Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7895–7899, September 1992
Biochemistry

Identification of a transcriptional repressor down-regulated during preadipocyte differentiation

(3T3-L1 cells/adipocyte/stearoyl-CoA desaturase 2)

ANDREW G. SWICK AND M. DANIEL LANE

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

Contributed by M. Daniel Lane, May 29, 1992

ABSTRACT **During differentiation of 3T3-L1 preadipocytes into adipocytes, the transcription of adipocyte genes, including the stearoyl-CoA desaturase 2 (SCD2) gene, is activated. Transfection experiments with chimeric SCD2 promoter-chloramphenicol acetyltransferase (CAT) reporter gene constructs revealed a preadipocyte repressor element (PRE) capable of repressing transcription of the reporter gene in preadipocytes but not in adipocytes. DNase I protection and gel retardation analyses were used to localize the PRE site between nucleotides −435 and −410 of the SCD2 promoter and to identify a nuclear PRE binding protein present at high levels in preadipocytes and HeLa cells but lacking or inactive in adipocytes. Southwestern blot analysis indicated that the PRE binding protein has an apparent molecular mass of ≈58 kDa. A single copy of the PRE site, inserted upstream of the simian virus 40 enhancer/promoter of pSV$_2$CAT, was capable of strongly repressing transcription of the reporter gene in preadipocytes and HeLa cells but not in adipocytes. Taken together these results suggest that the PRE site and binding protein may regulate transcription of SCD2 and possibly other adipocyte genes by inhibiting their transcription in preadipocytes.**

During differentiation of 3T3 preadipocytes into adipocytes there is a dramatic increase in expression of a number of adipocyte genes (1–4). Two such genes encoding the enzymes stearoyl-CoA desaturase 1 (SCD1) (5) and stearoyl-CoA desaturase 2 (SCD2) (6) are transcriptionally activated during the differentiation process (ref. 7; K. H. Kaestner and M.D.L., unpublished results). These enzymes catalyze the Δ^9 cis desaturation of C_{18} and C_{16} fatty acyl-CoAs leading to the production of oleoyl- and palmitoleoyl-CoA as the primary products (8). Expression of SCD1 and SCD2 differs with respect to tissue distribution and negative feedback response to dietary unsaturated fatty acids (5, 6).

The promoter of the SCD1 gene and its regulation have been partially characterized. This promoter contains a DNA binding site for and is transactivated by the transcription factor C/EBPα (9). C/EBPα is expressed only in terminally differentiated cells, including adipocytes, and is a positive regulator of gene transcription (10). ARF6, another positive regulator of adipocyte-specific transcription, binds to the enhancer region of the 422(aP2) gene and appears to be adipocyte-specific (11). Two examples of cis-acting negative sequence elements that prevent adipocyte gene promoter activity in preadipocytes have been reported. These include elements in the 422(aP2) (12, 13) and the adipsin gene promoters (14). In neither case have the proteins responsible for transcriptional repression been identified.

In this paper we describe the regulation of SCD2 gene transcription during preadipocyte differentiation. We identified a cis-acting DNA sequence element of the SCD2 promoter that has an inhibitory effect on reporter gene transcription in preadipocytes but not in adipocytes. This preadipocyte repressor element (PRE) is also capable of down-regulating transcription directed by the simian virus 40 (SV40) promoter-enhancer in preadipocytes and HeLa cells. The level or activity of a protein(s) that binds to the PRE site of the SCD2 gene appears to decrease during the process of preadipocyte differentiation.

MATERIALS AND METHODS

Cell Culture. 3T3-L1 preadipocytes were maintained in culture and differentiated as described (15). Preadipocytes were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum. Differentiation of preadipocytes into adipocytes was initiated by treating 2-day postconfluent cells (designated day 0) with 10% fetal bovine serum, 1.7 μM insulin, 1 μM dexamethasone, and 0.5 μM 3-isobutyl-1-methylxanthine in DMEM. On day 2 the medium was changed to DMEM containing 1.7 μM insulin with 10% fetal calf serum. On day 4 the medium was replaced with DMEM containing 10% calf serum. HeLa cells were grown and maintained in 1.5% fetal calf serum and 1.5% calf serum in DMEM.

Construction and Transfection of Promoter-Chloramphenicol Acetyltransferase (CAT) Constructs. A *Bgl* II–*Sac* II fragment (nucleotides −585 to +80) of the murine SCD2 gene (GenBank/EMBL data bank accession nos. M26269 and M26270) (6) was subcloned into the *Eco*RV site of pBlue-CAT1. The resultant construct was linearized 5′ to −585 and a series of 5′ deletions was generated by exonuclease III digestion. The extent of 5′ digestion was determined by DNA sequencing and the promoter-CAT constructs were designated accordingly (see Fig. 1*A*).

Oligonucleotides (corresponding to nucleotides −441 to −405) with *Bam*HI ends and containing the PRE (nucleotides −435 to −410) of the SCD2 gene, as shown in Fig. 4*A*, were synthesized. After annealing the single-stranded oligonucleotides and filling in the ends with Klenow polymerase in the presence of dNTPs, the double-stranded PRE oligonucleotide was subcloned 5′ to the SV40 promoter/enhancer of pSV$_2$CAT, which was linearized with *Acc* I and filled in with Klenow polymerase. A clone with a single copy of the PRE site in the same orientation as in the SCD2 gene was identified and designated PRESV$_2$CAT. Another clone with the PRE site in the reverse orientation (3′ → 5′) was isolated and designated ƎЯЧSV$_2$CAT. 422CAT contains −248 to +22 of the murine 422(aP2) gene fused to the CAT gene (16).

SCD2-CAT constructs (30–50 μg of $CsCl_2$-purified plasmid DNA) were transiently transfected into confluent day 0 preadipocytes or day 5 3T3-L1 adipocytes. To increase transfection efficiency cells were removed from 10-cm dishes

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: PRE, preadipocyte repressor element; SCD, stearoyl-CoA desaturase; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40; DTT, dithiothreitol.

by trypsinization, mixed with the DNA calcium phosphate precipitate (17), and replated. To ensure confluence 10 dishes were trypsinized and replated onto 8 dishes. The reattached cells achieved confluence within 4 hr, at which time the medium was removed and cells were shocked for 2.5 min with 12.5% glycerol in phosphate-buffered saline (PBS), washed, and refed with fresh medium. After 48 hr CAT activity was determined on cell extracts by the scintillation fluor diffusion method (18).

pSV_2CAT, $PRESV_2CAT$, and $ЭЯЧSV_2CAT$ were transiently transfected into dividing Hela cells by the calcium phosphate coprecipitation method. Eight micrograms of DNA was transfected into each 10-cm dish. Following exposure to the precipitate for 8 hr, cells were shocked with 12.5% glycerol in PBS, washed with PBS twice, and refed with fresh medium. After 48 hr cells were harvested and assayed for CAT activity.

To generate stable cell lines expressing SCD2-promoter-CAT constructs, 30% confluent 3T3-L1 preadipocytes were cotransfected with SCD2-CAT (either −585 SCD2-CAT or −413 SCD2-CAT) and pSV_2Neo DNA in a 10:1 molar ratio using the calcium phosphate coprecipitation method (17). After selection with G418, the foci (40–50 per dish) from each of three 10-cm dishes were pooled separately for triplicate analysis. Cells from each set of foci were replated, subjected to the preadipocyte differentiation protocol, and assayed for CAT activity at various times (18).

DNase I Protection and Gel Retardation Analysis. Nuclear extracts were prepared essentially as described (19) from day 0 preadipocytes and day 5 differentiated adipocytes. A fragment of the SCD2 promoter (nucleotides −585 to −260) for DNase I protection analysis was generated by digestion of −585 SCD2-CAT with *Ava* II and *Cla* I. The ends of the fragment were filled in with Klenow polymerase in the presence of dNTPs and subcloned into the *Eco*RV site of pBluescript. The orientation of the fragment was determined by DNA sequencing. DNase I footprint analysis was carried out as described (9, 20).

Gel retardation assays (21) were conducted in 25 μl containing 5 μg of nuclear extract protein, ^{32}P end-labeled DNA (0.5–1 ng), and 5 μg of poly[d(I-C)] in a buffer consisting of 10 mM Tris (pH 7.9), 50 mM NaCl, 0.5 mM EDTA, 1 mM dithiothreitol (DTT), 10% glycerol, and 1 mM $MgCl_2$. Following a 15-min incubation on ice protein/DNA complexes were separated on a 4% acrylamide gel (80:1 acrylamide: bisacrylamide) in 25 mM Tris/190 mM glycine/1.0 mM EDTA (pH 8.3).

Southwestern Blot Analysis. Southwestern blot analysis was performed by a modification of the method of Vinson *et al.* (22). Nuclear extract (≈50 μg) was mixed with 3× electrophoresis sample buffer containing 6% SDS and 60 mM DTT. After boiling for 5 min, samples were subjected to 10% SDS/PAGE. Following electrotransfer to nitrocellulose filters for 16 hr, filters were allowed to dry and then were immersed for 10 min at 4°C with shaking in 25 mM Hepes (pH 7.5), 25 mM NaCl, 5 mM $MgCl_2$, and 0.5 mM DTT containing 6 M guanidine hydrochloride. Partial renaturation of immobilized proteins was effected by five successive 5-min incubations of the filters in buffer containing progressive (2-fold) dilutions of guanidine hydrochloride and finally in buffer lacking the denaturant.

Filters were blocked with 5% nonfat dry milk in binding buffer containing 50 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, and 1 mM DTT for 45 min at room temperature and then were washed twice with 0.25% nonfat dry milk in binding buffer. Filters were hybridized in binding buffer containing ^{32}P end-labeled double-stranded PRE oligonucleotide (2×10^6 dpm/ml), 0.25% nonfat dry milk, and 1 μg of sonicated salmon sperm DNA per ml for 60 min at room temperature. Filters were then washed three times, 10 min each, with binding buffer containing 0.25% nonfat dry milk, dried, and subjected to autoradiography.

RESULTS

The SCD2 gene is differentially expressed during conversion of 3T3-L1 preadipocytes into adipocytes (6). To identify DNA sequence elements that are responsible for adipocyte-specific transcription of this gene, a segment of 5′ flanking sequence (nucleotides −585 to +80, Fig. 1*A*) was subcloned 5′ to the CAT gene in the promoterless pBlueCAT1 vector. A series of 5′ deletion constructs was generated (Fig. 1*A*) and the resultant CAT constructs were transiently transfected into confluent 3T3-L1 preadipocytes or adipocytes (day 5 after induction of differentiation). The cells were maintained in culture for 48 hr and then assayed for expression of CAT activity.

Transfection of −585 SCD2-CAT into preadipocytes led to very low levels of CAT activity (5 pmol of acetylchloramphenicol formed per hr per dish, Fig. 1*B*), whereas transfection of −413 SCD2-CAT or −285 SCD2-CAT gave rise to high levels of CAT activity (298 and 453 pmol of acetylchloramphenicol formed per hr per dish, Fig. 1*B*). Further deletion of the SCD2 promoter to within 52 base pairs of the start of transcription (−52 SCD2-CAT) completely abolished promoter-driven CAT expression (Fig. 1*B*). These results suggested the presence of a negative cis-acting element(s) between nucleotides −585 and −413 and a positive cis-acting element(s) between nucleotides −285 and −52.

To assess the function of these putative cis-acting elements in adipocytes, the SCD2-CAT constructs were transfected into fully differentiated day 5 3T3-L1 adipocytes. In contrast to the results obtained with preadipocytes, −585 SCD2-CAT was highly active in adipocytes (428 pmol of acetylchloram-

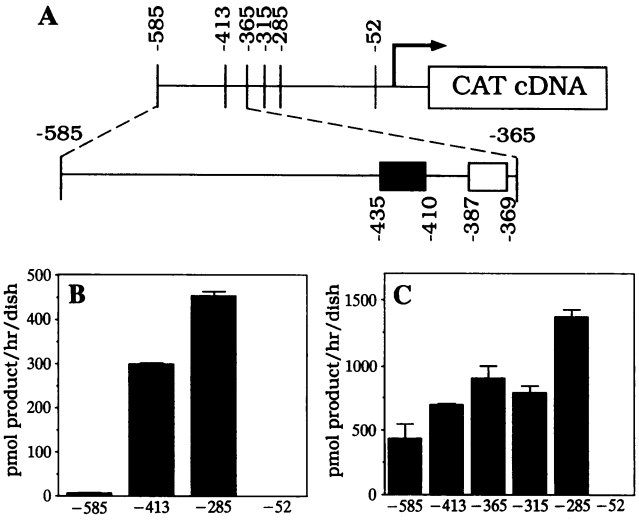

FIG. 1. Effect of 5′ deletions on the activity of the SCD2 promoter in 3T3-L1 preadipocytes and adipocytes. (*A*) Diagram of the −585 SCD2 promoter-CAT construct (−585 SCD2-CAT). Numbers refer to nucleotide position with respect to the start site (+1) of transcription (bent arrow). The boxes designate sites in the SCD2 promoter protected from DNase I digestion by nuclear extracts prepared from undifferentiated 3T3-L1 preadipocytes (filled box) or differentiated 3T3-L1 adipocytes (open box), respectively. (*B*) CAT activity 48 hr after transfection of undifferentiated (day 0) preadipocytes with SCD2-CAT constructs (30 μg of DNA per 10-cm dish) possessing different lengths of 5′ flanking sequence. Nucleotides −585 to +80 of the SCD2 gene were subcloned 5′ to the CAT gene of the promoterless pBlueCAT1 vector. The 5′ deletions were generated by exonuclease III digestion giving rise to the indicated 5′ delimitation of each construct. (*C*) CAT activity 48 hr after transfection of differentiated (day 5) adipocytes with SCD2-CAT constructs (50 μg of DNA per 10-cm dish) containing different lengths of 5′ flanking sequence.

phenicol formed per hr per dish, Fig. 1C) and, in fact, was only marginally less active than −413, −365, −315, or −285 SCD2-CAT (693, 782, 896, and 1369 pmol of acetylchloramphenicol formed per hr per dish, respectively, Fig. 1C). This finding suggested that the inhibitory effect of the putative negative cis-acting element (located between nucleotides −585 and −413 of the SCD2 gene) is lost upon conversion of preadipocytes into adipocytes. As in the case of preadipocytes, the shortest construct tested, −52 SCD2-CAT, was inactive in adipocytes.

To further examine the loss of repression by the putative cis-acting negative element during differentiation, cell lines (pooled foci) stably transfected with −413 or −585 SCD2-CAT were selected. Prior to the induction of differentiation, −413-CAT cell lines expressed ≈15-fold higher CAT activity than −585-CAT cell lines (Fig. 2A). During the course of differentiation CAT activity expressed by −585-CAT cell lines increased about 7-fold between day 0 and day 7, after which the activity remained elevated (Fig. 2B). During the same period the CAT activity of −413-CAT cell lines increased only ≈2.5-fold (Fig. 2B). Taken together these results support the view that a negative cis-acting element in the SCD2 promoter represses transcription of the gene in the preadipocyte and upon differentiation is derepressed allowing transcription of the gene in the adipocyte.

In view of these findings, we sought to identify the repressive element in the SCD2 promoter. Thus, DNase I protection analysis was performed on a fragment of the SCD2 gene encompassing nucleotides −585 to −260. Nuclear extracts, prepared from either undifferentiated 3T3-L1 preadipocytes or HeLa cells, protected the same site located between nucleotides −435 and −410 (Fig. 3). This site was not, however, protected by nuclear extract from differentiated (day 5) 3T3-L1 adipocytes, although an adipocyte-specific site, which spans nucleotides −387 to −369, was protected (Fig. 3). The region of DNA protected by preadipocyte nuclear extracts resides within the same region of the SCD2 promoter (nucleotides −585 to −413) responsible for the repression of transcription in preadipocytes (Fig. 1B) and thus is implicated as the negative cis-acting element. We designate this *p*readipocyte *r*epressor *e*lement as PRE.

To further characterize the binding interaction between the preadipocyte nuclear factor(s) and the PRE site, gel retardation assays were conducted with a synthetic double-stranded oligonucleotide (nucleotides −441 to −405, Fig. 4A) containing the PRE site. Incubation of the PRE oligonucleotide with nuclear extract from 3T3-L1 preadipocytes gave rise to one

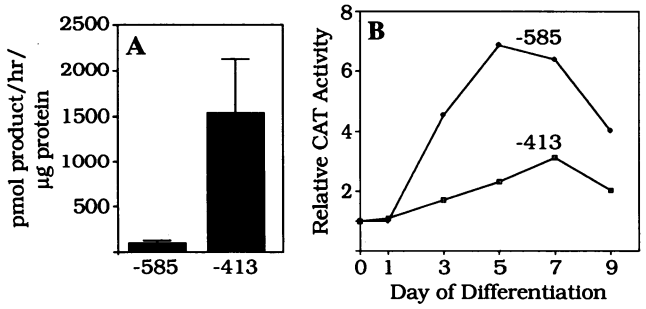

FIG. 2. Effect of differentiation on the expression of stably transfected −585-CAT and −413-CAT constructs. (A) Expression of CAT activity by confluent 3T3-L1 preadipocytes stably transfected with −585-CAT or −413-CAT prior to induction of differentiation (day 0). (B) Expression of CAT activity by 3T3-L1 cells stably transfected with −585-CAT or −413-CAT during the course (on days 0, 1, 3, 5, 7, and 9) of differentiation. Results are normalized to the CAT activity of each construct on day 0 shown in A. Each point in A and B represents the average of three different sets of pooled foci (40–50 foci per set).

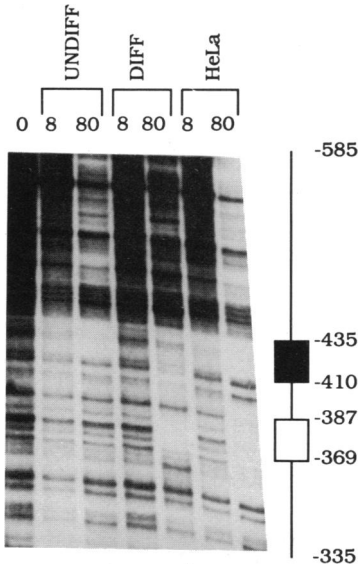

FIG. 3. DNase I protection analysis with nuclear extracts and a segment (nucleotides −585 to −335) of the SCD2 promoter. A ^{32}P end-labeled DNA fragment containing nucleotides −585 to −335 of the SCD2 promoter was incubated with either 0, 8, or 80 μg of nuclear extract prepared from undifferentiated day 0 preadipocytes (UNDIFF), differentiated day 5 adipocytes (DIFF), or HeLa cells (HeLa) and then subjected to DNase I protection analysis. The region spanning nucleotides −435 to −410 (the PRE site) was protected from DNase I digestion by UNDIFF and HeLa cell nuclear extracts and is denoted with a filled-in box, whereas an adipocyte-specific footprint encompassing −387 to −369 is denoted with an open box.

major and two minor gel shift complexes (Fig. 4B). These complexes appear to be specific as their formation is competitively prevented by unlabeled PRE oligonucleotide but not by an unlabeled irrelevant (Sp1) oligonucleotide (results not shown). A similar, but much weaker, pattern of complexes was observed with nuclear extracts from 3T3-L1 adipocytes (Fig. 4B). Hence, the factor(s) binding to the PRE site appears to be more abundant in preadipocytes than in adipocytes.

To estimate the subunit molecular mass(es) of the PRE binding protein(s), Southwestern blot analyses were performed using end-labeled double-stranded PRE oligonucleotide as probe. As illustrated in Fig. 5, the PRE oligonucleotide bound tightly to an ≈58-kDa protein present in nuclear extracts from 3T3-L1 preadipocytes and HeLa cells but not in nuclear extract from day 5 adipocytes. The fact that the 58-kDa PRE binding protein was found in greater abundance in preadipocytes and HeLa cells, which do not express the SCD2 gene, suggests that this nuclear protein functions as a transcriptional repressor.

To ascertain whether the PRE site can repress transcription of another gene, the PRE oligonucleotide was subcloned 5′ of the SV40 enhancer and promoter region of pSV_2CAT. A single copy of the PRE site was inserted into the *Acc* I site of pSV_2CAT in the 5′ → 3′ ($PRESV_2CAT$) and 3′ → 5′ ($ƎЯ¶SV_2CAT$) orientations (Fig. 6A). pSV_2CAT transfected into preadipocytes gave rise to a high level of expression of CAT activity (≈100 pmol of acetylated chloramphenicol formed per hr per dish), whereas CAT activity expressed by preadipocytes transfected with $PRESV_2CAT$ or $ƎЯ¶SV_2CAT$ was nil (Fig. 6B). However, all three constructs had similar activities after transfection into day 5 adipocytes (Fig. 6C). The levels of expression of CAT activity directed by these three constructs were similar to that achieved by day 5 3T3-L1 adipocytes transfected with a highly active 422(aP2) promoter-CAT construct containing the adipose-specific 422(aP2) pro-

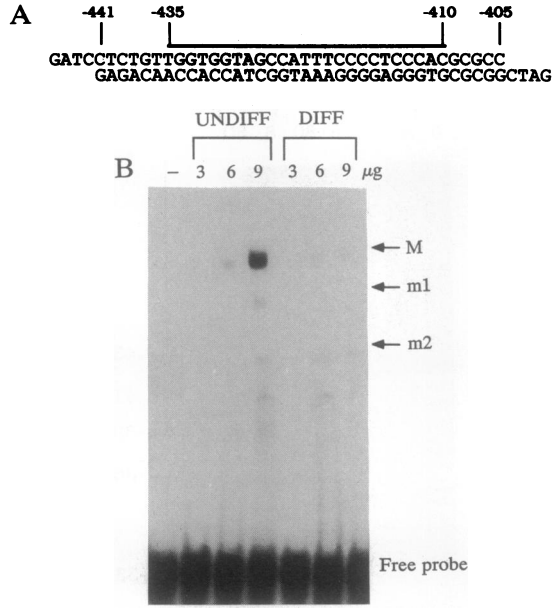

FIG. 4. Gel retardation analysis with nuclear extracts and an oligonucleotide containing the PRE site. (*A*) Oligonucleotide (nucleotides −441 to −405) encompassing the PRE site (nucleotides −435 to −410) of the SCD2 promoter and containing *Bam*HI ends. The heavy line denotes the region (the PRE site) protected from DNase I digestion by nuclear extracts from undifferentiated 3T3-L1 preadipocytes (UNDIFF) and HeLa cells (see Fig. 3). (*B*) The ^{32}P end-labeled oligonucleotide referred to in *A* was incubated with increasing amounts (0, 3, 6, or 9 μg of protein) of nuclear extracts from undifferentiated 3T3-L1 preadipocytes (UNDIFF) or differentiated 3T3-L1 adipocytes (DIFF). The resultant protein/DNA complexes were resolved by 4% nondenaturing PAGE. M denotes the major protein/DNA complex; m1 and m2 denote minor complexes. The intensity of m2 was similar to that of M with some nuclear extracts. The specificity of protein/oligonucleotide complex formation was verified (results not shown) using nonspecific (unlabeled Sp1) and specific (unlabeled PRE site) oligonucleotides as competitors. The quality of the nuclear extracts was judged as similar based on its capacity to bind other transcription factors, including Sp1, the expression of which does not change during differentiation (data not shown).

moter (Fig. 6*C*), which served as a positive control for adipocyte-specific transcription/expression.

Since HeLa cells appeared to express the same nuclear factor that binds to the PRE site (see Figs. 3 and 5), it was of interest to determine whether this element has a repressive effect in this cellular context. HeLa cells transfected with PRESV$_2$CAT and ЭЯЯSV$_2$CAT exhibited an ≈7-fold lower CAT activity than cells transfected with pSV$_2$CAT (Fig. 6*D*). Therefore, the PRE site of the SCD2 gene promoter markedly repressed transcription directed by the SV40 enhancer-promoter in HeLa cells. These results indicate that this negative cis-acting element is capable of repressing transcription of a strong viral promoter in an orientation-independent and cell-type-specific manner.

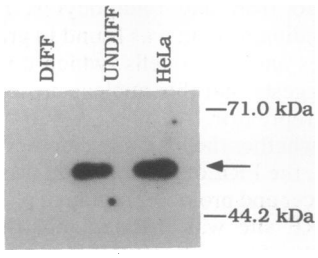

FIG. 5. Southwestern blot analysis of a preadipocyte nuclear protein with an oligonucleotide containing the PRE site. Nuclear extract (50 μg of protein), prepared from differentiated day 5 3T3-L1 adipocytes (DIFF), undifferentiated day 0 3T3-L1 preadipocytes (UNDIFF), or HeLa cells, was subjected to 10% SDS/PAGE and then transferred to nitrocellulose. Blots were probed with ^{32}P end-labeled double-stranded oligonucleotide containing nucleotides −441 to −405 of the SCD2 promoter (see Fig. 4). Positions of molecular mass markers and specific hybridization to the PRE site-containing oligonucleotide (arrow) are indicated. The subunit molecular mass of the PRE site binding protein is estimated to be 58 kDa.

DISCUSSION

The SCD2 gene is a member of a large group of genes whose expression is activated when 3T3-L1 preadipocytes differentiate into adipocytes (1–3). Several lines of evidence reported in this paper implicate a PRE in the SCD2 promoter (between nucleotides −435 and −410) and its cognate nuclear binding protein(s) in the differentiation-induced derepression of SCD2 gene transcription. (*i*) The presence of the PRE site in chimeric SCD2-CAT constructs markedly represses expression of the CAT reporter gene when transfected into preadipocytes but not in adipocytes (Figs. 1 and 2). Deletion of a segment of the SCD2 promoter that contains the PRE site gives rise to a high level of CAT expression in preadipocytes and adipocytes (Figs. 1 and 2). (*ii*) The insertion of a single copy of the PRE site upstream of the SV40 enhancer/promoter of pSV$_2$CAT strongly represses CAT expression when transfected into preadipocytes and HeLa cells but not into adipocytes. (*iii*) A DNA binding protein(s) present in nuclear extracts from preadipocytes and HeLa cells, but marginally detectable (or inactive) in adipocytes, footprinted the PRE site (Fig. 3) and gel-shifted (Fig. 4) the PRE site oligonucleotide. In addition, Southwestern blot analysis with a PRE oligonucleotide probe identified an ≈58-kDa PRE binding protein present in nuclear extracts prepared from preadipocytes and HeLa cells but not from adipocytes (Fig. 5).

Thus, it appears that the ≈58-kDa PRE binding protein is responsible for transcriptional repression of the SCD2 gene in preadipocytes and that the level and/or activity of this protein decreases upon differentiation. Possible explanations for this decrease include (*i*) the PRE binding protein is not expressed in adipocytes, (*ii*) another factor prevents the PRE binding protein from interacting with the PRE site in adipocytes, or (*iii*) a covalent modification(s) of the PRE binding protein in adipocytes changes its affinity for the PRE site. The possibility that the PRE binding protein(s) is regulated by phosphorylation/dephosphorylation requires further investigation. Preliminary evidence suggests that treatment of nuclear extracts prepared from preadipocytes or adipocytes with protein kinase A or alkaline phosphatase alters the binding of a protein(s) to the PRE site.

It has been suggested that transcriptional repression is involved in the differentiation-dependent expression of the adipocyte-specific 422(aP2) and adipsin genes (12–14); however, specific repressor proteins have not been identified. On the other hand, positive regulators of adipocyte gene transcription have been identified—i.e., C/EBPα (9) and ARF6 (11). C/EBPα appears to be essential for preadipocyte differentiation (23, 24) and is capable of accelerating adipocyte gene expression during differentiation (25). Moreover, C/EBPα can bind to and transactivate a number of adipocyte gene promoters including the SCD1 promoter (9). Unlike the SCD1 promoter, the region of the SCD2 promoter (nucleotides −585 to +80) examined in this paper does not contain a C/EBP binding site and is not transactivated by C/EBPα (unpublished results). Furthermore, the region of the SCD1 promoter that has been sequenced (5) does not contain a PRE site.

Although the amino acid sequences of the SCD1 and SCD2 are nearly 90% identical, these proteins are encoded by different genes and exhibit different tissue distribution, and

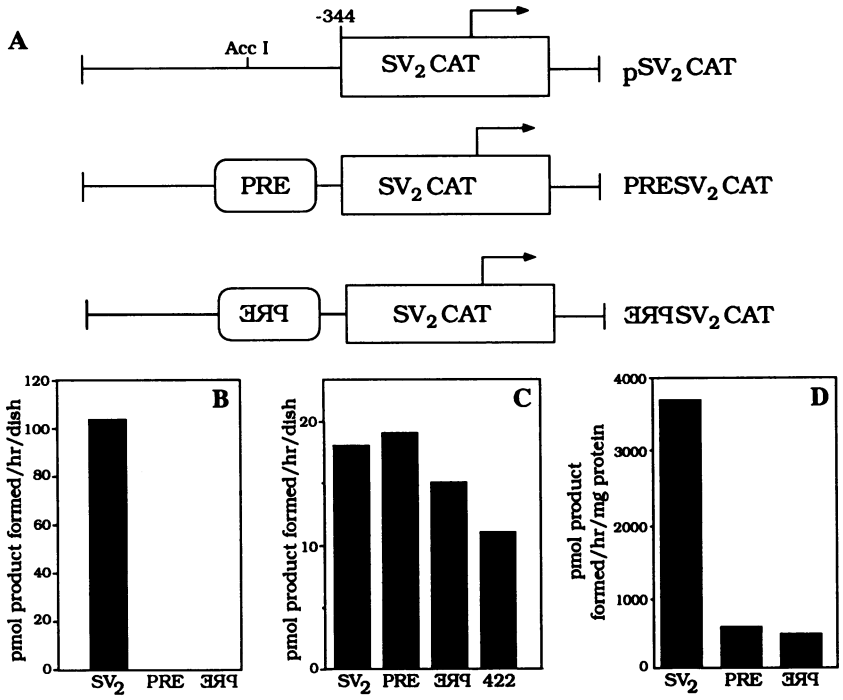

FIG. 6. Effects of the PRE site on the transcription directed by the SV40 late promoter-enhancer. (*A*) A single copy of the PRE oligonucleotide (Fig. 4*A*) was subcloned in the 5′ → 3′ (PRESV$_2$CAT) or 3′ → 5′ (ƎЯЧSV$_2$CAT) orientation into the *Acc* I site 5′ of the SV40 promoter-enhancer of pSV$_2$CAT. (*B*) Thirty micrograms of pSV$_2$CAT, PRESV$_2$CAT, or ƎЯЧSV$_2$CAT was transfected into confluent day 0 preadipocytes. (*C*) Fifty micrograms of pSV$_2$CAT, PRESV$_2$CAT, or ƎЯЧSV$_2$CAT was transfected into day 5 adipocytes. (*D*) Eight micrograms of pSV$_2$CAT, PRESV$_2$CAT, or ƎЯЧSV$_2$-CAT was transfected into dividing HeLa cells. In all cases cells were shocked with 12.5% glycerol, harvested 48 hr after transfection, and assayed for CAT activity.

their expression responds differently to dietary unsaturated fatty acids (5, 6). For example, SCD1 is expressed in liver and adipose tissue, but not in brain, whereas SCD2 is expressed in brain and adipose tissue but not in liver (6). Moreover, the expression of SCD1 is under negative feed-back control by dietary unsaturated fatty acids in liver but not in adipose tissue, whereas expression of SCD2 is inhibited by dietary unsaturated fatty acids in adipose tissue but not in brain (6). It is possible that C/EBPα and/or the PRE binding protein(s) are involved in this differential expression of the SCD1 and SCD2 genes. Although the identical nucleotide sequence of the PRE site is not present in other adipocyte gene promoters we have examined, sequences similar to the PRE site that are capable of repressing transcription may exist.

In this connection, the core DNA sequence of the PRE site is similar to that of the PRD1 element of the β-interferon gene promoter to which the repressor protein PRD1-BF1 binds (26). Like the PRE site (Fig. 6), the PRD1 element can repress SV40 promoter-enhancer-driven transcription (26). Further investigations will be necessary to determine whether the PRE binding protein is related to the PRD1-BF1.

That the PRE site alone is capable of regulating adipocyte-specific transcription of another gene is demonstrated by the fact that insertion of a single PRE site 5′ of the SV40 promoter/enhancer of pSV$_2$CAT confers adipocyte-specific CAT transcription. Thus, the PRE site has the capacity to repress CAT expression in preadipocytes and HeLa cells but not in adipocytes (Fig. 6). It is possible, therefore, that the PRE site and its binding protein(s) prevent transcription of certain adipocyte genes in inappropriate cell types.

We gratefully thank Dr. Peter Cornelius, Dr. Robert Christy, Dr. Katherine T. Landschulz, and Mireille Vasseur-Cognet for helpful discussions and comments. We acknowledge L. Sanders and Dr. D. Nathans for the generous gift of pBlueCAT1. The expert secretarial assistance of Natalie Tumminia is gratefully acknowledged. This work was supported by research grants from the National Institutes of Health (NIDDK-14575 and NIDDK-38418).

1. Spiegelman, B. M., Frank, M. & Green, H. (1983) *J. Biol. Chem.* **258**, 10083–10098.
2. Chapman, A. B., Knight, D. M., Dieckman, B. S. & Rimgold, G. M. (1984) *J. Biol. Chem.* **259**, 15548–15555.
3. Bernlohr, D. A., Bolanowski, M. A., Kelly, T. J. & Lane, M. D. (1985) *J. Biol. Chem.* **260**, 5563–5567.
4. Cook, K. S., Hunt, C. R. & Spiegelman, B. M. (1985) *J. Cell Biol.* **100**, 514–520.
5. 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.
6. Kaestner, K. H., Ntambi, J. M., Kelly, T. J. & Lane, M. D. (1989) *J. Biol. Chem.* **264**, 14755–14761.
7. Christy, R. C., Kaestner, K. H., Geiman, D. E. & Lane, M. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2593–2597.
8. Enoch, H. G., Catala, A. & Strittmatter, P. (1976) *J. Biol. Chem.* **251**, 5095–5103.
9. 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.
10. Birkenmeier, E. H., Gwynn, B., Howard, S., Jerry, J., Gordon, J. I., Landschulz, W. H. & McKnight, S. L. (1989) *Genes Dev.* **3**, 1146–1156.
11. Graves, R. A., Tontonoz, P. & Spiegelman, B. M. (1992) *Mol. Cell. Biol.* **12**, 1202–1208.
12. Distel, R. J., Ro, H., Groves, D. L. & Spiegelman, B. M. (1987) *Cell* **49**, 835–844.
13. Yang, V. W., Christy, R. J., Cook, J. S., Kelly, T. J. & Lane, M. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3629–3633.
14. Wilkinson, W. O., Min, H. Y., Claffey, K. P., Satterberg, B. L. & Spiegelman, B. M. (1990) *J. Biol. Chem.* **265**, 477–482.
15. Student, A. K., Hsu, R. Y. & Lane, M. D. (1980) *J. Biol. Chem.* **255**, 4745–4750.
16. Cook, J. S., Lucas, J. J., Sibley, E., Bolanowski, M. A., Christy, R. J., Kelly, T. J. & Lane, M. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2949–2953.
17. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
18. Neumann, J. R., Morency, C. A. & Russian, K. O. (1987) *BioTechniques* **5**, 444–447.
19. Swick, A. G., Blake, M. C., Kahn, J. W. & Azizkhan, J. C. (1989) *Nucleic Acids Res.* **17**, 9291–9304.
20. Graves, B. J., Johnson, P. F. & McKnight, S. L. (1986) *Cell* **44**, 565–576.
21. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9**, 6505–6525.
22. Vinson, C. R., LaMarco, K. L., Johnson, P. F., Landschulz, W. H. & McKnight, S. L. (1988) *Genes Dev.* **2**, 801–806.
23. Samuelsson, L., Stromberg, K., Vikman, K., Bjursell, G. & Enerback, S. (1991) *EMBO J.* **10**, 3787–3793.
24. Lin, F. & Lane, M. D. (1992) *Genes Dev.* **6**, 533–544.
25. Umek, R. M., Friedman, A. D. & McKnight, S. L. (1991) *Science* **251**, 288–292.
26. Keller, A. D. & Maniatis, T. (1991) *Genes Dev.* **5**, 868–879.