1989) *Genes Dev.* **3**, 1146–1156.
9. Friedman, A. D., Landschulz, W. H. & McKnight, S. L. (1989) *Genes Dev.* **3**, 1314–1322.
10. McKnight, S. L., Lane, M. D. & Gluecksohn-Waelsch, S. (1989) *Genes Dev.* **3**, 2021–2024.
11. Kaestner, K. H., Christy, R. J. & Lane, M. D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 251–255.
12. Herrera, R., Ro, H. S., Robinson, G. S., Zanthopoulos, K. G. & Spiegelman, B. M. (1989) *Mol. Cell. Biol.* **9**, 5531–5339.
13. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
14. Swick, A. G., Blake, M. C., Kahn, J. W. & Azizkhan, J. C. (1989) *Nucleic Acids Res.* **17**, 9291–9304.
15. Graves, B. J., Johnson, P. F. & McKnight, S. L. (1986) *Cell* **44**, 565–576.
16. Studier, F. W., Rosenberg, A. H. & Dunn, J. J. (1990) *Methods Enzymol.* **185**, 60–89.
17. Bohmann, D., Bos, T. J., Nishimura, T., Vogt, P. K. & Tjian, R. (1987) *Science* **238**, 1386–1392.
18. Descombes, P., Chojkier, M., Lichtsteiner, S., Falvey, E. & Schibler, U. (1990) *Genes Dev.* **4**, 1541–1551.
19. Kaestner, K. H., Flores-Riveros, J., McLenithan, J. C., Janicot, M. & Lane, M. D. (1990) *Proc. Natl. Acad. Sci. USA* **88**, 1933–1937.
20. Roman, C., Platero, S. J., Shuman, J. & Calame, K. (1990) *Genes Dev.* **4**, 1404–1415.
21. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. & Kishimoto, T. (1990) *EMBO J.* **9**, 1897–1906.
22. Wingender, E. (1988) *Nucleic Acids Res.* **16**, 1879–1902.
23. Christy, B. & Nathans, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8737–8741.
24. Blackwell, T. K., Kretzner, L., Blackwood, E. M., Eisenman, R. N. & Weintraub, H. (1990) *Science* **250**, 1149–1151.
25. Freytag, S. O. (1988) *Mol. Cell. Biol.* **8**, 1614–1624.