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Transcriptional Activation of the Stearoyl-CoA Desaturase 2 Gene by Sterol Regulatory Element-binding Protein/Adipocyte Determination and Differentiation Factor 1*

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To identify genes that are transcriptionally activated by sterol regulatory element-binding proteins (SREBPs), we utilized mRNA differential display and mutant cells that express either high or low levels of transcriptionally active SREBP. This approach identified stearoyl-CoA desaturase 2 (SCD2) as a new SREBP-regulated gene.

Cells were transiently transfected with reporter genes under the control of different fragments of the mouse SCD2 promoter. Constructs containing >199 base pairs of the SCD2 proximal promoter were activated following incubation of cells in sterol-depleted medium or as a result of co-expression of SREBP-1a, SREBP-2, or rat adipocyte determination and differentiation factor 1 (ADD1). Electromobility shift assays and DNase I footprint analysis demonstrated that recombinant SREBP-1a bound to a novel cis element (5′-AGCGAGATTGTTG-3′) in the proximal promoter of the SCD2 gene. The finding that the endogenous SCD2 mRNA levels were induced when wild-type Chinese hamster ovary fibroblasts were incubated in sterol-deficient medium is consistent with a role for SREBP in regulating transcription of the gene.

These studies identify SCD2 as a new member of the family of genes that are transcriptionally regulated in response to changing levels of nuclear SREBP/ADD1. In addition, the sterol regulatory element in the SCD2 promoter is distinct from all previously characterized motifs that confer SREBP- and ADD1-dependent transcriptional activation.

Sterol regulatory element-binding proteins (SREBPs)1 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 (reviewed in Ref. 1). Subsequent isolation of the human SREBP cDNAs indicated that three proteins, SREBP-1a, SREBP-1c, and SREBP-2 are derived from two SREBP genes (1). SREBP-1a and -1c mRNAs result from alternative splicing of exon 1 of the SREBP-1 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). ADD1 expression was shown to be required for adipogenesis and for the induction of a number of mRNAs, including fatty acid synthase and glycerol-3-phosphate acyltransferase (2–4), that occurs during this differentiation process.

SREBPs/ADD1 are synthesized as 125-kDa proteins that contain two transmembrane domains that anchor the proteins to the endoplasmic reticulum (1). When cellular sterol levels are low, two distinct proteolytic events release a mature 68-kDa NH2-terminal fragment of SREBPs/ADD1, which then translocates to the nucleus, binds to the promoters of target genes, and activates transcription (1). 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).

A number of genes have been shown to be regulated at the transcriptional level by mature SREBPs/ADD1. These include genes involved in cholesterol homeostasis (e.g. HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase, squalene synthase, and the LDL receptor) (1, 5, 6), in fatty acid metabolism (e.g. fatty acid synthase, acetyl-CoA carboxylase, and glycerol-3-phosphate acyltransferase) (2–4, 7, 8) as well as the SREBP-2 gene itself (9). Recent studies have shown that ADD1/SREBP1 also activates peroxisomal proliferator-activated receptor γ through production of an endogenous ligand (10) and stimulates expression of the leptin gene (11).

The mature, transcriptionally active SREBPs/ADD1 contain basic-helix-loop-helix-leucine zipper (bHLH-Zip) domains that are required for DNA binding and transactivation (1, 2). bHLH-Zip domains are found in a number of other transcription factors, including Myc and Max, that bind to E-box motifs (CANNTG) (12). In contrast, ADD1/SREBP bind to both E-box and non-E-box motifs (13). The ability of ADD1/SREBP to bind to non-E-box motifs is a result of an atypical tyrosine replacing an arginine that is conserved in the basic domain of all other bHLH-containing proteins (13).

Promoters from 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 sterol response elements (SREs), vary considerably; many, but not all SREs, contain two half-sites (direct repeats of C/TACG) separated by...
two nucleotides (1, 7). However, a number of SREs do not conform to this sequence motif (5–7).

A number of mutant cell lines have been isolated that do not regulate the expression/transcription of the HMG-CoA reductase, HMG-Coa synthase, and the LDL receptor genes in response to altered cholesterol levels (14–19). Such mutants include sterol regulatory defective (SRD) cell lines that constitutively express high (SRD-1, SRD-2) or low (SRD-6) levels of nuclear SREBP irrespective of the sterol status of the medium or of the cells (14–16). Subsequently, it was shown that the 5’ part of the SREBP-2 gene, that contains the bHLH-Zip domains, had undergone a translocation event in SRD-1 and SRD-2 cells. This resulted in the synthesis of distinct fusion proteins that contain no transmembrane sequences and were translocated to the nucleus where they transactivate target genes (16). In contrast, SRD-6 cells produce little or no mature SREBP as a result of a defect at the site-2 cleavage (20). As a result, the mRNA levels of a number of SREBP-responsive genes are extremely low, even when SRD-6 cells are incubated in the absence of sterols (15, 21).

We hypothesized the existence of novel genes and/or known genes not previously ascribed to be under the transcriptional control of SREBP and cellular sterols. To identify such genes, we utilized mRNA differential display (22), using RNAs isolated from SRD-2 and SRD-6 cells.

This approach identified a number of transcripts expressed at high levels in SRD-2 cells as compared with SRD-6 cells. Among these, we identified stearoyl-CoA desaturase 2 (SCD2), an enzyme involved in the synthesis of unsaturated fatty acids. SCD2 catalyzes the Δ9-cis desaturation of fatty acyl-CoAs such as stearoyl-CoA and palmitoyl-CoA to produce oleic and palmitoleic acid, respectively (23, 24). The current studies demonstrate that a gene (SCD2) encoding an enzyme involved in fatty acid desaturation is transcriptionally regulated by SREBPs/ADD1 or sterols. The finding provides a plausible explanation as to why certain mutant cells that are defective in SREBP ADD1 or sterols. The finding provides a plausible explanation for the reduced expression of stearoyl-CoA desaturase 2 in SRD cells as compared with cholesterol-fed control cells (24, 25).

SCD2 promoter-Reporter Gene Constructs—The 5’ end of the SCD2 gene (nt –588 to +81) was obtained by polymerase chain reaction using C57Bl/6J mouse genomic DNA as a template. The 5’ primer contained nucleotides that corresponded to –588 to –567 of the published SCD2 genomic sequence (24) and additional nucleotides (5’-TACCTCCCGAG-3’) containing an XhoI restriction site. The 3’ primer contained nucleotides that corresponded to +81 to +60 of the published genomic sequence (24) and additional nucleotides (5’-ACTTAAGCTT-3’) containing an HindIII restriction site. The PCR product was cloned into the pGL2 basic vector, utilizing XhoI and HindIII restriction sites to produce pSCD2-588. This 670-bp sequence of the SCD2 gene (–588 to +81) was identical to that reported by Kaestner et al. (24). This DNA was used as template for additional PCRs that utilized 5’ primers corresponding to –199 to –167, –150 to –129, and –110 to –89 of the SCD2 genomic sequence in combination with the common 3’ primer. These reactions generated a series of SCD2 promoter fragments that were cloned into the pGL2 basic vector to produce pSCD2-199, pSCCD2-150, and pSCCD2-110, respectively. Oligonucleotides (28mers) were used to introduce mutations into pSCD2-199 using QuikChange (Stratagene). The resulting reporter genes each contained three A to G mutations at nt –151, –148 and –146 (pSCD2-199mut A) or at nt –157 to –155 (pSCD2-199mut B), respectively. The promoter of each construct was sequenced to confirm sequence.

Northern Blot Analysis—Total RNA was isolated from SRD-1, SRD-2, SRD-6, and CHO cells using Trizol reagent (Life Technologies, Inc.). Ten μg of each RNA sample was fractionated by agarose/formaldehyde gel electrophoresis, transferred to nylon membranes, and crosslinked by exposure to UV light as described (26). [α-32P]dCTP-labeled DNA probes were generated by the random priming labeling method (Amersham Pharmacia Biotech). Hybridization and quantification, using a PhosphorImager (Molecular Dynamics), were as described (5). To correct for differences in DNA 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, pCI-SREBP-1a (encoding mature SREBP-1a) pCI-SREBP-2 (encoding mature SREBP-2), 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 sterol-free medium (repressing medium). The cells were lysed and the luciferase activities determined and normalized to the β-galactosidase activity to correct for minor differences in transfection efficiency (5).

Electromobility Shift Assays—Double-stranded DNA fragments corresponding to –588 to –278, –299 to –239, –259 to –178, –199 to –130, –150 to –91, –110 to +1, and –259 to –91 of the murine SCD2 proximal promoter were generated by PCR. The primers used to generate these SCD2 DNA fragments were flanked by the same 10 nucleotides, containing either an XhoI or HindIII restriction site, as described above. Single-stranded DNA (–164 to –137) containing wild-type or mutated sequences were annealed and the double-stranded DNA isolated and used in EEMs (5). Recombinant SREBP-1a was purified from E. coli extracts as described previously (5). The DNA was end-labeled with [32P] (20,000 cpm; 1.5 fmol) and used in EEMs as described (5).

DNA 1 Footprint Analysis—A DNA fragment corresponding to nucleotides –259 to –91 of the proximal SCD2 promoter (24) was used for DNase I footprinting. The fragment was cloned into the pCR2.1-TOPO cloning vector (Invitrogen). Purified plasmid DNA was cut with either BamHI or NotI and then end-labeled with [γ-32P]dATP. The labeled, linearized plasmid was digested with the reciprocal restriction enzyme, either NotI or BamHI, to liberate a fragment containing nucleotides –259 to –91, end-labeled at either end. The gel-purified, end-labeled fragments were incubated with purified SREBP-1a and treated with DNase I as described (5).
using the effects initiated by elevated nuclear SREBP, or nonphysiologically active SREBP-2 fusion proteins. To distinguish between these possibilities, wild-type CHO cells were incubated in medium supplemented with 10% LPDS and either mevinolin (inducing conditions) or sterols (repressing conditions). These conditions are known to regulate the nuclear localization of SREBPs and to result in changes in the mRNA levels of SREBP-regulated genes (1). The results in Fig. 3 demonstrate that the SCD2 mRNA levels were regulated 4.2-fold by the sterol status of the cells; the levels were elevated when cells were sterol-deprived and repressed when sterols were added to the medium.

Regulation of SCD2 Promoter-Reporter Genes by Sterols and Co-expressed SREBPs—We next sought to determine whether expression of the SCD2 promoter-reporter genes were regulated in response to changes in the levels of either nuclear SREBPs or cellular sterols. HepG2 cells were utilized, because this cell type exhibits sterol-regulated expression of transiently transfected SREB-responsive promoter-reporter genes (25). In contrast, sterol-regulated expression of many, if not all, such reporter genes is not observed in transiently transfected CHO cells (data not shown). For reasons that have yet to be determined, stably transfected CHO cells exhibit normal sterol-dependent regulation of these same SREBP-responsive reporter constructs (25).

Four different murine SCD2 promoter fragments were generated and cloned into a luciferase reporter vector (Fig. 4). Each SCD2 promoter-reporter construct was 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 the absence or presence of sterols, lysed, and the luciferase activities determined. The β-galactosidase activity was used to correct for differences in transfection efficiency.

Fig. 4 shows that incubation of cells in sterol-depleted medium resulted in a 5–12-fold increase in the activities of the reporter genes under the control of the proximal 588 or 199 bp of the SCD2 promoter. In contrast, reporter genes containing 150 or 110 bp of the proximal SCD2 promoter were unregulated and expressed at low levels (Fig. 4).

Taken together, the studies shown in Figs. 1–4 suggest that the transcription of the SCD2 gene is regulated by sterols and possibly by SREBPs. To determine the direct effect of SREBPs on transcription of the SCD2 promoter-reporter genes, the experiments illustrated in Fig. 5 were performed; HepG2 cells were transiently transfected with the indicated SCD2 promoter-reporter gene and plasmids that constitutively express either mature SREBP-1a or SREBP-2. The cells were then incubated for 20 h in the presence of excess sterols to prevent cleavage of endogenous SREBPs. Fig. 5A shows that pSCD2-588 was activated by co-expressed SREBP-1a or SREBP-2, in a dose-dependent manner. Maximal luciferase activities (>400-fold increase) were obtained when the cells were transfected with 50 ng of plasmid encoding either SREBP-1a or SREBP-2.
Duplicate dishes of HepG2 cells were transiently transfected with 100 ng of cDNA. radiolabeled probes specific for stearoyl-CoA desaturase 2 or 36B4 aldehyde gel, transferred to a nylon membrane and hybridized to the radiolabeled probes specific for stearoyl-CoA desaturase 2 or 36B4.

**Fig. 3.** SCD2 mRNA levels are repressed by sterols. RNA was isolated from duplicate dishes of Chinese hamster ovary cells incubated in media containing 10% LPDS supplemented with either sterols (+) (10 μg/ml cholesterol and 1 μg/ml 25-hydroxycholesterol) or 5 μM mevinolin in the absence of sterols (−) as described under “Experimental Procedures.” RNA (10 μg) was fractionated on a 1% agarose/formaldehyde gel, transferred to a nylon membrane and hybridized to the

**Fig. 4.** Regulation of SCD2 promoter-luciferase reporter genes by sterols. Duplicate dishes of HepG2 cells were transiently transfected with 1 μg of the indicated SCD2 promoter-reporter gene and a plasmid encoding β-galactosidase under the control of a cytomegalovirus promoter. The cells were incubated for 24 h in medium containing 10% LPDS with or without supplemented sterols, as described in the legend to Fig. 3. The normalized luciferase values, obtained from duplicate dishes, varied less than 10%. A value of 1 was given to the luciferase activity obtained with pSCD2-110 following incubation of cells in the sterol-supplemented repressing medium. The results are representative of three separate experiments.

**Fig. 5.** Stimulation of SCD2 promoter-reporter genes by co-expression of SREBP-1a or SREBP-2. SCD2 promoter-reporter genes were used as described under “Experimental Procedures.” One μg of the SCD2 promoter-reporter construct was transfected into HepG2 cells in duplicate along with a plasmid encoding β-galactosidase. In A, cells were co-transfected with the indicated amount of an expression plasmid encoding mature SREBP-1a (●) or SREBP-2 (○). In B, cells were co-transfected with plasmids encoding either SREBP-1a or SREBP-2 or carrier plasmid (−), as indicated. The cells were incubated for 24 h in medium supplemented with 10% fetal bovine serum and sterols. The cells were lysed, and the normalized luciferase activities were determined as described. The variation in the normalized luciferase activities between duplicate dishes was less than 10%. The results are representative of three separate experiments.

**Effect of Co-expression of a Dominant-negative SREBP-1a—**To further investigate the requirement for SREBP1a in the sterol-mediated induction of the SCD2 promoter-reporter constructs, we utilized a dominant-negative form of SREBP1a (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. The data in Fig. 6 demonstrate that the increased expression of the pSCD2-588 construct, in response to depletion of media sterols, was prevented by co-expression of this dominant-negative form of SREBP-1a. The normal induction of pSYSNSRE in response to sterol deprivation was also attenuated by co-expression of DN-SREBP-1a (Fig. 6), consistent with a functional role for SREBP in the transcriptional induction of the HMG-CoA synthase gene (1).

**Electromobility Shift Assays and DNase I Footprint Analyses—**Taken together, the studies illustrated in Figs. 1–6 demonstrate that the transcription of the SCD2 gene is regulated in response to changing levels of either cellular sterols or nuclear SREBP1a. However, examination of the SCD2 proximal promoter sequence did not reveal a sequence with high identity to any previously characterized SRE.

To identify a potential SRE in the SCD2 promoter, EMSAs were performed with different fragments of the proximal SCD2 promoter and recombinant SREBP-1a. DNA fragments −588 to −81, −299 to +81, −295 to +81, −199 to +81 and −199 to −130 all gave a single shifted complex with recombinant SREBP-1a (data not shown). In contrast, fragments −299 to −239, −259 to −178 and −110 to +1 gave no shifted complex (data not shown). The results of these EMSAs indicated that SREBP-1a likely bound to nucleotides located between −178 and −110 of the SCD2 promoter.
Consequently, a 169-bp fragment (−259 to −91) was end-labeled and utilized either in an EMSA (Fig. 7A) or subjected to DNase I digestion in the absence or presence of recombinant SREBP-1a (Fig. 7B). This 169-bp fragment gave a single shifted DNA-SREBP-1a complex (Fig. 7A) consistent with the presence of a single SREBP-1a binding site. The results of Fig. 7B show that SREBP-1a protected nucleotides −151 to −141 from DNase I digestion. Examination of the nucleotide sequence in the footprinted region does not reveal any identity to known sterol regulatory elements or to a consensus E-box motif, and we concluded that SREBP-1a bound to a novel and distinct sequence. The presence of a DNase I hypersensitive site at −140 (Fig. 7B) resulted in a decrease in the radioactivity associated with all faster migrating DNA species and made it difficult to delineate the 5′ border of the DNase I protected region. To better define the nucleotides that are necessary for formation of the SREBP-1a-DNA complex, additional EMSAs were performed. These studies utilized a fragment (nt −164 to −137) of the SCD2 promoter that overlapped the footprinted sequence and contained either wild-type or mutant sequences (Fig. 7C). The results indicate that both the wild-type and mutant B (mutations at −155 to −157) oligonucleotides formed a single shifted complex with recombinant SREBP-1a (Fig. 7C). In contrast, no shifted complex was formed with the mutant A oligonucleotide (Fig. 7C). These results demonstrate that SREBP-1a binds to nucleotides that lie between −151 and −141 (5′-AGCAGATTTG-3′) and that mutation of the three adenosines prevents SREBP-1a-DNA interactions in vitro.

A Functional Role for the Novel SRE in the SCD2 Promoter—Fig. 8 shows the results obtained when HepG2 cells were transiently transfected with reporter constructs pSCD2-199, pSCD2-199mut A (mutations at −151, −148, and −146) or pSCD2-199mut B (mutations at −155 to −157).

The expression of pSCD2-199 was increased 3–6-fold when cells were either incubated in lipid-depleted medium or were co-transfected with ADD1 and then incubated with excess sterols to inhibit the proteolytic release of endogenous SREBPs (Fig. 8A). ADD1 activation of pSCD2-199 was dependent on the amount of pADD1 co-transfected into the cells (Fig. 8A). In contrast, the activity of pSCD2-199 was not increased following co-transfection of ADD1-R (Fig. 8A), a protein that binds to an E-box motif but is unable to bind to SRE1 (13).

The results of Fig. 8B demonstrate that all three reporter genes were expressed at similar levels in sterol-treated cells. Incubation of the cells in lipid-depleted medium resulted in a
include CACGAG and CACGTTG. However, it was not established whether these noncanonical motifs function to bind bHLH-containing proteins, that contain bHLH domains, with the E-box motif but are unable to bind to SREs and E-box motifs (2, 13). All other proteins that contain bHLH and bind to E-box motifs have an arginine at the corresponding position (position 13 of the basic domain) (28). Conversion of the tyrosine at position 320 of ADD1 to an arginine results in the production of a protein (ADD1-R) that binds to an E-box motif but is unable to bind to SRE1 (13). Thus, the activation of a reporter gene by co-expressed ADD1 or ADD1-R can be used to determine, indirectly, whether the promoter contains a functional SRE and/or E-box motif.

The studies of Fig. 8 demonstrate that co-expression of ADD1, but not of ADD1-R, results in transcriptional activation of pSCD2-199. Thus, we conclude that the sequence between −151 and −141 of the SCD2 promoter contains a novel SRE but that it does not contain a functional E-box.

As indicated above, SREBP1c/ADD1 often bind to two direct repeats (CTCAC) that are separated by one nucleotide (1, 7). However, both the current and previous studies (5–7) indicate that other sequences that do not conform to this consensus sequence function as SREs in the promoters of various genes. A difference in the affinity of SREBP1c/ADD1, SREBP1c/ADD1-R, or SREBP2 for the various SRE motifs that have been identified may provide an additional level of transcriptional regulation that results in activation of distinct genes/pathways.

The observation that overexpression of SREBP1c or SREBP2 resulted in a weak, but definite, stimulation of an responsive promoter, that functions as an SRE. The nucleotide sequence (5‘-AGCAGATTGTG-3‘; −151 to −141) is distinct from previously described SREs. Specifically, this sequence does not contain direct repeats of C/TCAC that are separated by one nucleotide (1, 7). However, both nonconsensus E-box motifs are located between −152 and −143 of the SCD2 promoter, viz. 5’-CAGCA-3’ and 5’-CAGATG-3’. The nucleotides that vary from the classic E-box motif (CANNTG) are underlined. A number of bHLH-containing proteins, including Maf/c-Myc, Max, c-Myc, and USF have been shown to bind to both canonical and noncanonical E-box motifs in vitro (27). The noncanonical motifs, identified by EMSAs in this latter study, include CAGAG and CACGGTG. However, it was not established whether these noncanonical motifs function to bind bHLH-containing proteins in vivo and consequently activate transcription.
SCD2 promoter-reporter gene that terminated at −150 (pSCD2-150) (Fig. 5B) may indicate that additional upstream cis elements and transcription factors are necessary for full activation of the reporter gene. Under more physiological conditions, such as when cells are incubated in sterol-depleted medium, the nuclear levels of SREBs are insufficient to activate pSCD2-150, although they are sufficient to activate pSCD2-588, pSCD2-199 (Fig. 4) and pSYNSRE (data not shown).

mRNA levels of SCD1 are also induced during differentiation of preadipocytes to adipocytes (24, 30). Kaestner et al. reported that the proximal promoters of SCD2 and SCD1 contained 146 bp with 77% sequence identity (24). The SRE identified in the current study in the promoter of the SCD2 gene lies within this conserved sequence and shows 10/11 identity with nucleotides −423 to −413 in the SCD1 promoter (24). In addition, the mouse genes encoding SCD1 and SCD2 co-localize on chromosome 19, possibly as a result of gene duplication (31). Thus, the observation that hepatic SCD1 mRNA levels were induced in transgenic mice that overexpress SREBP-1a (32) is consistent with the direct transcriptional activation of the SCD1 gene by SREBP-1a. Further studies will be required to determine whether transcription of the mouse SCD1 gene, in response to increased levels of mature SREBPs, is dependent on the motif that has 10/11 identity with the novel SRE identified in the current study and/or on a more distal sequence (−495 to −486) (24) that shows 9/10 identity with SRE1.

More than 16 years ago Chin and Chang (33) demonstrated that there was a time-dependent increase in the enzymatic activity of stearoyl-CoA desaturase (presumably SCD1 and SCD2) when Chinese hamster ovary cells were incubated in media that contained lipid-depleted serum. They noted that the increase in stearoyl-CoA desaturase activity was prevented when the medium was supplemented with medium that contained cholesterol (33). Subsequently, Chang and co-workers (17) isolated a mutant Chinese hamster ovary cell that failed to induce cholesterol biosynthetic enzymes and the LDL receptor in response to cellular sterol depletion and was auxotrophic for both cholesterol and unsaturated fatty acids. Interestingly, revertants regained the capacity to synthesize cholesterol and unsaturated fatty acids concomitantly (17). Recently, Rawson et al. (34) used these mutant cells and complementation cloning to identify a putative metalloprotease that cleaves SREBP at site-2. The current demonstration that SCD2 is transcriptionally regulated by nuclear SREBP/ADD1 provides an explanation for the coordinate regulation of cholesterol synthesis and fatty acid desaturation that was noted in these earlier studies.

In summary, we have identified stearoyl-CoA desaturase 2 as a new member of the family of SREBP/ADD1-responsive genes. To date, these include genes involved in cholesterol biosynthesis and uptake, fatty acid synthesis, triglyceride synthesis, and now fatty acid desaturation. Sequence analysis of the SCD2 proximal promoter alone would not have predicted that it contained an SRE. The studies reported here demonstrate that the mRNA differential display technique may prove useful in identifying novel genes that are regulated by sterols and are important in the development of human disease.

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