CBP Is Required for Sterol-regulated and Sterol Regulatory Element-binding Protein-regulated Transcription*

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Cells were transfected with luciferase reporter genes, under the control of promoters derived from either the farnesyl diphosphate (FPP) synthase, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, or low density lipoprotein receptor genes. The increase in luciferase activity that occurred when cells were either incubated in sterol-depleted medium or cotransfected with a cDNA encoding sterol regulatory element-binding protein (SREBP)-1a was prevented by coexpression of wild-type E1A or a Gal4-CBP (1–451) fusion protein. The inhibitory effect of E1A was overcome by coexpression of CBP. The increase in reporter gene activity noted above was not affected when the cells were cotransfected with cDNAs that encoded either a mutant E1A that is unable to interact with the transcriptional activator CBP or Gal4-CBP fusion proteins encoding separate fragments of CBP, which span the remainder of the CBP molecule. A preformed SREBP-1a;[32P]DNA complex bound specifically to membrane-immobilized GST-CBP fusion proteins that contained amino-terminal portions of CBP.

In order to investigate the role of CBP in the regulation of endogenous genes, we isolated stable transfectants that express Gal4-CBP(1–451) in response to added doxycycline. Induction of endogenous FPP synthase and HMG-CoA synthase mRNAs, in response to cellular cholesterol depletion, was prevented when cells expressed Gal4-CBP(1–451).

We conclude that when cells are incubated in the absence of sterols, the transcriptional activation of the HMG-CoA synthase, HMG-CoA reductase, FPP synthase, and low density lipoprotein receptor genes is dependent on a specific interaction between SREBP, which is bound to the promoter DNA, and the amino-terminal domain (amino acids 1–451) of CBP.

Cellular cholesterol homeostasis is maintained, in part, as a result of coordinate transcriptional regulation of genes that encode both enzymes involved in cholesterol synthesis (e.g. HMG-CoA1 synthase, HMG-CoA reductase, FPP synthase, and squaleone synthase) and the LDL receptor, a protein required for endocytosis of LDL (reviewed in Ref. 1). The transcription of these genes is induced in cells deprived of cholesterol and repressed in cholesterol-loaded cells (1). Transcriptional induction is absolutely dependent on the binding of SREBP to the promoters of these genes (1–5). This conclusion is supported by the observation that the mRNAs of many of these genes are elevated in mutant cells that constitutively overexpress nuclear SREBP-2 (6–8) and are barely detectable in mutant cells that are unable to produce mature, nuclear-targeted SREBPs (7, 8). This conclusion is also supported by studies that have utilized reporter constructs controlled by the promoters derived from these same genes; induction of these reporter constructs is dependent on the interaction of SREBP with the promoter (1–5). However, promoter-reporter genes are inactive if the sequences upstream of the TATA box contain only SREBP binding sites (9, 10). A number of studies have demonstrated that additional transcription factors, so far limited to NF-Y, Sp1, and Sp3, are necessary for high levels of transcription of SREBP-dependent genes. Detailed studies that utilized the FPP synthase proximal promoter demonstrated that the interaction of NF-Y heterotrimers with two motifs (ATTGG and CCAT) resulted in a synergistic increase in the binding of SREBP to an adjacent motif (10, 11). We have hypothesized that, in sterol-deprived cells, the NF-Y-dependent binding of SREBP to the FPP synthase promoter results in enhanced transcription of the gene. NF-Y and SREBP are also required for the sterol-regulated transcription of the HMG-CoA synthase (12), GPAT (13), and SREBP-2 (14) genes. In contrast, high transcription of the LDL receptor (4), fatty acid synthase (9), and acetyl CoA carboxylase (15) genes requires that SREBP and Sp1 (or Sp3) (16) bind to the promoters. Detailed studies with the LDL receptor promoter have demonstrated that SREBP binds to SRE-1 and results in a synergistic increase in the binding of Sp1 to an adjacent motif (4).

The human SREBP proteins were purified and cloned based on their ability to bind to SRE-1, a functionally important 10-base pair element previously identified in the promoter of the LDL receptor gene (reviewed in Ref. 1). Subsequently, two SREBP genes were identified, SREBP-1 and SREBP-2 (1). As a result of alternate splicing of the SREBP-1 mRNA, these two genes can produce three separate proteins, i.e., SREBP-1a, SREBP-1c, and SREBP-2 (1). Adipocyte determination- and differentiation-dependent factor 1, the rat homologue of SREBP-1c, was shown to have an important role in the differentiation of preadipocytes to adipocytes (17). It was originally cloned as a result of experiments designed to identify transcription factors that bound to the E-box motif located in the promoter regulatory element; SREBP, SRE-binding protein; NF-Y, nuclear factor Y; LDL, low density lipoprotein; CBP, CREB-binding protein; GST, glutathione S-transferase; GPAT, glycerol-3-phosphate acyltransferase; LPDS, lipoprotein-deficient serum.

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The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; FPP, farnesyl diphosphate; CMV, cytomegalovirus; SRE, sterol regulatory element; SREBP, SRE-binding protein; NF-Y, nuclear factor Y; LDL, low density lipoprotein; CBP, CREB-binding protein; GST, glutathione S-transferase; GPAT, glycerol-3-phosphate acyltransferase; LPDS, lipoprotein-deficient serum.
moter of the fatty acid synthase gene (17). Subsequent studies demonstrated that two separate SREBP complexes could form on the fatty acid synthase promoter through interactions with four distinct half sites, two of which were present in the E-box motif (18).

SREBPs are unique transcription factors that are synthesized as 125-kDa precursor proteins, anchored in the endoplasmic reticulum via two transmembrane domains (reviewed in Ref. 1). Cellular sterol deprivation results in activation of an as yet unidentified protease that cleaves SREBPs at a specific site within the luminal loop (1). A second proteolytic event then occurs in the amino-terminal transmembrane domain of SREBP and results in the release of a 68-kDa amino-terminal fragment (1). This portion of the protein contains the basic helix-loop-helix-leucine zipper transactivation/dimerization domain and a nuclear targeting motif (1). The 68-kDa SREBP fragment enters the nucleus, binds to sterol regulatory elements, and activates transcription of target genes (see above). The mechanism of transactivation of these target genes and the role of other proteins/factors in this process have not been clearly elucidated.

CBP and p300 fall into a class of proteins termed coactivators that are thought to form a bridge between DNA-specific transcriptional activators and the basal transcriptional machinery (reviewed in Refs. 19–21). CBP was originally identified as a result of its interaction with the cAMP response element-binding protein (22). More recently, it has been established that different domains of CBP interact with a number of different transcription factors, with components of the pol II complex and with additional coactivators, such as p300- and CBP-associated factor (reviewed in Refs. 19–21 and 23). p300- and CBP-associated factor and CBP have acetyltransferase activity and, as such, are thought to be involved in the acetylation of histones and alteration of chromatin structure (21, 23–25). In a recent report, CBP was also shown to be capable of acetylating p53, a DNA-specific transcription factor (26).

E1A, derived from adenovirus, is known to be a multifunctional protein that affects diverse cellular functions as a result of alterations in the transcription of a large number of genes (reviewed in Refs. 19–21 and 25). Recent studies have demonstrated that E1A and p300- and CBP-associated factor bind to a similar domain of CBP (19–21). Thus, overexpression of E1A in cells can lead to displacement of p300- and CBP-associated factor from CBP and a decrease in the transcription of CBP-dependent genes, presumably due to the decrease in histone/protein acetylation (19–21).

In a recent study, Oliner et al. (27) demonstrated that recombinant SREBP interacted with an amino-terminal domain (amino acids 1–682) of CBP. In addition, transient transfection of cells with a plasmid encoding CBP resulted in an additional 2–4-fold increase in the activity of an LDL receptor-promoter gene that had been activated by cotransfection with a plasmid encoding the 68-kDa amino-terminal fragment of SREBP (27). These results implicate CBP in SREBP-dependent transcription of the LDL receptor gene, a process that is known to require both SREBP and Sp1 (1, 4). The role, if any, of Sp1, in the CBP-mediated activation has not been determined.

In the current study, we investigated the role of CBP in the physiological regulation of a number of genes, the transcription of which is regulated in response to alterations in cellular sterols.

**EXPERIMENTAL PROCEDURES**

**Materials**—DNA restriction and modification enzymes were obtained from Life Technologies, Inc. 32P-Labeled nucleotide triphosphates were obtained from Amersham Pharmacia Biotech. pSETB (Invitrogen) containing both a partial sequence of SREBP-1a (amino acids 1–490), both T7 and polyhistidine tags, and pCMV-CSA10, which encodes amino acids 1–490 of SREBP-1a, were kindly provided by Dr. T. Osborne (Department of Molecular Biology and Biochemistry, University of California, Irvine, CA). Constructs encoding E1A or Gal4- and GST-CBP fusion proteins under control of the CMV promoter were generous gifts of Drs. B. Lüschér and M. Austen (Institut für Molekularbiologie, Heinrich-Heine-Universität, Düsseldorf, Germany) (28). The construct encoding the mutant E1A protein (pCMV-E1AΔ64–68) in which amino acids 64–68 have been deleted was originally described by Bannister and Kouzariades (25) and was kindly provided by Dr. J. Darnell, Rockefeller University, New York, NY. pCMV-CBP (29) was a generous gift from Dr. C. Glass (Department of Cellular and Molecular Medicine, University of California, San Diego, CA). Lipoprotein-deficient fetal calf serum (LPDS) was purchased from HyClone. The sources of all other reagents and plasmids have been given (2, 11–13).

**Cell Culture, Transient Transfections, and Reporter Gene Assays**—HepG2 cells were cultured and transiently transfected as described previously (13). Luciferase reporter constructs (1 μg/plate) under the control of promoters derived from genes encoding FPP synthase (pFPSS0.319L), HMG-CoA synthase (pSYNSRE), HMG-CoA reductase (pRED), the LDL receptor (pLDLr), and an expression vector encoding β-galactosidase (pCMV-β-gal) (0.5 μg/plate) were added as indicated (12). Additional plasmids encoding SREBP-1a (pCMV-CSA10), E1A (pCMV-E1A), mutant E1A (pCMV-E1AΔ64–68), and CBP (pCMV-CBP) were added as indicated in the figure legends. Following transfection, the cells were incubated for 24 h in medium supplemented with either 10% lipoprotein-deficient calf serum in the absence (inducing medium) or presence (repressing medium) of sterols (10 μg/ml cholesterol and 1 μg/ml 25-hydroxycholesterol) (13) or 10% fetal bovine serum, as indicated in the figure legends. Cells were then lysed, and the luciferase and β-galactosidase activities were determined (13). The β-galactosidase activity was used to normalize for transfection efficiencies (13). Each experimental point was performed in duplicate (variation, <1%), and each experiment was repeated three or more times with similar results.

**Doxycycline-inducible Gene Expression**—Hek 293 cells that stably express the tetracycline-controlled transactivator (CLONTECH) were transfected with the empty pB-L vector or pB-L containing Gal4-CBP (1–451). Cells that stably express the latter plasmids were selected as described by the manufacturer. Cells were grown in the absence or presence of sterols and doxycycline (2 μg/ml) as described in the legend to Fig. 6.

**Purification of Recombinant SREBP-1a and GST-CBP Fusion Proteins**—Recombinant SREBP-1a, containing amino-terminal T7 and polyhistidine (His₆) tags, was purified to homogeneity from E. coli and crude lysates used as described.

**Far-Western Assay**—A fragment of the FPP synthase promoter containing nucleotides −293 to −233 and HindIII/BamHI flanking sequences was gel-purified and end-radiolabeled with [32P]dCTP. The radiolabeled probe (4 x 10⁵ cpm; 0.3 pml) was incubated in the presence of 30 μg of recombinant SREBP-1a (50 μg/ml) in 0.5 ml of Buffer A (29 mg/ml Hepes, pH 7.6, 75 mg/ml KCl, 2.5 mm MgCl₂, 0.05% Nonidet P-40, and 1% nonfat milk). GST-CBP fusion proteins were expressed and subjected to SDS-PAGE electrophoresis (25 μl of crude extract/lane) and blotted onto a nitrocellulose membrane. The membrane-bound proteins were renatured at room temperature in a buffer (25 mg/ml Hepes, pH 7.6, 25 mg/ml NaCl, 5 mg/ml MgCl₂, 0.05% Nonidet P-40, 1 mg/ml diethiothreitol, and 5% nonfat milk) that was changed four times. The membrane was incubated overnight in Buffer A (5 ml/membrane) containing the preformed SREBP-1a:radio-labeled DNA complex, washed twice at 4 °C in Buffer A (100 ml/membrane), and then exposed to film.

**Expression of Gal4-CBP (1–451)**—In Hek 293 Cells in Response to Doxycycline—Hek 293 cells that stably express the tet-controlled transcriptional activator were obtained from CLONTECH, CA. The cells were grown in the presence of G418 (100 μg/ml); transfected with pB-L or pB-L-Gal4-CBP (1–451) together with pTK-Hyg (CLONTECH). Cells were selected for resistance to hygromycin (100 μg/ml) as described by the manufacturer. Hygromycin-resistant cells were frozen or incubated for 24 h in medium supplemented with 10% LPDS in the absence or presence of sterols, as described above, and in the absence or presence of doxycycline (2 μg/ml) as indicated in the legend to Fig. 6.

**RESULTS**

**E1A Inhibits the Expression of a Number of Sterol-regulated Promoter-reporter Genes**—In order to begin to elucidate the mechanism involved in the transcriptional activation of sterol-
and SREBP-regulated genes, HepG2 cells were transiently transfected with separate plasmids containing luciferase reporter genes under the control of promoters derived from the FPP synthase (pFPPS), HMG-CoA synthase (pSYNSRE), LDL receptor (pLDRl), and HMG-CoA reductase (pRED) genes. HepG2 cells were transiently transfected with 1 μg of the indicated promoter-reporter gene together with plasmids encoding β-galactosidase and either E1A or mutant E1A (100 ng). The cells were then incubated for 24 h in medium supplemented with 10% LPDS in the absence (− sterols) or presence (+ sterols) of cholesterol (10 μg/ml) and 25-hydroxycholesterol (1 μg/ml). The relative luciferase activities (mean ± S.E., n = 4 or n = 6) are shown after normalization to account for differences in transfection efficiency.

As expected, in the absence of E1A, the luciferase activity of each reporter gene was induced when cells were incubated in sterol-depleted medium (Fig. 1, lanes 1 versus 2). Preliminary experiments demonstrated that the induction of pFPPS was attenuated, in a dose-dependent manner, when cells were cotransfected with 25–100 ng of the E1A-expressing plasmid (data not shown). The data in Fig. 1 demonstrate that the induction of each reporter gene was attenuated in cells transiently transfected with 100 ng of plasmid encoding wild-type E1A (Fig. 1, lanes 1 versus 3). In contrast, little or no inhibition of luciferase activity was observed when cells were cotransfected with the plasmid encoding the mutant E1A protein (Fig. 1, lanes 1 versus 5). Fig. 1 also shows that the luciferase activity of each reporter gene in sterol-treated cells was low (lanes 2) and was unaffected by coexpression of either wild-type (lanes 4) or mutant E1A (lanes 6) protein.

In other studies, HepG2 cells were transiently transfected with the FPP synthase promoter-luciferase reporter gene in the absence or presence of a plasmid encoding the nuclear-targeted amino-terminal domain of SREBP-1a and plasmids encoding wild-type or mutant E1A protein. The luciferase activity of the reporter gene increased 1.3-fold in response to the cotransfected SREBP-1a by a process that was unaffected by coexpression of the mutant E1A protein but was attenuated following coexpression of wild-type E1A, in a dose-dependent manner (data not shown).

**CBP Rescues E1A-dependent Transcriptional Inhibition of FPP Synthase Promoter-Reporter Genes**—The E1A protein is multifunctional (19). One cellular role of E1A may depend on its ability to bind CBP and thus interfere with the function of this transcriptional coactivator (19, 20). The results in Fig. 2 demonstrate that the inhibitory effect of E1A on the expression of the FPP synthase promoter-reporter gene were overcome when the cells were cotransfected with a plasmid encoding CBP (compare lanes 1, 3, and 5). In the absence of E1A, coexpressed CBP resulted in an additional increase in luciferase activity (Fig. 2, lane 6 versus lane 1), consistent with the proposal that the amount of endogenous CBP may be inadequate for maximal transcription of the reporter gene. In contrast, the luciferase activity in cells treated with sterols was not affected significantly by cotransfected E1A or CBP (Fig. 2, compare lane 2 with lanes 4, 6, and 8).

Taken together, the results shown in Figs. 1 and 2 are consistent with the hypothesis that transcriptional activation of a number of SREBP-dependent genes requires CBP. These results also indicate that under conditions of cellular sterol excess, basal transcription of these reporter genes is independent of CBP.

**The Amino-terminal Domain of CBP (Amino Acids 1–451) Both Interacts with an SREBP-1a:DNA Complex and Can Function as a Dominant Negative Regulator of SREBP-dependent Transcription**—A far-Southwestern assay was used to determine whether recombinant GST-CBP fusion proteins could bind to a preformed SREBP-1a:[32P]DNA complex. Fig. 3A demonstrates that an SREBP-1a:DNA complex bound to both GST-CBP(1–451) (lanes 3 and 4) and GST-CBP(451–721) (lanes 5 and 6) but not to GST (lanes 1 and 2), GST-CBP(721–1100) (lanes 7 and 8), or any of the other three GST-CBP fusion proteins used in these studies (data not shown). Because these studies were performed with recombinant proteins, we conclude that the interaction of SREBP with the two amino-terminal domains of CBP does not require any posttranslational modifications. A second, identical gel was stained with Coomassie Blue in order to demonstrate that the different GST-CBP fusion proteins were present at similar levels (Fig. 3B).

The results in Fig. 3A suggest that one or more amino-terminal domains of CBP may interact with SREBP in vivo. We hypothesized that expression of this CBP domain in cells would interfere with the physiological interaction of endogenous CBP with SREBP’s released into the nucleus in response to cellular sterol depletion. In order to test this hypothesis, we obtained six plasmids encoding various Gal4-CBP fusion proteins (28). The full-length CBP protein and the fragments (amino acids 1–451, 451–721, 721–1100, 1100–1460, 1460–1891, and 1891–2441) that were used to produce these six Gal4-CBP constructs...
are illustrated in Fig. 4A. Transient transfection of these plasmids into mammalian cells results in similar levels of expression of the fusion proteins (28). In addition, as a result of a nuclear targeting domain in Gal4, the fusion proteins are localized to the nucleus (28).

HepG2 cells were transiently transfected with the FPP synthase promoter-luciferase reporter gene in the absence or presence of plasmids encoding the indicated Gal4-CBP fusion proteins. The increase in luciferase activity that occurred when cells were incubated in the absence of sterols (Fig. 4B, lane 1 versus lane 2) was partially blocked by coexpression of Gal4-CBP(1–451)(Fig. 4B, lane 3 versus lane 1) but not by the other Gal4-CBP fusion proteins (Fig. 4B, lanes 5, 7, 9, 11, and 13). In other experiments, transient transfection of cells with higher levels of the plasmid encoding Gal4-CBP(451–721) did result in attenuation of the induced luciferase activity (data not shown), consistent with the observation that CBP(451–721) can interact with SREBP-1a (Fig. 3).

We conclude that the amino-terminal domain of CBP (amino acids 1–451) functions in a dominant negative manner and interferes with the physiological interaction between the endogenous SREBP, which is bound to the SRE-3 cis element of the FPPS promoter, and the endogenous full-length CBP. This conclusion is supported by additional studies, which demonstrated that the increase in luciferase activity in response to coexpressed SREBP-1a was inhibited 75% by Gal4-CBP(1–451) but was not affected by Gal4-CBP(721–1100) (data not shown).

In contrast, none of the six Gal4-CBP fusion proteins affected the luciferase levels in sterol-treated cells, a condition where there is little or no nuclear localized SREBP (Fig. 4B, lane 2 versus lanes 4, 6, 8, 10, 12, and 14). Thus, transcription of the reporter gene under sterol-repressed conditions is independent of CBP.

Qualitatively similar results were obtained when the experiments depicted in Fig. 4B were repeated with either an HMG-CoA synthase promoter-luciferase reporter gene (Fig. 5A) or an HMG-CoA reductase promoter-luciferase reporter gene (Fig. 5B). In each study, the induction of the reporter gene in response to cellular sterol depletion was attenuated by Gal4-CBP(1–451), whereas other Gal4-CBP fusion proteins had little or no inhibitory effect (Fig. 5). Further support for a specific inhibitory effect of Gal4-CBP(1–451) was obtained from other studies, which demonstrated that the 15-fold increase in the expression of the HMG-CoA synthase promoter-luciferase gene in response to coexpressed SREBP-1a was inhibited 70% by Gal4-CBP(1–451) but was unaffected by Gal4-CBP(721–1100) (data not shown). None of the promoter-reporter genes used in these studies were affected when the cells were cotransfected with a plasmid that expressed the DNA binding domain of Gal4 alone (data not shown).

The function of CBP in this and all other studies has been determined using either in vivo assays, in which cells are transfected with plasmids containing reporter-genes, or in vitro assays. The chromatin structure associated with transfected
plasmid DNA is not well defined but is thought to differ significantly from that associated with endogenous genes. In order to more clearly define the role of CBP in regulating endogenous genes, we performed the experiments depicted in Fig. 6. Hek 293 cells, which stably express the tetracycline activator, were stably transfected with either pBI-L or pBI-L-Gal4-CBP(1–451). Cells containing the latter two plasmids are expected to express luciferase or both luciferase and Gal4-CBP(1–451), respectively, in response to added doxycycline. Addition of doxycycline to the cells resulted in an 70–80-fold increase in luciferase expression, consistent with the presence of stably transfected, transcriptionally active plasmid (data not shown).

In the absence of doxycycline, the endogenous mRNA levels of FPP synthase and HMG-CoA synthase were regulated approximately 4-fold when the Hek 293 cells, containing either pBI-L-Gal4-CBP(1–451) or pBI-L, were incubated in the absence or presence of exogenous sterols (Fig. 6, lane 2 versus lane 1 and lane 6 versus lane 5). In control cells that contain pBI-L, the relative levels and fold regulation of these mRNAs by sterols were unaffected by doxycycline (Fig. 6, lane 8 versus lane 6 and lane 7 versus lane 5). In contrast, when cells containing pBI-L-Gal4-CBP(1–451) were incubated in sterol-depleted medium, the induction of both FPP synthase and HMG-CoA synthase mRNAs was prevented when doxycycline was added to the cells (Fig. 6, lane 4 versus lane 2). These results demonstrate that the induced transcription of endogenous FPP synthase and HMG-CoA synthase genes in response to sterol depletion is inhibited by Gal4-CBP(1–451). We conclude that CBP has an important physiological role in the SREBP-dependent transcriptional induction of these endogenous genes.

**DISCUSSION**

Cellular sterol deprivation is known to activate the transcription of a number of genes involved in cholesterol homeostasis (e.g., HMG-CoA synthase, HMG-CoA reductase, FPP synthase, squalene synthase, the LDL receptor, and SREBP-2) (1–5), in fatty acid metabolism (e.g., fatty acid synthase and acetyl CoA carboxylase) (9, 15, 17), and in triglyceride metabolism (e.g., GPAT) (13). Transcriptional activation of these genes is dependent on the binding of SREBP and either NF-Y or Sp1 to the proximal promoters. However, the exact mechanism(s) by which SREBP and either NF-Y or Sp1 activate transcription of these genes remains to be elucidated.

The current study demonstrates that in cells deprived of sterols, the elevated transcription of at least four SREBP-dependent reporter genes is dependent on an interaction between SREBP and the amino-terminal domain (amino acids 1–451) of CBP. The current study also utilized the doxycycline-inducible expression of a dominant negative form of CBP (to clearly demonstrate the important role of CBP in the physiological regulation of endogenous SREBP-responsive genes (Fig. 6). This latter approach may prove useful in delineating the role of CBP in the physiological regulation of other genes.

The stimulatory effect of coexpressed CBP on transcription of the FPP synthase promoter-reporter gene (Fig. 2) is consistent with the proposal that the endogenous levels of CBP may, under certain conditions, be a limiting factor in the expression of SREBP-regulated genes. Other investigators have reported that when cells are transiently transfected with constructs that express transcription factors that are known to interact with CBP, transcription of other CBP-dependent genes declines, presumably as a result of competition for limiting amounts of nuclear CBP (19–21). Thus, it seems likely that transcriptional activation of SREBP-dependent genes may be attenuated when other CBP-dependent signaling pathways are activated.

The current study confirms and extends an earlier report by Olner et al. (27). These authors utilized a far-Western assay to demonstrate that the amino-terminal domain of SREBP-1a or SREBP-2 bound to the amino-terminal domain (amino acids 1–682) of CBP in solution. They also demonstrated that the induced expression of an LDL receptor promoter-reporter gene that resulted from coexpression of a plasmid encoding either SREBP-1a or SREBP-2 was further enhanced 2–4-fold by coexpression of CBP (27).

CBP is thought to function in part by forming a bridge between transcription factors and components (TFIIB and TBP) of the polII complex (19–21). The dominant negative effect produced as a result of expression of Gal4-CBP(1–451) provides support for the physiological importance of the interaction of SREBP with CBP. However, interaction of CBP with TFIIB may not be required for transcription of the genes tested, because the reporter activity was unaffected by coexpression of Gal4 fusion proteins that contain carboxyl-terminal fragments of CBP known to interact with TFIIB (30, 31).

It is not known whether SREBP-CBP-dependent transcriptional activation is regulated by posttranslational modification of either protein, although such modifications are not necessary for protein-protein interaction in vitro (Fig. 3) (27). Recent studies utilizing either SREBP-1a- or SREBP-2-transgenic animals (1, 32, 33) or cotransfection of promoter-reporter genes together with constructs encoding either SREBP-1a or SREBP-2 (13) indicate that a number of genes are differentially regulated by SREBP-1 and SREBP-2. Further experimentation will be necessary to determine whether posttranslational modifications or additional coactivators or corepressors, such as those that have been shown to bind to nuclear hormone receptors (29, 34), differentially affect the function of CBP complexed with either SREBP-1a, SREBP-1c, or SREBP-2.

Under sterol-repressed conditions, the luciferase activities of all four promoter-reporter genes (HMG-CoA synthase, HMG-CoA reductase, FPP synthase, and the LDL receptor) were not significantly affected by coexpression of E1A, CBP, or Gal4-CBP fusion proteins. In addition, the sterol-repressed mRNA levels for endogenous HMG-CoA synthase and FPP synthase were unaffected by Gal4-CBP(1–451) expression (Fig. 6). Thus,
CBP appears to play no functional role in the basal (i.e. sterol-repressed) transcription of these four genes.

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