Regulation of Human Cytochrome P450 4F2 Expression by Sterol Regulatory Element-binding Protein and Lovastatin*

From the Division of Biochemistry, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037

Mei-Hui Hsu, Üzen Savas, Keith J. Griffin, and Eric F. Johnson

This report provides the first evidence that human P450 4F2 (CYP4F2) is induced by statins, which are widely used to treat hypercholesterolemia. Real time PCR and immunoblots indicate that lovastatin treatment increases expression of the endogenous CYP4F2 gene in human primary hepatocytes and HepG2 cells. The effects of lovastatin on gene expression are often mediated through sterol regulatory element-binding proteins (SREBPs). Immunoblots indicate that lovastatin-treated human hepatocytes display increased proteolytic processing of SREBP-2. In HepG2 cells, co-administration of a potent suppressor of SREBP-2 activation, 25-hydroxycholesterol, inhibits CYP4F2 mRNA induction by lovastatin. HepG2 cells transfected with an expression vector for the active nuclear form of SREBP-1a (nSREBP-1a) also display elevated endogenous CYP4F2 expression. Luciferase reporters containing the CYP4F2 proximal promoter are transactivated by nSREBPs (-1a, -1c, and -2) or a dominant positive form of the SREBP cleavage-activating protein (SCAP), which facilitates activation of endogenous SREBPs. Lovastatin-induced reporter expression is inhibited by overexpressed Insig-1, which prevents proteolytic activation of endogenous SREBPs. Electrophoretic mobility shift assays with in vitro translated nSREBP-1a identified two SREBP binding sites at −169/−152 and −109/−92, relative to the CYP4F2 transcription start site. Mutations in each site abolish SREBP binding. Chromatin immunoprecipitation experiments indicate that more SREBP-1 is associated with the CYP4F2 promoter after overexpression of nSREBP-1a. Transfection studies and mutagenesis indicate that the −109/−92 region is the primary site responsible for the effects of statins. Collectively, these results demonstrate that SREBPs transactivate CYP4F2 transcription and that CYP4F2 induction by statins is mediated by SREBP-2.

The capacity to oxidize the terminal carbon of aliphatic chains is a highly conserved enzymatic activity of cytochrome P450s in plant and animal species. In mammals, the ω-hydroxylation of fatty acids provides a means to remove potentially toxic excess concentrations of free fatty acids. This is the first step in the formation of dicarboxylic acids that can be more readily excreted or are further degraded by peroxisomal β-oxidation. The ω-hydroxylases also provide pathways for the degradation of signaling molecules such as prostanoids and leukotrienes. On the other hand, ω-hydroxylation of arachidonic acid forms 20-hydroxyeicosatetraenoic acid (20-HETE), which has been implicated in the regulation of vascular tone and blood pressure (1–3).

Cytochrome P450 4F2 (P450 4F2) is a fatty acid ω-hydroxylase that is expressed in human liver and kidney and contributes to free fatty acid catabolism and the conversion of arachidonic acid to 20-HETE. In humans, the predominant ω-hydroxylases in liver and kidney are microsomal P450s 4A11, 4F2, and 4F3B (4). The ω-hydroxylation of saturated fatty acids is generally associated with P450 4A enzymes in non-human species, and human P450 4A11 catalyzes the ω-hydroxylation of lauric acid > palmitic acid > arachidonic acid (5). Selective disruption of the murine Cyp4a10 (6) and Cyp4a14 (7) genes leads to hypertensive phenotypes that are thought to reflect changes in renal 20-HETE production arising through distinct mechanisms. Interestingly, an allelic variant encodes a human CYP4A11 with reduced catalytic activity that is associated with an increased risk for hypertension in studies of three independent cohorts (8, 9).

P450s 4F2 and 4F3B are highly similar in amino acid sequence and display overlapping catalytic activity profiles. Both enzymes efficiently oxidize arachidonic acid (10). An alternative transcript of the CYP4F3 gene, 4F3A, arises from differential promoter use leading to the incorporation of an alternative exon that alters substrate preferences to favor the ω-hydroxylation of leukotrienes (11). P450 4F3A is predominantly expressed in human leukocytes (12). Antibody inhibition experiments (13, 14) suggest that P450 4F2/4F3B in human liver and kidney microsomes contribute ~66% to the formation of 20-HETE. Recently, P450s 4F2 and 4F3B were found to ω-hydroxylate very long chain saturated fatty acids (15) as well as phytanic acid (16). Our preliminary experiments using com-

---

*This work was supported by National Institutes of Health Grant HD004445 (to E. F. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Mail Drop MEM255, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-784-7918; Fax: 858-784-7978; E-mail: Johnson@scripps.edu.

2 The abbreviations used are: 20-HETE, 20-hydroxyeicosatetraenoic acid; 25-OH, 25-hydroxycholesterol; C/EBP, CCAAT/enhancer-binding protein; CYP, cytochrome P450; DMEM, Dulbecco’s minimal essential medium; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; HMGCS1, 3-hydroxy-3-methylglutaryl-CoA synthase 1; Insig-1, insulin-induced gene 1; LDLR, low density lipoprotein receptor; PIC, protease inhibitor cocktail; PPIA, cyclophilin A; RT, reverse transcription; SREBP, sterol regulatory element-binding protein; SCAP, SREBP cleavage-activating protein; SRE, SREBP response element; VLCFA, very long chain-saturated fatty acid; X-ALD, X-linked adrenoleukodystrophy.
Statins Induce CYP4F2 Gene Expression

Regulated expression of CYP4 genes contributes to fatty acid homeostasis and prevention of lipotoxicity by increasing the capacity to remove excess free fatty acids. The expression of CYP4A genes in several mammalian species is elevated by conditions that increase the flux of free fatty acids, such as fasting and exposure to hypolipidemic drugs, such as fibrates. These responses are mediated by the peroxisome proliferator-activated receptor α (18, 19). In contrast to CYP4A genes, peroxisome proliferator-activated receptor α agonists generally suppress the hepatic expression of rat and mouse CYP4F mRNAs and these responses are peroxisome proliferator-activated receptor α independent (20, 21).

Statins, which are widely used in the treatment of hypercholesterolemia, also induce CYP4 gene expression in rat liver and rat hepatocytes (22). Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis. In addition to impairing the synthesis of isoprenoid intermediates, cholesterol, oxysterols, and bile acids, statins also increase cholesterol uptake via increased expression of the low density lipoprotein (LDL) receptor. These effects are mediated through activation of sterol regulatory element-binding proteins (SREBPs) by proteolytic cleavage.

SREBPs belong to the basic helix-loop-helix family of DNA-binding proteins. Three isoforms of SREBP have been identified, SREBP-1a, -1c, and -2. The DNA binding domains of SREBP-1a and -1c are identical, and they share over 70% identity with the corresponding domain of SREBP-2. Human SREBP-2 is encoded by a gene on chromosome 22. SREBP-1a and -1c are produced from a single gene on chromosome 17 through the usage of alternative transcription start sites that generate an alternative exon 1. SREBP-2 is predominantly involved in regulating the cholesterol synthesis pathway, whereas SREBP-1c mainly controls fatty acid synthesis (23, 24). SREBP-1a, the major SREBP-1 form found in many cell lines, regulates both cholesterol and fatty acid synthesis (25). After translation, the membrane bound precursor forms of SREBP bind to the SREBP cleavage-activating protein (SCAP) in the endoplasmic reticulum. In the presence of sterols, Insig (insulin-induced gene) protein binds to the sterol-sensing domain of SCAP and retains the SCAP-SREBP complex in the endoplasmic reticulum membrane. When intracellular sterol levels are depleted, the SCAP-SREBP complex is transferred to the Golgi where the precursor form of SREBP is proteolytically cleaved to produce the transcriptionally active N-terminal fragment that is transported into the nucleus and regulates gene transcription (26).

In transgenic mice that overexpress a transcriptionally active form of SREBP-1a, hepatic CYP4A expression was increased (27). In contrast, hepatic CYP4F13 and CYP4F14 mRNA levels were diminished in these mice, but CYP4F12 mRNA was up-regulated. Taken together, these results suggest a role for SREBP in the regulation of fatty acid ω-hydroxylases and in the effects of statins on CYP4 gene expression in mice.

The present study examined the effects of statins on the regulation of the CYP4F2 and CYP4F3B genes in human-derived HepG2 cells as well as in primary cultures of human hepatocytes. Our results indicate that P450 4F2 is selectively induced by statins and demonstrate that this effect is mediated by SREBPs through an enhancer in the proximal promoter of the CYP4F2 gene.

EXPERIMENTAL PROCEDURES

Constructs—pcDNA3-FLAG-nSREBP-1a was obtained from Dr. J. Ericsson at the Ludwig Institute for Cancer Research, Uppsala, Sweden. The pcDNA-FLAG-nSREBP-1c and pcDNA-FLAG-nSREBP-2 plasmids were obtained from Dr. T. Osborne, University of California, Irvine. The pCMV-INSIG-1-Myc, pTK-HSV-SCAP-T7(D443N), and pTK-HSV-SCAP-T7 expression plasmids were purchased from American Type Culture Collection (ATCC, Manassas, VA). PCR primers spanning various regions of the CYP4F2 5′-flanking region were used to amplify specific regions from the cosmids F20129 (AC003356) (MRC Geneservice, Cambridge, UK), which contains the CYP4F2 gene and the complete 5′ inter-gene region. The −753/+464 and −176/+464 PCR fragments were subcloned into a luciferase reporter plasmid (pLuc) and sequenced. The −176/−6 and −6/+464 pLuc reporter constructs were generated by digesting the −176/+464 reporter vector with StuI and BamHI and blunt end filling with Klenow large fragment followed by ligation. Oligonucleotides corresponding to the −67/−1 region of the CYP4F2 gene were inserted between the HindIII and BamHI sites of the pLuc reporter. For the luciferase constructs containing the CYP4F2 natural promoter, the mutant constructs were generated using the QuikChange® II site-directed mutagenesis kit (Stratagene, San Diego, CA). The correctness of each construct was confirmed by sequencing. Plasmid DNA for transfection experiments was prepared using the Qiagen Plasmid isolation kit (Qiagen, Valencia, CA) or the Qiagen Plasmid isolation kit (Qiagen, Valencia, CA) for transfection experiments was prepared using the Qiagen Plasmid isolation kit (Qiagen, Valencia, CA) or the Qiagen Plasmid isolation kit (Qiagen, Valencia, CA).

Cell Culture—Human hepatoma cells, HepG2, were obtained from ATCC and maintained in Dulbecco’s minimal essential medium (DMEM) (Mediatech, Herndon, VA) containing 10% fetal bovine serum (FBS) (Atlas Biologicals, Fort Collins, CO). The medium was supplemented with 10 mm HEPES, minimal essential medium nonessential amino acid mixture and penicillin/streptomycin. For non-transfected HepG2 cells, the cells were seeded at a density that would reach 70–80% confluence after 24 h. When the cells reached the appropriate density, they were maintained in DMEM containing 5% fetal bovine lipoprotein-deficient serum (Intracel, Frederick, MD) for 1 day before being incubated with 1 μM lovastatin or fluvastatin (EMD Biosciences, LA Jolla, CA) or mevastatin (Sigma) or 5 μg/ml 25-hydroxycholesterol (Sigma) or vehicle (Me2SO). After 24 h, cells were harvested. For nSREBP-1a transfection studies, the cells were transfected with pcDNA-FLAG-nSREBP-1a using Lipofectamine™ 2000 (Invitrogen). For each T25 flask, 1 μg of nSREBP-1a expression plasmid and 20 μl of Lipofectamine were used. After transfection...
tion, cells were maintained in DMEM with 10% FBS for 48 h. The cells were harvested and used for immunoblot, RT-PCR, and chromatin immunoprecipitation studies.

Primary Human Hepatocyte Culture—Primary human hepatocytes from two individuals were obtained from CellzDirect (Tucson, AZ). No known history of, or exposure to, hepatitis B, hepatitis C, cirrhosis, biliary diseases, or HIV was evident for these individuals. In addition, both were non-smokers with no known drug or alcohol abuse. One individual was a 77-year-old female Caucasian (Hu374), and the other was a 53-year-old female Caucasian (Hu419). The cells were seeded on collagen-coated 6-well plates with a Matrigel overlay. After receipt, the cells were maintained in modified Chee’s medium supplemented with dexamethasone (0.1 μM) and insulin, transferrin, and sodium selenite (ITS, Sigma). The media was replaced with fresh media daily. After 1–3 days, the cells were treated with 1 μM statins or Me2SO (vehicle). The cells were harvested for immunoblot and RT-PCR studies after a 24-h drug treatment. Total RNAs and proteins were isolated using TRIzol reagent (Invitrogen).

Reverse Transcription and PCR Amplification—TRIzol reagent or the E.N.Z.A. total RNA isolation kit (Omega Bio-tek, Atlanta, GA) were used for total RNA isolation. Omniscript reverse transcriptase (Qiagen) and an oligo(dt) primer (New England Biolabs, Beverly, MA) were used to generate first-strand cDNAs. Specific PCR primers for the CYP4F2, CYP4F3, and cyclophilin A (PPIA) cDNAs were used to generate gene-specific fragments. The PCR primer sets are: CYP4F2 (U02388: 244–730), upper primer 5'-GACACAGGGGCATG-GTCAACC-3' and lower primer 5'-GCCGCAATAATTCTCA-CTGGGTITC-3' and 3'-CYP4F3 (NM_000896: 756–1630), upper primer 5'-CACCAGCAGATCCTCCTGTACATA-3' and lower primer 5'-GTGGGTCTCTGCAGAACTCAGCTCAG-3'; PPIA (NM_021130: 274–536), upper primer 5'-CCATCTA-TGGGGGAGAAATTTGA-3' and lower primer 5'-GAAGCCGCATGAATTTGA-3'. PPIA was used as a control to normalize RNA levels. PCR were performed using the following conditions: 1 cycle at 97 °C for 3 min, multiple cycles (97 °C for 3 min, 57 °C for 2 min, and 72 °C for 3 min), and 1 cycle at 72 °C for 5 min. The number of amplification cycles used for each product was empirically determined based on the observed mRNA levels of the target genes.

Real Time PCR—Amplicons corresponding to the CYP4F2 (487 bp) and PPIA (263 bp) mRNAs were generated by RT-PCR from HepG2 cells, subcloned into the pCRII-TOPO vector (Invitrogen), and confirmed by DNA sequencing. Serial dilutions of each plasmid were used as a reference to determine the copy number of mRNA in each sample. Total RNA (5 μg) from HepG2 cells or human hepatocytes was reverse transcribed using an oligo(dt) primer and Stratascript (Stratagene) in a total reaction volume of 50 μl according to the supplier’s protocol. The reverse transcription mixture was diluted 2-fold with water and 2 μl of diluted reaction mixture were used as template for PCR. For real time thermal cycling, triplicate aliquots of serially diluted amplicon or RT sample were used in a reaction mixture that contained 250 nM of each primer in a reaction volume of 20 μl using the IQ™ SYBR Green Supermix (Bio-Rad). A Bio-Rad iCYCLER iQ real time PCR instrument was used for these experiments. The same primer sets used in RT-PCR were also used in this study. The thermal cycling was started with an initial 5 min at 95 °C, followed by 45 cycles of 95 °C for 30 s and 60 °C for 2 min. CYP4F2 mRNA concentration for each sample was calculated by mapping the threshold cycle to the plasmid concentration on the standard curve. Messenger RNA quantities were obtained in a similar manner for PPIA from the same samples using the PPIA amplicon.

Microsomal and Nuclear Protein Isolation—Harvested cells were washed with ice-cold phosphate-buffered saline twice and pelleted at 3,000 rpm for 5 min at 4 °C. Phenylmethylsulfonyl fluoride (0.2 μM), 4-(2-aminoethyl)benzenesulfonyl fluoride (0.1 μM), and protease inhibitor mixture (PIC) (Roche) were included in the ice-cold buffers prior to use in all of the following steps. The cells were resuspended in 10 mM Tris, pH 7.4, buffer containing 0.25 M sucrose, 1 mM EDTA, and 0.05% Nonidet P-40 (homogenization buffer). After incubation on ice for 5 min, the resuspended cells were homogenized using Radnoti tissue grinders (20 strokes). After centrifugation at 550 × g for 5 min at 4 °C, the supernatants were saved for microsomal fraction isolation. The pellets were washed with homogenization buffer and re-centrifuged at 550 × g for 5 min. The final pellets were resuspended in 50 mM HEPES buffer, pH 7.4, containing 0.1 mM EDTA, 400 mM NaCl, and 10% glycerol, prior to incubation on a rotator at 4 °C for 1 h. The nuclear extract was clarified by centrifugation at 15,000 × g for 15 min at 4 °C. The supernatants from the 550 × g centrifugation were pooled and centrifuged at 150,000 × g for 1 h at 4 °C. The resulting pellets represent the membrane fractions that were resuspended in 100 mM potassium phosphate buffer, pH 7.4, containing 0.05% Nonidet P-40, 1 mM EDTA, and 20% glycerol. The protein contents of the nuclear and membrane fractions were determined using Pierce BCA assay (Pierce). Aliquots of the nuclear and the membrane fractions were stored at −80 °C until used.

Immunoblots—Microsomal proteins, nuclear proteins, total cellular proteins, or commercially obtained Supersomes containing P450 4F2 (BD Gentest, Woburn, MA) were separated in 10% NuPAGE gels (Invitrogen) and transferred to nitrocellulose membranes. The blots were probed using a rabbit polyclonal antibody against P450 4F2/4F3 (a gift of Dr. Lasker) (13) or SREBP-1 (clone K-10) (Santa Cruz Biotechnology, Santa Cruz, CA) followed by a monoclonal anti-rabbit IgG conjugated with peroxidase (clone RG-96) (Sigma). For SREBP-2 detection, a mouse anti-SREBP-2 monoclonal antibody (IgG-1C6 clone) (BD Pharmingen) was used followed by a polyclonal anti-mouse IgG conjugated with peroxidase (Sigma). A rabbit polyclonal anti-cyclophilin A antibody (Upstate, Lake Placid, NY) or a mouse monoclonal anti-actin antibody (JLA20 clone) (EMD) or SREBP-1 (clone K-10) (Santa Cruz Biotechnology, Santa Cruz, CA) followed by a monoclonal anti-actin antibody (JLA20 clone) (EMD Bioscience) was used to normalize sample loading. The blots were developed with the Western Lightening Plus Chemiluminescence reagent (PerkinElmer Life Sciences).

Reporter Transfection—HepG2 cells were transfected with luciferase reporter plasmids containing various regions of the CYP4F2 gene using a modified calcium phosphate method as described previously (28). β-Galactosidase was used to normalize transfection efficiency. Costar 24-well plates were used for transfection studies. Details for each experiment are described in each figure legend.
Statins Induce CYP4F2 Gene Expression

Electrophoretic Mobility Shift Assays (EMSA)—Oligonucleotides spanning discrete areas of the −176/−67 region of CYP4F2 gene and oligonucleotides containing specific mutations in potential response elements were synthesized. An excess molar ratio of [γ-32P]ATP was used to end label both of the complementary oligonucleotide strands using T4 polynucleotide kinase (New England Biolabs). The 32P-labeled oligonucleotides were then annealed, gel purified, and the specific activities of each double-stranded probe were determined. Reaction mixtures containing 20 fmol of 32P-labeled double-stranded oligonucleotides were incubated with 5 µl of rabbit reticulocyte lysate programmed with pcDNA3-FLAG-nSREBP-1a or with control rabbit reticulocyte lysate (Promega). The reactions also contained 125 ng/µl of poly(dI-dC) and 20 ng/µl of sonicated salmon sperm DNA (Invitrogen). For supershift analysis, 0.5 µl of anti-FLAG® M2 monoclonal antibody (Stratagene) was included in the incubation mixture. After incubating for 30 min at room temperature, the reaction mixtures were loaded onto a 5% polyacrylamide (37.5:1) gel as described (29). The dried gels were analyzed using a GE Healthcare PhosphorImager, model SI.

Chromatin Immunoprecipitation Assays (ChiP)—A modification of the protocol described by Nowak et al. (30) was used. Briefly, nSREBP-1a transfected HepG2 cells and the corresponding control cells transfected with the empty vector were first exposed to a cross-linker, 2 mM disuccinimidyl glutarate (Pierce), at room temperature for 45 min. Then the cells were fixed with 1% (v/v) formaldehyde for 10 min. Glycine was added to 125 mM and incubated for another 5 min at room temperature to stop the cross-linking reaction. The cells were lysed by incubation in 50 mM Tris, pH 8.0, containing 2 mM EDTA, 0.1% IGEPAL 630 (Sigma), 10% glycerol, 1 mM dithiothreitol, PIC, and 1 mM phenylmethylsulfonyl fluoride, on ice for 15 min. Nuclei were pelleted at 12000 × g for 5 min and resuspended in SDS lysis buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 1% SDS). Samples were sonicated using 20-s pulses with 30 s rest on a setting of 4 for a total time of 8 min using a microtip and Misonix sonicator 3000. The degree of DNA fragmentation was verified by gel electrophoresis after reversing the cross-linking reaction as described below. If needed, sonication was repeated until the size distribution was predominantly between 200 and 500 bp. The CHIP-IT kit (Active Motif, Carlsbad, CA) and Protein G magnetic beads (New England Biolabs) were used to generate immunoprecipitated, protein-bound DNA using an anti-SREBP-1 rabbit polyclonal antibody (K-10, Santa Cruz Biotechnology). IgG obtained from non-immunized rabbit serum (Sigma) was used as a control. Eluted DNA was heated at 65°C overnight to reverse the cross-linking and then purified using the E.Z.N.A. Cycle Pure Kit (Biomiga). PCR analysis was used to analyze the resulting immunoprecipitated DNA. The PCR primers that were used are: LDL receptor oligonucleotides (L29401, 471 to 650) 5′-CGATGTCACTACCTGGCCGGCTTCG-3′ and 5′-CAGACCTGGTGTGCTAGCTGGAA-3′; and 4F2 oligonucleotides (AF67894, 1942–2137; −188 to +8, relative to the transcription start site) 5′-TGTGCTCATCTACCACTTCCATCTGGAA-3′ and 5′-TGTCTGCTGGGAGGCGCTTTGGAGA-3′. PCR were performed using the following conditions: 1 cycle at 97°C for 3 min, multiple cycles (97°C for 1 min, 58°C for 2 min, and 72°C for 3 min), and 1 cycle at 72°C for 5 min. The number of amplification cycles used for each target gene was empirically determined.

Statistical Analysis—A one-tailed Student’s t test was used to determine statistically significant differences between sample mean ± S.E. using Microsoft Excel software.

RESULTS

Statins Induce Endogenous CYP4F2 Gene Expression in Human Primary Hepatocytes and HepG2 Cells—Real time PCR results (Fig. 1A) indicate that CYP4F2 mRNA levels found in primary cultures of two independent preparations of human hepatocytes that had been treated for 24 h with lovastatin or mevastatin were elevated 2–6-fold relative to cells treated with Me2SO vehicle after normalization to PPIA mRNA levels. However, statin treatment did not affect CYP4F2 mRNA expression (data not shown). Similar results were obtained with the human hepatoma cell line, HepG2. Analysis of five independent HepG2 experiments indicates that CYP4F2 mRNA levels are elevated 2.9 ± 0.9-fold (mean ± S.E., p < 0.05) by a 24-h lovastatin treatment compared with the cells treated with Me2SO only (Fig. 1A). CYP4F3 mRNA levels were not appreciably altered by statin treatment (data not shown). Others have reported a similar -fold induction by lovastatin treatment in HepG2 cells for several known SREBP target genes, including: HMCR (~2.5-fold with 1 µM lovastatin) (31), HMGC5I (~2.7-fold with 1 µM lovastatin) (31), LDLR (1.5–2.5-fold with 1 µM lovastatin) (31–33), FASN (~2.5-fold with 0.3 µM lovastatin) (33), and squalene synthase (2.7–4-fold with 12 µM lovastatin) (34).

When a polyclonal antibody against P450 4F2/4F3 was used for immunoblot analysis, a band was observed in the microsomal fractions isolated from HepG2 cells that displayed a mobility similar to the band recognized in the Gentest 4F2 Supersomes that served as a positive control. Densitometry indicates that the antibody signal obtained from HepG2 cells treated with lovastatin is at least 2-fold higher than the signal obtained from control cells after normalization with loading controls (Fig. 1B). Endogenous CYP4F2 expression in HepG2 cells could also be induced by treatment with fluvastatin and mevastatin (data not shown). Similar results were also seen with protein preparations from lysates of cultured human hepatocytes treated with lovastatin or mevastatin for 24 h (Fig. 1C). As the CYP4F3B mRNA levels were not affected by lovastatin treatment, the increased antibody signal resulting from lovastatin treatment probably reflects increased P450 4F2 expression. Higher endogenous CYP4F3B mRNA levels are present in human liver (2–4-fold) (10) and HepG2 cells (1–2-fold) (data not shown), relative to CYP4F2 mRNA levels. Therefore, the actual -fold induction of P450 4F2 protein levels by statins in immunoblots is likely to be underestimated. These results indicate that statin treatment increases CYP4F2 expression in human primary hepatocytes and HepG2 cells, and suggest that HepG2 cells can provide a relevant model system to examine the mechanism of statin-induced CYP4F2 expression in hepatocytes.

Overexpressed nSREBP-1a Increases Endogenous CYP4F2 Expression in HepG2 Cells—To examine the potential regulatory role for SREBP in cells, an expression vector for nSREBP-1a was employed to study the effect of overexpressing the active,
Lovastatin induces expression of the CYP4F2 gene in human primary hepatocytes and HepG2 cells. Human hepatocytes and HepG2 cells were treated with 1 μM lovastatin or the vehicle MeSO (DMSO) for 24 h. Total RNAs and total cellular proteins or microsomal proteins were isolated as described under “Experimental Procedures.” A, real time PCR assay. RT-PCR was performed. The mRNA levels of CYP4F2 were normalized using PPIA mRNA levels. For human hepatocytes, at least two wells of a 6-well plate were used for each treatment and duplicate determinations were performed for each sample. The mean ± S.E. were determined from the replicate wells for each human hepatocyte preparation. For HepG2 cells, five independent treatments were performed and duplicate determinations were performed for each sample. The means ± S.E. were determined from five independent experiments. The mean value obtained from cells treated with lovastatin was compared with the mean obtained from the MeSO-treated cells to determine -fold activation. B, immunoblot for HepG2 cells. 50 μg of microsomal proteins or Supersomes containing 0.3 pmol of P450 4F2 were used. Five independent experiments were performed and the combined densitometry results are shown in the bar graph. A representative blot is shown. C, immunoblot for human hepatocytes (Hu374). 10 μg of total cellular protein or Supersomes containing 0.1 pmol of P450 4F2 were used. Four independent immunoblots were performed and a representative example is shown. Immunoblots were probed using a rabbit polyclonal antibody against P450 4F2/4F3, or with antibodies against actin or PPIA to monitor sample loading. Densitometry results are shown as bar graphs. The P450 4F2/4F3 immunoblot signals were normalized using the signals obtained with actin or PPIA antibodies. Normalized P450 4F2/4F3 signals from statin-treated samples were compared with the normalized values of the MeSO control and expressed as -fold activation. Means ± S.E are shown. Statistically significant differences are indicated as: *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

nuclear form of SREBP-1a (nSREBP-1a) on the regulation of the endogenous CYP4F2 gene in HepG2 cells. Based on other studies (35, 36), transfected nSREBP-1a displayed the strongest transactivation among the three nSREBP isoforms tested. In addition, SREBP-1a can activate target genes involved in both cholesterol and fatty acid synthesis (27). When cells were transfected with the pcDNA-nSREBP-1a expression vector, nuclear nSREBP-1a protein levels (Fig. 2A) and mRNA levels (data not shown) were significantly elevated. In addition, increased expression of nSREBP-1a produced elevated P450 4F2 protein levels relative to control cells that had been transfected with the empty pcDNA vector (Fig. 2B). These results clearly indicate that overexpression of the nuclear form of SREBP-1a can activate endogenous CYP4F2 gene transcription leading to increased protein levels in HepG2 cells.

Statins Induce CYP4F2 Gene Expression

FIGURE 1. Lovastatin induces expression of the CYP4F2 gene in human primary hepatocytes and HepG2 cells. Human hepatocytes and HepG2 cells were treated with 1 μM lovastatin or the vehicle MeSO (DMSO) for 24 h. Total RNAs and total cellular proteins or microsomal proteins were isolated as described under “Experimental Procedures.” A, real time PCR assay. RT-PCR was performed. The mRNA levels of CYP4F2 were normalized using PPIA mRNA levels. For human hepatocytes, at least two wells of a 6-well plate were used for each treatment and duplicate determinations were performed for each sample. The mean ± S.E. were determined from the replicate wells for each human hepatocyte preparation. For HepG2 cells, five independent treatments were performed and duplicate determinations were performed for each sample. The means ± S.E. were determined from five independent experiments. The mean value obtained from cells treated with lovastatin was compared with the mean obtained from the MeSO-treated cells to determine -fold activation. B, immunoblot for HepG2 cells. 50 μg of microsomal proteins or Supersomes containing 0.3 pmol of P450 4F2 were used. Five independent experiments were performed and the combined densitometry results are shown in the bar graph. A representative blot is shown. C, immunoblot for human hepatocytes (Hu374). 10 μg of total cellular protein or Supersomes containing 0.1 pmol of P450 4F2 were used. Four independent immunoblots were performed and a representative example is shown. Immunoblots were probed using a rabbit polyclonal antibody against P450 4F2/4F3, or with antibodies against actin or PPIA to monitor sample loading. Densitometry results are shown as bar graphs. The P450 4F2/4F3 immunoblot signals were normalized using the signals obtained with actin or PPIA antibodies. Normalized P450 4F2/4F3 signals from statin-treated samples were compared with the normalized values of the MeSO control and expressed as -fold activation. Means ± S.E are shown. Statistically significant differences are indicated as: *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

nuclear form of SREBP-1a (nSREBP-1a) on the regulation of the endogenous CYP4F2 gene in HepG2 cells. Based on other studies (35, 36), transfected nSREBP-1a displayed the strongest transactivation among the three nSREBP isoforms tested. In addition, SREBP-1a can activate target genes involved in both cholesterol and fatty acid synthesis (27). When cells were transfected with the pcDNA-nSREBP-1a expression vector, nuclear nSREBP-1a protein levels (Fig. 2A) and mRNA levels (data not shown) were significantly elevated. In addition, increased expression of nSREBP-1a produced elevated P450 4F2 protein levels relative to control cells that had been transfected with the empty pcDNA vector (Fig. 2B). These results clearly indicate that overexpression of the nuclear form of SREBP-1a can activate endogenous CYP4F2 gene transcription leading to increased protein levels in HepG2 cells.

FIGURE 2. Overexpression of the active, nuclear form of SREBP-1a increases endogenous CYP4F2 expression in HepG2 cells. HepG2 cells were transfected with an expression vector for the nuclear form of SREBP-1a (nSREBP-1a) or the empty vector (pcDNA) as described under “Experimental Procedures.” After overnight incubation with the Lipofectamine/DNA mixture, cells were washed and then incubated with media for another 48 h prior to isolation of microsomal and nuclear fractions. A, SREBP-1 immunoblot. The lanes contained 20 μg of crude nuclear protein. The blot was developed using a rabbit anti-SREBP-1 polyclonal antibody, clone K-10 (Santa Cruz). The mobilities of molecular weight markers are indicated on the left. NS indicates a nonspecific band. B, P450 4F2 immunoblot. The lanes contain 60 μg of microsomal protein or Gentest Supersomes containing 0.2 pmol of P450 4F2 (4F2). The blot was developed using the polyclonal antibody against P450 4F2/4F3 as described in the legend to Fig. 1.
Statins Induce CYP4F2 Gene Expression

A, overexpression of SCAP or a dominant positive form of SCAP (SCAP-D443N) transactivates the CYP4F2 (-179/+464) reporter. HepG2 cells were co-transfected with the CYP4F2 (-179/+464) reporter (1.5 μg/well) and the expression vector for wild type SCAP, the SCAP-D443N mutant, or the vector control (pTK-HSV) (30 ng/well). A β-galactosidase expression vector (0.19 μg/well) was also cotransfected and used to normalize transfection efficiency. The cells were incubated with 1 μM lovastatin or vehicle (DMSO) in DMEM containing 5% fetal bovine lipoprotein-deficient serum for 24 h. At least three independent experiments were conducted and a representative experiment is shown. The means ± S.E. were determined from three replicate samples for each data point. Between the Me2SO (vehicle) and lovastatin treatments, statistically significant differences, p < 0.05, are indicated (*). Among Me2SO-treated cells, (+) indicates statistically significant differences, p < 0.01, between pTK-HSV and SCAP or SCAP-D443N, and (4) indicates the statistical difference, p < 0.07, between SCAP and SCAP-D443N. B, overexpression of Insig-1 blocks lovastatin-induced expression of the CYP4F2 (-179/+464) reporter. The expression vector for Insig-1 or the vector control (pCMV) (7.5 ng/well) was co-transfected with the CYP4F2 (-179/+464) reporter (1.5 μg/well) into HepG2 cells. A β-galactosidase expression vector (0.19 μg/well) was also cotransfected and used to normalize transfection efficiency. The cells were treated with lovastatin or vehicle control as in A. Data were obtained and analyzed as described in A. For the pCMV-transfected cells, statistically significant differences, p < 0.05, between the Me2SO and lovastatin treatments are indicated (*). For lovastatin-treated cells, statistically significant differences, p < 0.05, between the Insig-1 co-transfected cells and the vector control co-transfected cells are indicated (+).

Overexpressed nSREBPs Transactivate CYP4F2 Reporter Constructs in HepG2 Cells—The therapeutic effects of lovastatin on target gene expression are primarily mediated through activation of SREBP-2 (33, 37). Unlike SREBP-1a, SREBP-2 predominantly regulates the sterol synthesis pathway and LDL uptake. Although SREBP-1c does not generally play a major role in regulating fatty acid synthesis in cell lines, it is the major isoform that regulates fatty acid synthesis in most mammalian tissues (25). Therefore, the potential role of each SREBP isoform to transactivate CYP4F2 expression was examined in co-transfection experiments. Two luciferase reporter plasmids containing portions of the 5’ proximal promoter region of the CYP4F2 gene were constructed, −753/+464 and −179/+464. Others have characterized regulatory sequences in intron 1 that affect reporter gene expression in HepG2 cells (38). Therefore, portions of the 5’ non-coding region, exon 1 and intron 1 were included in the initial CYP4F2 reporter constructs. When the reporters were co-transfected with each of the three expression vectors for nSREBP-1a, -1c, and -2, the results indicated that all three isoforms transactivated each reporter (Fig. 3). Transfected nSREBP-1c displays only 2-fold activation of the CYP4F2 reporters, whereas nSREBP-1a and nSREBP-2 show 19- and 9-fold activation, respectively.

Overexpressed SCAP-D443N Transactivates CYP4F2 Reporter Constructs in HepG2 Cells—An expression vector for a SCAP mutant, SCAP-D443N, was utilized to examine the effect of activating endogenous SREBP on the induction of CYP4F2 expression without overexpressing SREBP. The single amino acid substitution, D443N, in the sterol sensing domain of SCAP disables the sterol sensing function of SCAP and facilitates the SREBP cleavage pathway even in the presence of high intracellular sterol levels (39). An expression vector for either SCAP-D443N or wild type SCAP was co-transfected with the −179/+464 reporter construct into HepG2 cells and the cells were treated with MeSO or 1 μM Lovastatin for 24 h. In the MeSO-treated cultures, the cells co-transfected with wild type or mutant SCAP displayed higher luciferase levels than cells transfected with the vector control. In addition, the dominant positive SCAP-D443N mutant produced higher luciferase activity than wild type SCAP. When compared with cells treated with MeSO, lovastatin activation of the CYP4F2 reporter was seen in cells co-transfected with the vector control or the wild type SCAP. Although lovastatin further increases the reporter lucif-
erased expression levels in the presence of overexpressed SCAP-D443N, the activation extent is similar to the level seen with SCAP. These results indicate that increased nuclear SREBP levels transactivate CYP4F2 expression (Fig. 4A).

Overexpressed Insig-1 Inhibits Lovastatin Induction of CYP4F2 Reporter Constructs in HepG2 Cells—To further confirm that the induction of CYP4F2 expression by lovastatin requires SREBP activation, cells were transfected with an expression vector for Insig-1. A high ratio of Insig-1 to SCAP interferes with SREBP maturation even in the absence of exogenous sterols (40). Fig. 4B shows that lovastatin induction can be blocked by overexpressed Insig-1 further indicating that statin induction of CYP4F2 expression requires activation of SREBPs.

25-Hydroxycholesterol Abrogates Lovastatin Induction of Endogenous CYP4F2 Expression in HepG2 Cells—25-Hydroxycholesterol (25-OH) is a potent suppressor of SREBP activation in the regulation of cholesterol synthesis (41). This sterol derivative blocks transfer of the membrane-bound SREBP-2 precursor from endoplasmic reticulum to Golgi, and prevents proteolytic cleavage that produces the active nuclear form by preventing transfer of the membrane-bound SREBP-2 derivative blocks transfer of the membrane-bound SREBP-2 and prevents proteolytic cleavage that produces the active nuclear form by blocking expression levels in the presence of overexpressed SCAP-D443N, the activation extent is similar to the level seen with SCAP. These results indicate that increased nuclear SREBP levels transactivate CYP4F2 expression (Fig. 4A).

Overexpressed Insig-1 Inhibits Lovastatin Induction of CYP4F2 Reporter Constructs in HepG2 Cells—To further confirm that the induction of CYP4F2 expression by lovastatin requires SREBP activation, cells were transfected with an expression vector for Insig-1. A high ratio of Insig-1 to SCAP interferes with SREBP maturation even in the absence of exogenous sterols (40). Fig. 4B shows that lovastatin induction can be blocked by overexpressed Insig-1 further indicating that statin induction of CYP4F2 expression requires activation of SREBPs.

25-Hydroxycholesterol Abrogates Lovastatin Induction of Endogenous CYP4F2 Expression in HepG2 Cells—25-Hydroxycholesterol (25-OH) is a potent suppressor of SREBP-2 activation in the regulation of cholesterol synthesis (41). This sterol derivative blocks transfer of the membrane-bound SREBP-2 precursor from endoplasmic reticulum to Golgi, and prevents proteolytic cleavage that produces the active nuclear form by causing SCAP to bind to Insig (42). Although no statistically significant affect on endogenous CYP4F2 mRNA expression was seen when the HepG2 cells were treated with 25-OH alone, lovastatin induction of endogenous CYP4F2 gene expression was completely suppressed by the co-administration of 25-OH (Fig. 5). These results support the hypothesis that the induction of CYP4F2 expression by lovastatin is mediated through activation of SREBP-2.

FIGURE 6. Identification of lovastatin and SREBP-1a response regions in the CYP4F2 gene. A panel of reporter constructs corresponding to the CYP4F2 promoter (−753 + 464) was generated. A schematic of the CYP4F2 gene is shown to indicate the location of fragments relative to the start sites of transcription (+1) and translation (+463, ATG). The luciferase (Luc) reporter constructs (2 μg/well) were co-transfected with an expression vector for β-galactosidase (0.2 μg/well) to normalize transfection efficiency. Cells were then incubated in 1% FBS, DMEM containing the vehicle Me2SO (DMSO), or 1 μM lovastatin for another 24 h. The data were expressed as relative luciferase/β-galactosidase ratios for each reporter using the data obtained from Me2SO-treated cells transfected with the reporter vector control (pLuc) as 1. For the SREBP-1a experiments, the cells were also co-transfected with either the pcDNA-nSREBP-1a expression vector or the pcDNA vector control (20 ng/well). The data for each reporter are expressed relative to the data obtained from cells co-transfected with the reporter vector control (pLuc) and the expression vector control (pcDNA). At least three independent experiments were performed and a representative experiment is shown. Means ± S.E. were determined from three replicate samples for each data point. For the lovastatin experiment, statistical significance is indicated as: *, p < 0.05, and **, p < 0.005.

FIGURE 7. Two SREBP binding sites reside in the −176/−65 region of the CYP4F2 gene. Oligonucleotides spanning different areas of the −176/−65 region were synthesized. Double-stranded oligonucleotides were 32P-labeled and gel purified prior to incubation with rabbit reticulocyte lysates programmed with pcDNA-FLAG-nSREBP-1a or with the pcDNA vector control. For supershift analysis, anti-FLAG® M2 monoclonal antibody was used as positive controls. The nucleotide sequences were: HMGCS1, 5′-GGAAGTGGGGTGAGACTAG-3′; and LDLR, 5′-TTGCAGTGGGGTGATTTT-3′. The DNA-protein complexes are indicated (C). Complexes supershifted by the FLAG antibody are labeled (SC). The mobility of free probe is indicated (F).

Mapping the Lovastatin and SREBP-1a Response Elements in the CYP4F2 Gene—Serial deletion constructs were generated from the −179/+464 reporter to identify the lovastatin and
SREBP-1a response regions in the CYP4F2 gene. When these reporters were transfected into HepG2 cells, lovastatin-dependent activation was seen with the m24, m7, and m8 reporters. However, the lovastatin effect was lost when the m28 construct was used (Fig. 6). When these reporters were co-transfected with the nSREBP-1a expression vector, SREBP-1a responsiveness was also lost in the m28 construct (Fig. 6). Similar results were obtained when the SCAP-D443N expression vector was co-transfected with the deletion constructs (data not shown). This preliminary mapping suggested that the lovastatin and SREBP-1a response regions on the CYP4F2 gene are located in the m28 segment.

To identify SREBP binding elements, double-stranded oligonucleotides spanning the m28 region of the CYP4F2 gene were synthesized. In addition, oligos were also synthesized that correspond to characterized SREBP response elements (SREs)
in the LDL receptor (LDLR) and HMG-CoA synthase (HMGC51) genes (43). Rabbit reticulocyte lysate containing in vitro transcribed/translated FLAG-nSREBP-1a or control lysate was incubated with these $^{32}$P-labeled double-stranded oligonucleotides and analyzed using EMSA (Fig. 7). Two CYP4F2 double-stranded oligonucleotides, −169/−145 and −109/−83, display complexes with in vitro translated FLAG-nSREBP-1a that have a mobility similar to the complexes observed with the known SREs from the HMGC51 and LDLR genes. The complexes can be supershifted by the FLAG antibody confirming the presence of the in vitro translated nSREBP-1a in the binding complex. The minimal CYP4F2 SREBP binding sites were further localized using scanning mutagenesis and EMSA. Mutations located within the −169/−145 (site A) and −109/−83 (site B) regions that abolish SREBP binding define the minimal binding sites, which are indicated as “core” in Fig. 8.

**CHIP Assays**—To characterize the in vivo interaction of SREBP with the CYP4F2 gene promoter, ChiP analysis was performed utilizing an antibody against SREBP-1 and chromatin isolated from HepG2 cells transfected with either the expression vector for nSREBP-1a or the empty vector (pcDNA) (Fig. 9). Relative to the empty vector control samples, higher levels of anti-SREBP-1-immunoprecipitated CYP4F2 promoter DNA are present in samples isolated from cells transfected with the nSREBP-1a expression vector indicating that SREBP-1 binds to the promoter of the CYP4F2 gene. SREBPs interact with an SRE in the LDLR promoter (35, 44), and a specific PCR primer set for the appropriate region in the LDLR gene was used as a positive control. Significantly increased amounts of LDLR promoter DNA are also immunoprecipitated by anti-SREBP-1 antibody from nSREBP-1a transfected cells when compared with the levels seen for empty vector transfected controls. When the input chromatin samples from different treatments were examined to determine the available chromatin DNA, no significant differences in the input levels of the LDLR or CYP4F2 promoters were seen between the samples (Input in Fig. 9). In addition, control nonspecific IgG did not immunoprecipitate appreciable amounts of LDLR or CYP4F2 promoter DNA (IgG in Fig. 9). The ChiP data are concordant with the EMSA results (Fig. 7) and indicate that SREBP interacts with the CYP4F2 gene promoter. The ChiP results and the data obtained from transfection studies with Insig-1 as well as SCAP suggest that SREBP mediates the statin induction of CYP4F2 expression.

**Functional Analysis of CYP4F2 SREBP Binding Elements**—Oligonucleotides corresponding to both sites or harboring mutations that disrupted nSREBP binding in EMSA assays (Fig. 8, m11 and m24) were inserted into a reporter containing the thymidine kinase promoter (TK) and used for co-transfection studies in HepG2 cells. The results indicate that the two nSREBP binding sites identified in the CYP4F2 promoter region can be transactivated by overexpressing the nuclear form of either SREBP-2 (Fig. 10) or SREBP-1a (data not shown). Also, the reporters (m11 and m24) containing mutations in the two
binding sites displayed significantly diminished transactivation by nSREBPs. The data obtained with EMSA or the TK reporters indicate the potential for activation of both sites when the nSREBPs are overexpressed. However, the levels of mature, active SREBP in the normal cellular environment are low and tightly regulated (24, 45). To avoid the artificial and significantly higher levels of the normal cellular environment are low and tightly controlled overexpressed. However, the levels of mature, active SREBP in the potential for activation of both sites when the nSREBPs are by nSREBPs.

![Sequence comparison between CYP4F2 and CYP4F3 gene promoters](image)

**FIGURE 13. Sequence comparison between CYP4F2 and CYP4F3 gene promoters.** The transcription initiation site of the CYP4F2 gene is indicated (+1) and nucleotides are numbered relative to this location. Differences in the CYP4F3 gene are shown below the sequence of the CYP4F2 gene. A gap in the alignment at position −128 is indicated in the CYP4F2 sequence by dashes. The two SREBP binding sites identified in this study are boxed and labeled above the CYP4F2 gene as site A and site B. Potential transcription factor binding sites indicated by Zhang et al. (38) or identified by the MatInspector Program (50) are labeled: DR1, direct repeat 1; NF-Y, nuclear transcription factor Y; CREB, cAMP response element binding protein. A potential binding site forRAR-RXR complex implicated by Zhang et al. (38) overlaps the putative CREB site. Potential E-boxes are also indicated.

When the effects of lovastatin on reporter gene expression were investigated, the reporters containing mutations introduced into site B (Aw1/Bm and Am1/Bm) were no longer induced by lovastatin treatment (Fig. 11A). However, disruption of site A only (Am/Bwt) did not affect induction of the reporter by lovastatin, and the reporter displayed a response similar to the wild type constructs. When the reporter constructs harboring mutations were co-transfected with the constitutively active SCAP-D443N expression vector into HepG2 cells, similar results were observed for the mutants (Fig. 11B). Similar results were also obtained with the CYP4F2 reporter construct, −179/+464, which contains exon 1 and intron 1 (data not shown). Lovastatin has been reported to increase the nuclear form of SREBP-2 in HepG2 cells without activating SREBP-1 (33). Immunoblot results confirmed that lovastatin treatment also increased proteolytic processing of SREBP-2 (Fig. 12), but did not activate SREBP-1 (data not shown) in primary cultures of human hepatocytes. Our results suggest that site B (−109/−83) plays a dominant role in controlling −169/−152 (site A) and −109/−92 (site B) relative to the transcription start site. Mutations introduced into both sites abolished SREBP DNA binding. However, the transfection data suggest that site B is the primary element participating in statin induction and SCAP/SREBP transactivation. Our results support a regulatory role for SREBPs in CYP4F2 gene expression and indicate that the statin effect is mediated through activation of SREBP-2.

Although the statin-elevated protein band identified by the cross-reactive P450 4F2/4F3 antibody in immunoblots could reflect elevated expression of both CYP4F2 and CYP4F3B (Fig. 1, B and C), no significant statin effects on CYP4F3B mRNA levels were seen. Comparison of the CYP4F2 and CYP4F3 gene sequences reveals 80% sequence identity in the proximal promoter region (+1 to −168). However, the CYP4F2 SREBP binding site B, which is the principle site responsible for CYP4F2 transcriptional activation by statins and SREBPs, is located within a segment (−134 to −90) with divergent sequence (60% identity, Fig. 13). When a double-stranded oligonucleotide corresponding to the aligned CYP4F3 promoter sequence was synthesized and used in the EMSA, complexes were not seen with in vitro translated/transcribed nSREBP-1a (data not shown). The EMSA and real time RT-PCR results indicate that CYP4F2 gene expression is induced by statins and overexpressed SREBP, but the CYP4F3B gene is not.

The two CYP4F2 SREBP binding sites do not resemble the classical SRE sites, ATCCACCCCA or CTCAACAGGA, found in sterol-regulated genes, such as LDLR and HMGCS1. SREBPs also bind to other types of DNA elements (35) termed SRE-like sites whose consensus sequences are more diverse and are found in lipogenic genes, such as fatty acid synthase (FASN) and spot 14 (THSRP). The SREBPs isoform activation profile observed with SRE-like sequences in lipogenic gene targets is
shared with the site B identified in CYP4F2. Our transfection studies using reporters containing the CYP4F2 promoter indicate that SREBP-1a is the strongest transactivator, whereas SREBP-2 and SREBP-1c display progressively weaker activation (Fig. 3). Future studies will examine the potential role of SREBP-1a and -1c in the physiologic regulation of CYP4F2 expression. Several potential binding sites for transcription factors, such as nuclear transcription factor Y, C/EBP, CREB, and Sp1, can be identified by sequence similarity near the CYP4F2 site B (−109/−92), especially in the region between −134 to −109 (Fig. 13). It has been shown that these transcription factors can contribute to SREBP binding and to efficient target gene transactivation (44, 46–48). Experiments are in progress to identify the potential involvement of these transcription factors in SREBP-mediated CYP4F2 gene expression.

Recently, P450s 4F2 and 4F3B have been shown to be principle α-hydroxylases in human liver microsomes for metabolizing very long chain-saturated fatty acids (VLCFAs, i.e. C22:0, C24:0, and C26:0). This suggested that the induction of these enzymes could represent a potential therapeutic target for treatment of X-linked adrenoleukodystrophy (X-ALD) disease (15). X-ALD is a recessive genetic disease and is characterized by a defect in peroxisomal β-oxidation of very long chain fatty acids with secondary neuroinflammatory damage. X-ALD patients have elevated tissue and plasma levels of saturated and monounsaturated VLCFAs. Lovastatin has been used to treat X-ALD patients to reduce the accumulated plasma VLCFAs (49). Our results suggest that the effect of lovastatin on lowering plasma VLCFAs could be mediated, in part, through elevated expression of CYP4F2. Our preliminary studies using saturated fatty acids and Supersomes containing either recombinant P450 4F2 or P450 4A11 indicates that P450 4A11 prefers lauric acid (C12:0) and P450 4F2 prefers stearic acid (C18:0) (data not shown). Although the benefits of statin treatment for X-ALD have been questioned due to inconsistent outcomes, our CYP4F2 results suggest that early lovastatin treatment may offer potential benefits in controlling X-ALD disease as stearic acid is known to be the precursor for VLCFA synthesis.

SREBP-2 activation also provides a link between the regulation of CYP4F2 and the LDL receptor. This is consistent with the notion that CYP4F2 is up-regulated to oxidize excess fatty acids arising from the increased flux of cholesterol esters and triglycerides resulting from lipoprotein import. In addition to dietary fatty acids and phospholipids, chylomicrons transport a variety of lipids including vitamin E, which is a P450 4F2 substrate. The up-regulation of CYP4F2 may be analogous to the protection provided by the induction of P450 4A enzymes that is observed in animals when insulin levels decline, fatty acids are released from adipocytes, and liver fatty acid oxidation is induced by peroxisome proliferator-activated receptor α activation.

Acknowledgments—We thank Dr. Johan Ericsson for providing pcDNA-nSREBP-1a, Dr. Timothy Osborne for pcDNA-nSREBP-1c and pcDNA-nSREBP-2, and Dr. Jerome Lasker for rabbit anti-human CYP4F2 antibody. We also thank the Sam and Rose Stein trust for supporting the DNA Core Laboratory, Department of Molecular and Experimental Medicine, The Scripps Research Institute.
Statins Induce CYP4F2 Gene Expression

32. Liu, J., Zhang, F., Li, C., Lin, M., and Briggs, M. R. (2003) Arterioscler. Thromb. Vasc. Biol. 23, 90–96
33. Scharnagl, H., Schinker, R., Gierens, H., Nauck, M., Wieland, H., and Marz, W. (2001) Biochem. Pharmacol. 62, 1545–1555
34. Jiang, G., McKenzie, T. L., Conrad, D. G., and Shechter, I. (1993) J. Biol. Chem. 268, 12808–12824
35. Amemiya-Kudo, M., Shimano, H., Hasty, A. H., Yahagi, N., Yoshikawa, T., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Sato, R., Kimura, S., Ishibashi, S., and Yamada, N. (2002) J. Lipid Res. 43, 1220–1235
36. Datta, S., and Osborne, T. F. (2005) J. Biol. Chem. 280, 3338–3345
37. Shimomura, I., Bashmakov, Y., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12354–12359
38. Zhang, X., Chen, L., and Hardwick, J. P. (2000) Arch. Biochem. Biophys. 378, 364–376
39. Hua, X., Nohturfft, A., Goldstein, J. L., and Brown, M. S. (1996) Cell 87, 415–426
40. Yabe, D., Brown, M. S., and Goldstein, J. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 12753–12758
41. Yang, J., Sato, R., Goldstein, J. L., and Brown, M. S. (1994) Genes Dev. 8, 1910–1919
42. Adams, C. M., Reitz, J., De Brabander, J. K., Feramisco, J. D., Li, L., Brown, M. S., and Goldstein, J. L. (2004) J. Biol. Chem. 279, 52772–52780
43. Vallet, S. M., Sanchez, H. B., Rosenfeld, J. M., and Osborne, T. F. (1996) J. Biol. Chem. 271, 12247–12253
44. Bennett, M. K., and Osborne, T. F. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6340–6344
45. Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) J. Clin. Investig. 109, 1125–1131
46. Xiong, S., Chirala, S. S., and Wakil, S. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3948–3953
47. Nagai, M., Sakakibara, J., Nakamura, Y., Gejyo, F., and Ono, T. (2002) Biochem. Biophys. Res. Commun. 295, 74–80
48. Latasa, M. J., Griffin, M. J., Moon, Y. S., Kang, C., and Sul, H. S. (2003) Mol. Cell. Biol. 23, 5896–5907
49. Singh, I., Khan, M., Key, L., and Pai, S. (1998) N. Engl. J. Med. 339, 702–703
50. Quandt, K., Frech, K., Karas, H., Wingender, E., and Werner, T. (1995) Nucleic Acids Res. 23, 4878–4884