SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55γ

Anders Kallin,[1,*] Lene E. Johannessen,[1,2,†] Patrice D. Cani,[§] Catherine Y. Marbehant,[*] Ahmed Essaghir,[*] Fabienne Foufelle,[,††] Pascal Ferré,[**,††] Carl-Henrik Heldin,[†] Nathalie M. Delzenne,[§] and Jean-Baptiste Demoulin[3,*]**

Université catholique de Louvain,* Christian de Duve Institute of Cellular Pathology, Experimental Medicine Unit, Brussels, Belgium; Ludwig Institute for Cancer Research,† Uppsala, Sweden; Université catholique de Louvain,§ School of Pharmacy, Brussels, Belgium; Institut National de la Santé et de la Recherche Médicale (INSERM),** Unité Mixte de Recherche S872, Centre de Recherches des Cordeliers, Paris, France; and Université Pierre et Marie Curie,†† Paris, France

Abstract Sterol-regulatory element binding proteins (SREBPs) control the expression of genes involved in fatty acid and cholesterol biosynthesis. Using microarrays, we observed that mature SREBP-1 also induced the expression of genes unrelated to lipid metabolism, such as heme oxygenase 1 (HMOX1), plasma glutathione peroxidase, the phosphatidylinositol-3 kinase regulatory subunit p55γ, synaptic vesicle glycoprotein 2A, and COTE1. The expression of these genes was repressed upon addition of sterols, which block endogenous SREBP cleavage, and was induced by the statin drug mevinolin. Stimulation of fibroblasts with platelet-derived growth factor, which activates SREBP-1, had a similar effect. Fasted mice that were refed with a high-carbohydrate diet presented an increased expression of HMOX1 and p55γ in the liver. Overall, the transcriptional signature of SREBP-1 in fibroblasts stimulated by growth factors was very similar to that described in liver cells. We analyzed the HMOX1 promoter and found one SREBP binding site of the E-box type, which was required for regulation by SREBP-1a and SREBP-1c but was insensitive to SREBP-2. In conclusion, our data suggest that SREBP-1 regulates the expression of stress response and signaling genes, which could contribute to the metabolic response to insulin and growth factors in various tissues.—Kallin, A., L. E. Johannessen, P. D. Cani, C. Y. Marbehant, A. Essaghir, F. Foufelle, P. Ferré, C-H. Heldin, N. M. Delzenne, and J-B. Demoulin. **SREBP-1 regulates the expression of heme oxygenase 1 and the phosphatidylinositol-3 kinase regulatory subunit p55γ. *J. Lipid Res.* 2007.** 48: **1628–1636.**

Supplementary key words PIK3R3 • C1orf2 • GPx-3 • PDGF • HO1 • SV2A • stress response • growth factors • fatty acid • phospholipid and cholesterol synthesis • bilirubin • E-box • signal transduction

Sterol-regulatory element binding proteins (SREBPs) are ubiquitous transcription factors that control the expression of the enzymes involved in the biosynthesis of cholesterol and fatty acids (1). Three different SREBP proteins have been characterized: SREBP-1a, -1c, and -2. SREBP-1c is encoded by the same gene as SREBP-1a but has a shorter and weaker N-terminal transactivation domain as a result of the use of an alternative first exon. The DNA binding domain of SREBPs consists of a particular basic helix-loop-helix leucine zipper domain able to bind to two types of motifs found in SREBP target gene promoters: sterol-regulatory elements and E-boxes (2, 3).

SREBPs are produced as precursor proteins with two transmembrane domains inserted in the endoplasmic reticulum membrane (1). The C-terminal domain interacts with sterol-regulatory element binding protein cleavage-activating protein (SCAP), a cholesterol sensor. When the local concentration of cholesterol is low, SCAP escorts SREBPs via COPII-coated vesicles to the Golgi, where SREBPs are processed by two proteases, S1P and S2P, releasing the mature active transcription factor, which corresponds to the N-terminal half of the protein. When cells are loaded with cholesterol, SCAP interacts with Insig-1 and Insig-2 in the endoplasmic reticulum, thereby preventing SREBP translocation and processing.

Manuscript received 19 March 2007 and in revised form 16 April 2007.

Published, JLR Papers in Press, April 23, 2007.
DOI 10.1194/jlr.M700136-JLR200

Abbreviations: GPx-3, plasma glutathione peroxidase; HIF, hypoxia-inducible factor; HMOX1, heme oxygenase 1; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol-3 kinase; SCAP, sterol-regulatory element binding protein cleavage-activating protein; SCD, stearoyl-coenzyme A desaturase; SREBP, sterol-regulatory element binding protein; SV2A, synaptic vesicle glycoprotein 2A.

[1] A. Kallin and L. E. Johannessen contributed equally to this work.
[2] Present address of L. E. Johannessen: National Veterinary Institute, Section of Food and Feed Microbiology, Oslo, Norway.
[3] To whom correspondence should be addressed.
e-mail: jean-baptiste.demoulin@mexp.ucl.ac.be

SREBPs are key regulators of lipid metabolism in vivo. In the liver and the adipose tissue, SREBP-1c expression is strongly induced by dietary glucose and insulin (4, 5). In addition, it was suggested that insulin stimulates the processing of the SREBP-1 precursor and increases the stability of mature SREBP-1 (6, 7). SREBP-1c is responsible for lipid accumulation in the liver of mice that are fed a high-carbohydrate diet after fasting (4).

The regulation of lipid metabolism by SREBPs is not restricted to liver cells. Early studies showed that SREBP processing is required for cell proliferation in lipid-free medium (8). We and others have shown that growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor, and insulin-like growth factor-1 induce a strong accumulation of mature SREBPs in the nucleus of various cell types, resulting in increased membrane lipid synthesis associated with cell growth (9, 10). SREBP-1 has also been shown to be hyperphosphorylated and activated during mitosis (11). Activation of SREBPs by growth factors and insulin is mediated by the activation of the phosphatidylinositol-3 kinase (PI3K) and its downstream effector, protein kinase B (4, 9).

A large number of genes regulated by SREBPs in liver cells have been identified by systematic analysis of the expression of lipid metabolism enzymes and by microarray studies in mice carrying a transgene encoding an activated form of SREBP (1, 12, 13). SREBP-1c preferentially activates lipogenic genes (such as fatty acid synthase), whereas SREBP-2 acts more specifically on cholesterol biosynthesis genes (3). SREBP-1a regulates both metabolic pathways. In this study, we analyzed gene regulation by SREBP-1 in fibroblasts. Besides lipid metabolism genes, we found a number of SREBP-1-regulated transcripts that had not been connected to lipid metabolism previously. These include heme oxygenase 1 (HMOX1) and other stress response genes.

MATERIALS AND METHODS

Cell culture and reagents

AG01518 human foreskin fibroblasts (Coriell Institute for Medical Research, Camden, NJ) from passages 11–19 were cultured in modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum and glutamine. Cholesterol, 25-hydroxycholesterol, hemin, and mevinolin were from Sigma. HepG2 cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (Sigma) in the presence of 10% fetal calf serum. Active rat SREBP-1c[1–403] and mock adenoviruses were described elsewhere (5). Constructs encoding active human SREBP-1a, human SREBP-2, and rat SREBP-1c/ADD1 were kindly provided by Johan Ericsson (Uppsala, Sweden).

cDNA microarray analysis

Subconfluent AG01518 fibroblasts were infected with adenoviruses encoding SREBP-1c[1–403] or mock viruses at multiplicity of infection 60 per cell for 24 h, which was found to be optimal. Total RNA was isolated using the RNeasy kit (Qiagen) and used for microarray hybridization as described (9, 14). Briefly, total RNA (40 μg) from cells infected with SREBP-1c or mock viruses was labeled in reverse transcription reactions (SuperScript II kit; Invitrogen) with dCTP-Cy5 or dCTP-Cy3, respectively (Amersham). Purified cDNA probes labeled with Cy3 and Cy5 were mixed per pair and hybridized to cDNA microarray chips (Hver1.2.1) from the Sanger Institute/LICR/CRUK Consortium (see http://www.sanger.ac.uk/Projects/Microarrays/ for details and hybridization protocols). We performed three independent hybridizations, including one dye-swap control. Chips were scanned in a Perkin-Elmer/GSI Lumonics ScanArray 4000 scanner, and spot intensities were measured using QuantArray software (histogram method with background subtraction). Normalization and statistical analysis of the triplicate data sets were performed using GeneSpring 5.0 analysis software (Silicon Genetics). A Lowess nonlinear normalization was applied, and a statistical analysis of the data based on the global error model of GeneSpring was used. For all features selected using this protocol, the signal was significantly above the background, indicating that the expression of these genes was detectable. Finally, as Hver1.2.1 microarrays contain replicate spots (one to six) corresponding to the same gene, genes represented by spots that were not regulated in a similar manner were discarded. We show the average ratio of one representative spot for each regulated gene, with standard error calculated from three hybridizations and with the annotation provided by the microarray facility (Hver1.2.1_NCBI35). The data files were submitted to the databases ArrayExpress (accession number E-TABM-215) and GEO (accession number GSE6877).

Quantitative real-time PCR experiments were performed as described (9, 14).

Animal experiments

All studies were approved by the institutional review boards for the care and use of experimental animals. Male mice (25 g; NMRI, Harlan, The Netherlands) were divided into two groups (six mice per group): fasted (control) and fasted/refed with a high-carbohydrate diet. All animals had been fed a regular chow diet (A04; UAR, Villemoisson sur Orge, France) until the fasting and refeeding treatment started. The control group was fed regular chow diet ad libitum. Fasted/refed mice were fasted for 24 h (8:00 PM to 8:00 PM) and then refed with a high-carbohydrate/fat-free diet [40% (w/w) sucrose, 40% corn starch, 17% casein, 2% mineral mix, and 1% vitamin mix] for 24 h (8:00 PM to 8:00 PM). The fasting and refeeding cycle was repeated three times at 3 day intervals for the refeeding group to adapt the animals to the dietary manipulation. The final refeeding cycle was performed for 12 h (8:00 PM to 8:00 AM), and the control group was fasted for 24 h (8:00 AM to 8:00 AM) before euthanasia. Liver samples were immediately frozen in liquid nitrogen and stored at −80°C for further mRNA analysis.

Luciferase experiments

A fragment of the human HMOX1 promoter (311 bp including the transcription start) was amplified by PCR using the following oligonucleotides: 5′-TCAGATTTCCTTAAAGGTTTTGTGTG-3′ and 5′-CGAGAGGAGGCAGGCG-3′. The PCR product was purified, digested with *Kpn*I and *Hin*dIII (Fermentas), and cloned into pGL3basic (Promega). Mutants were produced using the Quickchange method (Stratagene). All constructs were verified by sequencing. HepG2 cells were seeded 1 day before transfection on 12 well plates (10^5 cells per well) and transfected with calcium phosphate precipitates, as described (9). The indicated promoters cloned into the pGL3 luciferase reporter (250 ng; Promega) were mixed with pDRIVE-chEF1-RU5′ (β-galactosidase reporter; Invitrogen; 250 ng) and pCDNA3 as carrier DNA. Cells

were washed, incubated in medium for 40 h, lysed, and processed as described (9). Data are presented as average ratios between the luciferase activity and the β-galactosidase content.

Western blots

Subconfluent AG01518 cells were either infected with adenoviruses as described above or starved for 24 h in the presence of 0.1% essentially fatty acid-free BSA (Sigma) and treated with PDGF-BB (25 ng/ml in starvation medium) or hemin (10 μM) for an additional 24 h. Cells were washed with phosphate-buffered saline and lysed [1% Triton, 0.2% sodium deoxycholate, 0.1% SDS, 140 mM NaCl, 10% glycerol, 50 mM Tris, pH 8, and 1 mM Pefabloc (Roche)]. Lysates were homogenized by passing through a 20 gauge needle and cleared by centrifugation. Equal amounts of total protein extracts were analyzed by SDS-PAGE followed by Western blotting with anti-p85 (UBI), anti-HMOX1 (Santa Cruz Biotechnology), anti-stearoyl-coenzyme A desaturase (SCD) (9), or anti-β-actin (Sigma) antibody, as described (15).

RESULTS

Gene regulation by SREBP-1 in human fibroblasts

Using cDNA microarrays, we have shown that growth factors activate SREBP-1 and induce the expression of SREBP target genes in normal human fibroblasts (9). To gain knowledge about gene regulation by SREBP-1 in fibroblasts, we expressed mature SREBP-1 in AG01518 cells. We infected these cells with an adenovirus encoding the active part of SREBP-1c [amino acids 1–403 (5)]. **Figure 1** shows that, 24 h after infection with the SREBP-1c virus, expression of SCD was strongly induced. The control adenovirus had no effect compared with noninfected cells (data not shown). We performed microarray analysis to identify other genes regulated by SREBP-1c. Cy3- and Cy5-labeled probes were prepared from RNA extracted from cells infected either with the control virus or with the SREBP-1c virus. These probes were hybridized onto Hver1.2.1 human cDNA microarrays provided by the Sanger Institute. We selected genes that were significantly regulated ($P < 0.05$) at least 2-fold based on an average of three independent experiments. Among the genes selected using these criteria, we found six genes that are involved in fatty acid biosynthesis and that have been shown to be regulated by SREBP-1c in liver cells (**Table 1**). Another 10 regulated genes are known targets of SREBP-1a and SREBP-2 (Table 1). It is likely that overexpression of SREBP-1c resulted in the activation of SREBP-1a target genes in our model, because these two factors share an identical DNA binding domain. The expression of most of these genes was increased by >3-fold. In conclusion, infection of fibroblasts with SREBP-1 adenoviruses induced the classical SREBP transcriptional signature described in hepatocytes.

We identified 11 other upregulated genes involved directly in lipid metabolism, such as mitochondrial citrate and carboxylate transport proteins (SLC25A1 and SLC16A1), lipoprotein receptors (LRP8 and OLR1), and genes involved in phosphatidylethanolamine synthesis (PCYT2, PISD, and PEBP1) (**Table 2**). These genes are likely novel targets of SREBP-1, although they are upregulated to a lesser extent compared with known SREBP target genes. Interestingly, expression of the citrate transporter SLC25A1 was increased in transgenic mice for SREBP-1a and SREBP-2 and was decreased in SCAP-deficient mice, according to published microarray results (see supplementary data in Ref. 13).

A surprisingly large number of genes that were significantly upregulated upon infection with the SREBP-1c virus are not directly related to lipid metabolism (**Table 3**). Because most known SREBP target genes were induced by >3-fold, we considered only the 25 genes that passed this threshold. Five genes that are not directly connected to lipid metabolism were also downregulated by SREBP-1c infection (data not shown). The induction of the top five upregulated genes in this category [phosphatidylinositol-3 kinase regulatory subunit p55γ (PIK3R3), synaptic vesicle glycoprotein 2A (SV2A), COTE1, plasma glutathione peroxidase (GPx-3), and HMOX1] in AG01518 fibroblasts was confirmed by quantitative PCR (Fig. 1A). None of these genes had previously been associated with lipid synthesis: PIK3R3 encodes the p55γ regulatory subunit of PI3K, an enzyme that plays a key role in insulin and growth factor signaling (16); SV2A is associated with exocytosis in neurones and endocrine cells (17); COTE1 encodes a hydrophobic protein of unknown function; GPx-3 encodes plasma glutathione peroxidase; and HMOX1 catalyzes the degradation of heme into biliverdin, releasing iron and carbon monoxide (18). Interestingly, biliverdin reductase B, an enzyme that converts biliverdin to bilirubin, was found to be upregulated in the liver of transgenic mice for SREBP-1a and SREBP-2 (13). HMOX1, biliverdin reductase, and GPx-3 exhibit strong antioxidant properties (18, 19).

Gene regulation by sterols and growth factors

We next showed that the SREBP-1c adenovirus also induced the expression of the selected genes in HepG2 hepatoma cells (Fig. 1C). To demonstrate that the selected genes are physiological targets of SREBP, we analyzed their expression in HepG2 cells cultured in the presence of delipidated serum. Cells were treated with 25-hydroxycholesterol, which blocks the endogenous processing of SREBP-1a and SREBP-2, or with mevinolin, a statin drug that inhibits HMG-CoA reductase, the rate-limiting enzyme of the cholesterol biosynthesis pathway, resulting in enhanced SREBP cleavage. As expected if these genes were controlled by the presence of mature SREBP in the nucleus, expression of p55γ, HMOX1, COTE1, GPx-3, and SV2A was enhanced by mevinolin and reduced by sterols, as observed for SCD, which was used as a positive control (Fig. 1C). The extent of regulation was lower compared with infection with SREBP-1c adenoviruses.

We have shown that PDGF and other growth factors activate SREBPs in fibroblasts and induce the expression of lipogenic enzymes (9). Stimulation of AG01518 cells with PDGF increased the expression of all potential SREBP-1 target genes except GPx-3 (Fig. 1). Together, our results

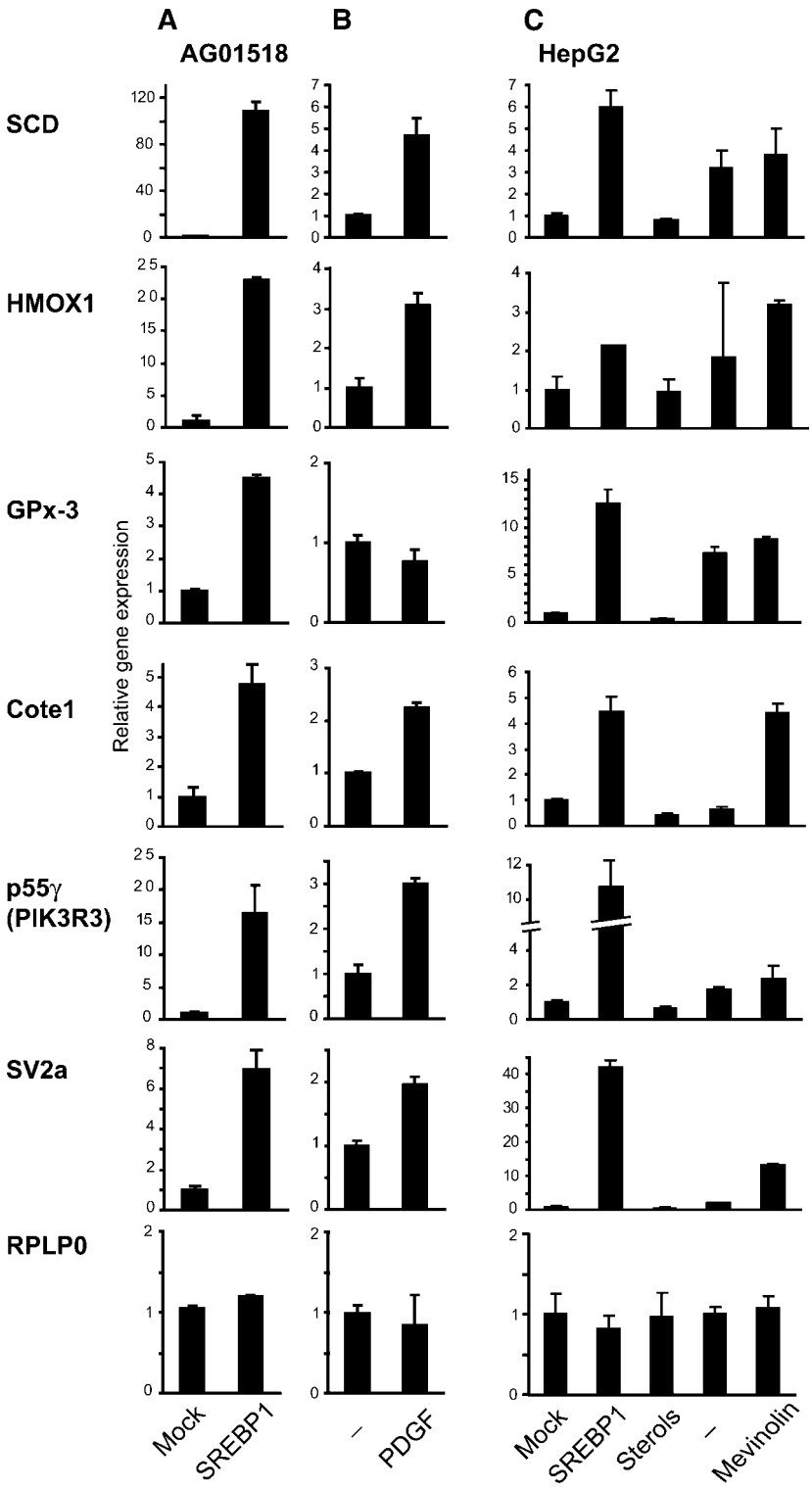

Fig. 1. Regulation of novel sterol-regulatory element binding protein-1 (SREBP-1) target genes by platelet-derived growth factor (PDGF), sterols, and mevinolin. A, B: AG01518 cells were infected with mock or SREBP-1c adenoviruses for 24 h (A) or starved for 24 h in the absence of serum and restimulated with PDGF-BB (25 ng/ml) for 24 h (B). RNA was extracted, and the indicated genes were analyzed by quantitative PCR. C: HepG2 cells were infected as described above or grown in medium containing delipidated serum and 25-hydroxycholesterol (0.5 μg/ml), mevinolin (0.1 μM), or vehicle (0.2% ethanol) (−) for 24 h. Results of one representative experiment with standard deviations based on triplicate PCR are shown. GPx-3, plasma glutathione peroxidase; HMOX1, heme oxygenase 1; PIK3R3, phosphatidylinositol-3 kinase regulatory subunit p55γ; RPLP0, 60S acidic ribosomalprotein P0; SCD, stearoyl-coenzyme A desaturase; SV2A, synaptic vesicle glycoprotein 2A.

TABLE 1. Known SREBP target genes induced by SREBP-1c adenovirus infection

Gene	Ratio	Ensembl Identifier	Description	PDGF
Fatty acid syznthesis				
SCD	54.6 ± 1.9	ENSG00000099194	Stearoyl-CoA desaturase	+[a]
ACAS2	20.2 ± 5.2	ENSG00000131069	Acetyl-CoA synthetase, cytoplasmic	+
FASN	14.3 ± 0.2	ENSG00000169710	Fatty acid synthase	+
ACLY	7.8 ± 0.2	ENSG00000131473	ATP-citrate lyase	+
ACAT2	6.4 ± 1.7	ENSG00000120437	Acetyl-CoA acetyltransferase 2	+
ELOVL5	4.7 ± 0.2	ENSG00000012660	Long-chain polyunsaturated fatty acid elongation	
Cholesterol synthesis				
HMGCR	27.9 ± 6.7	ENSG00000113161	3-Hydroxy-3-methylglutaryl-CoA reductase	+
HMGCS1	10.0 ± 3.2	ENSG00000112972	Hydroxymethylglutaryl-CoA synthase	+
SC4MOL	7.7 ± 1.4	ENSG00000052802	C-4 methyl sterol oxidase	+
EBP	5.4 ± 1.6	ENSG00000147155	3-β-Hydroxysteroid-delta(8),delta(7)-isomerase	+
LSS	4.0 ± 0.3	ENSG00000160285	Lanosterol synthase	+
DHCR7	3.9 ± 0.5	ENSG00000172893	7-Dehydrocholesterol reductase	+
SQLE	3.7 ± 0.1	ENSG00000104549	Squalene monooxygenase	+
DHCR24	2.2 ± 0.2	ENSG00000116133	24-Dehydrocholesterol reductase	+
Others				
ME1	8.7 ± 2.8	ENSG00000065833	NADP-dependent malic enzyme	
CYCS	2.0 ± 0.1	ENSG00000172115	Cytochrome C	+

PDGF, platelet-derived growth factor; SREBP, sterol-regulatory element binding protein. Fibroblasts were infected with SREBP-1c or control adenoviruses for 24 h. RNA was extracted and used to prepare fluorescent probes hybridized onto Hver1.2.1 microarrays. Average ratios of the expression in the presence of SREBP-1c versus controls are shown with SEM calculated from three independent experiments. This table includes genes that were significantly regulated ($P < 0.05$) by >2-fold and were previously shown to be regulated by SREBP factors. A complete list of regulated genes is available on our website (www.icp.be/mexp/pdgf).

[a] + indicates genes that are regulated by PDGF in the same cells (9).

show that the expression of p55γ, HMOX1, COTE1, and SV2A was correlated with endogenous SREBP-1 activation in vitro.

For two of the selected gene products, HMOX1 and p55γ, commercially available antibodies were suitable for Western blotting. Using an antibody directed against p85α/β, which cross-reacts with p55γ, we found that the expression of p55γ was induced in AG01518 cells upon infection with SREBP-1c virus and upon treatment with PDGF (**Fig. 2A**), in agreement with the RNA data. SREBP-1 did not change the expression of p85α and p85β. This was also confirmed by quantitative PCR (data not shown). HMOX1 protein level was also increased by infection of AG01518 cells with SREBP-1 virus (Fig. 2B). As a positive control, we treated cells with hemin, a well-characterized inducer of HMOX1 expression. Although HMOX1 was previously reported to be induced by PDGF (20), we did not observe any significant effect of PDGF on HMOX1 protein in AG01518 cells (data not shown).

Regulation of HMOX1 and p55γ in vivo

To determine whether p55γ, SV2A, COTE1, and HMOX1 were regulated in vivo, fasted mice were refed a high-carbohydrate diet, which induces SREBP-1c expression and activation in the liver. We used a pool of RNA extracted from livers of six mice in each group to hybridize to mouse microarrays. Genes upregulated to the highest extent (5- to 20-fold) included long-chain fatty acid elongase 5,

TABLE 2. Potential novel SREBP target genes involved in lipid metabolism

Gene	Ratio	Ensembl Identifier	Description
Phosphatidylethanolamine biosynthesis			
PCYT2	5.0 ± 1.3	ENSG00000185813	Ethanolamine-phosphate cytidylyltransferase
PISD	2.6 ± 0.8	ENSG00000100141	Phosphatidylserine decarboxylase
PEBP1	2.2 ± 0.3	ENSG00000089220	Phosphatidylethanolamine binding protein
Lipoprotein receptors			
LRP8	2.3 ± 0.2	ENSG00000157193	Apolipoprotein E receptor 2, isoform 3 precursor
OLR1	2.1 ± 0.4	ENSG00000173391	Oxidized low density lipoprotein receptor 1
Carboxylate transporters			
SLC25A1	2.9 ± 0.1	ENSG00000100075	Citrate transport protein, mitochondrial
SLC16A1	2.1 ± 0.4	ENSG00000155380	Monocarboxylate transporter 1
Cholesterol metabolism			
NPC1	3.2 ± 0.9	ENSG00000141458	Niemann-Pick C1 protein
NSDHL	2.4 ± 0.1	ENSG00000147383	NADP-dependent steroid dehydrogenase
Others			
DHRS3	3.1 ± 0.4	ENSG00000162496	Short-chain dehydrogenase/reductase 3
DLAT	4.6 ± 1.3	ENSG00000150768	Component of pyruvate dehydrogenase complex

Significantly regulated genes were selected as described in Table 1 and in Materials and Methods.

TABLE 3. Genes unrelated to lipid metabolism and significantly induced by SREBP-1c in human fibroblasts

Gene	Ratio	Ensembl Identifier	Description
PIK3R3[a]	10.3 ± 0.1	ENSG00000117461	Phosphatidylinositol 3-kinase regulatory γ subunit
Cote1[a]	7.5 ± 2.3	ENSG00000160767	
SV2A[a]	7.0 ± 0.1	ENSG00000159164	Synaptic vesicle glycoprotein 2a
GPx-3[a]	6.0 ± 2.6	ENSG00000211445	Plasma glutathione peroxidase
HMOX1[a]	5.4 ± 0.1	ENSG00000100292	Heme oxygenase 1
ANK2	4.7 ± 0.7	ENSG00000145362	Ankyrin 2
TAC1	4.1 ± 0.4	ENSG00000006128	Protachykinin 1
HB-EGF[a]	3.8 ± 1.2	ENSG00000113070	Heparin binding EGF-like growth factor
PTMS	3.7 ± 0.5	ENSG00000159335	Parathymosin
AF1q	3.7 ± 1.3	ENSG00000143443	Protein af1q
NEU1	3.6 ± 0.9	ENSG00000204386	Sialidase 1
LGALS7	3.6 ± 0.1	ENSG00000178934	Galectin-7
PRPS1	3.5 ± 1.1	ENSG00000147224	Ribose-phosphate pyrophosphokinase I
HRASLS3	3.5 ± 0.7	ENSG00000176485	h-rev 107 protein homolog
SGCD	3.5 ± 0.1	ENSG00000170624	Δ-Sarcoglycan
COL11A1	3.4 ± 0.3	ENSG00000060718	Collagen α1(xi)
PSCD1	3.4 ± 1.1	ENSG00000108669	Cytohesin 1
CABYR	3.4 ± 0.1	ENSG00000154040	Fibrous heathin II (FSP-2)
NUPR1	3.3 ± 0.4	ENSG00000176046	Nuclear protein p8, candidate of metastasis 1
HFE	3.3 ± 0.1	ENSG00000010704	Hereditary hemochromatosis protein precursor (HLA H)
KIAA0367	3.3 ± 0.6	ENSG00000106772	
ST4S6	3.2 ± 0.6	ENSG00000182022	N-Acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase
PDGFRB[b]	3.2 ± 1.0	ENSG00000113721	Platelet-derived growth factor receptor β
PPFIA3	3.1 ± 1.1	ENSG00000177380	Protein tyrosine phosphatase receptor type f
TSSC3[a]	3.0 ± 0.4	ENSG00000181591	Tumor-suppressing subtransferable candidate 3
SMG7	3.0 ± 1.0	ENSG00000116698	C1orf16

Significantly regulated genes were selected as described in Table 1 and in Materials and Methods.
[a] Confirmed by quantitative PCR.
[b] Not confirmed.

fatty acid synthase, glycerol-3-phosphate acyltransferase, and SREBP-1 (data not shown), which were all expected to be part of the lipogenic response (21). HMOX1 was the only new common hit between the lists of genes regulated by SREBP-1 in mouse liver and in human fibroblasts. We confirmed the regulation of HMOX1 in the liver of mice that received a high-carbohydrate diet by quantitative PCR (**Fig. 3**). The other selected genes were not represented on the mouse microarray and were tested by quantitative PCR. PIK3R3/p55γ RNA was also increased, with an average of ~2-fold. The expression of ribosomal protein gene 36B4, used as a control, was not changed. SV2A and COTE1 RNA were not detected in the liver, and GPx-3 was not regulated in these experiments (data not shown). As a control, we measured by quantitative PCR the expression of SCD2, which was strongly induced in three of six mice (Fig. 3). Together, these data show that the expression of HMOX1 and p55γ was correlated with SREBP-1c activation in vivo.

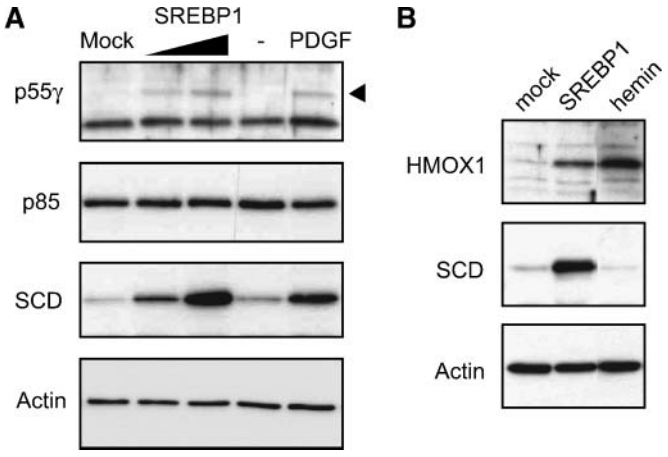

Fig. 2. SREBP-1 increases HMOX1 and p55γ protein expression. AG01518 cells were infected with SREBP-1c or mock adenoviruses for 24 h. Alternatively, cells were starved for 24 h in serum-free medium and treated with PDGF-BB (25 ng/ml; A) or hemin (10 μM; B) for an additional 24 h. Extracted proteins were analyzed by Western blotting with antibodies directed against p85α/β (which cross-reacts with p55γ; A), HMOX1 (B), SCD, and β-actin. The p55γ radiography was obtained from the p85 blot after longer exposure.

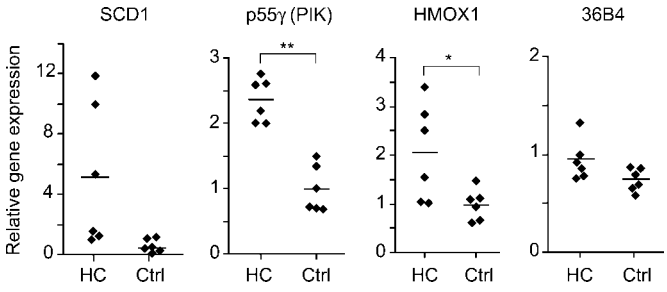

Fig. 3. HMOX1 and p55γ are regulated by a high-carbohydrate diet in the liver of fasted mice. Mice (six per group) were fasted for 24 h and then refed with a high-carbohydrate (HC) diet for 12 h. In the control (Ctrl) group, mice were euthanized after fasting. RNA was extracted from the liver and used for reverse transcription and quantitative PCR analysis. Student's *t*-test was performed (* $P < 0.05$, ** $P < 0.01$).

SREBP-1 activates the HMOX1 promoter

We next analyzed the proximal promoters of the selected genes using Genomatix softwares www.genomatix.de). Potential promoter regions were screened for SREBP binding sites that matched the Transfac matrices and that were conserved in mouse and human genes. We found one potential E-box-type SREBP binding site in the HMOX1 promoter, which was conserved in rat, mouse, human, chimpanzee, and chicken (**Fig. 4A** and data not shown). Putative sterol-regulatory element sites were also found in PIK3R3 and GPx-3 promoters but were not conserved. A 311 bp fragment of the human HMOX1 promoter including this site and the transcription start was cloned in front of a luciferase reporter gene. In HepG2 cells transfected with this construct, luciferase activity was induced by SREBP-1c and SREBP-1a but not by SREBP-2 (Fig. 4B). The difference between the two SREBP-1 isoforms was not statistically significant. The E-box was required for HMOX1 promoter regulation by SREBP-1, because a double mutation of the consensus CACGTG abolished the effect of SREBP-1 (Fig. 4). By contrast, the regulation of the HMG-CoA synthase promoter, which contains a classical sterol-regulatory element, was more sensitive to SREBP-1a and SREBP-2 than to SREBP-1c, as described (3). Similar observations were reported for the interleukin-8 gene promoter, which also includes a canonical sterol-regulatory element (22).

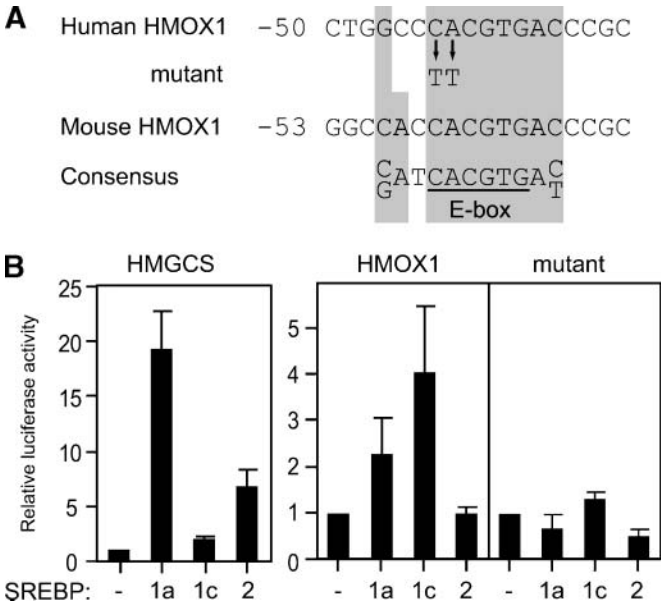

Fig. 4. SREBP-1 induces HMOX1 promoter activity. A: The HMOX1 promoter was analyzed in silico, and one potential SREBP binding site was found to be conserved between human and mouse sequences, which were aligned with a consensus SREBP binding site of the E-box type (2). B: A 311 bp fragment of the HMOX1 promoter containing the consensus E-box site was cloned in front of a luciferase reporter gene and transfected into HepG2 cells together with a plasmid encoding the active form of SREBP-1a, SREBP-1c, SREBP-2, or an empty vector. A mutant promoter in which the HMOX1 E-box was mutated to TTCGTG (as shown in A) was also analyzed. The HMG-CoA synthase (HMGCS) promoter was used as a control. Average normalized luciferase activities of three experiments are shown with standard errors.

DISCUSSION

In this report, we identified novel target genes for the SREBP-1 transcription factors, including HMOX1, p55γ, SV2A, and COTE1, based on the following observations: *i*) the corresponding transcripts were enriched in human fibroblasts infected with an adenovirus encoding mature SREBP-1c; *ii*) their expression was upregulated by mevinolin and repressed by sterols, which modulate the processing of endogenous SREBP-1a and SREBP-2; *iii*) all transcripts were also induced by stimulation of AG01518 fibroblasts with PDGF, which activates SREBP-1 (9); *iv*) finally, expression of HMOX1 and p55γ was increased in the liver of fasted mice refed with a high-carbohydrate diet, a condition known to activate SREBP-1c in vivo. GPx-3 expression was also regulated by SREBP-1c adenovirus and sterols but not by PDGF. Although GPx-3 was expressed in the liver, it was not affected by the carbohydrate diet, suggesting that the regulation of this gene is more complex.

The promoter region of the HMOX1 gene contains a conserved consensus SREBP binding site. We were unable to find such sites in the proximal promoter region of the other selected genes. Therefore, we cannot exclude the possibility that these genes are regulated in an indirect manner. Analysis of the proximal promoter of HMOX1 confirmed the direct regulation by both SREBP-1a and SREBP-1c and led to the identification of one potential regulatory E-box. SREBP-2 was not able to regulate the HMOX1 promoter, by contrast with the classical sterol-regulatory element-driven promoter of the HMG-CoA synthase gene. These results are in agreement with previous studies showing that SREBP-2 is not able to activate E-box-driven promoters (3). Because SREBP-2 can bind to E-box sequences, at least in gel-shift assays, it has been suggested that it is not able to cooperate with cofactors that are essential for the transactivation of E-box-driven lipogenic promoters (3).

The site identified in HMOX1 overlaps with a previously described hypoxia-inducible factor (HIF) binding site. Two other genes induced by SREBP-1c in AG01518 cells are also known to be induced by hypoxia: cyclin G2 (data not shown) and GPx-3 (23); other classical hypoxia response genes, such as vascular endothelial growth factor and adrenomedullin, were regulated in an opposite manner (data not shown), indicating that SREBP-1 did not activate HIF in fibroblasts. Whether SREBP can cooperate or compete with HIF on certain gene promoters, such as HMOX1 or GPx-3, should be studied further. Interestingly, yeast SREBP has been shown to act as an oxygen sensor (24).

HMOX1 catalyzes the degradation of heme into biliverdin, which is subsequently transformed into bilirubin by biliverdin reductase. The latter enzyme may also be regulated by SREBP-1, according to microarray data published by Horton and colleagues (13). HMOX1, which is

TABLE 4. Summary of atypical SREBP target genes unrelated to lipid metabolism

Gene Name (Regulation)	Function	Reference
IRS-2 (down)	Phosphatidylinositol-3 kinase signaling	34
PIK3R3/p55γ (up)	Phosphatidylinositol-3 kinase signaling	#
CDKN1A/WAF1/p21 (up)	Cell cycle inhibitor/ p53 response	35
DNAJA4 (up)	Stress response/heat shock	28
HMOX1 (up)	Stress response/heme degradation	#
Biliverdin reductase B (up)	Stress response/heme degradation	13
GPx-3 (up)	Oxidative stress response	#
Glutathione-*S*-transferase (up)	Oxidative stress response	13
SV2A (up)	Vesicle sorting	#
Cote1 (up)	Hydrophobic protein	#
Interleukin-8 (up)	Chemokine	22

Based on published reports and (#) on the present results.

upregulated by different types of cellular stresses, also presents potent antioxidant properties that are associated with the release of carbon monoxide, iron, and biliverdin from the heme molecule (18, 19). HMOX1-deficient cells are more sensitive to oxidative stress. Knockout animals are vulnerable to hepatic necrosis and die when challenged with endotoxin (19). Because SREBP-1 overexpression can lead to liver steatosis and stress (25, 26), we speculate that HMOX1 induction by SREBP-1 may be part of a protective response. In agreement with this hypothesis, increased HMOX1 expression was observed by Malaguarnera et al. (27) in nonalcoholic fatty liver disease patients. Together, our data support the hypothesis that SREBPs play a role in stress responses, as suggested previously by Robichon and colleagues (28, 29), who showed that SREBP-2 regulates the expression of the heat-shock protein DNAJA4. Strikingly, most of the genes that have been reported to be regulated by SREBPs and that are not related to lipid metabolism are involved in stress responses (**Table 4**).

Activation of SREBP-1 by insulin and growth factors depends on PI3K, an enzymatic complex composed of a p110 catalytic subunit associated with a regulatory subunit, which acts as an adaptor protein. There are five regulatory subunits for PI3K encoded by three genes: p85α, p55α, and p50α are encoded by PIK3R1, p85β is encoded by PIK3R2, and p55γ is encoded by PIK3R3. Differences in the expression of p85α and p85β were shown to affect PI3K activation. These regulatory subunits are required for PI3K activation, but when their concentration exceeds that of the catalytic subunit p110, free p85 may compete with PI3K complexes for binding to activated receptors (30, 31). Therefore, the upregulation of p55γ may represent a negative or positive feedback mechanism, depending on the local regulatory subunit concentration. It is also possible that p55γ transduces a signal that is slightly different from p85α and p85β, as p55γ has a unique N-terminal sequence that is able to bind to tubulin (32) and Rb (33). This is the second example of PI3K regulation by SREBP-1, which was previously shown to decrease the expression of IRS-2 (34), an adaptor protein that is essential for PI3K activation by insulin. Inhibition of IRS-2 expression by SREBP decreases the hepatocyte response to insulin in a negative feedback manner (34).

The potential roles of SV2A and COTE1 in the SREBP response are unknown. SV2A is a membrane protein associated with exocytosis in neurones and endocrine cells (17). Whether SREBP can regulate certain exocytic processes should be studied further. The COTE1 gene product does not contain any known motif except four potential transmembrane domains. This hydrophobic protein may play a role in lipid metabolism.

In conclusion, we have identified novel nonclassical mediators of the SREBP-1 response, including HMOX1 and p55γ, further supporting the hypothesis that SREBP-1 regulates stress response and signaling genes.

J-B.D. was the recipient of a Marie Curie Fellowship (European Commission). A.K. was supported by a Lacroix Fellowship from the Christian de Duve Institute of Cellular Pathology. L.E.J. was the recipient of a postdoctoral fellowship from the Norwegian Cancer Society. This work was supported in part through grants from the Belgian Federation against Cancer (a nonprofit organization) la Région Wallonne (WALEO), and the Funds for Scientific Research, Belgium. The authors thank Johan Ericsson for advice and reagents. The microarray consortium was funded by the Wellcome Trust, Cancer Research UK, and the Ludwig Institute for Cancer Research. The authors thank the staff of the Sanger Institute Microarray Facility http://www.sanger.ac.uk/Projects/Microarrays/) for the supply of arrays, laboratory protocols, and technical advice (David Vetrie, Cordelia Langford, Adam Whittaker, and Neil Sutton), Quantarray/GeneSpring data files, and all data analyses and databases relating to elements on the arrays (Kate Rice, Rob Andrews, Adam Butler, and Harish Chudasama). The human I.M.A.G.E. cDNA clone collection was obtained from the Medical Research Council Human Genome Mapping Project Resource Centre (Hinxton, UK). All cDNA clone resequencing was performed by Team 56 at the Sanger Institute.

REFERENCES

1. 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.
2. Kim, J. B., G. D. Spotts, Y. D. Halvorsen, H. M. Shih, T. Ellenberger, H. C. Towle, and B. M. Spiegelman. 1995. Dual DNA binding specificity of ADD1/SREBP1 controlled by a single amino acid in the basic helix-loop-helix domain. *Mol. Cell. Biol.* **15:** 2582–2588.
3. Amemiya-Kudo, M., H. Shimano, A. H. Hasty, N. Yahagi, T. Yoshikawa, T. Matsuzaka, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, et al. 2002. Transcriptional activities of nuclear SREBP-1a, -1c, and -2 to different target promoters of lipogenic and cholesterogenic genes. *J. Lipid Res.* **43:** 1220–1235.
4. Foufelle, F., and P. Ferre. 2002. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem. J.* **366:** 377–391.
5. Foretz, M., C. Guichard, P. Ferre, and F. Foufelle. 1999. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc. Natl. Acad. Sci. USA.* **96:** 12737–12742.

6. Hegarty, B. D., A. Bobard, I. Hainault, P. Ferre, P. Bossard, and F. Foufelle. 2005. Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c. *Proc. Natl. Acad. Sci. USA.* **102:** 791–796.
7. Sundqvist, A., M. T. Bengoechea-Alonso, X. Ye, V. Lukiyanchuk, J. Jin, J. W. Harper, and J. Ericsson. 2005. Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCF(Fbw7). *Cell Metab.* **1:** 379–391.
8. 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.
9. Demoulin, J. B., J. Ericsson, A. Kallin, C. Rorsman, L. Ronnstrand, and C. H. Heldin. 2004. Platelet-derived growth factor stimulates membrane lipid synthesis through activation of phosphatidylinositol 3-kinase and sterol regulatory element-binding proteins. *J. Biol. Chem.* **279:** 35392–35402.
10. Swinnen, J. V., H. Heemers, L. Deboel, F. Foufelle, W. Heyns, and G. Verhoeven. 2000. Stimulation of tumor-associated fatty acid synthase expression by growth factor activation of the sterol regulatory element-binding protein pathway. *Oncogene.* **19:** 5173–5181.
11. Bengoechea-Alonso, M. T., T. Punga, and J. Ericsson. 2005. Hyperphosphorylation regulates the activity of SREBP1 during mitosis. *Proc. Natl. Acad. Sci. USA.* **102:** 11681–11686.
12. Sakakura, Y., H. Shimano, H. Sone, A. Takahashi, N. Inoue, H. Toyoshima, S. Suzuki, N. Yamada, and K. Inoue. 2001. Sterol regulatory element-binding proteins induce an entire pathway of cholesterol synthesis. *Biochem. Biophys. Res. Commun.* **286:** 176–183.
13. Horton, J. D., N. A. Shah, J. A. Warrington, N. N. Anderson, S. W. Park, M. S. Brown, and J. L. Goldstein. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA.* **100:** 12027–12032.
14. Demoulin, J. B., M. Enarsson, J. Larsson, A. Essaghir, C. H. Heldin, and K. Forsberg-Nilsson. 2006. The gene expression profile of PDGF-treated neural stem cells corresponds to partially differentiated neurons and glia. *Growth Factors.* **24:** 184–196.
15. Demoulin, J. B., J. K. Seo, S. Ekman, E. Grapengiesser, U. Hellman, L. Ronnstrand, and C. H. Heldin. 2003. Ligand-induced recruitment of Na^+/H^+-exchanger regulatory factor to the PDGF (platelet-derived growth factor) receptor regulates actin cytoskeleton reorganization by PDGF. *Biochem. J.* **376:** 505–510.
16. Pons, S., T. Asano, E. Glasheen, M. Miralpeix, Y. Zhang, T. L. Fisher, M. G. Myers, Jr., X. J. Sun, and M. F. White. 1995. The structure and function of p55PIK reveal a new regulatory subunit for phosphatidylinositol 3-kinase. *Mol. Cell. Biol.* **15:** 4453–4465.
17. Xu, T., and S. M. Bajjalieh. 2001. SV2 modulates the size of the readily releasable pool of secretory vesicles. *Nat. Cell Biol.* **3:** 691–698.
18. Otterbein, L. E., M. P. Soares, K. Yamashita, and F. H. Bach. 2003. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol.* **24:** 449–455.
19. Poss, K. D., and S. Tonegawa. 1997. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc. Natl. Acad. Sci. USA.* **94:** 10925–10930.
20. Durante, W., K. J. Peyton, and A. I. Schafer. 1999. Platelet-derived growth factor stimulates heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2666–2672.
21. Achouri, Y., B. D. Hegarty, D. Allanic, D. Becard, I. Hainault, P. Ferre, and F. Foufelle. 2005. Long chain fatty acyl-CoA synthetase 5 expression is induced by insulin and glucose: involvement of sterol regulatory element-binding protein-1c. *Biochimie.* **87:** 1149–1155.
22. Yeh, M., A. L. Cole, J. Choi, Y. Liu, D. Tulchinsky, J. H. Qiao, M. C. Fishbein, A. N. Dooley, T. Hovnanian, K. Mouilleseaux, et al. 2004. Role for sterol regulatory element-binding protein in activation of endothelial cells by phospholipid oxidation products. *Circ. Res.* **95:** 780–788.
23. Bierl, C., B. Voetsch, R. C. Jin, D. E. Handy, and J. Loscalzo. 2004. Determinants of human plasma glutathione peroxidase (GPx-3) expression. *J. Biol. Chem.* **279:** 26839–26845.
24. Hughes, A. L., B. L. Todd, and P. J. Espenshade. 2005. SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast. *Cell.* **120:** 831–842.
25. Qi, N. R., J. Wang, V. Zidek, V. Landa, P. Mlejnek, L. Kazdova, M. Pravenec, and T. W. Kurtz. 2005. A new transgenic rat model of hepatic steatosis and the metabolic syndrome. *Hypertension.* **45:** 1004–1011.
26. Horton, J. D., I. Shimomura, S. Ikemoto, Y. Bashmakov, and R. E. Hammer. 2003. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J. Biol. Chem.* **278:** 36652–36660.
27. Malaguarnera, L., R. Madeddu, E. Palio, N. Arena, and M. Malaguarnera. 2005. Heme oxygenase-1 levels and oxidative stress-related parameters in non-alcoholic fatty liver disease patients. *J. Hepatol.* **42:** 585–591.
28. Robichon, C., M. Varret, X. Le Liepvre, F. Lasnier, E. Hajduch, P. Ferre, and I. Dugail. 2006. DnaJA4 is a SREBP-regulated chaperone involved in the cholesterol biosynthesis pathway. *Biochim. Biophys. Acta.* **1761:** 1107–1113.
29. Robichon, C., and I. Dugail. 2007. De novo cholesterol synthesis at the crossroads of adaptive response to extracellular stress through SREBP. *Biochimie.* **89:** 260–264.
30. Ueki, K., D. A. Fruman, S. M. Brachmann, Y. H. Tseng, L. C. Cantley, and C. R. Kahn. 2002. Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. *Mol. Cell. Biol.* **22:** 965–977.
31. Mauvais-Jarvis, F., K. Ueki, D. A. Fruman, M. F. Hirshman, K. Sakamoto, L. J. Goodyear, M. Iannacone, D. Accili, L. C. Cantley, and C. R. Kahn. 2002. Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. *J. Clin. Invest.* **109:** 141–149.
32. Inukai, K., M. Funaki, M. Nawano, H. Katagiri, T. Ogihara, M. Anai, Y. Onishi, H. Sakoda, H. Ono, Y. Fukushima, et al. 2000. The N-terminal 34 residues of the 55 kDa regulatory subunits of phosphoinositide 3-kinase interact with tubulin. *Biochem. J.* **346:** 483–489.
33. Xia, X., A. Cheng, D. Akinmade, and A. W. Hamburger. 2003. The N-terminal 24 amino acids of the p55 gamma regulatory subunit of phosphoinositide 3-kinase binds Rb and induces cell cycle arrest. *Mol. Cell. Biol.* **23:** 1717–1725.
34. Ide, T., H. Shimano, N. Yahagi, T. Matsuzaka, M. Nakakuki, T. Yamamoto, Y. Nakagawa, A. Takahashi, H. Suzuki, H. Sone, et al. 2004. SREBPs suppress IRS-2-mediated insulin signalling in the liver. *Nat. Cell Biol.* **6:** 351–357.
35. Inoue, N., H. Shimano, M. Nakakuki, T. Matsuzaka, Y. Nakagawa, T. Yamamoto, R. Sato, A. Takahashi, H. Sone, N. Yahagi, et al. 2005. Lipid synthetic transcription factor SREBP-1a activates p21WAF1/CIP1, a universal cyclin-dependent kinase inhibitor. *Mol. Cell. Biol.* **25:** 8938–8947.