Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 1049–1053, February 1996
Biochemistry

Sterol regulation of acetyl coenzyme A carboxylase: A mechanism for coordinate control of cellular lipid

JOSE M. LOPEZ, MARY K. BENNETT, HUGO B. SANCHEZ, JOHN M. ROSENFELD, AND TIMOTHY F. OSBORNE*

Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717

Communicated by Jan L. Breslow, The Rockefeller University, New York, NY, October 24, 1995 (received for review August 9, 1995)

ABSTRACT **Transcription from the housekeeping promoter for the acetyl coenzyme A carboxylase (ACC) gene, which encodes the rate-controlling enzyme of fatty acid biosynthesis, is shown to be regulated by cellular sterol levels through novel binding sites for the sterol-sensitive sterol regulatory element binding protein (SREBP)-1 transcription factor. The position of the SREBP sites relative to those for the ubiquitous auxiliary transcription factor Sp1 is reminiscent of that previously described for the sterol-regulated low density lipoprotein receptor promoter. The experiments provide molecular evidence that the metabolism of fatty acids and cholesterol, two different classes of essential cellular lipids, are coordinately regulated by cellular lipid levels.**

Fatty acids and cholesterol are both major constituents of animal cell membranes and their cellular levels must rise and fall together to provide a balanced supply for membrane biosynthesis and turnover during normal cellular growth. In addition, the liver plays a special role in lipid homeostasis since cholesterol and fatty acids are packaged into very low density lipoprotein (VLDL) particles that deliver their lipid to other sites in the body to maintain fat homeostasis (1). It is likely that there is a feedback mechanism to modulate the accumulation of both cholesterol and fatty acids for optimal cell growth and VLDL assembly.

Coordinate regulation could be accomplished at least in part through the AMP-dependent protein kinase, which phosphorylates and inactivates both 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and acetyl coenzyme A carboxylase (ACC), the rate-controlling enzymes of cholesterol and fatty acid biosynthesis, respectively (2). While it is clear that a high ratio of AMP to ATP stimulates the kinase, other metabolic factors that modulate its activity are not well understood. Therefore, a role for the kinase in coordinating regulation in response to cellular lipid levels is unclear.

Transcriptional control of genes that encode important enzymes of both cholesterol and fatty acid metabolism would be an alternative level for coordinate regulation to occur, and recent work has identified a family of activator proteins that could link the two pathways together. These sterol regulatory element binding proteins (SREBPs) activate transcription of the low density lipoprotein (LDL) receptor and HMG CoA synthase genes, which are essential for cholesterol uptake and biosynthesis, respectively (3, 4). cDNAs for SREBP-1 and -2 were cloned with specific oligonucleotide probes predicted by the amino acid sequence of the purified polypeptides (3, 4). Both SREBP proteins bind the same cis-acting elements in the LDL receptor and HMG CoA synthase promoters and activate expression only when cellular sterol levels fall below that required for optimal cell function (5).

Independently, SREBP-1 was cloned from an adipocyte cDNA expression library with a DNA recognition site probe containing a special "E-box" sequence (6). Its mRNA was expressed in several different tissues, at exceedingly high levels in brown fat, and it was induced during adipocyte differentiation in cell culture. Hence, SREBP-1 may be a regulator of genes that are important for lipid accumulation, and it was alternatively called the adipocyte determination and differentiation-dependent factor 1 (ADD1).

The above studies indicate that SREBP-1/ADD1 may provide a direct link between regulation of cholesterol and fatty acid metabolism. ACC, the rate-controlling enzyme of fatty acid biosynthesis, forms the three-carbon malonyl CoA molecule, which donates the two-carbon unit during each cycle of fatty acid chain growth (7). The ACC gene is transcribed from two promoters, PI and PII, which result in alternatively spliced mRNAs containing different 5′ noncoding regions (8). PI is active in white fat and is activated during lipogenesis in the liver (9). In contrast, PII is a housekeeping type of promoter, which is active in all tissues at a low level (10). Here we report that ACC mRNA levels are subject to down-regulation identically to mRNA levels for genes that control intracellular cholesterol levels. We further show that the PII promoter for ACC is specifically regulated by sterols through the action of transcription factor Sp1 and the SREBP proteins in a manner reminiscent of the LDL receptor.

MATERIALS AND METHODS

Cloning ACC DNA Fragments. A 409-bp human cDNA fragment for ACC was cloned by PCR with cDNA prepared from HepG2 mRNA with a cDNA synthesis kit from Invitrogen according to the manufacturer's instructions. PCR primers were designed to hybridize to 30-bp regions close to the N-terminus of the protein. The resulting PCR product corresponds to bases 61–570 of the published human ACC mRNA coding sequence (11). The PCR product was then cloned into the PCR II cloning vector (Invitrogen), which was subsequently excised by *Eco*RI and inserted into M13mp18. A clone containing the strand equal to the mRNA was used to prepare a single-stranded probe for S1 nuclease protection analysis as described (12).

The rat PII promoter from −994 to −238 was isolated by PCR from rat genomic DNA. This fragment was linked to its own transcription initiation region (−6 to +10) and inserted into the multiple cloning site of basic pGL2 (Promega) upstream of the luciferase reporter coding sequence. The DNA sequence of the entire insert was confirmed and shown to be identical to the published rat sequence (10). Mutant derivatives of the wild-type ACC PII promoter were made by PCR-assisted deletion from the 5′ side (mutants A and B). Mutant C was made by using PCR to remove bases from the 3′ side of mutant B. Mutant D was made by cutting at a natural *Sac* I site at −281 and deleting all sequence 3′ to this site. ACC fragments from mutants C and D were inserted upstream of a

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ACC, acetyl coenzyme A carboxylase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; SREBP, sterol regulatory element binding protein; HLHzip, basic helix–loop–helix zipper; HMG, 3-hydroxy-3-methylglutaryl.

*To whom reprint requests should be addressed.

basal TATA box upstream of the luciferase coding sequence (13).

Cell Culture and DNA Transfections. HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and plated on day 0 in 100-mm dishes at 225,000 cells per dish. On day 2, the cells were fed either induced (DMEM containing 10% lipoprotein-depleted serum plus 1 μM mevacor) or suppressed (the same but containing 10 μg of cholesterol per ml and 1 μg of 25-OH cholesterol per ml) medium. After a 24-hr incubation, the cells were harvested and total RNA was prepared as described (14).

All plasmids were analyzed for sterol regulation by a transient DNA transfection assay in CV-1 cells. This assay has been described (13). The fold regulation is the ratio of normalized luciferase activity expressed when transfected cells were cultured in the absence or presence of regulatory sterols. The standard error and number of individual experiments performed on duplicate dishes for each plasmid are shown in Fig. 2. The non-sterol-regulated cytomegalovirus promoter β-galactosidase expression construct was included as an internal control for normalization.

Drosophila SL2 cells were cultured at 25°C in Shields and Sang *Drosophila* medium (Sigma) containing 10% heat-inactivated fetal bovine serum. They were seeded at 1.2×10^6 cells per 60-mm dish and transfected by a standard calcium phosphate coprecipitation method (13).

DNase I Footprinting and Gel Mobility-Shift Analyses. The recombinant human SREBP-1 (amino acids 1–490) and native human Sp1 proteins were purified as described (13). Briefly, a cDNA fragment encoding the above-mentioned fragment was inserted into the pRSET vector (Invitrogen) and expressed as a fusion protein to the 6-histidine tag sequence. The resulting fusion protein was purified by nickel chelation chromatography. Sp1 was purified by DNA recognition site affinity chromatography after elution from wheat germ agarose fractionation beginning with a nuclear extract prepared from HeLa cells. The ACC promoter fragment used as the footprint probe was labeled on the top DNA strand at position −400. The conditions for DNase footprinting were essentially as described (13).

The binding conditions and electrophoresis for the gel mobility-shift studies have also been described (13). The LDL receptor probe contained a single copy each of repeats 2 and 3 of the wild-type human LDL receptor promoter. The ACC PII promoter probe corresponds to the sequence from −294 to −238 and is underlined in Fig. 4*C*.

Enzyme Assays. Luciferase activities were measured in a luminometer with a luciferin reagent from Promega Biotec. The protein concentration of all samples was determined with the Bio-Rad kit. β-Galactosidase assays were performed by a standard colorimetric procedure with *o*-nitrophenyl β-galactoside as substrate (15). The ratio of luciferase activity in relative light units was divided by β-galactosidase activity (activity/hr) for each extract.

RESULTS

Sequence inspection of the rat PII promoter revealed a potential SREBP binding site beginning at nucleotide position −977 relative to the mRNA start site that was confirmed by gel mobility-shift experiments with recombinant SREBP-1 protein (data not shown). This suggested that ACC mRNA might be regulated by sterols similar to other SREBP-activated genes such as the LDL receptor and HMG CoA synthase. Therefore, we analyzed the expression of ACC mRNA in human HepG2 cells, which were cultured in the presence or absence of regulatory sterols (Fig. 1).

The results demonstrate that ACC mRNA is high in cells cultured in the absence of regulatory sterols and is suppressed when cells are fed sterols (Fig. 1 *Top*). This expression pattern

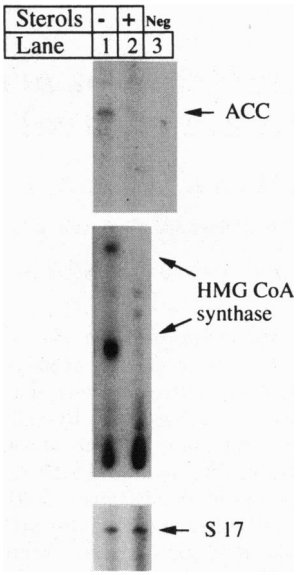

FIG. 1. ACC mRNA expression is regulated by cellular sterol levels. Total cell RNA was prepared from HepG2 cells cultured in the presence or absence of regulatory sterols as described. (*Top*) Fifty-microgram aliquots of RNA from treated (lane 1) or untreated (lane 2) cells were analyzed for ACC mRNA by an S1 nuclease assay (12, 16). Probe alone (neg) digested with S1 nuclease is in lane 3. (*Middle*) Fifty-microgram aliquots of the same RNA as above were used for HMG CoA synthase mRNA detection by a primer-extension assay, which generates two 5′ ends (16). (*Bottom*) Twenty-microgram aliquots of the same RNA as above were used to detect transcription of ribosomal protein S17 by a primer-extension assay (16).

is characteristic of genes that are regulated by intracellular sterol levels, such as HMG CoA synthase whose mRNA level was also analyzed by a primer-extension assay (16) with aliquots of the same RNA samples (Fig. 1 *Middle*). In contrast, the transcript for the non-sterol-regulated ribosomal protein S17 was present at similar levels in the two RNA samples (Fig. 1 *Bottom*). Therefore, ACC mRNA expression is regulated by intracellular sterol levels similarly to the major genes that control cholesterol metabolism.

To further evaluate regulation of ACC expression by SREBP and sterols, the sequence of the rat PII promoter from −994 to −238, which contains the SREBP binding site at −977, an E-box beginning at −931, and other potential important promoter elements, was fused to the luciferase reporter gene as described. This fragment was chosen since it contains the elements we wished to evaluate, but it lacked a more proximal part of the minimal promoter that contains a basal level enhancer element (10) and could mask potential sterol regulation in a transient transfection assay. We have noted a similar basal element in the promoter for HMG CoA synthase (S. M. Vallett, K. A. Dooley, L. Yieh, and T.F.O., unpublished data).

The full-length promoter was analyzed by a transient DNA transfection assay for sterol regulation and the results are shown in Fig. 2. When transfected cells were cultured in the absence of sterols, the reporter was expressed 4.4-fold higher than in the presence of sterols. This magnitude of sterol regulation was similar to that observed for the endogenous ACC mRNA (Fig. 1) and for the wild-type HMG CoA synthase and LDL receptor promoters analyzed similarly (see legend to Fig. 2). A deletion of all of the sequence upstream of −400 did not diminish regulation (plasmids A and B in Fig. 2). In addition, the activity of the −400 construct was significantly stimulated by cotransfection with a mammalian expression vector encoding a transcriptionally active fragment of the SREBP-1 protein (Fig. 3). In fact, the degree of activation by SREBP-1 was similar to that observed for the wild-type LDL

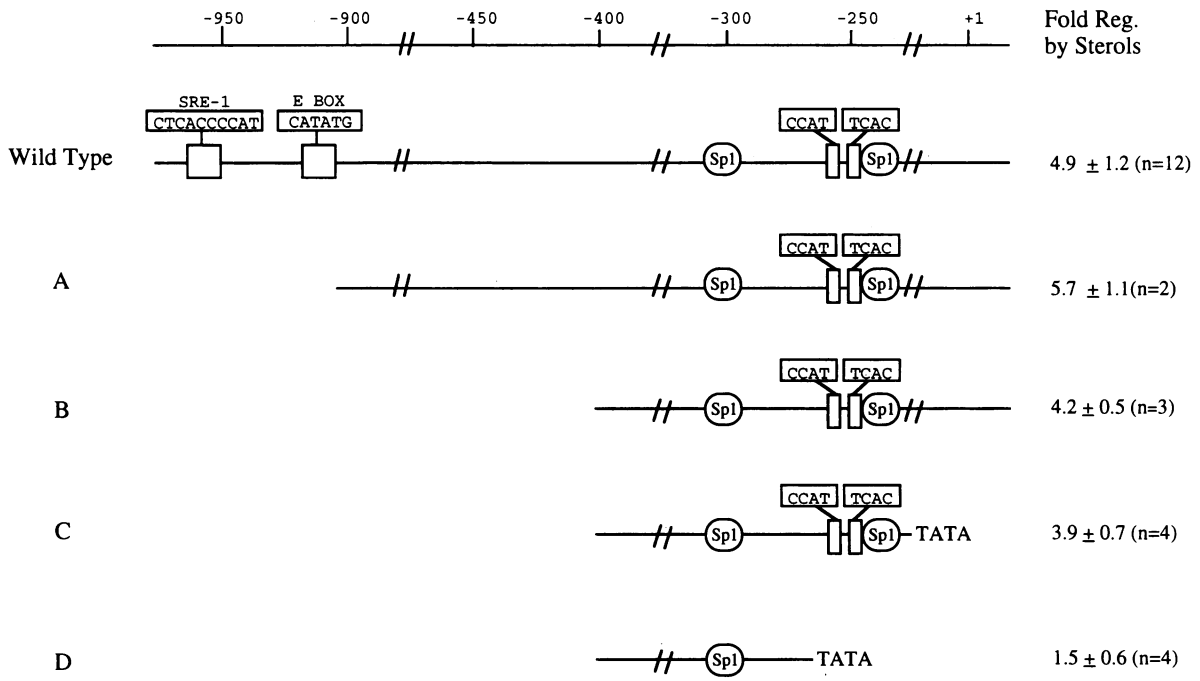

FIG. 2. Regulation of ACC PII promoter by sterols and identification of novel sterol regulatory elements that are activated by SREBP. Promoter deletion series is shown. Scale for the DNA sequence of the rat ACC PII promoter is shown at the top (10). Notable sequence features are diagrammed on the line corresponding to the wild-type promoter. There is a 10-bp SRE-1-like sequence at −977 (5′-CTCACCCCAT-3′), which differs from the wild-type human LDL receptor SRE-1 at positions 1 and 10. SREBP-1 binds this site with an affinity comparable to that for the LDL receptor SRE-1 (S. M. Vallett, K. A. Dooley, L. Yieh, and T.F.O., unpublished observations). An E-box motif (5′-CATATG-3′) is present beginning at −931. Two binding sites for Sp1 and two potential half-site consensus SRE-1 sites are indicated. The wild-type HMG CoA synthase and LDL receptor promoters were analyzed similarly and -fold regulation values for these were 7.9 and 3.7, respectively.

receptor promoter (Fig. 3). These studies indicated that the consensus SRE site at −977 and the E-box at −931 are not crucial for sterol regulation and suggested that there were cryptic sterol regulatory DNA elements between −400 and −238 that bind SREBP.

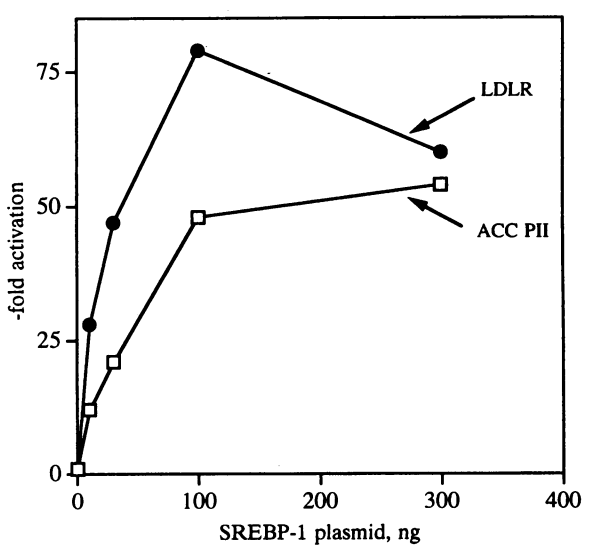

FIG. 3. Activation of ACC PII promoter by overexpression of SREBP-1. HepG2 cells were cultured in DMEM and 10% (vol/vol) fetal bovine serum and transfected with ACC PII plasmid C or wild-type LDL receptor (LDLR) promoter by a standard protocol except that a cytomegalovirus promoter-based construct expressing amino acids 1–490 of the human SREBP-1 protein was included in the amounts indicated on the abscissa. The fold activation is the ratio of normalized activity (luciferase/β-galactosidase) obtained with SREBP-1 cotransfection divided by the normalized activity in the absence of cotransfection.

To evaluate SREBP binding in this region, we performed a standard DNase I protection assay with recombinant SREBP-1 protein (13) and a DNA fragment labeled at −400 of the ACC promoter. This experiment identified a region of SREBP binding between −286 and −240 (Fig. 4*A*, compare lanes 1–3). This large footprint and the presence of a hypercleavage site in the middle suggested that there might be two or more separate sites that bind SREBP. Therefore, we prepared oligonucleotide probes containing the sequence from −294 to −238 for use in gel mobility-shift experiments (13). We consistently observed two SREBP–DNA complexes with this DNA probe (Fig. 4*B*, lanes 1–5), whereas only one SREBP–DNA complex formed with a probe containing the LDL receptor sterol regulatory element (Fig. 4*B*, lanes 6–10). These results are consistent with two separate sites for SREBP in this region of the ACC promoter. It is noteworthy that this sequence interval does not contain a recognizable SRE-like site or an E-box motif. However, there are two "half sites" (noted in Fig. 2) that may each be the core recognition site for SREBP (3, 18).

This SREBP binding region is close to a binding site for Sp1 that was also detected by the DNase I footprint assay with purified Sp1 (Fig. 4*A*, lane 5). There is another Sp1 binding site upstream of the SREBP binding region (see Fig. 4 *A* and *C*). A similar grouping of a SREBP binding site between two Sp1 sites is critical for sterol regulation of the LDL receptor promoter (19). When binding of SREBP and Sp1 were analyzed together on the ACC promoter, bases normally protected by SREBP were more accessible to digestion by DNase I (Fig. 4*A*, compare lanes 2–4). An analogous result was obtained when SREBP and Sp1 were both bound to the LDL receptor (13) and suggests that Sp1 partially displaces SREBP from the DNA. This parallelism suggests that SREBP and Sp1 activate the ACC and LDL receptor promoter by a similar mechanism. Competition studies with oligonucleotides containing either the LDL receptor SREBP or unrelated nonspecific DNA

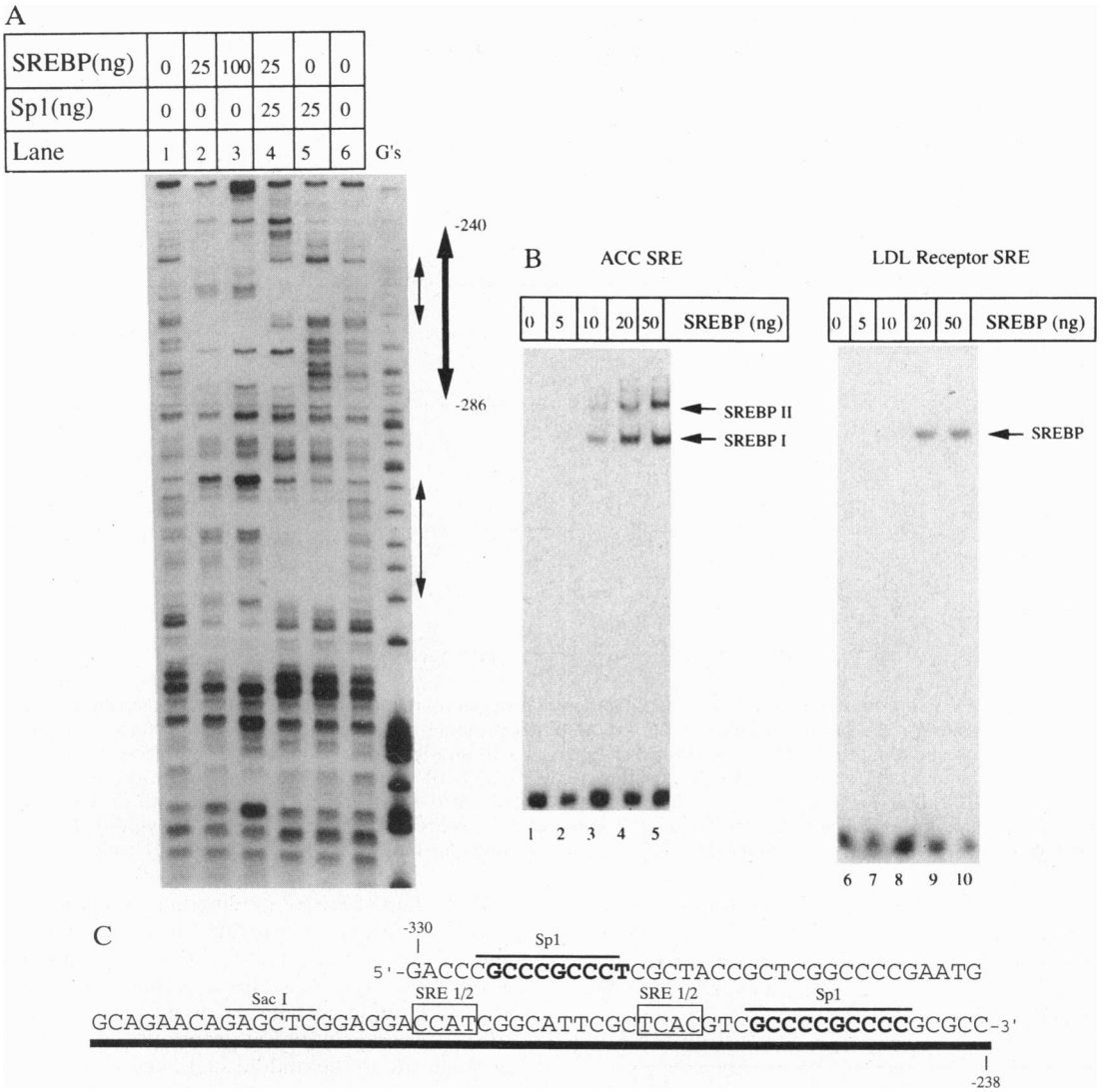

FIG. 4. Binding sites for SREBP and Sp1 in the sterol regulatory region of ACC PII promoter. (*A*) Purified recombinant SREBP-1 (amino acids 1–490) or Sp1 purified from HeLa cell extracts used in a standard DNase I footprint analysis with a probe labeled on the top strand of the ACC promoter essentially as described (13). Amounts of each protein used are indicated. A chemical DNA sequencing track for G residues (17) was included as a marker. Thick vertical line denotes the SREBP binding area; thin lines mark the Sp1 binding regions. (*B*) Oligonucleotides representing bases −294 to −238 of the PII promoter (10) were used as the ACC probe. A probe for the LDL receptor promoter SREBP binding site and methods for mobility-shift assays have been described (13). ACC (lanes 1–5) or LDL receptor (lanes 6–10) probes were incubated with the indicated amounts of purified recombinant SREBP-1 protein (amino acids 1–490). Positions of the SREBP–DNA complexes are indicated. (*C*) Sequence of rat ACC PII promoter top strand from −330 to −238. Recognition sites for Sp1 are overlined and are in boldface. SRE half sites CCAT and TCAC are boxed. The natural *Sac* I site used to delete sequence from the 3′ side of plasmid C to generate plasmid D in Fig. 2 is shown. Sequence of the oligonucleotide used in the mobility-shift experiment in *B* is underlined.

binding sites also confirmed that SREBP binding to the ACC promoter fragment was the result of a specific interaction (data not shown).

The potential involvement of these noncanonical SREBP recognition sites in sterol regulation of the ACC promoter was evaluated by analyzing two additional promoter fusion plasmids. The sequence from −400 to −238, which contains the two SREBP sites and the adjacent Sp1 site was inserted next to a generic TATA site to provide a minimal promoter. This construct was regulated by sterols as efficiently as the plasmid that was deleted from the 5′ side down to −400 (Fig. 2, plasmid C). Next, we deleted 47 bases from the 3′ side, which included the proximal Sp1 site and the adjacent SREBP recognition sites. When this plasmid was transfected into cells, it was not regulated by sterols (Fig. 2, plasmid D). Therefore, the region of the ACC PII promoter that contains the two noncanonical SREBP sites and the adjacent Sp1 site is crucial for sterol regulation.

To directly evaluate activation of the ACC PII promoter by SREBP and Sp1 together, we performed cotransfection studies with SREBP and Sp1 expression plasmids in *Drosophila* SL2 cells. These cells are devoid of both SREBP and Sp1, yet they retain all of the accessory regulatory proteins to mount a response to exogenous Sp1 (20). We have used this system previously to demonstrate that SREBP and Sp1 synergistically activate the LDL receptor promoter (13). When the ACC promoter plasmid C was transfected into SL2 cells alone, a very low level of promoter activity was observed (Fig. 5, lane 1). The inclusion of plasmids that express either Sp1 or SREBP alone did not significantly alter this low level of activity (Fig. 5, lanes 2 and 3); however, when both expression constructs were added, a 300-fold activation was observed (lane 4). In contrast, mutant D was not activated (Fig. 5, lanes 5–8). These results indicated that SREBP-1 and Sp1 synergistically activate the ACC PII promoter through the elements that are essential for sterol regulation.

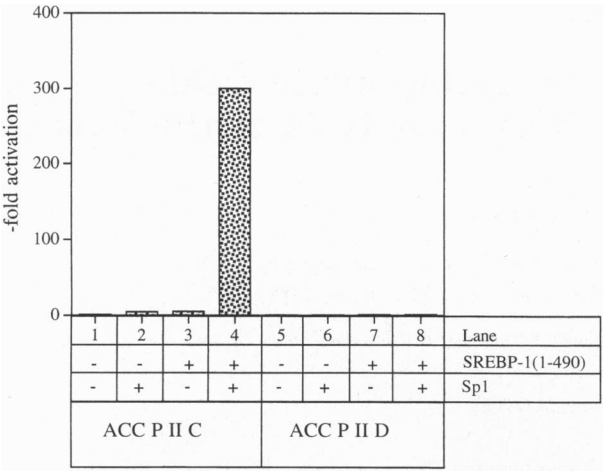

FIG. 5. Activation of ACC PII promoter by SREBP-1 and Sp1 in SL2 cells. ACC PII promoter mutant C (lanes 1–4) or D (lanes 5–8) was transfected (2 μg of reporter plasmid per dish) and analyzed for reporter enzyme activity. Plasmids were used alone (lanes 1 and 5) or with 50 ng of an expression vector encoding Sp1 (lanes 2 and 6) or SREBP-1 (lanes 3 and 7) alone or with 50 ng of both expression vectors (lanes 4 and 8). Activity obtained when the reporter plasmid was transfected alone was set at 1.0 and all values are plotted relative to this value. These results show a typical experiment performed on duplicate dishes.

DISCUSSION

These experiments demonstrate that ACC, the gene encoding the rate-controlling step of fatty acid biosynthesis, is regulated by cellular sterols in a manner similar to important genes of cholesterol homeostasis. The housekeeping promoter for ACC contains novel sterol regulatory DNA binding sites for SREBP that are adjacent to a binding site for the ubiquitous transcription factor Sp1. This is reminiscent of the LDL receptor promoter where it was previously shown that Sp1, and SREBP are both essential for sterol regulation.

SREBP-1 is an unusual basic helix–loop–helix zipper (bHLHzip) transcription factor that binds to a subset of consensus palindromic E-box motifs as well as to the direct repeat of the LDL receptor SRE-1. This dual binding specificity results from a tyrosine substitution for an arginine, which is highly conserved in the DNA binding domain of bHLHzip proteins (21). The sequence of the essential SREBP binding sites in the ACC promoter does not closely resemble either the SRE-1 consensus element of the LDL receptor promoter or an E-box consensus site. Interestingly, other bHLHzip proteins bind non-E-box (and non-SRE-1) sites with high affinity (22). However, because these additional sites were identified by *in vitro* site selection, they did not represent known physiologic targets. Since SREBP is a related bHLHzip protein, the identification of a functionally significant noncanonical target region in the ACC promoter is unique. There are two potential SREBP half sites in the region (3, 18) and it will be interesting to investigate the binding site specificity for SREBP in more detail in the future. The ACCAT half-site sequence (Fig. 4*C*) is identical to a SREBP binding site that is crucial for sterol regulation in the promoter for HMG CoA reductase that was identified with single nucleotide precision (unpublished data).

The involvement of SREBP-1 in transcriptional control of both fatty acid synthesis and cholesterol homeostasis would ensure regulated membrane metabolism by maintaining a balance in the cellular level of two essential membrane lipids. Also, this would provide a mechanism to coordinate accumulation of cholesteryl ester and triglyceride for the assembly of VLDL particles in the liver.

SREBP-2, a second member of the SREBP family, has been identified (4). The reason for the existence of two SREBPs, however, is not yet clear. Since SREBP-1 is highly expressed in fat (6) and SREBP-2 is activated by cholesterol deprivation in the liver at the expense of SREBP-1 (23), the regulation for these two proteins and their physiologic targets in the body may be partially distinct. Further studies analyzing the role of each SREBP in the activation of distinct target genes of cholesterol or fatty acid regulation both under different physiological conditions and in different organs will help identify the separate roles for these two closely related transcriptional activator proteins.

We thank J. Goldstein, M. Brown, and P. Edwards for helpful comments on the manuscript. This work was supported in part by grants from the Lucille P. Markey Charitable Trust (91-8) and the National Institutes of Health (HL48044) and by a gift from ZENECA (ICI Pharmaceuticals). H.B.S. is the recipient of a minority fellowship from the National Institutes of Health, T.F.O. is an Established Investigator of the American Heart Association, and J.M.R. is the recipient of a predoctoral fellowship from the American Heart Association California Affiliate.

1. Glickman, R. M. & Sabesin, S. M. (1988) in *Lipoprotein Metabolism*, eds. Arias, I. M., Jakoby, W. B., Popper, H., Schachter, D. & Shafritz, D. A. (Raven, New York), pp. 331–354.
2. Hardie, D. G. (1992) *Biochim. Biophys. Acta* **1123,** 231–238.
3. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Wa, X., Goldstein, J. L. & Brown, M. S. (1993) *Cell* **75,** 185–197.
4. Hua, X., Yokoyama, C., Wu, J., Briggs, M. R., Brown, M. S., Goldstein, J. L. & Wang, X. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11603–11607.
5. Wang, X., Sato, R., Brown, M. S., Hua, X. & Goldstein, J. L. (1994) *Cell* **77,** 53–62.
6. Tontonoz, P., Kim, J. B., Graves, R. A. & Spiegelman, B. M. (1993) *Mol. Cell. Biol.* **13,** 4753–4759.
7. Kim, K.-H. (1983) *Curr. Top. Cell. Regul.* **22,** 143–175.
8. Kim, K.-H. & Tae, H.-J. (1994) *J. Nutr.* **124,** 1273S–1283S.
9. Lopez-Casillas, F., Ponce-Castenada, V. & Kim, K.-H. (1991) *Endocrionolgy* **129,** 1049–1058.
10. Luo, X. & Kim, K. H. (1990) *Nucleic Acids Res.* **18,** 3249–3254.
11. Ha, J., Daniel, S., Kong, I.-S., Park, C.-K., Tae, H.-J. & Kim, K.-H. (1994) *Eur. J. Biochem.* **219,** 297–306.
12. Osborne, T. F., Gil, G., Brown, M. S., Kowal, R. C. & Goldstein, J. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3614–3618.
13. Sanchez, H. B., Yieh, L. & Osborne, T. F. (1995) *J. Biol. Chem.* **270,** 1161–1169.
14. Chirgwin, J. M., Przbyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
16. Metherall, J. E., Goldstein, J. L., Luskey, K. L. & Brown, M. S. (1989) *J. Biol. Chem.* **264,** 15634–15641.
17. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
18. Smith, J. R. (1990) *Feedback Regulation of Genes Controlling Cholesterol Metabolism* (Univ. of Texas Southwestern Medical School, Dallas).
19. Sudhof, T., Van der Westhuyzen, D., Goldstein, J., Brown, M. & Russell, D. (1987) *J. Biol. Chem.* **262,** 10773–10779.
20. Courey, A. J. & Tjian, R. (1988) *Cell* **55,** 887–898.
21. Kim, J. B., Spotts, G. D., Halvorsen, Y.-D., Shih, H.-M., Ellenberger, T., Towle, H. C. & Spiegelman, B. M. (1995) *Mol. Cell. Biol.* **15,** 2582–2588.
22. Blackwell, T. K., Huang, J., Ma, A., Kretzner, L., Alt, F. W., Eisenman, R. N. & Weintraub, H. (1993) *Mol. Cell. Biol.* **13,** 5216–5224.
23. Sheng, Z., Otani, H., Brown, M. S. & Goldstein, J. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 935–938.