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Identification of Conserved cis-Elements and Transcription Factors Required for Sterol-regulated Transcription of Stearoyl-CoA Desaturase 1 and 2*

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We previously identified stearoyl-CoA desaturase 2 (SCD2) as a new member of the family of genes that are transcriptionally regulated in response to changing levels of nuclear sterol regulatory element binding proteins (SREBPs) or adipocyte determination and differentiation factor 1 (ADD1). A novel sterol regulatory element (SRE) (5′-AGCAGATTGTG-3′) identified in the proximal promoter of the mouse SCD2 gene is required for induction of SCD2 promoter-reporter genes in response to cellular sterol depletion (Tabor, D. E., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1998) J. Biol. Chem. 273, 22052–22058).

In this report, we demonstrate that this novel SRE is both present in the promoter of the SCD1 gene and is critical for the sterol-dependent transcription of SCD1 promoter-reporter genes. Two conserved cis elements (5′-CCAAT-3′) lie 5 and 48 base pairs 3′ of the novel SREs in the promoters of both the SCD1 and SCD2 murine genes. Mutation of either of these putative NF-Y binding sites attenuates the transcriptional activation of SCD1 or SCD2 promoter-reporter genes in response to cellular sterol deprivation. Induction of both reporter genes is also attenuated when cells are cotransfected with dominant-negative forms of either NF-Y or SREBP. In addition, we demonstrate that the induction of SCD1 and SCD2 mRNAs that occurs during the differentiation of 3T3-L1 preadipocytes to adipocytes is paralleled by an increase in the levels of ADD1/SREBP-1c and that the SCD1 and SCD2 mRNAs are induced to even higher levels in response to ectopic expression of ADD1/SREBP-1c. We conclude that transcription of both SCD1 and SCD2 genes is responsive to cellular sterol levels and to the levels of nuclear SREBP/ADD1 and that transcriptional induction requires three spatially conserved cis elements, that bind SREBP and NF-Y. Additional studies demonstrate that maximal transcriptional repression of SCD2 reporter genes in response to an exogenous polyunsaturated fatty acid is dependent upon the SRE and the adjacent CCAAT motif.

Sterol regulatory element binding proteins (SREBPs) were purified based on their ability to bind to a 10-bp sequence, the sterol regulatory element-1 (SRE-1), that had been identified in the promoter of the LDL receptor gene (1). Subsequent isolation of the human SREBP cDNAs indicated that there are three proteins, SREBP-1a, SREBP-1c, and SREBP-2 (1). SREBP-1a and SREBP-1c are derived from the SREBP-1 gene, resulting from the use of two different promoters plus alternative splicing of mRNAs (1). SREBP-2 is the only known product derived from the SREBP-2 gene (1). Adipocyte determination and differentiation factor 1 (ADD1), the rat homologue of SREBP-1c, was cloned independently as a result of studies aimed at identifying a nuclear protein that bound the E-box motif in the promoter of the fatty acid synthase gene (2).

SREBPs/ADD1 are synthesized as 125-kDa proteins that contain three distinct domains; an NH2-terminal domain containing a basic-helix-loop-helix-leucine zipper, a central domain that contains two hydrophilic segments that anchor the proteins to the endoplasmic reticulum, and a COOH-terminal, regulatory domain (1). The latter domain can interact with SREBP cleavage-activating protein, which is itself anchored to the endoplasmic reticulum by means of multiple putative transmembrane domains (3). When cellular sterol levels are low, two distinct proteolytic events result in the release a mature 68-kDa NH2-terminal fragment of SREBPs/ADD1 (1). The mature protein translocates to the nucleus, binds to the promoters of target genes, and activates transcription (1, 4). Conversely, when levels of cellular sterols are high, proteolytic processing of SREBPs/ADD1 is diminished, nuclear levels of the mature proteins decline and transcription of target genes is low (1, 4).

SREBP responsive genes include those involved in cholesterol homeostasis (e.g. HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase, squalene synthase, and the LDL receptor) (1, 5, 6), fatty acid synthase (e.g. fatty acid synthase, acetyl-CoA carboxylase) (7, 8), fatty acid desaturation (SCD2) (9), phospholipid and triglyceride synthesis (glycerol-3-phosphate acyltransferase) (10), as well as the SREBP-2 gene itself (11). In each example, transcriptional induction of the gene requires mature SREBP and an additional transcriptional induction factor(s), so far limited to either NF-Y or Sp1. Detailed studies using sequences derived from the proximal promoters of the LDL receptor (12), farnesyl diphosphate synthase (13), fatty acid synthase (8), and acetyl-CoA carboxylase genes (14) have demonstrated the importance of synergistic binding of SREBP/ADD1 and either NF-Y or Sp1 to the DNA. In contrast, 

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reporter genes that contain intact SREs, but mutations in the NF-Y or Sp1 binding sites are transcriptionally crippled even in the presence of mature SREBP (10, 12, 15). SREBP/ADD1 can also activate transcription indirectly; overexpression of SREBP/ADD1 results in activation of peroxisome proliferator-activated receptor γ (PPAR γ), apparently through stimulated production of an endogenous ligand for this nuclear receptor (16).

Promoters of a number of SREBP/ADD1-responsive genes have been analyzed to identify the nucleotide sequences necessary for SREBP/ADD1-DNA interaction. The results indicate that the nucleotide sequence of the different SREs varies considerably; many, but not all SREs, contain two half-sites (direct repeats of C/TCAC/T) separated by one nucleotide (8). However, a number of SREs, including those identified in the promoters of farnesyl diphosphate synthase (5), squalene synthase (6), glycerol-3-phosphate acyltransferase (10), and SCD2 (9) do not conform to this sequence motif. Other studies have demonstrated that SREBP/ADD1, unlike other proteins that contain a classic helix-loop-helix motif, has an atypical tyrosine in the DNA binding domain (17). The result is a protein that binds to both an E-box motif (CANNTG) as well as varied SREs (17).

To identify genes not previously assayed to be under the transcriptional control of SREBP's and cellular sterols, we recently utilized two mutant cell lines and mRNA differential display. This approach identified stearyl-CoA desaturase 2 (SCD2) as an SREBP-regulated gene (9). SCD2 catalyzes the Δ⁹- desaturation of fatty acyl-CoAs (18). The preferred substrates, stearyl-CoA and palmitoyl-CoA, are converted to oleoyl-CoA and palmitoleoyl-CoA, respectively (19). A second fatty acid desaturase gene, SCD1, encodes an enzyme that catalyzes the identical reaction (20).

Murine SCD1 and SCD2 genes colocalize to chromosome 19 (21) consistent with gene duplication. In mice fed a triacylglycerol-free diet, both mRNAs are expressed in lung, kidney, and adipose tissue (19). However, there are some differences in the tissue expression of SCD1 and SCD2; for example, SCD1 mRNA, but not SCD2 mRNA is expressed in the liver, whereas SCD2 mRNA, but not SCD1 mRNA is expressed in the brain (19). SCD1 and SCD2 mRNA levels are repressed in all tissues in response to dietary polyunsaturated fatty acids (PUFAs) with the exception that SCD2 is not repressed in the brain (19).

Consistent with these observations, exogenous PUFAs have been shown to decrease the transcription of the SCD1 gene (22). Preliminary characterization of the SCD1 gene proximal promoter identified a 60-bp sequence that was termed a "PUFA response region" (23).

In this study, we demonstrate that the novel SRE, previously identified in the promoter of the SCD2 gene (9), is conserved in the promoter of the SCD1 gene and is required for sterol-regulated expression. We also demonstrate that sterol-regulated expression of both SCD1 and SCD2 promoter-reporter genes is dependent upon two additional cis elements (CCAAAT) that are adjacent to the SRE. The finding that the novel SRE is located within the 60-bp PUFA response region, previously identified by Waters et al. (23), raises the possibility that exogenous PUFAs repress transcription of the SCD1 and SCD2 genes by a process that requires an intact SRE.

**EXPERIMENTAL PROCEDURES**

**Materials**—DNA modification and restriction enzymes were obtained from Life Technologies, Inc. ³²P- and ³⁵S-labeled nucleotide triphosphates were obtained from ANDOTEK Life Sciences Company. Lipoprotein-deficient serum (LPDS) was purchased from Permimmune. The HMG-CoA synthase reporter gene (pSYNSRE) and all other reagents have been described elsewhere (10, 24).

**Oligonucleotides**—Oligonucleotides were synthesized by Life Technologies, Inc.

DNA Sequencing—DNA fragments generated by PCR were subcloned into the pCR1.1TOPO cloning vector (Invitrogen) as described for the supplier. Escherichia coli, strain DH5α, were transformed and selected for growth on ampicillin-containing media. β-Galactosidase-deficient colonies were screened for the presence of inserts following EcoRI digestion of the plasmid DNA and electroeluted from 1% agarose gels. Inserts were sequenced by the Sanger chain-termination method using the T7 Sequenase v2.0 kit (Amersham Pharmacia Biotech).

**Cell Culture**—HepG2 and CV-1 cells were cultured as described previously (25).

**Directed Expression of Either ADD1 or ADD1-DN in 3T3-L1 Cells**—BOSC 23 cells were transfected with the pBabe retroviral vector (26, 27) containing either ADD1 or ADD1-DN. 3T3-L1 preadipocytes were transfected with the supernatant. After 10–14 days, puromycin-resistant cells were pooled, replated, and grown to confluence (day 0 of differentiation). The cells were then induced to differentiate and subsequent media changes were performed as described (28).

**SCD Promoter-Reporter Gene Constructs**—The construction of a SCD2 promoter-reporter gene containing nucleotides −588 to +81 of the published SCD2 genomic sequence has been described (9). The 5′ end of the SCD1 gene (nucleotides −589 to +81) was obtained by polymerase chain reaction using C57Bl/6J mouse genomic DNA as a template. The 5′ primer contained nucleotides (5′-TACCTCTGAG-3′) corresponding to mouse SCD1−134 (XhoI site) followed by the nonvariant sequence according to nucleotides to +5 to +427 of the published SCD1 genomic sequence (20). The 3′ primer contained nucleotides (5′-ACTTAAGCTT-3′) containing a HindIII restriction site followed by nucleotides that corresponded to +25 to +1 of the published genomic sequence (20). The PCR product was cloned into the pGL2 basic vector, using XhoI and HindIII restriction sites to produce pSCD1−589. The 614-bp promoter sequence of pSCD1−589 (−589 to +25) was identical to that reported by Ntambi et al. (20). This DNA was used as template in an additional PCR, which used a 5′ primer corresponding to −454 to −427 of the SCD1 genomic sequence in combination with the same 3′ primer described above. The PCR product was cloned into the pGL2 basic vector, using XhoI and HindIII restriction sites to produce pSCD2−454. The sequences of PCR products were confirmed and shown to contain the expected sequence.

Oligonucleotides (29-mers) were used to introduce mutations into pSCD1−589 and pSCD2−199 using QuikChange (Stratagene). The resulting SCD1 reporter genes contained T to G and C to A mutations at positions −494 and −493 (pSCD1−589-SREMut), three A to C mutations at positions −423, −420, and −418 (pSCD1−589-MutA), C to A and C to A mutations at positions −406, −405, and −363, −362 (pSCD1−589-NF-Y1 and pSCD1−589-NF-Y2, respectively) and two C to T mutations at positions −213 and −211 (pSCD1−589-Sp1).

The resulting SCD2 reporter genes contained C to A and C to A mutations at positions −134, −133, and −91, −90 (pSCD2−199-NF-Y1 and pSCD2−199-NF-Y2), and two G to A mutations at positions −174 and −172 (pSCD2−199-Sp1).

**Northern Blot Analysis**—Total RNA was isolated from HepG2 cells using Trizol reagent (Life Technologies, Inc). 10 µg of each RNA sample was fractionated by agarose/formaldehyde gel electrophoresis, transferred to nylon membranes, and cross-linked by exposure to UV light as described (29). SCD1 and SCD2 gene-specific DNA probes corresponding to nucleotides 2910 to 3797 and 3623 to 4685, respectively, were generated as described (21). [α-³²P]dCTP-radiolabeled DNA probes were generated by the random priming labeling method (Amersham Pharmacia Biotech). Hybridization and quantification, using a PhosphorImager (Molecular Dynamics) were as described (30). To correct for differences in RNA loading in each lane, blots were also hybridized to a probe, 36B4, that hybridized to a constitutively expressed mRNA.

**Transient Transfections and Reporter Gene Assays**—Transient transfections were performed using minor modifications of the transfection MBS kit (Stratagene). Details of the transient transfection of HepG2 cells with luciferase reporter genes, a plasmid encoding β-galactosidase, and pCl-SREBP-1a (encoding mature SREBP-1a) appear in previous publications (5, 25). Following transfection, cells were incubated for 20 h in medium containing 10% LPDS supplemented with either 5 µM mevinolin (inducing medium) or steroids (10 µg/ml cholesterol and 1 µg/ml 25-hydroxycholesterol) (repressing medium). The cells were subjected to nuclear and the luciferase and β-galactosidase activities determined by the β-galactosidase activity to correct for minor differences in transfection efficiency. The normalized values from duplicate dishes varied less than 10%.

**Electromobility Shift Assays**—Complementary single-stranded DNAs containing wild-type or mutated sequences were annealed, the double-stranded DNA was isolated and end-labeled with ³²P as de-
SCD1 and SCD2 mRNA Levels Are Regulated Upon Adipocyte Differentiation and Ectopic Expression of SREBP/ADD1—Analysis of the published nucleotide sequence of the proximal promoters of the murine SCD1 and SCD2 genes revealed the presence of a number of conserved elements (Fig. 1). These conserved elements include the novel SRE previously identified and characterized in the SCD2 promoter (9) (Fig. 1, solid triangle) and two putative binding sites (CCAAT) for NF-Y that are 5- and 48-bp 3′ of the novel SRE (Fig. 1, solid triangles). The novel SRE of the SCD1 promoter shows 9/10 identity with the corresponding sequence in the SCD2 promoter (Fig. 1) (9). In addition, both proximal promoters contain a putative binding site for Sp1 (Fig. 1, solid circle). The two CCAAT elements and the putative Sp1 binding site had been previously identified, but not functionally characterized by Waters et al. (23) in studies designed to identify a PUFA response element. Interestingly, the novel SRE and the most adjacent NF-Y binding site lie within a highly conserved 60-bp that was termed a PUFA response sequence (Fig. 1, solid underline). The SCD1 promoter also contains a sequence with 9/10 identity with the well characterized SRE-1 originally identified in the promoter of the LDL receptor gene (Fig. 1, open square).

Based on the sequence and spatial conservation of the SRE and CCAAT motifs in the promoters of the SCD1 and SCD2 genes, we hypothesized that the transcription of both genes would be activated in an SREBP/ADD1- and NF-Y-dependent manner. However, because both promoters also contain putative Sp1 binding sites and the transcription of a number of SREBP-dependent genes is known to require Sp1 (4), we hypothesized that Sp1 might also be important in transcriptional activation.

Fig. 2 shows that the mRNA levels for SCD1 and SCD2 increase 20- and 1.8-fold, respectively, during differentiation of 3T3-L1 adipocytes to adipocytes (compare lanes 1 and 4). These data are consistent with a previous report (19). We have previously demonstrated that the levels of ADD1 mRNA, the rat homolog of SREBP-1c, increase during this differentiation process (10). To determine whether these increases in SCD mRNA levels are affected by levels of mature ADD1, the adipocytes were infected with a retrovirus that expressed either wild-type ADD1 or a dominant-negative form of ADD1, termed ADD1-DN (28). As compared with uninfected cells, ectopic expression of ADD1 led to further increases in SCD1 and SCD2 mRNA levels; after 6 days of differentiation, the mRNA levels of SCD1 and SCD2 increased an additional 2.7- and 1.4-fold, respectively (Fig. 2, lanes 4 versus 5). Thus, in the presence of ectopically expressed ADD1, the mRNA levels of SCD1 and SCD2 increased 55- and 2.5-fold during differentiation. In contrast, ectopic expression of ADD1-DN attenuated the normal increases in SCD1 and SCD2 mRNA levels (Fig. 2, lanes 4 versus 6). Taken together, these results are consistent with a stimulatory role for ADD1/SREBP-1c in the transcriptional regulation of the SCD1 and SCD2 genes during adipocyte differentiation.

Regulation of SCD1 and SCD2 Promoter-Reporter Genes in Response to Sterols and Coexpression of SREBP—We next sought to determine whether expression of an SCD1 promoter-reporter gene was regulated in response to either coexpression of transcriptionally active SREBP or to alterations in the cellular sterol status of the cell. An SCD2 reporter construct served as a positive control (9). SCD1 and SCD2 promoter-reporter constructs were transiently transfected into HepG2 cells together with a plasmid encoding β-galactosidase under the control of a nonregulated, cytomegalovirus-derived promoter. The cells were then incubated for 20 h in medium containing 10% LPDS and either 5 μM mevinolin or steroids (10 μg/ml cholesterol, 1.0 μg/ml 25-hydroxycholesterol), lysed, and the luciferase activities determined. The β-galactosidase activity was used to normalize luciferase activities for minor differences in transfection efficiencies.

The data show that SCD1 and SCD2 promoter-reporter genes are induced approximately 20-fold (Fig. 3A, lanes 1 versus 2) and 60-fold (Fig. 3B, lanes 1 versus 2), respectively, when HepG2 cells are cultured in medium supplemented with 10% LPDS and mevinolin, as compared with 10% LPDS and steroids. To determine the direct effect of SREBP on transcription of the SCD promoter-reporter genes, HepG2 cells were transiently transfected with either an SCD1 (pSCD1–589) or an SCD2 (pSCD2–588) promoter-reporter gene and low levels of a plasmid that constitutively expresses transcriptionally active, mature SREBP-1a (Fig. 3). The cells were then incubated for 20 h...
in the presence of excess sterols to prevent cleavage and maturation of endogenous SREBPs. The data show that both pSCD1–589 (Fig. 3A, lanes 2–5) and pSCD2–588 (Fig. 3B, lanes 2–5) were activated following coexpression of SREBP-1a, in a dose-dependent manner. When cells were transfected with 500 ng of the plasmid encoding mature SREBP-1a, the SCD1 and SCD2 reporter genes were induced to supraphysiological levels that were >300-fold higher than basal levels (data not shown).

The Novel SRE, but not the Classical SRE-1, Is Required for Sterol-Regulated Expression of SCD1 Promoter-Reporter Genes—The data from Figs. 2 and 3 indicate that sterols repress and SREBP/ADD1 induces the expression of SCD1. To determine whether the novel SRE and/or the SRE-1 element are required for the sterol-dependent regulation of the SCD1 promoter-reporter genes, the experiments illustrated in Fig. 4 were carried out. HepG2 cells were transiently transfected with the indicated reporter genes and incubated in medium containing 10% LPDS and either 5 μM mevinolin (solid bars) or sterols (open bars). The results demonstrate that deletion (pSCD1–454) or mutation (pSCD1–589 SREMut) of the classic SRE-1 motif had no effect on the expression or regulation of the reporter gene (Fig. 4). In contrast, incorporation of a 3-bp mutation into the novel SRE (pSCD1–589 MutA) attenuated both the absolute level of activity of the reporter gene and the fold regulation in response to sterols/sterol deprivation (Fig. 4). In a previous study, we demonstrated that a similar 3-bp mutation of the novel SRE in the promoter of the SCD2 gene impaired the regulation of a reporter gene in response to sterols (9). Thus, we conclude that the novel SRE, but not the SRE-1 motif in the SCD1 promoter, is required for sterol-regulated transcription.

To further investigate the requirement for SREBPs in the induction of the SCD1 promoter-reporter constructs, we used a dominant-negative form of SREBP-1a (DN-SREBP-1a). As a result of deletion of the amino-terminal 90 amino acids, the DN-SREBP-1a is capable of binding DNA (data not shown), but is incapable of transcriptional activation (data not shown). The data in Fig. 5A demonstrate that the increase in expression of the pSCD1–589 construct, in response to depletion of medium sterols, was attenuated by coexpression of this dominant-negative form of SREBP-1a. pSYNSRE, a reporter gene under the control of a promoter derived from the HMG-CoA synthase gene, was used as a positive control. Induction of pSYNSRE in response to sterol depletion is known to require SREBP (1). In response to cellular sterol deprivation, and consistent with an earlier report (9), the induction of pSYNSRE was also attenuated by coexpression of DN-SREBP-1a (Fig. 5B).

Electrophoretic Mobility Shift Assays—Taken together, the studies illustrated in Figs. 3–5 demonstrate that reporter genes under the control of the proximal promoters derived from the SCD1 and SCD2 genes are regulated in an SREBP-dependent manner. Analysis of the SCD1 and SCD2 proximal promoters reveals that the novel SRE, first identified in the SCD2 promoter (9) is conserved (9/10 identity) in the SCD1 promoter (Fig. 1). The SCD1 promoter also contains an element with 9/10 identity with the SRE-1 first identified in the promoter of the LDL receptor gene (31). However, the SRE-1 sequence in the proximal promoter of the SCD1 gene (−445 to −454) is not necessary for the sterol-dependent regulation of SCD1 promoter-reporter constructs (Fig. 4).

The data of Fig. 6 demonstrate that recombinant SREBP-1a can bind in vitro to the SRE-1 sequence (lane 2 versus 1) and the novel SRE (lane 6 versus 5) identified in the SCD1 promoter (Fig. 1). The formation of the SREBP-1a-DNA complexes was abolished when the DNA contained the indicated mutations in the SRE-1 (Fig. 6, compare lanes 4 and 2) or the novel SRE (lanes 8 and 6). Consistent with an earlier study (9), recombinant SREBP-1a formed a complex with DNA containing the novel SRE identified in the SCD2 promoter (Fig. 6, lanes 10 versus 9) but not with the DNA containing three point mutations in the SRE (Fig. 6, lanes 12 versus 10). Based on these results, we conclude that SREBP-1a can bind to both SRE sequences in the SCD1 promoter. However, the data of Figs. 5 and 6 indicate that within the proximal promoter of the SCD1 gene, only the novel SRE, that shows 9/10 identity with the SRE in the SCD2 promoter, has a role in sterol-regulated expression. Mutations in the novel SRE of the SCD1 promoter abolish both the sterol regulation of the reporter gene (Fig. 5) and the formation of an SREBP-1a-DNA complex (Fig. 6, lanes 8 versus 6). Taken together, these results demonstrate that the single nucleotide difference between the novel SREs in the SCD1 and SCD2 promoters does not affect function.

Two CCAAT Motifs in the Proximal SCD1 and SCD2 Promoters Are Required for Sterol Regulation of Reporter Genes—The sterol-regulated transcription of the LDL receptor and fatty acid synthase genes requires both SREBP and Sp1 (7, 12). In contrast, the sterol-regulated transcription of the farnesyl diphosphate synthase, HMG-CoA synthase, glycerol-3-phosphate acyltransferase, and the SREBP-2 genes has been shown to require both SREBP and NF-Y (10, 11, 13, 24).

Both the SCD1 and SCD2 proximal promoters contain two putative NF-Y binding sites (5′–CCAAT-3′ that are located 5′ and 48–52 bp of the critical SRE (Fig. 1). We next sought to determine whether these putative NF-Y binding sites were required for regulation of SCD1 and SCD2 promoter-reporter genes in response to alterations in the sterol content of cells. The results shown in Fig. 7 indicate that mutation of either CCAAT motif results in a >90% decrease in reporter gene activity. In addition, both SCD1 and SCD2 promoter-reporter genes showed reduced responsiveness to regulation by sterols (Fig. 7). These results suggest that both CCAAT motifs are...
critical for the high expression of the reporter genes that occurs upon cellular depletion. In contrast, mutation of the putative Sp1 binding sites in the SCD1 or SCD2 promoters did not result in an attenuated response (Fig. 7). Surprisingly, mutation of the putative Sp1 binding site in the SCD1 promoter resulted in a significant increase in reporter gene activity when the cells were incubated under inducing conditions (Fig. 7). The reason for this latter induction has not been investigated. Nonetheless, the studies from Fig. 7 demonstrate that the putative Sp1 binding sites in the SCD1 and SCD2 promoters are not necessary for normal induction of SCD1 and SCD2 reporter genes in response to cellular sterol depletion.

Expression of a Dominant-Negative NF-Y Attenuates SCD1 and SCD2 Reporter Gene Induction by Sterol Depletion—The observation that mutation of either of the two CCAAT sequences results in attenuated induction of SCD1 and SCD2 reporters strongly suggests that induction is dependent on NF-Y, because NF-Y is known to bind to this motif. To test this hypothesis, CV-1 cells were transiently transfected with SCD1, SCD2, LDL receptor, or HMG-CoA synthase reporter genes in the absence or presence of a plasmid encoding β-galactosidase. The classical SRE ( ), novel SRE ( ), CCAAT boxes ( ), and GC boxes ( ) are indicated. A mutated motif is indicated ( ). The cells were incubated for 20 h in medium containing 10% LPDS and either 5 μM mevinolin (solid bars) or sterols (open bars), lysed, and the normalized luciferase activities determined as described under “Experimental Procedures.” The luciferase activity of pSCD1–589 under sterol repressed conditions was given an arbitrary value of 1.0.

NF-YA protein is unable to bind DNA, but does bind and sequester NF-YB and NF-YC (32). This results in a decrease in the nuclear levels of the functional heterotrimer of NF-YA, NF-YB, and NF-YC (32). pSYNSRE, a luciferase reporter construct under control of sequences derived from the promoter of the HMG-CoA synthase gene, was used as a positive control, because induction in response to sterol deprivation is known to require both SREBP and NF-Y (1, 15, 24).

The results demonstrate that induction of SCD1, SCD2, and...
pSYNSRE reporter genes in response to sterol depletion was attenuated by coexpression of NF-YA29 (Fig. 8). In contrast, the dominant-negative NF-YA29 had little effect on the induction of the LDL receptor reporter gene (Fig. 8), consistent with the known requirement for SREBP and Sp1, but not NF-Y (12). Taken together, the results in Fig. 8 indicate that sterol-regulated induction of SCD1 and SCD2 is dependent upon NF-Y.

The Same cis Elements Are Required for Transcriptional Repression of SCD2 by Sterols and PUFAs—Previous studies have shown that PUFAs repress the transcription of the SCD1 gene (22). Characterization of the promoter of the SCD1 gene identified a 60-bp sequence, termed a PUFA response region, that imparted PUFA-dependent repression when placed in a heterologous promoter-reporter gene (23). This 60-bp region is highly conserved between the promoters of the SCD1 and SCD2 genes (19). Interestingly, this 60-bp sequence contains the novel SRE and adjacent CCAAT motif characterized in the current study. Based on these observations, we hypothesized that the novel SRE and/or the adjacent CCAAT motif might also be necessary for PUFA-dependent repression.

Fig. 9 shows the data obtained when HepG2 cells were transiently transfected with an SCD2 promoter-reporter gene (pSCD2-199), and the cells then incubated for 20 h in medium supplemented with 2.5% LPDS and 1% fatty acid-free bovine serum albumin in the absence or presence of exogenous linolenic acid (0.3 mM). As expected (23), the activity of the reporter gene was decreased 55% when the fatty acid was added to the medium (Fig. 9, lanes 1 versus 2). In the absence of linolenic acid, the activities of reporter genes containing mutations in either the SRE (pSCD2–199 MutA) or the NF-Y binding site (pSCD2–199-NF-Y1) were approximately 5 and 2%, respectively, of the control. Addition of linolenic acid repressed the activity of the mutant constructs less than the wild-type reporter gene (pSCD2–199 MutA and pSCD2–199-NF-Y1 were repressed by linoleic acid 30 and 6%, respectively (Fig. 9, lanes 3–6). These results demonstrate that maximum repression of the SCD2 reporter gene by linoleic acid requires that the promoter contain a wild-type CCAAT box motif and the adjacent SRE. However, mutations of either the SRE or the CCAAT box motif result in a 95–98% decrease in the SCD2 reporter gene activity (Fig. 9, lanes 3 and 5 versus 1). Thus, we cannot discount the possibility that the inability of an exogenous PUFA to maximally repress the mutant reporter genes might be a consequence of the near basal activity of these reporter genes. We demonstrate that SCD1 mutant promoter-reporter genes were expressed at even lower levels than mutant SCD2 genes. Consequently, it was not possible to assess the role of linolenic acid on luciferase expression of mutant SCD1 reporter genes (data not shown).

DISCUSSION

This report demonstrates that transcription of the murine SCD1 and SCD2 genes is regulated by cellular sterols; transcription is repressed by excess sterols and induced when cellular sterol levels are depleted. Detailed studies demonstrate that transcriptional induction of both genes is dependent upon a novel SRE and two adjacent CCAAT motifs that are conserved in the promoters of SCD1 and SCD2 genes. It is of interest that transcription of SCD1 and SCD2 genes is also induced by fatty acids (24, 25). The current study demonstrates that the transcriptional induction of both SCD1 and SCD2 genes in response to fatty acids is dependent upon SREs and CCAAT motifs.

2 D. E. Tabor and P. A. Edwards, unpublished results.
transcription of SREBP/ADD1 (28) and is activated following the addition to the cells of exogenous unsaturated fatty acids that are converted to ligands for PPARγ (16).

In these studies, we demonstrate that the increase in SCD1 and SCD2 mRNA levels that occurs during the differentiation of 3T3-L1 preadipocytes is enhanced when cells express excess ADD1 and is attenuated following the expression of a dominant-negative form of ADD1 (Fig. 2). Based on these results, we propose that during the differentiation of 3T3-L1 cells, induction of SCD1 and SCD2 mRNAs, like those of glycerol-3-phosphate acyltransferase (10), is dependent on the increased expression of SREBP/ADD1.

In mice, both SCD1 and SCD2 mRNAs are expressed in lung, kidney, and adipose tissue (19). In contrast, liver and brain normally express the mRNAs encoding only SCD1 or SCD2, respectively (19). However, transgenic mice that express elevated hepatic levels of truncated dominant-negative forms of SREBP-1a, SREBP-1c, or SREBP-2 (36), or a sterol-resistant form of SREBP cleavage-activating protein (37), have detectable hepatic mRNA levels of SCD2, as well as induced levels of SCD1. These latter results are consistent with the transcriptional activation of both genes by SREBP/ADD1, a process that we have detailed mechanistically in the current study.

In rats, the activity of the SCD enzymes is regulated by diet, hormone balance, developmental state, and various drugs (reviewed in Refs. 18 and 38). Regulation of the desaturases is a result of diverse mechanisms that include altered transcription of the genes (18, 39) and altered stability of the mRNAs (40). One mode of regulation, which is incompletely understood, is end-product repression in response to exogenous unsaturated fatty acids. Early studies demonstrated that both SCD1 and SCD2 mRNA levels were induced in a number of mouse tissues when animals were fasted and then refed a triacylglycerol-free diet (19). Conversely, excess exogenous unsaturated fatty acids resulted in repression of hepatic SCD1 mRNA levels and in a decrease in the activity of an SCD1 promoter-reporter gene (18, 23). A 60-bp sequence in the proximal promoter of the SCD1 gene was termed a polyunsaturated fatty acid response region, because it conferred responsiveness to polyunsaturated fatty acids when placed in a heterologous promoter (23). Interestingly, this 60-bp sequence is highly conserved (>80%) in both the SCD1 and SCD2 promoters (19) and contains the novel SRE and one adjacent CCAAT motif (Fig. 1). The current studies demonstrate the importance of these two motifs in sterol-regulated transcription of both genes.

Two recent publications (41, 42) demonstrated that unsaturated fatty acids inhibit the cleavage of the precursor form of SREBP and result in decreased levels of nuclear SREBP and decreased transcription of an HMG-CoA synthase promoter-reporter gene. Based on these observations, we hypothesize that unsaturated fatty acids might inhibit the transcription of SCD1 and SCD2 genes in an SREBP-dependent process. Consistent with this hypothesis, the data of Fig. 9 indicate that maximum repression of SCD2 reporter genes, in response to PUFAs, is dependent upon the SRE and adjacent CCAAT box.

The transcription of a number of other genes, including S14, glucose-6-phosphate dehydrogenase, and fatty acid synthase, are also known to be inhibited by exogenous PUFAs (43–45). The promoters of the fatty acid synthase (8, 28) and S14 (5) genes contain elements to which SREBP binds. Recent studies have demonstrated that the mRNAs of S14, glucose-6-phosphate dehydrogenase, and fatty acid synthase are all increased in the livers of transgenic mice that overexpress mature SREBP (46). Taken together, these results indicate that a number of genes that are transcriptionally repressed by PUFAs also contain an SREBP-binding element in their promoters.
Consequently, we hypothesize that PUFAs repress transcription of a number of genes, including SCD1, SCD2, S14, glucose-6-phosphate dehydrogenase, and fatty acid synthase, by inhibiting the formation of an SREBP-NF-Y or Sp1 complex on the DNA. Demonstration that transcriptional activation of the S14 or the glucose-6-phosphate dehydrogenase genes is dependent on the interaction of SREBP/ADD1 with a functional SRE in the promoters of these two genes would provide further support for the hypothesis.

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