Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 2949–2953, May 1988
Biochemistry

Expression of the differentiation-induced gene for fatty acid-binding protein is activated by glucocorticoid and cAMP

(3T3-L1/preadipocyte/adipocyte)

JONATHAN S. COOK*, JOHN J. LUCAS*†, ERIC SIBLEY*, MARK A. BOLANOWSKI‡§, ROBERT J. CHRISTY*, THOMAS J. KELLY‡, AND M. DANIEL LANE*¶

Departments of *Biological Chemistry and ‡Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

Contributed by M. Daniel Lane, January 11, 1988

ABSTRACT **We have isolated and characterized a fragment of the gene encoding adipose fatty acid-binding protein (gene 422) from a 3T3-L1 adipocyte genomic library. The 5′-flanking sequence of the 422 gene contains potential regulatory regions for adipose-specific expression. At position −120 there is a fat-specific element that occurs in several genes expressed as preadipocytes differentiate, and at position −393 there is a glucocorticoid regulatory element core sequence. Chimeric constructs were prepared by ligating 858 base pairs or 248 base pairs of 5′-flanking sequence and 22 nucleotides of 5′-untranslated sequence of the 422 gene to the bacterial gene encoding chloramphenicol acetyltransferase (CAT); these constructs (Δ858.CAT and Δ248.CAT) were transfected into 3T3-L1 preadipocytes. When differentiation was initiated by the adipogenic agents methylisobutylxanthine (a cAMP phosphodiesterase inhibitor), dexamethasone, and insulin, expression of both constructs increased, reaching maximal levels within 24 hr. Both constructs were maximally induced 48 hr before appreciable accumulation of the endogenous 422 mRNA. Expression of Δ858.CAT, but not of Δ248.CAT, was induced by dexamethasone, which correlates with deletion of the potential glucocorticoid regulatory element. Expression of both constructs was induced by 8-bromoadenosine 3′,5′-cyclic monophosphate, thus implicating the first 248 base pairs of 5′-flanking sequence of the 422 gene in the response to cAMP. Indirect effects by the adipogenic factors on CAT protein or mRNA synthesis and turnover were ruled out, since replacing the 5′-flanking region of the 422 gene constructs with viral promoters abolished the effects of dexamethasone and 8-bromoadenosine 3′,5′-cyclic monophosphate on CAT expression. We conclude that the first 858 base pairs of 5′-flanking sequence of the 422 gene contains elements that mediate activation by dexamethasone and cAMP.**

Green and Kehinde (1, 2) have established several 3T3 cell lines that differentiate into adipocytes in response to appropriate stimuli. The expression of adipocyte morphology by these cells is accompanied by the specific reprogramming of enzymatic and regulatory functions to those typical of adipose cells. The accumulation of cytoplasmic triglyceride that occurs during differentiation is correlated with a coordinate rise in the activity of every enzyme of the pathways of *de novo* fatty acid and triglyceride synthesis (3–7). In addition, 3T3 preadipocytes acquire the enzymatic capacity to mobilize triglyceride (8) and respond with great sensitivity to lipogenic (9–11) and lipolytic hormones (12). There is now compelling evidence (13–15) that the increase in the levels of the specific translatable mRNAs for these differentiation-induced proteins is responsible for the observed increases in their expression during 3T3 preadipocyte differentiation, which in all cases thus far examined depends on increased transcription of the corresponding genes (16–18).

We (19) and others (20, 21) have isolated a group of cDNAs corresponding to messages whose levels are greatly increased during adipocyte conversion. One of these cDNAs, pAL422, encodes an adipocyte homologue of myelin P2 (19). This protein (also referred to as aP2) has sequence homology to a class of fatty acid-binding proteins found in lipogenic tissues (19). Expression of the 422 gene is transcriptionally activated during differentiation, leading to a 50- to 100-fold accumulation of its 0.7-kilobase (kb) mRNA (16, 17). The complete gene has been isolated and sequenced (22); the 422 gene contains four exons encompassing 4.5 kb of genomic DNA. Distel *et al.* (23) showed that 168 base pairs (bp) of 422 gene 5′-flanking sequence are sufficient to drive the differentiation-dependent expression of a heterologous reporter gene in 3T3-F442A preadipocytes. Furthermore, a 28-nucleotide element was identified in this region that inhibits the expression of the 422 gene in preadipocytes and that appears to bind a fos-like protein.

We report here the isolation from a 3T3-L1 library, a genomic fragment that encodes the differentially expressed 422 protein. We have sequenced the 5′-flanking region of our genomic clone and verified the start site of transcription. In addition, we have demonstrated that in 858 nucleotides of the 5′-flanking region there are sequences that mediate increased expression of a heterologous gene in 3T3-L1 cells treated with cAMP or dexamethasone.

MATERIALS AND METHODS

Materials. Restriction enzymes, T4 DNA ligase, DNA polymerase (Klenow fragment), the dideoxynucleotide sequencing kit, and calf intestinal alkaline phosphatase were from Boehringer Mannheim or Pharmacia. Double-stranded form of M13 DNA and pUC vectors were from New England Biolabs. The simian virus 40-based expression plasmids used in this study were those described (24–26). [^{14}C]Chloramphenicol, [*acetyl*-^{3}H]acetyl-CoA, and deoxyadenosine 5′-[α-^{35}S]thiotriphosphate were from New England Nuclear. 8-Bromoadenosine 3′,5′-cyclic monophosphate (8BrcAMP) was from Sigma, and G418 sulfate was from GIBCO.

Cell Culture. 3T3-L1 preadipocytes were maintained and induced to differentiate as described (7, 27). Briefly, differentiation was induced by exposure of confluent 3T3-L1 preadipocytes to medium containing 1.7 μM insulin, 1 μM

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.

Abbreviations: iBuMeXan, 3-isobutyl-1-methylxanthine; 8BrcAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; CAT, chloramphenicol acetyltransferase; GRE, glucocorticoid regulatory element; FSE, fat-specific element.

†Present address: Department of Biochemistry and Molecular Biology, State University of New York Health Science Center, Syracuse, NY 13210.
§Present address: Howard Hughes Medical Institute, Duke University Medical Center, Department of Medicine, Durham, NC 27710.
¶To whom reprint requests should be addressed.

dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (iBuMeXan). After 2 days the medium containing dexamethasone and iBuMeXan was replaced by medium supplemented with only 1.7 μM insulin. Within 24 hr, i.e., on day 3, 422 mRNA increased to virtually its maximal level, and >95% of the cells had begun to accumulate cytoplasmic triglyceride (16).

Isolation and Analysis of p422 Genomic Clone. Genomic DNA from 3T3-L1 cells was digested with *Eco*RI and fractionated to enrich for a 4.3-kb species that hybridizes to the 5′ end of the full-length cDNA pAL422 (19). This fraction was cloned into λgt10, and 4×10^5 plaques were screened with a 5′ fragment of pAL422. A positive clone was identified and shown to encode a portion of the 422 protein (Fig. 1*B*). Restriction fragments were isolated and subcloned into the appropriate vector (28). M13 single-stranded DNA was sequenced by the dideoxynucleotide chain-termination method (29), with deoxyadenosine 5′-[α-^{35}S]thiotriphosphate as substrate (30). The S1-nuclease analysis was carried out as described by Favaloro *et al.* (31).

Stable and Transient Transfections. Cells were stably transfected with a 5:1 molar ratio of chimeric construct/SV2neo (25) DNAs (15 μg per dish) by calcium phosphate precipitation (32), followed by a 10% (vol/vol) dimethyl sulfoxide shock. Neomycin-resistant foci appeared 10–12 days after transfection. To measure the transient expression of transfected constructs, we developed an electroporation protocol that used the Gene Pulser (Bio-Rad). This protocol gives high levels of transfected gene expression, while maintaining the adipose morphology. Confluent monolayers of 3T3-L1 adipocytes, 4 days after inducing differentiation, or preadipocytes maintained for an identical period, were removed from 100-mm dishes by treatment with 0.05% trypsin in isotonic phosphate-buffered saline (PBS). Cell suspensions were centrifuged through fetal bovine serum (adipocytes) or calf serum (preadipocytes), and the pellets were washed three times with PBS. Cells were resuspended in ice-cold electroporation buffer (0.8 mM $CaCl_2$/0.48 mM $MgSO_4$/1.3 mM KH_2PO_4/7.36 mM Na_2HPO_4/2.44 mM KCl/124 mM NaCl/5 mM glucose, pH 7.2), and 0.7-ml aliquots, each containing the cells from one 100-mm plate, were added to sterile chambers containing 30 μg of 422.CAT construct (see Fig. 1) in 100 μl of the same buffer. This was mixed and incubated on ice for 10 min. Adipocytes were electroporated at 650 V at a capacitance of 25 μF; for preadipocytes the voltage was 550 V. The cells were further incubated for 10 min on ice, for 5 min at room temperature, and then at 37°C. The cells from each 100-mm dish transfected were cultured on two 60-mm dishes in medium supplemented as required. Cell extracts were prepared 48 hr after transfection.

Cell Extracts and Chloramphenicol Acetyltransferase (CAT) Assays. Extracts of transfected 3T3-L1 cells were prepared, and CAT activity was assayed as described by Gorman *et al.* (24) or by Neumann *et al.* (33) as indicated in the figure legends. The results from both methods are standardized to a plate (60 mm in diameter) equivalent of cell extract added.

RESULTS

Structure of a 422 Genomic Clone. We have reported (19) the isolation and sequence of the full-length cDNA (pAL422) for the 422 protein, the adipocyte homologue of myelin P2, which is expressed upon differentiation of 3T3-L1 preadipocytes. To further investigate the differentiation-induced transcription of the 422 gene (16), we have cloned a 4.3-kb segment of 3T3-L1 DNA that contains exon 1, 122 nucleotides of exon 2, and 1.7 kb of 5′-flanking sequence of the 422 gene (Fig. 1*B*).

We obtained the sequence of the 5′-flanking region of the 422 genomic clone, 868 bp of which is shown in Fig. 1*A*. Phillips *et al.* (22) reported the sequence of the 422 gene isolated from a BALB/c mouse genomic library beginning at position −509. There is good agreement between the two sequences in the 5′-flanking region of the 422 gene (98.4% homology); eight nucleotides are different and there are two deletions. This slight variation in sequence may reflect the various sources of genomic DNA used to prepare the libraries.

By using S1-nuclease analysis we identified the adenine residue at position +1 as the major start site of transcription (data not shown), which agrees with previous observations (22, 34). A minor initiation site was also detected at an adenine nucleotide at position −8; the significance of this was not further investigated. Transcripts beginning at either nucleotide occur only 3T3-L1 adipocytes, not in preadipocytes (data not shown). At position −33 is an A+T-rich region, and an inverted CAAT homology occurs at position −66. The fat-specific element (FSE) identified by Spiegelman and coworkers (23, 34) occurs at position −120. In addition to the FSE, we have also noted a potential glucocorticoid regulatory element (GRE) (for reviews, see refs. 35 and 36) at position −393 in the 5′-flanking region of the 422 gene (Fig. 1*A*).

Differentiation-Induced Expression of 422.CAT Constructs. To assess the activity of adipogenic factor-dependent regulatory elements in the 422 gene, putative promoter regions, including the start site of transcription, were spliced in front of CAT coding sequences (see Fig. 1*C* and *D*). These con-

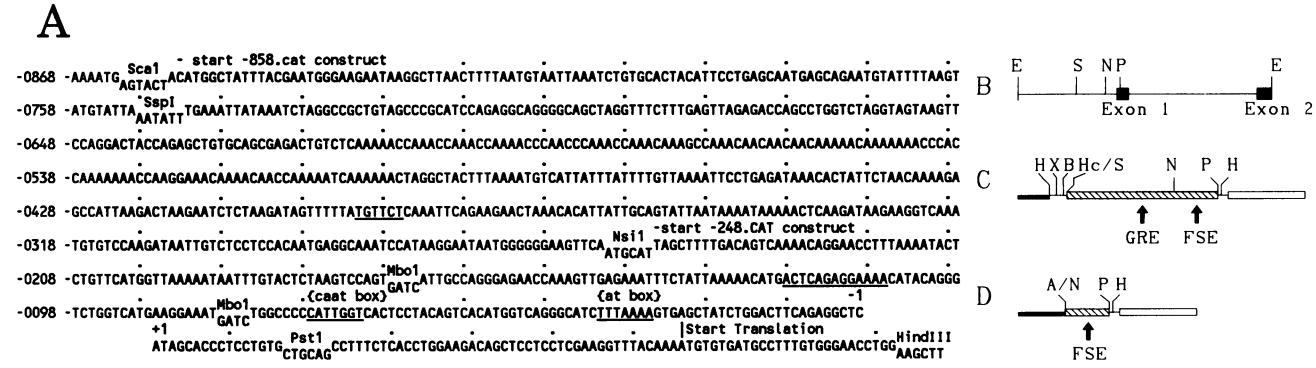

FIG. 1. Partial 422 genomic clone, 5′-flanking sequence, and construction of 422.CAT chimeric genes. (*A*) Sequence of 858 bp of 422 gene 5′-flanking region. Relevant restriction sites are shown in subscript, and potential regulatory sequences are underlined. (*B*) Partial genomic clone isolated from a 3T3-L1 genomic library. Exons are in solid bars. Δ858.CAT (*C*) and Δ248.CAT (*D*) are chimeric constructs. Sequences were derived as follows: thin lines, M13 cloning vector; thick lines, pBR322; hatched bars, 422 gene; and open bars, CAT coding region. E, *Eco*RI; H, *Hin*dIII; X, *Xba* I; B, *Bam*HI; Hc, *Hinc*II; S, *Sca* I; N, *Nsi* I; P, *Pst* I; A, *Ava* I.

structs were cotransfected with pSV2neo into 3T3-L1 preadipocytes, and the activity of CAT was assayed in cell extracts of stable transformants. Low levels of CAT activity were observed when promoterless pSV0CAT (24) or Δ858(−).CAT (the 422 promoter region is in the antisense orientation relative to the CAT coding sequence) was introduced into 3T3-L1 preadipocytes that were then induced to differentiate (data not shown). Low levels of expression of the Δ858.CAT and Δ248.CAT were maintained by 3T3-L1 cells kept in a nondifferentiating confluent state for periods up to 12 days (Fig. 2).

Two days after confluence, 3T3-L1 preadipocytes that had been transfected with either Δ858.CAT or Δ248.CAT were induced to differentiate by the addition of iBuMeXan, dexamethasone, and insulin for 48 hr, after which time the iBuMeXan and the dexamethasone were removed. No loss of adipogenic potential was seen in these transfected 3T3-L1 cells. Within 24 hr, the adipogenic factors caused a maximal increase in CAT activity in cells that contained either of the 422.CAT constructs; this level of CAT activity was maintained for 2–3 days. The activity then declined and increased after day 8 (Fig. 2). The decrease in CAT activity correlates with removal of iBuMeXan and dexamethasone from the medium. This time course of CAT expression differs from the accumulation of endogenous 422 mRNA, which does not appear until iBuMeXan and dexamethasone are removed from the medium; maximal levels of expression of the endogenous gene are not observed for 72–96 hr (16).

Dexamethasone and cAMP Regulatory Regions in the 422.CAT Constructs. As shown above, the expression of 422.CAT constructs is rapidly increased by the combined addition of adipogenic factors. To assess the contribution of each agent to this induction, individual and various combinations of adipogenic factors were added 2 days after confluence to 3T3-L1 preadipocytes transfected with either Δ858.CAT or Δ248.CAT (Fig. 3). Insulin alone had no effect on expression of either of the two constructs under the conditions of these experiments (data not shown). This is consistent with the decline in CAT activity when iBuMeXan and dexamethasone are withdrawn, and the cells are incubated in medium supplemented with insulin alone (Fig. 2).

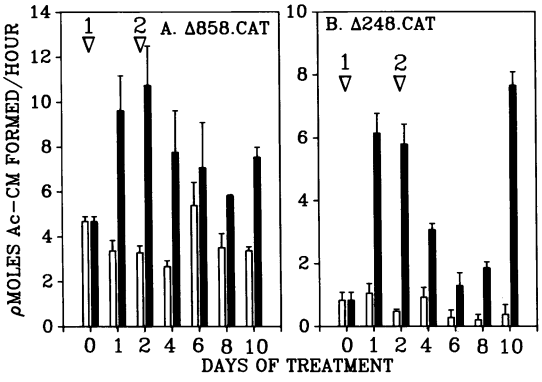

FIG. 2. Differentiation-dependent expression of transfected 422.CAT constructs. 3T3-L1 preadipocytes were transfected with a 1:5 molar ratio of pSV2neo to either Δ858.CAT (*A*) or Δ248.CAT (*B*). Pools of neomycin-resistant clones were expanded, and CAT activity was assayed through 10 days of a differentiation program. The addition of iBuMeXan, dexamethasone, and insulin (arrowhead 1) and the removal of dexamethasone and iBuMeXan (arrowhead 2) are indicated. Open bars show the activity in 3T3-L1 preadipocytes maintained in an undifferentiated state, and solid bars show the activity in preadipocytes induced to differentiate. CAT activity was assayed in extracts prepared at the times indicated as described (33). Results are the mean of two independent observations ± the range. CAT activity is expressed as pmol of acetylated chloramphenicol (Ac-CM) formed per hr per plate (60 mm) equivalent.

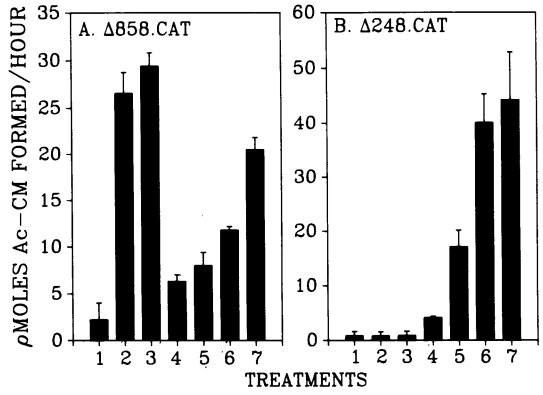

FIG. 3. Factors affecting the expression of 422.CAT constructs. Pools of neomycin-resistant 3T3-L1 preadipocytes transfected with either Δ858.CAT (*A*) or Δ248.CAT (*B*) were maintained at confluence for 2 days, after which time they were treated for 48 hr as follows. Treatments: 1, none; 2, 1 μM dexamethasone; 3, 1 μM dexamethasone plus 1.7 μM insulin; 4, 1 μM dexamethasone, 1.7 μM insulin, plus 0.5 mM iBuMeXan; 5, 1 mM 8BrcAMP; 6, 1 mM 8BrcAMP plus 1 μM dexamethasone; 7, 1 mM 8BrcAMP, 1 μM dexamethasone, plus 1.7 μM insulin. Cell extracts were prepared, and CAT activity was assayed (33). CAT activity is expressed as pmol of acetylated chloramphenicol (Ac-CM) formed per hr per plate (60 mm) equivalent. Results are the mean of two independent observations ± the range.

Dexamethasone caused a rapid increase in CAT activity when the transfected construct contained 858 bp of the 5′-flanking region (12-fold, Fig. 3*A*) but not when it was deleted to position −248 (Fig. 3*B*). Dexamethasone plus insulin caused a slightly greater induction of Δ858.CAT expression (13-fold, Fig. 3*A*), but again there was no significant increase in CAT activity in Δ248.CAT-transfected cells (Fig. 3*B*). The effect of dexamethasone on Δ858.CAT expression was first seen within 4 hr and was maintained for ≈4 days, after which time it decreased (data not shown). As Δ248.CAT does not respond to dexamethasone, an element between positions −858 and −248 must mediate the induction by glucocorticoid. The results obtained in stably transformed cells were confirmed by transient expression experiments (Fig. 4). Dexamethasone induced the transient expression of Δ858.CAT in both preadipocytes and adipocytes; thus all information required for the dexamethasone response is present in the 858 bp of the 422 gene. In

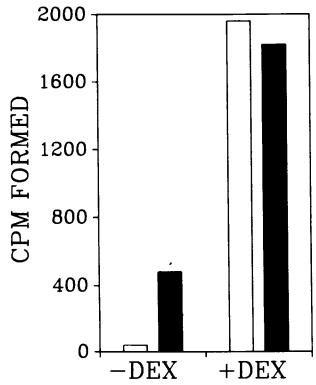

FIG. 4. Regulation by dexamethasone (Dex) of transiently expressed Δ858.CAT. 3T3-L1 preadipocytes (open bars) and adipocytes (solid bars) were transfected by electroporation. After electroporation, cells were carried in culture in medium with 10% (vol/vol) fetal bovine serum with (+) or without (−) 1 μM dexamethasone. Incubations were continued for 48 hr, at which time cell extracts were prepared, and CAT activity was assayed (24). Results are expressed as total cpm of chloramphenicol acetylated per plate (60 mm) equivalent.

addition, basal CAT activity in these transient expression experiments was greater in adipocytes (cells subjected to the differentiation protocol) than preadipocytes, consistent with observations made above (Fig. 2*A*).

When iBuMeXan was included along with dexamethasone and insulin, the effect on the expression of the two transfected constructs differed. This adipogenic factor reduced the expression of Δ858.CAT, whereas it induced expression of Δ248.CAT (Fig. 3). The 13-fold increase in Δ858.CAT expression induced by dexamethasone plus insulin was decreased to a 3-fold increase when iBuMeXan was also added. Comparison of the same two treatments on Δ248.CAT expression (Fig. 3*B*) showed that iBuMeXan caused a 5-fold increase.

iBuMeXan, a potent cAMP phosphodiesterase inhibitor, may accelerate differentiation of 3T3-L1 cells by increasing the level of cAMP (37). Therefore, we also tested the effect of 8BrcAMP on the expression of the two 422.CAT constructs. The expression of Δ858.CAT was increased 4-fold by 8BrcAMP (Fig. 3*A*). Like iBuMeXan, 8BrcAMP reduced the induction caused by dexamethasone (a 12- to a 5-fold increase) and by dexamethasone plus insulin (a 13- to a 9-fold increase). 8BrcAMP caused a 19-fold increase in CAT activity when added to 3T3-L1 cells transfected with the Δ248.CAT (Fig. 3*B*). This induction by 8BrcAMP was potentiated by dexamethasone (a 19- to a 45-fold increase, Fig. 3*B*) and by dexamethasone plus insulin (a 19- to a 50-fold increase, Fig. 3*B*).

During differentiation of 3T3-L1 preadipocytes induced by the adipogenic factors, major changes in mRNA and protein metabolism occur (16). It seemed possible, therefore, that the observed changes in CAT activity in cells transfected with Δ858.CAT or Δ248.CAT and treated with adipogenic factors were a result of nonspecific changes in mRNA or protein metabolism—i.e., synthesis or turnover. To rule out this possibility, we measured CAT activity in 3T3-L1 preadipocytes transfected with either pSV2CAT or pRSVCAT (26) and subsequently treated for 48 hr with adipogenic factors (Fig. 5). These constructs differ from Δ248.CAT and Δ858.CAT only in the promoter sequence. Negligible changes (maximum 1.5-fold) in CAT activity were observed in response to adipogenic agents added to these cells. Thus, we conclude that the cAMP- and dexamethasone-induced changes in expression of the 422.CAT constructs are mediated through elements present in the promoter region of the 422 gene.

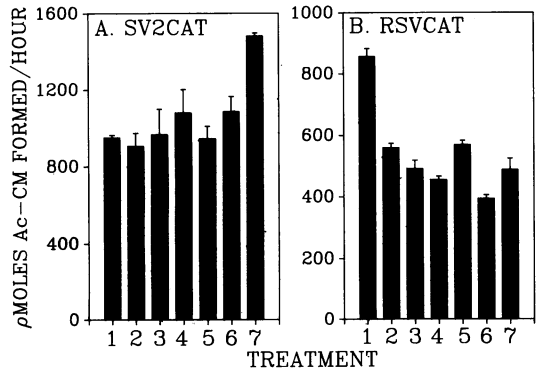

FIG. 5. Effects of adipogenic agents on expression of CAT constructs with viral promoters. 3T3-L1 preadipocytes were transfected with a 1:5 molar ratio of pSV2neo to either pSV2CAT (*A*) or pRSVCAT (*B*). Pools of stable transformants were maintained at confluence for 2 days, after which time they were treated for 48 hr as follows. Treatments: 1, none; 2, 1 μM dexamethasone; 3, 1 μM dexamethasone plus 1.7 μM insulin; 4, 1 μM dexamethasone, 1.7 μM insulin, plus 0.5 mM iBuMeXan; 5, 1 mM 8BrcAMP; 6, 1 mM 8BrcAMP plus 1 μM dexamethasone; 7, 1 mM 8BrcAMP, 1 μM dexamethasone, plus 1.7 μM insulin. Cell extracts were prepared, and CAT activity was assayed (33). CAT activity is expressed as pmol of acetylated chloramphenicol (Ac-CM) formed per hr per plate (60 mm) equivalent. Results are the mean of two independent observations ± the range.

DISCUSSION

The differentiation of 3T3-L1 preadipocytes and the expression of adipose-specific genes are accelerated by iBuMeXan and dexamethasone (16, 27, 38). Preadipocyte differentiation induced by glucocorticoids has been observed consistently (7, 27, 38) with 3T3-L1 preadipocytes and with the TA1 preadipocyte line (21). Furthermore, differentiation of TA1 preadipocytes and transcription of the 422 gene are greatly increased by glucocorticoid (39). A GRE core sequence (TGTTCT) at position −393 in the 5′-flanking region (Fig. 1*A*) may be responsible for the induction of expression of the 422 gene by dexamethasone. The ability of transfected Δ858.CAT, which possesses the GRE core element, to respond rapidly (<4 hr) to dexamethasone is consistent with this interpretation. This is further supported by the fact that deleting this element, as in the Δ248.CAT construct, eliminates the response to glucocorticoid. Furthermore, preliminary studies in this laboratory (R.J.C., unpublished results), with more detailed deletions within this region of the 422 gene, are consistent with the GRE at position −393 mediating the effects of dexamethasone. In the 5′-flanking region of the 422 gene there are two other potential GREs at positions −1470 and −1520 (J.S.C., unpublished observations).

It has been shown that iBuMeXan accelerates the differentiation of 3T3-L1 preadipocytes by inhibiting soluble cAMP phosphodiesterase (37). Thus elevated levels of cAMP may affect this developmental process. In support of this view, cAMP can augment the differentiation of 3T3-L1 preadipocytes (40). In agreement with a role for this agent in promoting adipocyte conversion and activating the expression of adipose-specific genes, we have shown here that 8BrcAMP activates the expression of both 422.CAT constructs in 3T3-L1 cells. As this agent increases the expression of both Δ858.CAT and Δ248.CAT constructs (Fig. 3), our data suggest the first 248 bp of the 5′-flanking sequence of the 422 gene contains the regulatory element(s) that mediates the effect of cAMP. In this region of the 422 gene, there are no sequences with >75% sequence identity to the identified cAMP regulatory elements (41). Moreover, the rather long lag (4–12 hr) required for the induction of 422.CAT expression by 8BrcAMP (data not shown) suggests that the effect may be indirect, i.e., mediated by some factor whose synthesis or modification is induced by cAMP. A candidate for this factor is c-*fos* that is believed to bind to this region of the 422 gene (23) and is known to be induced by cAMP in certain cell types (42). This possibility is rendered less likely by our observation that insulin has no effect on the expression of the 422.CAT constructs, since it is known that insulin can cause the induction of c-*fos* in 3T3-L1 cells (43). The combination of dexamethasone and iBuMeXan is more effective in promoting differentiation of 3T3-L1 preadipocytes than either agent alone (38). Consistent with this observation is the ability of dexamethasone to potentiate the effect of 8BrcAMP on Δ248.CAT expression, despite the fact that it has no effect by itself (Fig. 3*B*). Glucocorticoid may either stabilize increased cAMP levels or induce a factor that acts synergistically with a cAMP-induced effector.

Our results indicate the presence in the promoter region of the 422 gene elements that mediate the response to the adipogenic agents dexamethasone and iBuMeXan (through cAMP). Both these agents greatly accelerate the differentiation of 3T3-L1 preadipocytes and, in their absence, the endogenous adipose-specific genes are not expressed (16). Consistent with a role for these various cis-acting elements in coordinately regulating the expression of several adipose-specific genes, GRE core sequences also occur in the

differentially regulated gene 122, which encodes stearoyl-CoA desaturase (J. Ntambi, personal communication). Furthermore, a potential GRE is found at position −150 in the sequence of the gene encoding glycerol-3-phosphate dehydrogenase (22). The FSE identified by Spiegelman and coworkers occurs with various degrees of sequence identity in the promoter regions of adipose-specific genes, including those coding for glycerol-3-phosphate dehydrogenase (13 of 14 match) (23), adipsin (6 of 14 match) (34), and stearoyl-CoA desaturase (7 of 14 match) (J. Ntambi, personal communication). We do not find sequences with homology to a GRE in the adipsin gene (22), although there is a potential FSE (34). Interestingly, the adipsin mRNA appears later in the differentiation program of 3T3 preadipocytes than the mRNAs encoding 422 and glycerol-3-phosphate dehydrogenase (20). Thus the combination or spatial arrangement of regulatory elements in adipose-specific genes may be important in determining not only the level of expression but also the time when a gene is expressed in the developmental program. It seems likely that such differences in the number and organization of various regulatory signals explains the differences in the response of Δ858.CAT and Δ248.CAT to adipogenic agents. Similar considerations could also explain why the time course of both chimeric constructs differs from the endogenous gene. This work and studies by Distel *et al.* (23) indicate that the regulation of adipose-specific genes during differentiation may involve complex interactions between multiple regulatory elements within promoter regions.

We thank Dr. Vincent W. Yang for helpful discussions and comments on the manuscript and Deborah E. Geiman for excellent technical assistance. This work was supported by a research grant from the National Institutes of Health (DK 38418). R.J.C. is a recipient of an NRS award from the National Institutes of Health (DK 08019).

1. Green, H. & Kehinde, O. (1974) *Cell* **1**, 113–116.
2. Green, H. & Kehinde, O. (1976) *Cell* **7**, 105–113.
3. Green, H. (1979) *INSERM Symp.* **87**, 15–24.
4. Mackall, J. C., Student, A. K., Polakis, S. E. & Lane, M. D. (1976) *J. Biol. Chem.* **251**, 6462–6464.
5. Mackall, J. C. & Lane, M. D. (1977) *Biochem. Biophys. Res. Commun.* **79**, 720–725.
6. Coleman, R. A., Reed, B. C., Macall, J. C., Student, A. K., Lane, M. D. & Bell, R. M. (1978) *J. Biol. Chem.* **253**, 7256–7261.
7. Student, A. K., Hsu, R. Y. & Lane, M. D. (1980) *J. Biol. Chem.* **255**, 4745–4750.
8. Kawamura, M., Jensen, D. F., Wancewicz, E. V., Joy, L. L., Khoo, J. C. & Steinberg, D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 732–736.
9. Rubin, C. S., Hirsch, A., Fung, C. & Rosen, O. M. (1978) *J. Biol. Chem.* **253**, 7570–7578.
10. Reed, B. C., Ronnett, G. V., Clements, P. R. & Lane, M. D. (1981) *J. Biol. Chem.* **256**, 3917–3925.
11. Resh, B. C. (1982) *J. Biol. Chem.* **257**, 6978–6986.
12. Rubin, C. S., Lai, E. & Rosen, O. M. (1977) *J. Biol. Chem.* **252**, 3554–3557.
13. Wise, G. H., Sul, H. S. & Rubin, C. S. (1984) *J. Biol. Chem.* **259**, 4827–4832.
14. Angus, C. W., Lane, M. D., Rosenfeld, P. & Kelly, T. J. (1981) *Biochem. Biophys. Res. Commun.* **103**, 1216–1222.
15. Spiegelman, B. M. & Green, H. (1980) *J. Biol. Chem.* **255**, 8811–8818.
16. Bernlohr, D. A., Bolanowski, M. A., Kelly, T. J. & Lane, M. D. (1985) *J. Biol. Chem.* **260**, 5563–5567.
17. Cook, K. S., Hunt, C. R. & Spiegelman, B. M. (1985) *J. Cell Biol.* **100**, 514–520.
18. Djian, P., Phillips, M. & Green, H. (1985) *J. Cell. Physiol.* **124**, 554–556.
19. Bernlohr, D. A., Angus, C. W., Lane, M. D., Bolanowski, M. A. & Kelly, T. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5468–5472.
20. Spiegelman, B. M., Frank, M. & Green, H. (1983) *J. Biol. Chem.* **258**, 10083–10089.
21. Chapman, A. B., Knight, D. M., Dieckman, B. S. & Ringold, G. M. (1984) *J. Biol. Chem.* **259**, 15548–15555.
22. Phillips, M., Djian, P. & Green, H. (1986) *J. Biol. Chem.* **261**, 10821–10827.
23. Distel, R. J., Ro, H.-S., Rosen, B. S., Groves, D. L. & Spiegelman, B. M. (1987) *Cell* **49**, 835–844.
24. Gorman, C. M., Moffat, L. F. & Howard, H. F. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
25. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
26. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. & Howard, B. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6777–6781.
27. Reed, B. C. & Lane, M. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 285–289.
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
29. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
30. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3963–3965.
31. Favaloro, J., Triesman, R. & Kamen, R. (1980) *Methods Enzymol.* **65**, 718–749.
32. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–457.
33. Neumann, J. R., Morency, C. A. & Russian, K. O. (1987) *Biotechniques* **5**, 444–447.
34. Hunt, C. R., Ro, J. H.-S., Dobson, D. E., Min, H. Y. & Spiegelman, B. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3786–3790.
35. Yamamoto, K. R. (1985) *Annu. Rev. Genet.* **19**, 209–252.
36. Ringold, G. (1985) *Annu. Rev. Pharmacol. Toxicol.* **25**, 529–566.
37. Elks, M. L. & Manganiello, V. C. (1985) *J. Cell. Physiol.* **124**, 191–198.
38. Rosen, O. M., Smith, C. J., Hirsch, A., Lai, E. & Rubin, C. S. (1979) *Recent Prog. Horm. Res.* **35**, 477–499.
39. Ringold, G. M., Chapman, A. B. & Knight, D. M. (1986) *J. Steroid Biochem.* **24**, 69–75.
40. Williams, I. H. & Polakis, S. E. (1977) *Biochem. Biophys. Res. Commun.* **77**, 175–186.
41. Silver, B. J., Boker, J. A., Virgin, J. B., Vallen, E. A., Milsted, A. & Nilsen, J. H. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2198–2202.
42. Bravo, R., Neuberg, M., Burckhardt, J., Almendral, J., Wallich, R. & Muller, R. (1987) *Cell* **48**, 251–260.
43. Stumpo, D. J. & Blackshear, P. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9453–9457.