IDH1 gene transcription is sterol regulated and activated by SREBP-1a and SREBP-2 in human hepatoma HepG2 cells: evidence that IDH1 may regulate lipogenesis in hepatic cells

Ishaiahu Shechter,[1] Peihua Dai, Liang Huo, and Guimin Guan

Department of Surgery, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799

Abstract **The mRNA level for cytosolic NADP-dependent isocitrate dehydrogenase (IDH1) increases 2.3-fold, and enzyme activity of NADP-isocitrate dehydrogenase (IDH) 63%, in sterol-deprived HepG2 cells. The mRNA levels of the NADP- and NAD-dependent mitochondrial enzymes show limited or lack of regulation under the same conditions. Nucleotide sequences that are required, and sufficient, for the sterol regulation of transcription are located within a 67 bp region of an IDH1-secreted alkaline phosphatase promoter-reporter gene. The IDH1 promoter is fully activated by the expression of SREBP-1a in the cells and, to a lesser degree, by that of SREBP-2. A 5′-end truncation of 23 bp containing a CAAT and a GC-Box results in 6.5% residual activity. The promoter region involved in the activation by the sterol regulatory element binding proteins (SREBPs) is located at nucleotides −44 to −25. Mutagenesis analysis identified within this region the IDH1-SRE sequence element GTGGGCTGAG, which binds the SREBPs. Similar to the promoter activation, electrophoretic mobility shifts of probes containing the IDH1-SRE element exhibit preferential binding to SREBP-1a, as compared with SREBP-2. These results indicate that IDH1 activity is coordinately regulated with the cholesterol and fatty acid biosynthetic pathways and suggest that it is the source for the cytosolic NADPH required by these pathways.**—Shechter, I., P. Dai, L. Huo, and G. Guan. **IDH1 gene transcription is sterol regulated and activated by SREBP-1a and SREBP-2 in human hepatoma HepG2 cells: evidence that IDH1 may regulate lipogenesis in hepatic cells.** ***J. Lipid Res.*** **2003.** 44: **2169–2180.**

Supplementary key words cytosolic NADP-dependent IDH • sterol regulatory element binding protein • fatty acid synthesis • transcriptional regulation • cholesterogenesis

Eukaryotic cells express three different forms of isocitrate dehydrogenase (IDH). These enzymes catalyze the oxidative decarboxylation of isocitrate into α-ketoglutarate utilizing either NAD or NADP as cosubstrates (1, 2). The role of the mitochondrial NAD-dependent IDH (IDH3) is well known, as it is the IDH that catalyzes a step in the tricarboxylic acid cycle (3). In contrast, the biochemical role of the two NADP-dependent IDHs (E.C. 1.1.1.42) is not entirely clear. These latter forms of the enzyme are encoded in the nucleus and function as homodimers (4, 5). One NADP-IDH is localized in the mitochondria (IDH2), but gene disruption studies in yeast have shown that it cannot replace the function of the IDH3 (6). The human cytoplasmic NADP-specific enzyme (IDH1) is encoded by the IDH1 gene, and its mRNA sequence was reported (7). The subcellular localization of the enzyme's protein was shown to be in both peroxisomes and the cytoplasm (8). Interestingly, the rat enzyme was shown to localize exclusively in peroxisomes (9). The subcellular localization of IDH1 in peroxisomes is consistent with the presence of the peroxisomal targeting PST-1 sequence (Ala-Lys-Leu-COOH).

An additional physiological role for peroxisomal IDH1 may be to provide cytosolic NADPH for several NADPH-dependent enzymes, such as 3-hydroxy-3-methylglutaryl-CoA reductase (10–13), acyl-CoA reductase (14), and 2,4-dienoyl-CoA reductase (15, 16). The activity of IDH1 is the only known source for peroxisomal NADPH.

Another physiological role of peroxisomal IDH1 is to provide α-ketoglutarate, which is required as a cosubstrate for the phytanoyl-CoA α-hydroxylase reaction (17–20).

Manuscript received 27 June 2003 and in revised form 8 August 2003.

Published, JLR Papers in Press, August 16, 2003.
DOI 10.1194/jlr.M300285-JLR200

Abbreviations: HSS, human squalene synthase; IDH, isocitrate dehydrogenase; IDH1, cytosolic NADP-dependent IDH; IDH2, mitochondrial NADP-dependent IDH; IDH3, mitochondrial NAD-dependent IDH; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; SCAP, SREBP cleavage-activating protein; SEAP, secreted alkaline phosphatase; SQS, squalene synthase; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein.

[1] To whom correspondence should be addressed.
e-mail: ishechter@usuhs.mil

This enzyme catalyzes a step in the degradation of phytanoic acid, a by-product of the plant-derived phytol. Several metabolic functions for phytanic acid have been reported: regulation of glucose metabolism in primary rat hepatocytes by acting as an agonist to peroxisome proliferator-activated receptors (PPARs) (21, 22) and retinoid X receptor (RXR) (23); white adipocyte differentiation (24); and activation of uncoupling protein-1 gene transcription and brown adipocyte differentiation (25). In the autosomal disorder Refsum's disease, α-oxidation of phytanic acid is affected in some, but not all, cases, and accumulation of this acid is observed in various tissues (26).

The likely function of the cytoplasmic IDH1 is to produce NADPH for reductive reactions (27). Although the hexose monophosphate shunt is considered to be the major source of NADPH in the cytoplasm, genetic defects in glucose-6-phosphate dehydrogenase (G6PD), which catalyzes the first step in the shunt, do not affect sterol or fatty acid metabolism in humans (28). Population studies provide no evidence that any disorders other than hemolytic anemia are associated with G6PD deficiency (29), and both Chinese hamster ovary cells and human fibroblasts with less than 5 percent of normal G6PD activity grow normally (30, 31). This result suggests that the contribution of IDH1 to NADPH production may be significant. There are growing indications that IDH1 participates in cytosolic NADPH production and in fatty acid biosynthesis (32, 33). In support of this idea, IDH1 was also identified as a major source of cytosolic NADPH needed for the regeneration of reduced glutathione, critically important in cellular defense against oxidative damage (32, 34). Finally, an interesting and unexpected role for IDH1 was shown in bovine eyes, where the enzyme protein fulfills the criteria for a corneal epithelial crystallin, which may be involved in maintaining corneal epithelial transparency (35). Because the activity of IDH1 serves as a major source for nonmitochondrial NADPH required in multiple metabolic pathways, it is important to understand how IDH1 activity is regulated. The regulation in the peroxisomes and cytoplasm is not known. The 5′ promoter sequence for the gene encoding IDH1 is also not known, and information on the transcriptional regulation of this gene has not been reported.

Cholesterol and fatty acid synthesis is regulated mainly by coordinated transcription involving sterol regulatory elements (SREs), which are the targets of three basic-helix-loop-helix leucine zipper transcription factors called sterol regulatory element binding proteins (SREBPs) (36). These proteins are synthesized as membrane-bound endoplasmic reticulum (ER) precursors (M-SREBPs) with two membrane-spanning domains (37). After synthesis, the SREBPs are bound to an SREBP cleavage-activating protein (SCAP). The NH_2-terminal region of SCAP contains eight membrane-spanning helices that serve as the sterol-sensing domain; the COOH-terminal region contains several copies of a WD40 sequence that binds to the SREBPs (37–41). At low membrane cholesterol levels, SCAP escorts the SREBPs to the Golgi compartment (42), where the SREBPs are processed by site-1 and site-2 proteases. Proteolytic cleavage releases the C-terminal SREBPs and the soluble transcriptionally active N-terminal SREBPs (N-SREBPs) into the cytosol. The latter can then enter the nucleus and activate sterol and fatty acid synthesis. The sterol-regulated movement of the SCAP-SREBP complex from the ER to the Golgi is a pivotal event that controls cholesterol homeostasis in eukaryotic cells (37, 43–45). Recently, two additional ER-bound proteins, INSIG-1 (46) and INSIG-2 (47), were shown to be involved in the regulated transport of the SCAP-SREBP complex to the Golgi. These two proteins have 59% identity with five putative transmembrane domains. In the presence of high membrane cholesterol content, the INSIG proteins bind to the sterol-sensing domain of SCAP, causing its retention in the ER and preventing SREBP-SCAP complex transport to the Golgi. At low cholesterol levels, the INSIGs dissociate from the SCAP, allowing the migration of the SREBP-SCAP complex to the ER budding region for transport to the Golgi (46–49). We have recently demonstrated that the ER-to-Golgi transport of the SREBP-SCAP complex is inhibited at 20°C and that this inhibition in transport can be overcome by the overexpression of SCAP (50).

The N-SREBPs display differential activation of gene transcription. It was first reported that various *cis* elements in the human hepatic squalene synthase (HSS) promoter differentially bind N-SREBP-1a and N-SREBP-2. Based on that, distinct functional specificity for the two transcription factors was predicted (51, 52). A subsequent study by Goldstein and colleagues (53) established the differential stimulation of cholesterol and unsaturated fatty acid biosynthesis in Chinese hamster ovary cells expressing the individual SREBPs. There again, the mRNA for squalene synthase (SQS) was differentially expressed by SREBP-2 and not by the two isoforms of SREBP-1. These observations, together with results from studies in genetically manipulated mice (54), indicate that SREBP-1 is selectively involved in the activation of genes associated with fatty acid metabolism while SREBP-2 is more specific in cholesterol homeostasis, primarily through its selective activation of the SQS gene. The number of genes found to be activated by the SREBPs is constantly increasing, and includes enzymes involved in cellular cholesterol homeostasis and fatty acid synthesis (55–57). Our study describes the promoter for the IDH1 gene, its transcriptional regulation, and the relationship between this regulation and the regulation of fatty acid and sterol biosynthesis.

EXPERIMENTAL PROCEDURES

Cell cultures and transient transfections

Human hepatoma HepG2 cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 1 mM glutamine, 1 mM pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37°C under 5% CO_2 atmosphere. Transient transfections were conducted in 12-well plates. Used for each transfection were 0.5 μg of a given IDH1-SEAP reporter plasmid and 0.1 μg of pCMV-β-galactosidase (as a control for transfection efficiency) plus 25 ng of one of the plasmids pCDNA3.1 (as pCMV vector control), pCMV-CSA10 (con-

stitutively expressing the nuclear form of human SREBP-1a), or pCMV-CS2 (constitutively expressing the nuclear form of human SREBP-2). The transfections were performed using Fugene6 Reagent (Roche). At the time of treatment (24 h after transfection), the culture media were changed to sterol (+) or sterol (−) as defined below. After an additional 24 h incubation, the culture medium from each plate was collected and assayed for secreted alkaline phosphatase (SEAP) activity by luminometry using the Great EscAPe SEAP Reporter System kit from Clontech. The cells were then lysed, and the whole-cell extracts were assayed for β-galactosidase activity as previously described (52). Relative SEAP activity is expressed as the ratio of SEAP activity (light unit) to β-galactosidase activity. Sterol (+) and sterol (−) conditions were achieved by addition of either 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol or 5 μg/ml lovastatin, respectively, to MEM supplemented with 10% lipid-depleted serum (LDS).

Preparation of IDH1 promoter-reporter constructs

The 5′ flanking region of the *IDH1* gene was amplified by PCR from HepG2 genomic DNA using a pair of primers set for the desired region, based on sequence information of the *IDH1* gene in human chromosome 2 (GenBank accession number AC016697). The sequences of the two primers used are: 5′-GTGGTACCTCCACCGTTTTCTAAGGCTTCACATC-3′(forward) and 5′-CTCAAGCTTGATGATATGCTGGCGAAGAGTTGGGG-3′(reverse). The amplified DNA fragment is in the region −962 to +170 relative to the *IDH1* gene transcription initiation site (see below). The DNA was then ligated into the *Kpn*I (5′) and *Hind*III (3′) sites of the vector pTAL-SEAP (Clontech) utilizing the two linker sequences built into the primers. This resulting construct is designated pIDH1-962-SEAP and was used as the longest SEAP promoter-reporter for the human *IDH1* gene. For 5′ truncation of the promoter, a PCR-based, site-directed mutagenesis procedure was employed. Briefly, the plasmid pIDH1-962-SEAP was used as the template. A series of oligonucleotides that correspond to the desired 5′ end of the *IDH1* promoter were used as forward primers, all containing a *Kpn*I site. The reverse primer was an oligonucleotide located 3′ to the cloning *Kpn*I site. The PCR reactions were performed using the Expand Long Template PCR System (Roche). The resulting PCR products were digested with *Kpn*I and self-ligated to produce the SEAP-reporter plasmids of *IDH1* with various 5′ promoter ends. The sequences of the forward primers used to generate the different promoter-reporters and the designations of the plasmids are: pIDH1-481-SEAP, 5′-GCTAGGTACCGCATTAGGCAGCGCGGAACCCCCTAG-3′; pIDH1-219-SEAP, 5′-GCTAGGTACCTTCGCTGTCGGGATTCGGGACTGAATC-3′; pIDH1-91-SEAP, 5′-GCTAGGTACCATCCCACGGGAATTGGCGTGTGGCG-3′; pIDH1-67-SEAP, 5′-GCTAGGTACCGGCGATTGGAGGCGTGTCGGGGGCG-3′; pIDH1-44-SEAP, 5′-GCTAGGTACCGGGGCTGGGGGAGGTGGGCTGAGGA-3′; pIDH1-25-SEAP, 5′-GCTAGGTACCTGAGGAGGCGGGGCCTGGGAGGGG-3′; and pIDH1-6-SEAP, 5′-GCTAGGTACCGAGGGGACAAAGCCGGGAAGAGGAAA-3′.

Site-directed mutagenesis of IDH1 promoter

Replacement mutation of the IDH1-SRE-1 element in the human IDH1 promoter was obtained using the QuikChange™ Site-Directed Mutagenesis Kit (Stratagene). The primers used in this procedure were the pair of primers used in the electrophoretic mobility shift assay to produce the IDH1−40/−5 m5 probe (see Fig. 7B), in which the sequence TGAT of the proposed IDH1-SRE element was replaced by the sequence GTTT. This mutation procedure produced the IDH1-67m5-SEAP mutant promoter-reporter construct.

Determination of transcription initiation sites

To determine the transcription initiation site(s) of the *IDH1* gene, a 5′-rapid amplification of cDNA ends (RACE) was performed (58) using the 5′-Race System kit from Invitrogen. The gene-specific primers GSP1 (5′-TCCTCAACCCTCTTCTCATC-3′) and GSP2 (5′-TTCGTCTCATTTCATCTCCT-3′) used are located at +42 and +234 3′ to the AUG translation initiator site. The various RACE clones isolated were sequenced, and the most 5′ end sequences were determined.

Preparation of poly(A$^+$) RNA and Northern blot analysis

HepG2 cells (1×10^6) were plated onto 150 mm plates and grown for 2 days at 37°C then treated as described above. Two sets of plates were treated. One set was kept at 37°C, and the other was transferred and kept at 20°C. At the end of the 24 h treatment period, the cells were harvested and total RNA was prepared using Tryzol Reagent (Invitrogen). Poly(A$^+$) RNA was extracted from total RNA using the FastTrack® 2.0 Kit (Invitrogen). For Northern blots, 4 μg poly(A$^+$) RNA from each sample was separated on 1.2% agarose gel, transferred onto nylon membranes, and probed with different ^{32}P-radiolabeled cDNA probes. IDH1 and IDH2 cDNA probes were generated by RT-PCR based on known mRNA sequences, and the probes for IDH3-β and IDH3-γ were excised from clones containing corresponding cDNA sequences obtained from American Type Culture Collection. Quantitation of specific signal intensities was performed by densitometry using ImageQuant software (Molecular Dynamics).

Assay for NADP-dependent IDH activity

HepG2 cells were cultured and treated with sterol (+) and sterol (−) medium as described above. Whole-cell lysates were prepared by sonication of cell suspensions in a buffer containing 50 mM Tris-HCl (pH 7.9), and 1 mM EDTA, followed by centrifugation to obtain clear extracts. NADP-dependent IDH enzymatic activity assays were performed at 25°C using the Farrell procedure (59). Enzyme activity is expressed in μmol increase of NADPH per minute.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was carried out as described previously (60). Briefly, annealed oligo probes were end-labeled with γ-^{32}P-ATP using T4 polynucleotide kinase. For the binding reaction, 8×10^4 dpm of each probe was incubated with ∼50 ng of purified SREBP protein (60) in a buffer containing 25 mM Tris-HCl (pH 8.0), 50 mM KCl, 6.25 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 μg/ml of polydeoxyinosinic-deoxycytidylic acid, and 10% glycerol for 30 min on ice. The mixtures were then separated by electrophoresis on a nondenaturing 30% polyacrylamide gel. Detection of radiolabeled signals was done by autoradiography. The probes used in the EMSA were all synthetic oligonucleotides; sequences are described in each experiment (below). For positive control, an oligonucleotide probe containing the HSS-SRE-1 sequence in the human SQS promoter was used [5′-TAGAGTGTTATCACGCCAGTCTCCTT-3′ (60)].

RESULTS

Sterol-mediated regulation of IDH1 mRNA level

The possible regulatory role of sterols in IDH1 expression was investigated by the addition of sterols to HepG2 cells, and analysis of IDH1 mRNA level. At 37°C, a 2.3-fold induction of the IDH1 mRNA level was observed after 24 h in sterol-depleted cells, as compared with sterol-loaded

cells (**Fig. 1**). Under the same conditions, the sterol regulation of the NADP-dependent mitochondrial IDH2 was only 1.3-fold, and no sterol regulation was observed for the mRNA levels of either the β- or the γ-subunits of the NAD-dependent mitochondrial IDH3. We have recently demonstrated that sterol regulation of enzymes in the cholesterol and fatty acid biosynthetic pathways is diminished or completely absent in cells maintained at 20°C, due to lack of processing of the SREBPs at this temperature (50). It appears that a similar low-temperature effect is observed for the regulation of IDH1. The sterol-regulated increase in IDH1 mRNA level is inhibited in sterol-deprived cells maintained at 20°C, and the mRNA levels are lower than those observed in cells at 37°C, regardless of the presence of sterols. The limited sterol regulation of IDH2 is also absent at 20°C, but the level of mRNA for this enzyme is still rather substantial. No temperature effect or sterol regulation was observed for the mRNA of either the β- or the γ-subunits of IDH3 (Fig. 1). These observations, together with our earlier studies (50), indicate a coordinated regulation between cholesterol and fatty acid synthesis and the expression of the IDH1 gene. They also point to SREBPs as the likely common regulatory transcription factors for all of these genes. Lack of, or limited, sterol regulation of the mRNA for the other IDH genes may suggest that IDH1 is the major source for NADPH for sterol and fatty acid biosynthesis.

Sterol regulation of NADP-dependent IDH enzyme activity

The 24 h sterol effect on the NADP-dependent IDH activity in HepG2 extracts is shown in **Fig. 2**. Sterol deprivation of cells for this period of time results in a 63% increase in NADP-dependent IDH activity compared with the activity in cells grown in the presence of sterols. Because this enzyme assay cannot distinguish between the activities of IDH1 and IDH2, it is not possible to assess the contribution of each to the observed activity and the participation of either in the sterol regulation. Nevertheless, a significant sterol effect on the NADP-dependent activity in total cell extract is observed.

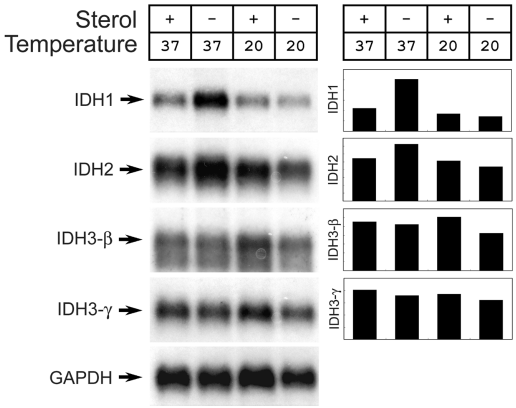

Fig. 1. Sterol regulation of IDH1 mRNA level in HepG2 cells. HepG2 cells were treated with 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol [sterol(+)], or 5 μg/ml lovastatin (sterol(−)] in Eagle's minimum essential medium (MEM) supplemented with 10% lipid-depleted serum (LDS). The cells were incubated at either 37°C or 20°C for 24 h. Poly(A$^+$) RNA was then prepared from the cells under the various conditions. Quantitation of specific mRNAs was done by Northern blot analyses (left section) using as probes the ^{32}P-radiolabled human cDNAs indicated at left. Quantitation of the specific signals was performed by densitometry, using ImageQuant software (Molecular Dynamics). The bar graphs (at right) represent the relative amounts of the mRNA for each target gene normalized to the human GAPDH mRNA level.

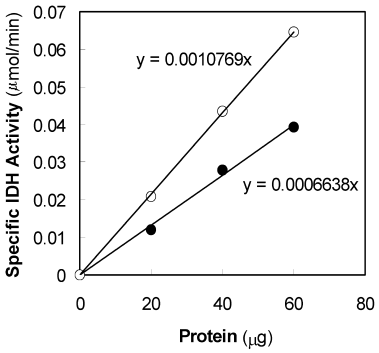

Fig. 2. Sterol regulation of NADP-dependent isocitrate dehydrogenase (IDH1) enzymatic activity in HepG2 cells. HepG2 cells were treated with 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol (closed circle), or 5 μg/ml lovastatin (open circle) in MEM medium supplemented with 10% LDS. The cells were then incubated at 37°C for 24 h. IDH1 activity was measured in whole-cell lysate as described in Experimental Procedures. The enzyme activity is expressed as μmol NADPH formed per minute at pH 7.4.

Analysis of gene structure and the cloning of the promoter for IDH1

Blast analysis of GenBank was performed using different sequences of the cDNA structure for IDH1 (accession number NM005896). All sequences of the cDNA were identified in a 172,304 bp BAC clone, RP11-140C4 (accession number AC016697). The intron-exon organization for the IDH1 gene was determined by comparing the cDNA sequence with that of the BAC clone, applying the GT-AG rule for intron splicing (61). **Figure 3** depicts the intron-exon organization and the codons at the various splice sites. The first two 5′ exons (black boxes) are located at the 5′ untranslated region (see transcription start site below). The largest exon is located at the very 3′ end of the cDNA (941 bp) and contains the polyA tail (Fig. 3A).

Five distinct transcription start sites were determined by RACE analysis at 122, 157, 167, 180, and 211 bp 5′ to the ATG codon (Fig. 3B). The major site, at 167 bp 5′ to the ATG codon, was also identified by primer extension analysis, as well as a minor site at 122 bp (data not shown). The sequences of all the RACE-generated clones fully matched sequences in the genomic RP11-140C4 BAC clone, indicating genuine transcription sequences. In separate experiments, some clones at the size indicated below were not found. However, in a single experiment in which 11 clones were sequenced, four different isolated RACE clones with 5′ untranslated sequences of 122, 157, 180, and 211 bp were identified, whereas seven identical clones

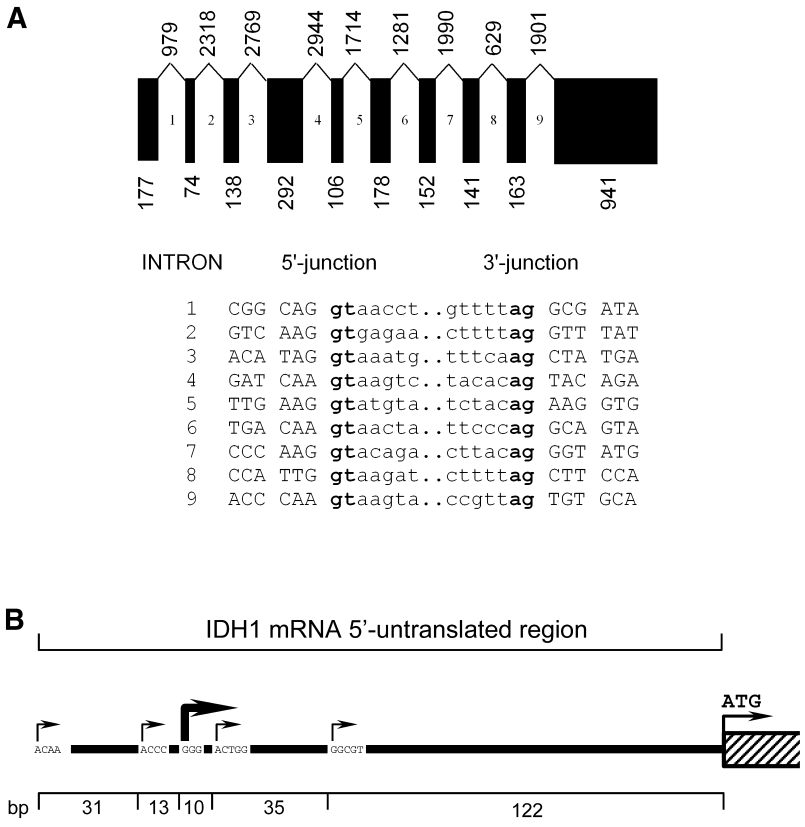

Fig. 3. The intron-exon organization for the *IDH1* gene and transcription initiation sites. The intron-exon organization for the IDH1 gene was determined by comparing the IDH1 cDNA sequence with that of the *IDH1* BAC clone RP11-140C4 (accession number AC016697) applying the GT-AG rule for intron splicing. The intron-exon organization and the codons at the various splice sites are shown (A). The first two 5′ exons (black boxes) are located at the 5′ untranslated region (see 5′-UTR below). The largest exon is located at the very 3′ end of the cDNA (941 bp) and contains the polyA tail. Nucleotide sequences of five 5′-UTRs of the human IDH1 mRNA are shown (B). The five distinct transcription start sites were determined by rapid amplification of cDNA ends (RACE) analysis at 122, 157, 167, 180, and 211 bp 5′ to the ATG codon and are indicated by arrows. The first few nucleotides for each initiation site are also shown. In a single representative experiment, in which 11 clones were sequenced, four different isolated RACE clones with 5′ untranslated sequences of 122, 157, 180, and 211 bp were identified, whereas seven identical clones with a 5′ untranslated sequence of 167 bp (bold arrow) were found. Distances between the initiation sites as well as the size of the shortest 5′-UTR are labeled as base pair at the bottom of the scheme.

with a 5′ untranslated sequence of 167 bp were found. Thus, it appears that clones with a 5′ untranslated sequence of 167 bp are at approximately ×7 frequency. The most 5′ adenosine of the longest clone (211 bp 5′ to the ATG) was designated 1. Based on the above, the sequence of −962 to +170 was amplified for the construction of the pIDH1-962-SEAP promoter-reporter.

Sterol regulation of pIDH1-962-SEAP promoter-reporter activity

The IDH1 promoter activity is enhanced 2-fold in cells grown in media containing 10% LDS, as compared with the activity in cells grown in the presence of 10% FBS (**Fig. 4**). The promoter activity in cells grown in the presence of FBS is suppressed by the addition of sterols to the media. Addition of sterols completely diminishes the activation by LDS, and the IDH1 promoter activity is suppressed regardless of the type of serum in the media. As expected for a sterol-mediated regulation, addition of lovastatin to the growth media greatly enhanced the IDH1 promoter activity. A 5.4-fold enhancement in activity is observed in lipid-deprived cells in which the promoter is fully activated (LDS + lovastatin), compared with suppressed conditions (LDS + sterols). Thus, the regulation of the IDH1 promoter activity is sterol mediated, similar to the observed variations in IDH1 mRNA levels seen in Fig. 1.

Regulation of pIDH1-962-SEAP promoter-reporter activity by SREBP

Because the SREBP transcription factors are involved in sterol-mediated regulation of genes involved in cholesterol and fatty acid biosynthesis, it is anticipated that activation of the sterol-responsive IDH1 promoter is similarly regulated by these transcription factors. Indeed, the activity of the pIDH1-962-SEAP promoter increases in re-

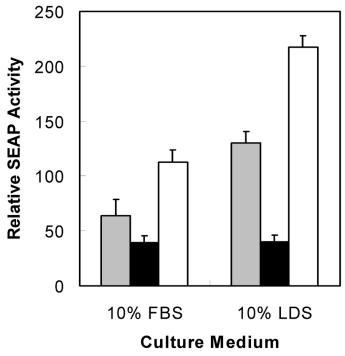

Fig. 4. Sterol regulation of human IDH1 gene promoter activity. HepG2 cells were transiently transfected with pIDH1-962-SEAP and a β-galactosidase expression vector. Two main experimental groups were formed, one of cells maintained in 10% FBS and the other of cells maintained in 10% LDS. The media in one-third of the cultures of each experimental group were supplemented with either 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol (black bars), 5 μg/ml lovastatin (white bars), or no additional treatment (gray bars), and the cells were further incubated for 24 h. The culture media were assayed for secreted alkaline phosphatase (SEAP) activity, and the whole-cell extracts were assayed for β-galactosidase activity. The relative SEAP activity is expressed as the ratio of SEAP to β-galactosidase activities (n = 6). Error bars indicate SD.

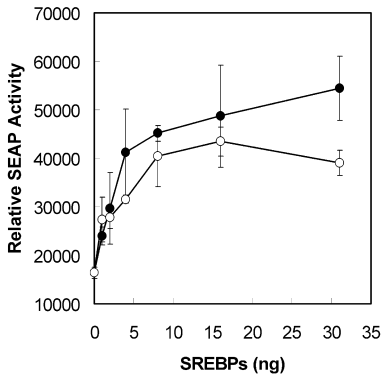

Fig. 5. Activation of the IDH1 promoter by SREBP-1a and SREBP-2. HepG2 cells (10^5) were transiently cotransfected with 0.5 μg of pIDH1-962-SEAP, 0.1 μg of β-galactosidase expression vector, and either of two plasmids, pCMV-CSA10 and pCMV-CS2, which express the nuclear form of human SREBP-1a (closed circles) or the human SREBP-2 (open circles), respectively. The amount of transfected DNA encoding the sterol regulatory element binding proteins (SREBPs) varied between 2 to 31 ng per transfection, as indicated. After 24 h, the growth media were replaced with media supplemented with 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol and the cells were incubated for an additional 24 h. The media were then collected for SEAP assay and the whole-cell lysates were assayed for β-galactosidase activity. Relative SEAP activity is expressed as the ratio of SEAP to β-galactosidase activities (n = 3). Error bars indicate SD.

sponse to increasing amounts of pCMV-CSA10 (SREBP-1a) and pCMV-CS2 (SREBP-2) DNA in transiently transfected HepG2 cells (**Fig. 5**). The dose-response activation curves for SREBP-1a and SREBP-2 are similar, but activation by SREBP-2 is consistently lower. Almost identical activation of the IDH1 promoter was observed by transfection of pCMV-CSA10 and pCMV-CS1c, which encodes expression of SREBP-1c, indicating a similar response of the reporter gene to the two SREBP-1 expression vectors (data not shown).

Sterol regulation and activation by SREBPs of fusion genes containing successively 5′-truncated IDH1 promoter

A series of successively 5′-truncated IDH1 promoter-SEAP reporter genes was prepared by PCR methodology (**Fig. 6A**). Significant sterol-dependent regulation of promoter activity is observed in fusion genes containing promoter sequences of 67 bp or longer (Fig. 6B). In fact, IDH1-67-SEAP shows the most response (7.9-fold) to sterol regulation compared with longer promoters. The promoter-reporter genes longer than 67 bp also show a significant response to an expressed SREBP-1a or SREBP-2. The activation of each of the promoters by SREBP-1a is consistently higher than the activation by SREBP-2. For IDH1-67-SEAP, the activation by SREBP-1a is >2-fold higher than the activation by SREBP-2. Additional 23 bp 5′ truncation (IDH1-44-SEAP) resulted in a dramatic loss of promoter activity and response to sterols. However, residual response to the expressed SREBPs is still observed. Within this truncated 23 nucleotide (nt) sequence reside an inverted CCAAT box [−62]ATTGG-[−58] and a GC-Box sequence [−55]CGGGGGCGGGGCT-[−43] (Fig. 6A). Thus, the loss of promoter activity in this reporter gene may be due to a lack of sequences for the binding of NF1/NFY, or to an SP1 transcription factor, or both. A truncation of additional 19 nts resulted in an almost complete loss of promoter activity. This latter truncation (IDH1-25-SEAP) was so designed in order to cleave at an inverted SRE-1-like element, designated IDH1-SRE [−30]GTGGGCTGA-[−22] (Fig. 6A) suspected for the binding of and the activation by the SREBP transcription factors (see below for gel retardation studies).

Mutation analyses of the interaction of the SREBPs with promoter sequences of IDH1

For the analyses of the interaction of the SREBPs with the IDH1 promoter, different synthetic radiolabeled probes were prepared based on the promoter sequences. The binding of the SREBPs to these probes was assayed in electrophoretic mobility shift assays, and the results were compared with the binding of the SREBPs to a probe containing the SRE-1 element in the HSS promoter (HSS-SRE-1) (52). SREBP-1a and SREBP-2 similarly retard the HSS-SRE-1 probe, resulting in radioactive signals of approximately the same intensity (**Fig. 7A**). A significantly weaker binding of SREBP-1a to the probe IDH1−71/−21 is observed. This probe contains at its very 3′ end the IDH1-SRE-1 sequence. Relatively weak signals in the mobility shift assay indicate that the IDH1−71/−21 probe does not bind to the SREBP-2 protein in a significant way. The IDH1−49/+1 probe displays a much stronger binding to the SREBP-1a protein. This probe contains the IDH1-SRE-1 sequence element at its core, and its binding to SREBP-1a is comparable in signal intensity to that of

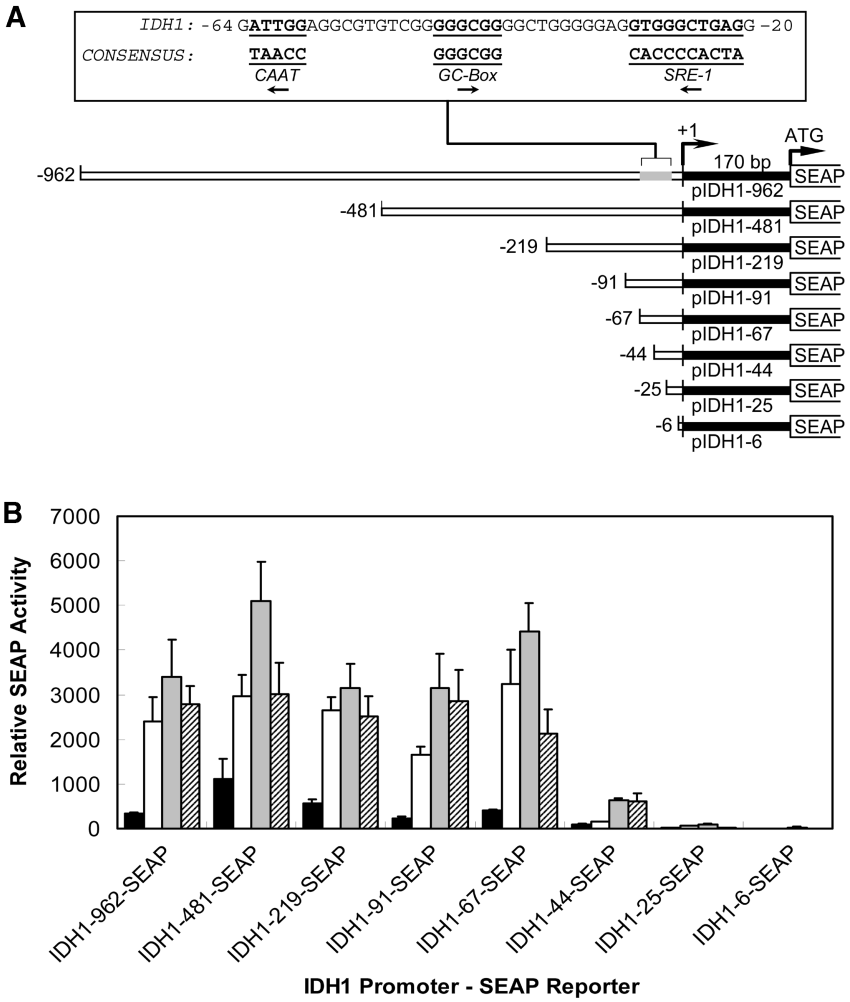

Fig. 6. Sterol regulation and activation by SREBPs of successively 5′-translated IDH1-SEAP promoter-reporter genes. Successively 5′-truncated promoter fragments of the human IDH1 gene fused to a SEAP reporter were generated by site-directed mutagenesis. A schematic representation of the various truncations is shown in A. The white bars represent different lengths of human IDH1 promoter, and the black bars represent the 5′-UTR of the human IDH1 mRNA. Promoter lengths, relative to transcription initiation site, are indicated at the left of each construct. The DNA sequence between nucleotides −64 and −20 is shown above, with three potential regulatory elements underlined and in bold. The names and consensus sequences of these three elements are given below each sequence, with arrows indicating their normal orientation. Promoter activities of the various constructs in transiently transfected HepG2 cells grown under different conditions are shown in B. The cells were transfected with 0.5 μg of the IDH1-SEAP reporters, 0.1 μg of β-galactosidase control plasmid for transfection efficiency, and 25 ng of either SREBP-1a or SREBP-2 expression vectors (pCMV-CSA10 or pCMV-CS2, respectively). The cells were treated with either 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol [sterol(+)] or with 5 μg/ml lovastatin [sterol(−)] in 10% LDS-containing MEM medium for 24 h, and the culture media were assayed for SEAP activity. The relative SEAP activity is expressed as the ratio of SEAP to β-galactosidase activity in the cell lysates (n = 3). Black bar, sterol(+); white bar, sterol(−); gray bar, sterol(+) plus pCMV-CSA10 (human n-SREBP-1a); hatched bar, sterol(+) plus pCMV-CS2 (human n-SREBP-2). Error bars indicate SD.

the HSS-SRE-1 probe. Only a weak retardation signal is observed for the IDH1−49/+1 probe with the SREBP-2 protein, indicating a weaker interaction with this protein compared with that with SREBP-1a (Fig. 7A).

On the basis of these results, the IDH1−40/−5 synthetic probe was designed for mutation analysis of the interaction of the SREBPs with sequence elements in the IDH1 promoter. This shorter probe also contains the IDH1-SRE sequence element at its core and displays retardation by the SREBPs similar to the retardation observed for the longer IDH1−49/+1 probe (Fig. 7B). A successive 4 nt scanning mutation analysis indicates that the eight 5′ bp (mutations m1 and m2) and the sixteen 3′ bp (mutations m6 to m9) of the probe are not essential for the retardation and are therefore not involved in the binding to the SREBPs. However, mutations m3, m4, and m5 of the IDH1−40/−5 probe completely abolish the mobility shift. These three mutations are within the sequence

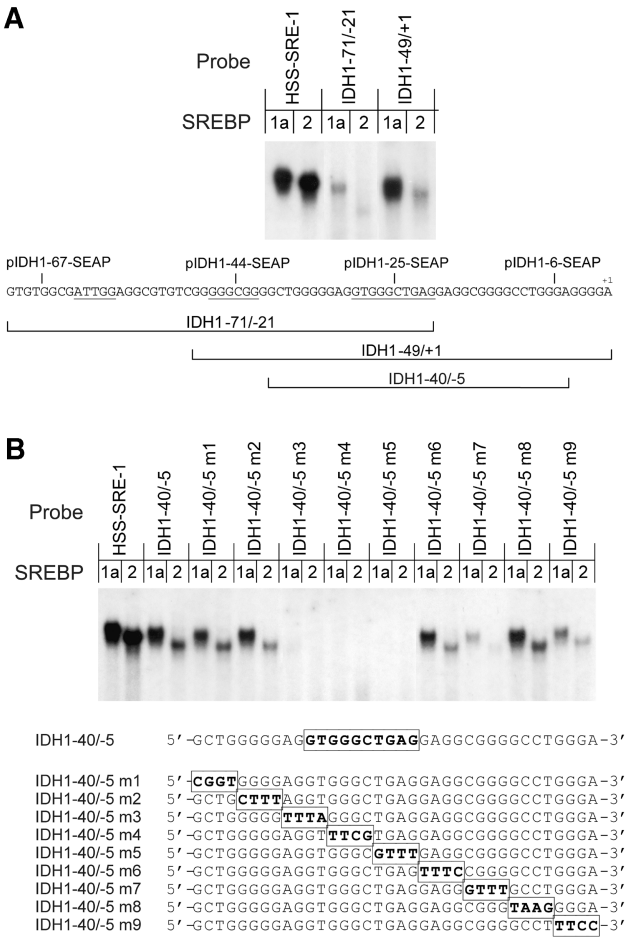

Fig. 7. Identification of a sequence element in the human IDH1 promoter that binds SREBPs. Various ^{32}P-end-labeled synthetic oligonucleotide probes (8×10^4 cpm) were used in the electrophoretic mobility shift assay. The various probes were incubated with 50 ng of the purified human nuclear form of either SREBP-1a or SREBP-2 proteins, and binding was assayed in electrophoresis mobility shift retardation assay as described in Experimental Procedures. Mobility shift of a probe with the HSS SRE-1 element (HSS-SRE-1), known to bind equally well to SREBP-1a and SREBP-2, is shown in the left two lanes of each gel. The name of each probe is given at the top of the images. A: Mobility shift of two long overlapping probes (IDH1-71/-21 and IDH1-49/+1) spanning the shortest sterol-regulated promoter sequence of IDH1 (IDH1-67-SEAP). The sequence, the region of the probes, and the three potential *cis*-regulatory elements (underlined) are depicted in the scheme. Retardation intensities indicate that sequences that bind to the SREBPs are located primarily at the 3′ end of the promoter and bind preferentially to SREBP-1a as compared with SREBP-2. The spanning region of IDH1−40/−5, which contains the IDH1-SRE element, is also indicated. B: Mobility shift assay of wild type and mutant variants of the IDH1−40/−5 probe. The succession of 4 bp replacement transversion mutations of the IDH1−40/−5 probe (designated m1 to m9) is indicated in the scheme below (bolded and boxed). The three mutant probes, m3 to m5, all located within the IDH1-SRE element, fail to bind SREBPs. All other mutant probes, as well as the wild-type IDH1−40/−5 probe, bind SREBP-1a preferentially.

GTGGGCTGAG (or the antisense sequence 5′-CTCAGCCCAC), designated the IDH1-SRE element. Interestingly, retardation signals of the IDH1−40/−5 and all its mutants outside of the IDH1-SRE element display high affinity to SREBP-1a and a much weaker signal for the binding to SREBP-2.

Functional analysis of the IDH1-SRE element

To verify the function of the IDH1-SRE element within the context of the IDH1 promoter, a replacement mutation was introduced into this sequence element in the IDH1-67-SEAP reporter. We have introduced the m5 mutation, because it was demonstrated to hinder binding of the SREBPs to the IDH1−40/−5 probe containing the IDH1-SRE element (Fig. 7B). The activity of the resulting mutant promoter-reporter, IDH1-67m5-SEAP, expressed in HepG2 cells, is very low and is only 4.6% that of the wild-type promoter in sterol-deprived cells (**Fig. 8**). As anticipated, the SREBPs also fail to activate the IDH1-67m5-SEAP promoter significantly. Only 5.5% and 6.6% of the activation of the IDH1-67-SEAP promoter by SREBP-1a and SREBP-2, respectively, are observed for the IDH1-67m5-SEAP promoter.

DISCUSSION

The results in this report suggest an involvement of IDH1 in lipogenesis and provide a possible mechanistic rationale for this involvement, using HepG2 cells as a model. The enzyme's specific role in supplying NADPH for the reduction reactions in the cholesterol and fatty acid biosynthetic pathways is suggested by the sterol-mediated regulation of the IDH1 gene transcription, which is

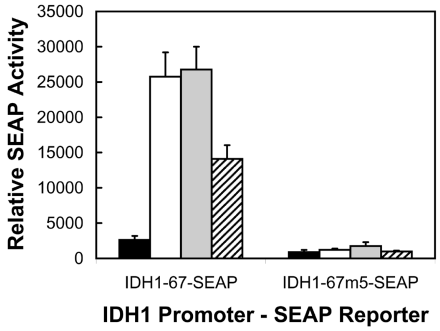

Fig. 8. Mutation of the IDH1-SRE sequence element decreases the human IDH1 promoter activity. A replacement mutation, in which an m5 sequence (see Fig. 7B) was introduced into the IDH1-SRE element in the IDH1-67-SEAP promoter-reporter, was obtained by site-directed mutagenesis. The activity of the resulting mutant promoter, IDH1-67m5-SEAP, is compared with that of the wild-type IDH1-67-SEAP. HepG2 cells were transfected with 0.5 μg of either IDH1-67-SEAP or IDH1-67m5-SEAP reporters, 0.1 μg of β-galactosidase expression plasmid, and 25 ng of either SREBP-1a or SREBP-2 expression vector as indicated. The cells were treated with 1 μg/ml 25-OH cholesterol plus 10 μg/ml cholesterol [sterol(+)] or 5 μg/ml lovastatin [sterol(−)] in 10% LDS-containing MEM medium for 24 h. Samples of the culture media were taken for SEAP assay following the treatment period. The relative SEAP activity is the ratio of SEAP activity to β-galactosidase activity in total cell lysates. Black bar, sterol(+); white bar, sterol(−); gray bar, sterol(+) plus pCMV-CSA10 (human n-SREBP-1a); hatched bar, sterol(+) plus pCMV-CS2 (human n-SREBP-2). Error bars indicate SD.

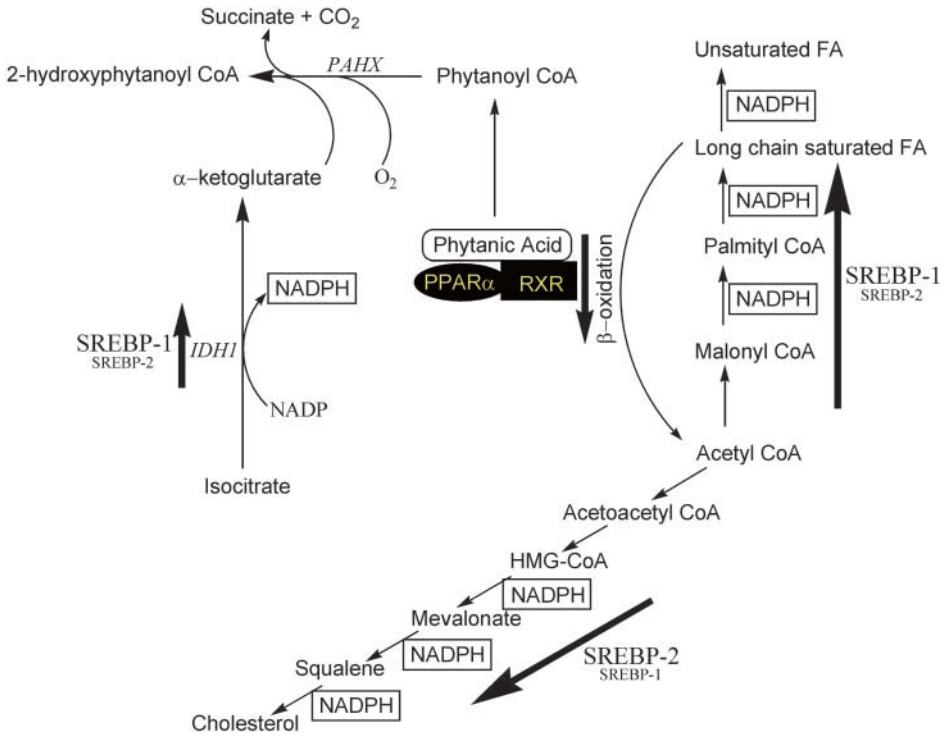

Fig. 9. Involvement of IDH1 in the regulation of cholesterol and fatty acid biosynthesis. The diagram shows the major metabolic intermediates in the biosynthesis of cholesterol and fatty acid. The activity of IDH1 is proposed as the major source of the NADPH required at the indicated parts of the pathway. SREBPs activate various genes in the pathway (bold arrows). In vivo, SREBP-2 preferentially activates genes in the cholesterol biosynthetic pathway, whereas, SREBP-1 (a and c) preferentially activates genes involved in fatty acid synthesis, as well as the IDH1 gene. α-Ketoglutarate, a product of the IDH1 reaction, is a cosubstrate in the phytanoyl-CoA α-hydroxylase reaction and is required for the catabolic removal of phytanic acid. Phytanic acid is an agonist for the activity of the functional heterodimer retinoid X receptor/peroxisome proliferator-activated receptor α (RXR/PPARα), which is involved in the catabolism of fatty acid through β-oxidation. Thus, activation of IDH1 by SREBPs will have a compounded effect on the production and maintenance of the pool of hepatic fatty acids, by first supplying NADPH and by then lowering the phytanic acid levels, which, in turn, will diminish the PPARα/RXR effect on the β-oxidative degradation process.

coordinated with the transcription of all other regulated genes in these pathways. This coordinated regulation is achieved through the SREBP transcription factors, which are long recognized to have a pivotal role in the lipid-mediated regulation of the lipogenic pathways (37, 43–45).

The biochemical source of the cytosolic NADPH required for the cholesterol and fatty acid biosynthetic reactions has not been firmly established. Recently, it was suggested that the reactions catalyzed by the three cytosolic enzymes malic enzyme, G6PD, and 6-phosphogluconate dehydrogenase supply the necessary NADPH (43). However, there is no evidence to suggest that the regulation of any of these enzymes is linked to the production of sterols and fatty acids. In fact, there are several reports suggesting that G6PD, or the reactions catalyzed by the hexose monophosphate shunt, may not be the source of the NADPH needed to drive lipogenesis in humans or in cultured cells (28–31). Because the exact cellular function of IDH1 has not been established, its potential in generating significant amounts of NADPH in the cytosol may be underestimated. The significant contribution of IDH1 in producing cytosolic NADPH was actually demonstrated in one early comparative study in which IDH1 in rat liver was shown to be 16- and 18-fold more active in producing NADPH than were G6PD and malic enzyme, respectively (62).

The regulation of IDH1 gene transcription by sterols is now demonstrated in HepG2 cells (Fig. 1). The magnitude of the regulation of the IDH1 mRNA level by sterols exceeds by far that of the mitochondrial dehydrogenase IDH2 mRNA, which displays limited response, or that of the β- and γ-subunits of IDH3 mRNA, which do not display any response to sterols. The regulatory response to sterols is also demonstrated in the modulation of enzyme activity (Fig. 2). Because this enzyme assay, performed with total-cell extracts, cannot distinguish between the activities of IDH1 and IDH2, the level of the sterol regulation of IDH1 cannot be determined precisely, because of a background contribution of activity by IDH2. However, based on the data shown in Fig. 1, the activity of IDH2 is not likely to be highly regulated and, thus, the sterol response could be attributed mostly to IDH1. Future production of IDH1-specific antibodies should resolve this issue. The 20°C suppression of IDH1 mRNA level and its

sterol regulation (Fig. 1) provided the first evidence that this regulation, similar to the sterol-mediated regulation of all other enzymes in the cholesterol biosynthetic pathway, is effected through the SREBPs (50). The function of SREBPs in the sterol regulation of the IDH1 gene transcription is demonstrated by the activation of the IDH1 promoter-reporter genes by the SREBPs (Figs. 4–6). In all of these experiments, consistently, the activation by SREBP-1a is significantly higher than that obtained by the expression of SREBP-2. For IDH1-67-SEAP, the shortest sterol-responsive reporter gene, the activation by SREBP-1a is more than 2-fold higher than that obtained with SREBP-2 (Fig. 6B).

On the basis of mutation analyses of electrophoretic mobility shift (Fig. 7B) and promoter activity (Fig. 8), the *cis*-regulatory element in the IDH1 promoter targeted by the SREBPs is most likely the IDH1-SRE sequence [−30]5′-GTGGGCTGAG[−21] (or the antisense sequence 5′-CTCAGCCCAC), which resembles (but in a reverse orientation to) the SRE-1 element in the LDL receptor gene (63). Unlike the HSS-SRE-1 sequence element in the SQS gene, this sequence element preferentially binds to SREBP-1a, as compared with a much weaker binding to SRRBP-2 (Fig. 7). This difference in binding may explain the differential activation of the IDH1 promoter by the two SREBPs (Figs. 4–6). The most pronounced difference in sequence between the consensus SRE-1 and the IDH1-SRE elements is an AT to CT change in the most 5′ end of the latter (Fig. 6A). Yet, this seemingly minute change in sequence uniquely results in a preferential binding of the IDH1-SRE sequence to SREBP-1a. In an earlier report, we have demonstrated that the sequence 5′-TCCACCCCAC, present in the HSS promoter [originally designated HSS-SRE(8/10)], exclusively binds to SREBP-2 and not to SREBP-1a (52). Here again, the most pronounced difference in the sequence is at the 5′ end, where a TC replaces the AT in the consensus SRE-1 element. Thus, it appears that the specificity in binding to (and presumably activation by) the various SREBPs resides, in part, in the two 5′ nucleotides of the SRE motif. This observation, which appears to be correct for the HSS, IDH1, and LDL receptor promoters, will have to be further examined by mobility shift assays of variant SRE motifs. If correct, specific response in transcription to the various SREBPs may be predicted by promoter sequence analysis. Whatever the explanation, the activation of the IDH1 promoter is effected in lipid-depleted cells mostly by SREBP-1a and SREBP-1c (data not shown for the latter).

The shortest IDH1 promoter-reporter gene that displays sterol regulation is IDH1-67-SEAP (Fig. 6). IDH1-44-SEAP, which is a 23 bp shorter promoter-reporter gene, still contains the intact IDH1-SRE element but lacks the CAAT and the GC-Box elements at its 5′ end. These elements are known to bind the NFY/NF1and SP1 transcription factors, respectively. The IDH1-44-SEAP promoter-reporter activity is very low, is no longer responsive to sterols, and in the presence of SREBPs, is only a fraction of the activity of the longer promoters. Thus, the earlier observation that activation of gene transcription by the SREBPs requires the coactivators NFY/NF1 or SP1 (51, 64–67) is also correct for the IDH1 gene, which further supports the idea that, functionally, this gene is a member of the SREBP-activated gene family.

The preferential activation of the IDH1 promoter by SREBP-1a compared with SREBP-2 may indicate that the regulation of IDH1 activity may be coordinated more with fatty acid biosynthesis than with sterol production (43). Thus, it is expected that activation of IDH1 is a response to a shortage in hepatic fatty acid supply. The subcellular localization of IDH1 in both cytosol and peroxisomes ideally situates it to respond to the lipogenic requirement of the cell. The coproduct of the reaction, α-ketoglutarate, which is required as a cosubstrate for the phytanoyl-CoA α-hydroxylase reaction (17–20), is required for the catabolic removal of phytanic acid, a known agonist to PPARα (21, 22). PPARα is highly expressed in heart, muscle, kidney, and endothelial cells, but mostly in liver (27, 34), where its function is in the catabolism of fatty acids through the regulation of genes encoding mitochondrial and peroxisomal enzymes important for β-oxidation (68). In addition, phytanic acid was also found to bind and activate the RXR (23), which forms a functional heterodimer with PPARα. Therefore, an increase in IDH1 activity will have a compounded effect on the production and maintenance of the hepatic fatty acid pool, by first supplying NADPH, which will enhance fatty acid biosynthesis, and by then lowering the phytanic acid levels, which, in turn, will diminish the PPARα/RXR effect on the β-oxidative degradation process (**Fig. 9**). Indeed, the expression of IDH1 in rats is primarily in liver, where the above processes are likely to occur (27, 34).

The available data, and the results presented herein, strongly suggest that the activity of IDH1 is required as a major source for cytosolic NADPH, which is specifically targeted as a substrate in the biosynthesis of cellular fatty acids and cholesterol. This activity also produces α-ketoglutarate, which indirectly regulates the β-oxidation of fatty acids through the degradation of the PPARα/RXR agonist phytanic acid. Thus, IDH1 activity is involved in maintaining cellular cholesterol and fatty acid homeostasis through synthesis and degradation. Additional data, from studies using gene overexpression and knockout methodologies in cultured cells and animals, are needed to assess the specific function of IDH1 under various lipogenic conditions. If the activity of IDH1 is proven to be the major biochemical NADPH-producing reaction for these processes, it may be worthwhile to consider IDH1 as a target enzyme for lipid-lowering pharmacological strategies.

This work was supported by Grant HL-048540 from the National Institutes of Health.

REFERENCES

1. Illingworth, J. A., and K. F. Tipton. 1970. Purification and properties of the nicotinamide-adenine dinucleotide phosphate-depen-

dent isocitrate dehydrogenase from pig liver cytoplasm. *Biochem. J.* **118:** 253–258.
2. Plaut, G. W., M. Cook, and T. Aogaichi. 1983. The subcellular location of isozymes of NADP-isocitrate dehydrogenase in tissues from pig, ox and rat. *Biochim. Biophys. Acta.* **760:** 300–308.
3. Plaut, G. W., C. M. Smith, and W. L. Alworth. 1974. Biosynthesis of water-soluble vitamins. *Annu. Rev. Biochem.* **43:** 899–922.
4. Ramachandran, N., and R. F. Colman. 1980. Chemical characterization of distinct subunits of pig heart DPN-specific isocitrate dehydrogenase. *J. Biol. Chem.* **255:** 8859–8864.
5. Keys, D. A., and L. McAlister-Henn. 1990. Subunit structure, expression, and function of NAD(H)-specific isocitrate dehydrogenase in Saccharomyces cerevisiae. *J. Bacteriol.* **172:** 4280–4287.
6. Haselbeck, R. J., and L. McAlister-Henn. 1993. Function and expression of yeast mitochondrial NAD- and NADP-specific isocitrate dehydrogenases. *J. Biol. Chem.* **268:** 12116–12122.
7. Nekrutenko, A., D. M. Hillis, J. C. Patton, R. D. Bradley, and R. J. Baker. 1998. Cytosolic isocitrate dehydrogenase in humans, mice, and voles and phylogenetic analysis of the enzyme family. *Mol. Biol. Evol.* **15:** 1674–1684.
8. Geisbrecht, B. V., and S. J. Gould. 1999. The human PICD gene encodes a cytoplasmic and peroxisomal NADP(+)-dependent isocitrate dehydrogenase. *J. Biol. Chem.* **274:** 30527–30533.
9. Yoshihara, T., T. Hamamoto, R. Munakata, R. Tajiri, M. Ohsumi, and S. Yokota. 2001. Localization of cytosolic NADP-dependent isocitrate dehydrogenase in the peroxisomes of rat liver cells: biochemical and immunocytochemical studies. *J. Histochem. Cytochem.* **49:** 1123–1132.
10. Keller, G. A., M. C. Barton, D. J. Shapiro, and S. J. Singer. 1985. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase is present in peroxisomes in normal rat liver cells. *Proc. Natl. Acad. Sci. USA.* **82:** 770–774.
11. Keller, G. A., M. Pazirandeh, and S. Krisans. 1986. 3-Hydroxy-3-methylglutaryl coenzyme A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine-treated animals: quantitative biochemical and immunoelectron microscopical analyses. *J. Cell Biol.* **103:** 875–886.
12. Krisans, S. K. 1996. Cell compartmentalization of cholesterol biosynthesis. *Ann. N. Y. Acad. Sci.* **804:** 142–164.
13. Biardi, L., and S. K. Krisans. 1996. Compartmentalization of cholesterol biosynthesis. Conversion of mevalonate to farnesyl diphosphate occurs in the peroxisomes. *J. Biol. Chem.* **271:** 1784–1788.
14. Wanders, R. J., and J. M. Tager. 1998. Lipid metabolism in peroxisomes in relation to human disease. *Mol. Aspects Med.* **19:** 69–154.
15. Fransen, M., P. P. Van Veldhoven, and S. Subramani. 1999. Identification of peroxisomal proteins by using M13 phage protein VI phage display: molecular evidence that mammalian peroxisomes contain a 2,4-dienoyl-CoA reductase. *Biochem. J.* **340:** 561–568.
16. Geisbrecht, B. V., X. Liang, J. C. Morrell, H. Schulz, and S. J. Gould. 1999. The mouse gene PDCR encodes a peroxisomal delta(2), delta(4)-dienoyl-CoA reductase. *J. Biol. Chem.* **274:** 25814–25820.
17. Jansen, G. A., S. J. Mihalik, P. A. Watkins, H. W. Moser, C. Jakobs, S. Denis, and R. J. Wanders. 1996. Phytanoyl-CoA hydroxylase is present in human liver, located in peroxisomes, and deficient in Zellweger syndrome: direct, unequivocal evidence for the new, revised pathway of phytanic acid alpha-oxidation in humans. *Biochem. Biophys. Res. Commun.* **229:** 205–210.
18. Mihalik, S. J., A. M. Rainville, and P. A. Watkins. 1995. Phytanic acid alpha-oxidation in rat liver peroxisomes. Production of alpha-hydroxyphytanoyl-CoA and formate is enhanced by dioxygenase cofactors. *Eur. J. Biochem.* **232:** 545–551.
19. Croes, K., M. Casteels, E. De Hoffmann, G. P. Mannaerts, and P. P. Van Veldhoven. 1996. Alpha-oxidation of 3-methyl-substituted fatty acids in rat liver. Production of formic acid instead of CO2, cofactor requirements, subcellular localization and formation of a 2-hydroxy-3-methylacyl-CoA intermediate. *Eur. J. Biochem.* **240:** 674–683.
20. Verhoeven, N. M., and C. Jakobs. 2001. Human metabolism of phytanic acid and pristanic acid. *Prog. Lipid Res.* **40:** 453–466.
21. Heim, M., J. Johnson, F. Boess, I. Bendik, P. Weber, W. Hunziker, and B. Fluhmann. 2002. Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. *FASEB J.* **16:** 718–720.
22. Ellinghaus, P., C. Wolfrum, G. Assmann, F. Spener, and U. Seedorf. 1999. Phytanic acid activates the peroxisome proliferator-activated receptor alpha (PPARalpha) in sterol carrier protein 2-/sterol carrier protein x-deficient mice. *J. Biol. Chem.* **274:** 2766–2772.
23. Kitareewan, S., L. T. Burka, K. B. Tomer, C. E. Parker, L. J. Deterding, R. D. Stevens, B. M. Forman, D. E. Mais, R. A. Heyman, T. McMorris, and C. Weinberger. 1996. Phytol metabolites are circulating dietary factors that activate the nuclear receptor RXR. *Mol. Biol. Cell.* **7:** 1153–1166.
24. Schluter, A., P. Yubero, R. Iglesias, M. Giralt, and F. Villarroya. 2002. The chlorophyll-derived metabolite phytanic acid induces white adipocyte differentiation. *Int. J. Obes. Relat. Metab. Disord.* **26:** 1277–1280.
25. Schluter, A., M. J. Barbera, R. Iglesias, M. Giralt, and F. Villarroya. 2002. Phytanic acid, a novel activator of uncoupling protein-1 gene transcription and brown adipocyte differentiation. *Biochem. J.* **362:** 61–69.
26. Wierzbicki, A. S., M. D. Lloyd, C. J. Schofield, M. D. Feher, and F. B. Gibberd. 2002. Refsum's disease: a peroxisomal disorder affecting phytanic acid alpha-oxidation. *J. Neurochem.* **80:** 727–735.
27. Jennings, G., S. Sechi, P. Stevenson, R. Tuckey, D. Parmelee, and L. McAlister-Henn. 1994. Cytosolic NADP(+)-dependent isocitrate dehydrogenase. Isolation of rat cDNA and study of tissue-specific and developmental expression of mRNA. *J. Biol. Chem.* **269:** 23128–23134.
28. Luzzatto, L., A. Mehta, and T. Vulliamy. 2001. Glucose 6-phosphate dehydrogenase deficiency. *In* The Metabolic and Molecular Bases of Inherited Diseases. 8th edition. Vol. 3. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 4517–4553.
29. Heller, P., W. R. Best, R. B. Nelson, and J. Becktel. 1979. Clinical implications of sickle-cell trait and glucose-6-phosphate dehydrogenase deficiency in hospitalized black male patients. *N. Engl. J. Med.* **300:** 1001–1005.
30. Rosenstraus, M., and L. A. Chasin. 1975. Isolation of mammalian cell mutants deficient in glucose-6-phosphate dehydrogenase activity: linkage to hypoxanthine phosphoribosyl transferase. *Proc. Natl. Acad. Sci. USA.* **72:** 493–497.
31. Town, M., M. Athanasiou-Metaxa, and L. Luzzatto. 1990. Intragenic interspecific complementation of glucose 6-phosphate dehydrogenase in human-hamster cell hybrids. *Somat. Cell Mol. Genet.* **16:** 97–108.
32. Winkler, B. S., N. DeSantis, and F. Solomon. 1986. Multiple NADPH-producing pathways control glutathione (GSH) content in retina. *Exp. Eye Res.* **43:** 829–847.
33. Belfiore, F., and S. Iannello. 1995. Fatty acid synthesis from glutamate in the adipose tissue of normal subjects and obese patients: an enzyme study. *Biochem. Mol. Med.* **54:** 19–25.
34. Lee, S. M., H. J. Koh, D. C. Park, B. J. Song, T. L. Huh, and J. W. Park. 2002. Cytosolic NADP(+)-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. *Free Radic. Biol. Med.* **32:** 1185–1196.
35. Sun, L., T. T. Sun, and R. M. Lavker. 1999. Identification of a cytosolic NADP+-dependent isocitrate dehydrogenase that is preferentially expressed in bovine corneal epithelium. A corneal epithelial crystallin. *J. Biol. Chem.* **274:** 17334–17341.
36. Yokoyama, C., X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, J. L. Goldstein, and M. S. Brown. 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell.* **75:** 187–197.
37. Brown, M. S., and J. L. Goldstein. 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc. Natl. Acad. Sci. USA.* **96:** 11041–11048.
38. Nohturfft, A., M. S. Brown, and J. L. Goldstein. 1998. Topology of SREBP cleavage-activating protein, a polytopic membrane protein with a sterol-sensing domain. *J. Biol. Chem.* **273:** 17243–17250.
39. Sakai, J., R. B. Rawson, P. J. Espenshade, D. Cheng, A. C. Seegmiller, J. L. Goldstein, and M. S. Brown. 1998. Molecular identification of the sterol-regulated luminal protease that cleaves SREBPs and controls lipid composition of animal cells. *Mol. Cell.* **2:** 505–514.
40. Sakai, J., A. Nohturfft, J. L. Goldstein, and M. S. Brown. 1998. Cleavage of sterol regulatory element-binding proteins (SREBPs) at site-1 requires interaction with SREBP cleavage-activating protein. Evidence from in vivo competition studies. *J. Biol. Chem.* **273:** 5785–5793.
41. Hua, X., A. Nohturfft, J. L. Goldstein, and M. S. Brown. 1996. Sterol resistance in CHO cells traced to point mutation in SREBP cleavage-activating protein. *Cell.* **87:** 415–426.
42. Nohturfft, A., D. Yabe, J. L. Goldstein, M. S. Brown, and P. J. Espenshade. 2000. Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes. *Cell.* **102:** 315–323.

43. Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109:** 1125–1131.
44. Edwards, P. A., D. Tabor, H. R. Kast, and A. Venkateswaran. 2000. Regulation of gene expression by SREBP and SCAP. *Biochim. Biophys. Acta.* **1529:** 103–113.
45. Goldstein, J. L., R. B. Rawson, and M. S. Brown. 2002. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. *Arch. Biochem. Biophys.* **397:** 139–148.
46. Yang, T., P. J. Espenshade, M. E. Wright, D. Yabe, Y. Gong, R. Aebersold, J. L. Goldstein, and M. S. Brown. 2002. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell.* **110:** 489–500.
47. Yabe, D., M. S. Brown, and J. L. Goldstein. 2002. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc. Natl. Acad. Sci. USA.* **99:** 12753–12758.
48. Espenshade, P. J., W. P. Li, and D. Yabe. 2002. Sterols block binding of COPII proteins to SCAP, thereby controlling SCAP sorting in ER. *Proc. Natl. Acad. Sci. USA.* **99:** 11694–11699.
49. Loewen, C. J. R., and T. P. Levine. 2002. Cholesterol homeostasis: not until the SCAP lady INSIGs. *Curr. Biol.* **12:** R779–R781.
50. Shechter, I., P. Dai, M. A. Roseman, S. D. Gupta, B. B. Boyer, and G. Guan. 2003. Low-temperature effect on the sterol-dependent processing of SREBPs and transcription of related genes in HepG2 cells. *J. Lipid Res.* **44:** 1581–1590.
51. Guan, G., P. H. Dai, and I. Shechter. 1998. Differential transcriptional regulation of the human squalene synthase gene by sterol regulatory element-binding proteins (SREBP) 1a and 2 and involvement of 5′ DNA sequence elements in the regulation. *J. Biol. Chem.* **273:** 12526–12535.
52. Guan, G., P. H. Dai, T. F. Osborne, J. B. Kim, and I. Shechter. 1997. Multiple sequence elements are involved in the transcriptional regulation of the human squalene synthase gene. *J. Biol. Chem.* **272:** 10295–10302.
53. Pai, J. T., O. Guryev, M. S. Brown, and J. L. Goldstein. 1998. Differential stimulation of cholesterol and unsaturated fatty acid biosynthesis in cells expressing individual nuclear sterol regulatory element-binding proteins. *J. Biol. Chem.* **273:** 26138–26148.
54. Horton, J. D., I. Shimomura, M. S. Brown, R. E. Hammer, J. L. Goldstein, and H. Shimano. 1998. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J. Clin. Invest.* **101:** 2331–2339.
55. Shimano, H. 2001. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog. Lipid Res.* **40:** 439–452.
56. Tabor, D. E., J. B. Kim, B. M. Spiegelman, and P. A. Edwards. 1998. Transcriptional activation of the stearoyl-CoA desaturase 2 gene by sterol regulatory element-binding protein/adipocyte determination and differentiation factor 1. *J. Biol. Chem.* **273:** 22052–22058.
57. Fielding, C., and P. Fielding. 2000. Cholesterol and caveolae: structural and functional relationships. *Biochim. Biophys. Acta.* **1529:** 210–222.
58. Frohman, M. A., M. K. Dush, and G. R. Martin. 1988. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA.* **85:** 8998–9002.
59. Farrell, H. M., Jr. 1980. Purification and properties of NADP+: isocitrate dehydrogenase from lactating bovine mammary gland. *Arch. Biochem. Biophys.* **204:** 551–559.
60. Guan, G., G. Jiang, R. L. Koch, and I. Shechter. 1995. Molecular cloning and functional analysis of the promoter of the human squalene synthase gene. *J. Biol. Chem.* **270:** 21958–21965.
61. Ohshima, Y., and Y. Gotoh. 1987. Signals for the selection of a splice site in pre-mRNA. Computer analysis of splice junction sequences and like sequences. *J. Mol. Biol.* **195:** 247–259.
62. Veech, R. L., L. V. Eggleston, and H. A. Krebs. 1969. The redox state of free nicotinamide-adenine dinucleotide phosphate in the cytoplasm of rat liver. *Biochem. J.* **115:** 609–619.
63. Smith, J. R., T. F. Osborne, J. L. Goldstein, and M. S. Brown. 1990. Identification of nucleotides responsible for enhancer activity of sterol regulatory element in low density lipoprotein receptor gene. *J. Biol. Chem.* **265:** 2306–2310.
64. Magana, M. M., S. H. Koo, H. C. Towle, and T. F. Osborne. 2000. Different sterol regulatory element-binding protein-1 isoforms utilize distinct co-regulatory factors to activate the promoter for fatty acid synthase. *J. Biol. Chem.* **275:** 4726–4733.
65. Jackson, S. M., J. Ericsson, R. Mantovani, and P. A. Edwards. 1998. Synergistic activation of transcription by nuclear factor Y and sterol regulatory element binding protein. *J. Lipid Res.* **39:** 767–776.
66. Ericsson, J., S. M. Jackson, and P. A. Edwards. 1996. Synergistic binding of sterol regulatory element-binding protein and NF-Y to the farnesyl diphosphate synthase promoter is critical for sterol-regulated expression of the gene. *J. Biol. Chem.* **271:** 24359–24364.
67. Jackson, S. M., J. Ericsson, T. F. Osborne, and P. A. Edwards. 1995. NF-Y has a novel role in sterol-dependent transcription of two cholesterogenic genes. *J. Biol. Chem.* **270:** 21445–21448.
68. Desvergne, B., A. IJpenberg, P. R. Devchand, and W. Wahli. 1998. The peroxisome proliferator-activated receptors at the cross-road of diet and hormonal signalling. *J. Steroid Biochem. Mol. Biol.* **65:** 65–74.