Sterol Regulation of 3-Hydroxy-3-Methylglutaryl-coenzyme A Synthase Gene through a Direct Interaction Between Sterol Regulatory Element Binding Protein and the Trimeric CCAAT-binding Factor/Nuclear Factor Y*

(Received for publication, October 14, 1997, and in revised form, November 4, 1997)

Kimberly A. Dooley‡, Shawn Millinder, and Timothy F. Osborne§

From the Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92697-3900

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase, a key regulatory enzyme in the pathway for endogenous cholesterol synthesis, is a target for negative feedback regulation by cholesterol. The promoter for HMG-CoA synthase contains two binding sites for the sterol regulatory element-binding proteins (SREBPs). When cellular sterol levels are low, the SREBPs are released from the endoplasmic reticulum membrane, allowing them to translocate to the nucleus and activate SREBP target genes. In all SREBP-regulated promoters studied to date, additional co-regulatory transcription factors are required. In the HMG-CoA synthase promoter there are several potential co-regulatory transcription factor binding sites, including an inverted CCAAT box. A similar element has been shown to function with SREBP to mediate sterol regulation of another gene involved in cholesterol metabolism, farnesyl diprophosphate synthase. Here, we show that CCAAT binding factor/nuclear factor Y (CBF/NF-Y) binding to the CCAAT box is required for sterol-regulated transcription of HMG-CoA synthase. The SREBP sites and the inverted CCAAT box are normally separated by 17 base pairs, and we show that increasing this distance results in a decrease in the level of transcriptional regulation by sterols. Furthermore, we provide evidence that there is a direct interaction between CBF/NF-Y and the basic helix-loop-helix-zipper region of SREBP. Interestingly, this interaction does not occur efficiently with any of the isolated subunits and appears to require all three nonidentical CBF/NF-Y subunits in a preassembled complex. Since CBF/NF-Y only binds to DNA when all three subunits are in a complex, this would prevent SREBP from forming nonproductive associations with the individual subunits.

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)1 synthase is an early enzyme in the cholesterol biosynthetic pathway and is a target for negative feedback regulation by cholesterol (1, 2). When cellular sterol levels are low, the transcription of the HMG-CoA synthase gene is turned on, and when cellular sterol levels rise, transcription is turned off. This negative feedback regulation is mediated in part by the sterol-regulatory element-binding proteins (SREBPs). The SREBPs are synthesized as 125-kDa membrane-spanning proteins. When sterol levels fall, these proteins are released from the endoplasmic reticulum membrane by a two step proteolytic process that results in the release of the mature ~68-kDa amino-terminal portion (3). This mature protein translocates to the nucleus and activates genes involved in both cholesterol and fatty acid metabolism through binding to sterol-regulatory elements (SREs) present in the promoters of target genes (4–10).

By themselves, SREBPs are very inefficient transcriptional activators and therefore require additional co-regulatory transcription factors to achieve high level activation. In the low density lipoprotein receptor, acetyl CoA carboxylase, and fatty acid synthase promoters, the co-regulatory factor is Sp1 (5, 7, 11, 12). In the case of farnesyl diprophosphate (FPP) synthase, squalene synthase, and HMG-CoA synthase, the co-regulatory factor is the CCAAT binding factor (CBF), also called nuclear factor Y (NF-Y) (6, 10, 13).

CBF/NF-Y is a ubiquitously expressed, trimeric transcriptional activator that binds to CCAAT motifs in a number of eukaryotic promoters (14). In most cases, it functions together with more specific transcriptional activators to achieve high levels of transcriptional activation in specific tissues or under certain conditions. In the albumin promoter, for example, CBF/NF-Y functions together with liver specific members of the CCAAT/enhancer-binding protein (C/EBP) family to achieve a high level of liver specific expression (15).

The purpose of the current studies was to further define the role of CBF/NF-Y in the sterol regulated transcription of the HMG-CoA synthase gene. The sterol regulatory region of the HMG-CoA synthase promoter is contained within a 100-bp segment of the promoter, from -324 to -295 and that

*† Support by National Institutes of Health Grant GM07311.
§ Established Investigator of the American Heart Association. To whom correspondence should be addressed. Tel.: 714-824-2979; Fax: 714-824-8551.

1 The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; bp, base pair(s); SRE, sterol regulatory element; FPP, farnesyl diprophosphate synthase; SREBP, sterol regulatory element-binding protein; CBF, CCAAT-binding factor; NF-Y, nuclear factor Y; bHLH, basic helix-loop-helix; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; C/EBP, CCAAT/enhancer-binding protein.

This paper is available online at http://www.jbc.org
Sterol Regulation of HMG-CoA Synthase Promoter

CBF/NF-Y in solution in the absence of DNA. Interestingly, this interaction does not occur with any of the individual CBF/ NF-Y subunits, but requires all three subunits to be present at the same time.

MATERIALS AND METHODS

Cells and Media—CV-1 cells were obtained from Dr. K. Cho (University of California, Irvine). HepG2 cells were obtained from the ATCC. HeLa nuclear extracts were purchased from Endotronics (Minneapolis, MN). All cell culture materials were purchased from Life Technologies Inc. Lipoprotein-deficient serum was prepared by ultrafiltration as described previously (16). Cholesterol and 25-hydroxycholesterol were obtained from Steraloids Inc., and stock solutions were dissolved in absolute ethanol.

Plasmids—pGL2 basic was purchased from Promega and was used as the source of the luciferase reporter gene in all constructs. Standard techniques were used in all cloning procedures (17). pSsynWT contains nucleotides −488 to +38 relative to the mRNA start site of the hamster HMGG-CoA synthase promoter inserted upstream of the luciferase reporter gene in pGL2 basic (18). To construct the plasmid pSynSRE, polymerase chain reaction amplification of the sequence corresponding to −324 to −225 of the HMGG-CoA synthase promoter was performed using oligonucleotides containing SacI and Nhel restriction sites attached to the 5′- and 3′-flanking synthase oligonucleotides, respectively. The resulting fragment was inserted upstream of the minimal HMGG-CoA synthase TATA box (−28 to +39) of the plasmid “TATA only” described previously (11). The insertion mutations, pSynSRE +5 and pSynSRE +10 were constructed using the Altered Sites mutagenesis kit (Promega) according to the manufacturer’s protocol. The oligonucleotides used were complementary to the 5′ and 3′ sequences relative to the insertion site at −290 and contained the following insertion sequences: 5′-CATGG-3′, 5′-CATGGTGGAC-3′, for the +5 and +10 mutants, respectively. All mutants were confirmed by sequencing. pSynNF-Y was constructed in the same way using an oligonucleotide containing the scramble mutation at the site of the inserted CCAAT box (5′-ATTGCG-3′ → 5′-AGATCT-3′) beginning at position −295 relative to the mRNA start site.

The plasmid pNF-YA29, which encodes a dominant-negative version of the A subunit of NF-Y (19) expressed from the simian virus 40 early promoter, was a kind gift of Dr. Robert Mantovani from the Universita Degli Studi Di Milano, Italy. pGex2T (Pharmacia) was used to construct all glutathione S-transferase (GST) fusion genes for protein expression. The pCiteCBF-A, pCiteCBF-B, and pCiteCBF-C constructs for use in vitro translation reactions were generated by polymerase chain reaction amplification with appropriate oligonucleotide primers. The fusion constructs were made by polymerase chain reaction amplification with Pfu polymerase (Stratagene, Inc.) of the coding region for amino acids 1–490 of SREBP 1a, the corresponding region of SREBP 1c, or amino acids 1–481 of SREBP-2, and inserting the resulting fragments in frame with the GST-coding region of pGex2T. Truncated SREBP 1a constructs encoding amino acids 1–320 or 320–420 were generated in the same way with appropriate oligonucleotide primers. The fusion proteins were expressed in E. coli strain BL21DE3. 500-nl cultures were grown to an OD600 of 0.6 to 0.8, induced with isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 mM, and grown for an additional 3 h at 37 °C. Cells were then harvested by ultracentrifugation.

The cell pellets were resuspended in NETN (100 mM NaCl, 20 mM Tris, pH 8, 1 mM EDTA, 0.5% Nonidet P-40) and lysed by sonication, and cell debris was removed by ultracentrifugation. Glycerol was added to the supernatant to a final concentration of 1 mM, and stored at −70 °C. The expression of the fusion proteins was verified by SDS-PAGE analysis followed by Coomassie Blue staining, as well as by ECL Western blot (Pierce) analysis using anti-GST antibody and horseradish peroxidase-conjugated anti-rabbit IgG (Sigma).

RESULTS

In an effort to gain further insight into how SREBP's function together with unique co-regulators in the different SREBP-regulated promoters we have been studying how the SREBP's function together with CBF/NF-Y to activate the promoter for HMGG-CoA synthase. A schematic representation of the promoter for hamster HMGG-CoA synthase is shown in Fig. 1A. When the synthase promoter was fused to the luciferase re-
porter gene and transfected into CV-1 cells, the promoter was seven times more active in cells cultured in the absence of sterols compared with cells cultured in the presence of sterols.

To simplify further analysis of regulatory elements involved in this process, we made a smaller version of the synthase promoter, pSynSRE, which lacks bases from −248 to −235 and from −224 through −29 as shown in Fig. 1. When this truncated version was fused to the luciferase reporter gene and analyzed, it was subject to efficient sterol regulation as well.

The HMG-CoA synthase promoter region in pSynSRE contains two consensus binding sites for the SREBP proteins, as well as binding sites for the ATF/AP-1 family of transcription factors and an inverted CCAAT box that is a consensus binding site for the CBF/NF-Y protein. The two SRE elements were previously shown to be required for sterol regulation (2). To directly determine if they both bind SREBP proteins, we performed a DNase I footprinting experiment with a probe from the synthase promoter and purified recombinant SREBP-1a protein (Fig. 1B). When increasing amounts of SREBP-1a were added to the synthase probe, both predicted sites were protected from DNase I cleavage consistent with both sites binding SREBP protein.
Sterol Regulation of HMG-CoA Synthase Promoter

To determine if the CCAAT sequence might function as a co-regulatory site for sterol regulation by the SREBPs, we made a mutant derivative of pSynSRE, which simultaneously alters all bases of the inverted CCAAT box beginning at position −295. The mutant construct was designated pSynΔNF-Y. When it was transfected into CV-1 cells and compared directly to the wild-type pSynSRE plasmid, it was completely defective for sterol regulation (Fig. 2A and B, compare lanes 1 and 6). Since NF-Y is one protein that has been shown to bind to CCAAT elements, we transfected a dominant-negative expression construct for the A subunit of NF-Y, pNF-YA29, along with the wild-type pSynSRE plasmid. When the cells were cultured in medium containing lipoprotein-depleted serum, a dose-dependent decrease in transcriptional activity was observed (Fig. 2A and B, lanes 1–5). Taken together, these data show that the inverted CCAAT box at −295 is required for the sterol-regulated transcription of the HMG-CoA synthase promoter, and consistent with an earlier report (13), support a role for CBF/NF-Y in sterol regulation of the HMG-CoA synthase promoter.

Several transcription factors have been shown to bind to CCAAT box sequences, including CBF/NF-Y, C/EBP, and NF-1 (14). To determine if CBF/NF-Y could specifically bind to the HMG-CoA synthase CCAAT box sequence, we performed gel shift experiments with HepG2 nuclear extract and 32P-end-labeled probes encompassing this region of the synthase promoter corresponding to either the wild-type (SynSRE) or CCAAT box mutant plasmid (SynΔNF-Y). There were two complexes that were produced only with the wild-type probe (Fig. 3, compare lanes 2 and 6), indicating that they contain proteins specific for the CCAAT box sequence. The migration of the upper of these two complexes was further retarded in the presence of an antibody specific for the A subunit of NF-Y (lane 3) but not in the presence of an antibody that is specific for Sp1 (lane 4). This, thus, upper CCAAT box-specific complex is due to the binding of NF-Y.

The lower CCAAT box-specific complex observed with the wild-type probe has not been identified and probably represents another CCAAT box-binding protein. Several shifted bands were observed with both the wild-type probe and the mutated probe (NS), indicating that these complexes are not specific for the CCAAT box sequence. These results extend the observations of an earlier report (13), and taken together with the results of Fig. 2, further support a role for NF-Y in sterol regulation of the HMG-CoA synthase promoter.

The binding of CBF/NF-Y to a site within the FPP synthase promoter enhances the binding of SREBP-1a at an adjacent SRE element (22). This stimulatory effect and sterol regulation were both lost when 4 bp were inserted between the CCAAT box and the SRE element in FPP synthase. In the wild-type HMG-CoA synthase promoter, the inverted CCAAT box and the SRE 1.5′ are separated by 17 bp. To determine if the relative spacing of these two sites was important for sterol regulation of the HMG-CoA synthase promoter, we inserted 5 or 10 bp between the synthase SRE and the inverted CCAAT element as diagrammed in Fig. 4C. These insertion mutants were then transfected into CV-1 cells and assayed for sterol regulation as described above. The activities from the +5 and +10 promoters under induced conditions were significantly reduced, indicating that the relative spacing between the SRE and CCAAT elements is important for sterol regulation of the synthase promoter. These results support the notion that the CCAAT box and SRE sites must function together to achieve optimal sterol regulation.

The experiments presented so far suggest there may be a functional interaction between the CBF/NF-Y protein, which binds to the inverted CCAAT site, and SREBP, which binds to the SRE element. To test this hypothesis, we constructed GST-SREBP fusion proteins for use in in vitro protein-protein interaction assays (Fig. 5). For these experiments we fused the entire mature forms of SREBP-1a, -1c, or -2 to the glutathione S-transferase coding sequence, and as a control we used a vector that expressed the GST portion without any fused sequence.

Plasmids encoding each of the GST proteins were induced in E. coli, and the crude extracts were bound to glutathione-agarose beads and then incubated either alone or in the presence of nuclear extract protein from HeLa cells. Material that

Fig. 2. NF-Y is involved in the sterol-mediated regulation of the HMG-CoA synthase promoter. CV-1 cells were transiently transfected with luciferase reporter constructs pSynSRE or pSynSRE containing a mutation that scrambles the inverted CCAAT box sequence beginning at position −295 from 5′-ATTGGC-3′ to 5′-AGATCT-3′ (pSynΔNF-Y). After transfection, the cells were cultured in medium containing 10% lipoprotein-depleted serum in the absence (ind) or presence (sup) of 10 μg/ml cholesterol and 1 μg/ml 25-hydroxycholesterol. Where indicated (lanes 2–5), increasing amounts (100 ng, 300 ng, 1 μg, or 3 μg) of pNF-YA29, a dominant-negative expression construct for the A subunit of NF-Y (19), were co-transfected with pSynSRE. For all transfections, a CMV β-galactosidase construct was co-transfected as a control for transfection efficiency. The average of the luciferase reporter activity under induced (ind) and suppressed (sup) conditions for three individual experiments is shown in panel A. The luciferase activity is plotted as “normalized” luciferase activity, which was obtained by dividing the relative luciferase units (RLU) by the β-galactosidase activity (Aβgal) to obtain the normalized values. The data from panel A is represented as “fold regulation” by taking the normalized luciferase activity under induced growth conditions divided by that under suppressed growth conditions (B).
bound specifically to the beads was retained after extensive washing and then analyzed by SDS-PAGE followed by Western blot analysis using an antibody specific for the A subunit of NF-Y. A single, ~42-kDa immunoreactive band corresponding to NF-YA was specifically detected in the HeLa nuclear extract (lane 1). An identical band was present in the sample eluted from the GST-SREBP-1a (lanes 3 and 4) GST-SREBP-1c (lanes 5 and 6) and GST-SREBP-2 (lanes 7 and 8) reactions. In contrast, NF-YA was not present in the sample incubated with only the GST protein (lanes 2 and 3). These data are consistent with a direct interaction between the SREBP proteins and NF-Y.

CBF/NF-Y is a trimeric protein complex consisting of A, B, and C subunits, which are simultaneously required for DNA binding (21). The experiment shown in Fig. 5 indicates that SREBPs interact with at least the A subunit of NF-Y (B subunit of CBF). However, since CBF/NF-Y is a trimeric complex, the results from Fig. 5 are also consistent with SREBP interacting only with the preassembled trimeric complex, perhaps via the B or C subunit, and thereby indirectly interacting with the A subunit. To address this question, we transcribed/translated the subunits of CBF/NF-Y in vitro in the presence of 35S-labeled amino acids either individually or in several combinations and used the crude translation mixes in GST-interaction assays with the SREBPs similar to the experiments described in Fig. 5.

When GST-SREBP-1a-bound agarose beads were incubated with the translated reactions for either CBF-A or CBF-C, there was no detectable interaction (Fig. 6A, lanes 5 and 7). There was a low level of CBF-B retained, but this was also observed for the GST control resin (Fig. 6C). In contrast, when all three CBF subunits were co-translated and incubated with the GST-SREBP-1a resin, all were strongly retained (Fig. 6A, lane 9). When the GST-SREBP-1c or GST-SREBP-2-bound material was analyzed, similar results were obtained (Fig. 6A, lanes 11 and 13). This indicates that SREBP and CBF/NF-Y interact in solution in the absence of DNA, and all three subunits must be present for an efficient interaction to occur.

To identify which regions of the SREBPs interact with CBF/NF-Y, we created mutant derivatives of SREBP-1a. In one version aa 1–320 were fused to GST, and in the other version, amino acids 320–420 were fused to GST. As depicted at the bottom of Fig. 6B, the first 320 amino acids of SREBP-1a contain both its acidic activation domain and a serine-threonine rich region, whereas amino acids 320–420 includes the...
bHLH/leucine zipper (bHLH-Zip) region responsible for DNA binding and dimerization (4).

These fusion proteins were individually expressed in E. coli and used in the GST-protein interaction assay as described above. When incubated with all three co-translated CBF/NF-Y subunits, the truncated SREBP-1a including the bHLH-Zip domain interacted very strongly with CBF/NF-Y, as evidenced by the presence of three 35S-labeled bands corresponding to individual CBF/NF-Y subunits, the truncated SREBP-1a including the bHLH-Zip and used in the GST-protein interaction assay as described under “Materials and Methods.” The fusion proteins (GST only, lanes 2 and 3; GST-SREBP-1a, lanes 4 and 5; GST-SREBP-1c, lanes 6 and 7; GST-SREBP-2, lanes 8 and 9) were incubated with (lanes 3, 5, 7, and 9) or without (lanes 2, 4, 6, and 8) 150 μg total of nuclear protein from HeLa cells. After binding to glutathione-agarose beads and extensive washing, aliquots were analyzed by SDS-PAGE followed by ECL Western blot analysis using anti-NF-YA antibody. Lane 1 corresponds to 12 μg of HeLa nuclear extract loaded directly as a control for the presence of NF-YA.

**DISCUSSION**

The region of the HMG-CoA synthase promoter necessary for sterol-regulated transcription includes two SRE I elements and an inverted CCAAT box (Fig. 1A). The two SRE I elements were previously shown to be simultaneously required for sterol-regulated transcription (2), and we have shown that mature SREBP-1a binds to both the 5’ and the 3’ SRE I sites (Fig. 1B). SREBP-2 also binds to both the 5’ and 3’ SRE I sites (data not shown).

Although both SRE I sites are required, by themselves they are not sufficient for sterol regulation of the HMG-CoA synthase promoter (2, 11, 12). This is similar to the situation for all other SREBP-regulated promoters that have been carefully studied to date; additional co-regulatory factors are required for efficient transcriptional activation (5, 7, 9–11, 22). In HMG-CoA synthase at least one of the co-regulatory sites is an inverted CCAAT element, and we have shown that this site is necessary for sterol-regulated transcription (Fig. 2).

The promoter for FPP synthase, another gene encoding a cholesterol synthetic enzyme, also contains a CCAAT box in addition to an SRE element. In FPP synthase, this CCAAT box is required for sterol-regulated transcription of this gene as well (22). Several transcription factors bind to CCAAT box sequences (14). Here we have shown that the CBF, also termed NF-Y, binds to the CCAAT box at –295 in the HMG-CoA synthase promoter (Fig. 3). This is consistent with earlier studies where overexpression of a dominant-negative version of one CBF/NF-Y subunit decreased sterol regulation of both FPP synthase and HMG-CoA synthase (13).

To extend these earlier studies we made a mutation that specifically altered the CCAAT element in the HMG-CoA synthase promoter, and we demonstrated that this mutation eliminated the binding of CBF/NF-Y (Fig. 3) and abolished regulation by sterols (Fig. 2). Additionally, we have shown that when increasing amounts of a vector expressing the dominant-negative NF-YA was transfected along with the HMG-CoA synthase promoter a dose-dependent decrease in promoter activity was observed (Fig. 2).

CBF/NF-Y is a ubiquitously expressed transcription factor that is composed of three nonidentical subunits all of which must be present in a complex for specific DNA recognition. CBF/NF-Y binding to CCAAT boxes present in several eukaryotic promoters is important in their transcriptional regulation (23–26). In the case of the albumin promoter, CBF/NF-Y cooperates with a member of the C/EBP transcription factor family to activate a high level of albumin expression specifically in the liver (15). In this case, the positive synergism between C/EBP and NF-Y does not appear to occur at the level of DNA binding but rather at a subsequent step in transcriptional activation. In contrast, in the FPP synthase promoter NF-Y appears to stimulate the binding of SREBP to an adjacent SRE (22). These results are consistent with a physical interaction between CBF/NF-Y and SREBP.

To study this further in the HMG-CoA synthase promoter, first we inserted additional bases between the 5’ SRE I and the inverted CCAAT box to separate the two elements and possibly decrease the potential for an interaction between proteins bound at the two sites. As shown in Fig. 4, the insertion of 5 or 10 bp did result in a loss of regulated transcription, which is also consistent with an interaction occurring between SREBP and CBF/NF-Y bound at the two sites. Because one turn of the DNA helix is roughly 10 bp, this experiment also provides evidence that any interaction that occurs is not strictly dependent on helical phasing of the DNA.

Furthermore, we have shown by in vitro protein-protein interaction assays, that GST-SREBP fusion proteins interact specifically with CBF/NF-Y (Fig. 5). Therefore, in the case of the HMG-CoA synthase promoter, it appears that there is a physical interaction between SREBP and CBF/NF-Y that is required for maximal transcriptional activation. These results do not exclude the possibility, however, that CBF/NF-Y is also important at a subsequent step in transcription, as was seen in the case of the albumin promoter mentioned above.

The three subunits of CBF/NF-Y assemble in an ordered fashion prior to binding DNA (21). First CBF-A and CBF-C, each of which contains a histone-fold motif (27), form a dimer that is then recognized by CBF-B. This heterotrimer is then competent to recognize DNA. The Western blot procedure used to show SREBP interacted with CBF/NF-Y present in the crude HeLa extract was performed with an antibody specific for the A subunit of NF-Y (analogous to the B subunit of CBF). From this experiment, it was impossible to determine if SREBP interacted specifically with the A subunit of NF-Y or indirectly via interaction with one or more of the subunits of the complex.

To distinguish between these possibilities, protein-protein interaction assays with the GST-SREBP fusion proteins and individual CBF/NF-Y subunits or various combinations were performed. The results demonstrated that GST-SREBP-1a did
Sterol Regulation of HMG-CoA Synthase Promoter

The interaction between SREBP and CBF/NF-Y requires all three subunits of CBF/NF-Y and the bHLH-Zip region of SREBP. CBF-A, CBF-B, CBF-C, or combinations thereof were translated in vitro in the presence of [35S]translabel as described under "Materials and Methods." The figures are from autoradiograms of dried gels from SDS-PAGE analysis. A, 2 μl of the 50-μl crude transcription/translation reaction for CBF-A, CBF-B, or CBF-C, were loaded in lanes 1, 2, and 3, respectively. GST-SREBP-1a was bound to glutathione-agarose beads, then incubated with mock-translated extract (lane 4) or with aliquots of in vitro translated CBF subunits (lanes 5–9) alone or in combinations, and followed by extensive washing before analysis. The CBF subunit(s) present in the translation reactions corresponding to each lane are indicated (+). The same procedure was followed for GST-SREBP-1c (lanes 10 and 11) or GST-SREBP-2 (lanes 12 and 13) as indicated. B, 2 μl of the 50-μl crude transcription/translation reaction for CBF-A, CBF-B, or CBF-C, were loaded in lanes 1, 2, and 3, respectively. Protein-protein interaction assays for GST fusion proteins of the truncated forms of SREBP-1a containing either amino acids (aa) 1–320 or amino acids 320–420 (lanes 6 and 7) were performed as well. Reactions included mock extract (−) or extract from reactions containing all three CBF subunits as indicated (+). A diagram of the mature transcriptionally active SREBP-1a protein is shown at the bottom with the location and amino acid positions of the acidic activation and bHLH-leucine zipper (bHLH-Zip) DNA binding domains indicated. C, the simple GST protein was used as a control in protein-protein interaction assays as described above and under "Materials and Methods." The translated CBF subunit(s) used in each reaction are indicated (+).
REFERENCES
1. Gil, G., Goldstein, J. L., Slaughter, C. A., and Brown, M. S. (1986) J. Biol. Chem. 261, 3710–3716
2. Smith, J. R., Osborne, T. F., Brown, M. S., Goldstein, J. L., and Gil, G. (1988) J. Biol. Chem. 263, 18480–18487
3. Brown, M. S., and Goldstein, J. L. (1997) Cell 89, 331–340
4. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L., and Brown, M. S. (1993) Cell 75, 185–197
5. Bennett, M. K., Lopez, J. M., Sanchez, H. B., and Osborne, T. F. (1995) J. Biol. Chem. 270, 25578–25583
6. Ericsson, J., Jackson, S. M., Lee, B. C., and Edwards, P. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 845–850
7. Lopez, J. M., Bennett, M. K., Sanchez, H. B., Rosenfeld, J. M., and Osborne, T. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1049–1053
8. Sato, R., Inoue, J., Kawahe, Y., Kodama, T., Takano, T., and Maeda, M. (1996) J. Biol. Chem. 271, 26461–26464
9. Ericsson, J., Jackson, S. M., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1997) J. Biol. Chem. 272, 7298–7305
10. Guan, G., Dai, P.-H., Osborne, T. F., Kim, J. B., and Shechter, I. (1997) J. Biol. Chem. 272, 10295–10302
11. Sanchez, H. B., Yieh, L., and Osborne, T. F. (1995) J. Biol. Chem. 270, 1161–1169
12. Briggs, M. R., Yokoyama, C., Wang, X., Brown, M. S., and Goldstein, J. L. (1993) J. Biol. Chem. 268, 14490–14496
13. Jackson, S. M., Ericsson, J., Osborne, T. F., and Edwards, P. A. (1995) J. Biol. Chem. 270, 21445–21448
14. Johnson, P. F., and McKnight, S. L. (1989) Annu. Rev. Biochem. 58, 799–839
15. Miles, P. M., and Zaret, K. S. (1992) Genes Dev. 6, 991–1004
16. Goldstein, J., Basu, S., and Brown, M. (1983) Methods Enzymol. 98, 241–260
17. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Vallet, S. M., and Osborne, T. F. (1994) Nucleic Acids Res. 22, 5184–5189
19. Mantovani, R., Li, Y.-X., Pessara, U., van Huisjduijnen, R. H., Benoist, C., and Mathis, D. (1994) J. Biol. Chem. 269, 20340–20346
20. Attardi, L. D., and Tjian, R. (1993) Genes Dev. 7, 1341–1353
21. Sinha, S., Maity, S. N., Al, J., and De Crombrugghe, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1624–1628
22. Ericsson, J., Jackson, S. M., and Edwards, P. A. (1996) J. Biol. Chem. 271, 24359–24364
23. Maity, S. N., Golombek, P. T., Karsenty, G., and De Crombrugghe, B. (1988) Science 241, 582–585
24. Mantovani, R., Pessara, U., Trouche, F., Li, X.-Y., Knapp, A., Pasquali, J.-L., Benoist, C., and Mathis, D. (1992) EMBO J. 11, 3315–3322
25. Wright, K. L., Vilen, B. J., Itob-Lindstrom, Y., Moore, T. L., Li, G., Cricciello, M., Cogswell, P., Clarke, J. B., and Ting, J. P.-Y. (1994) EMBO J. 13, 4042–4053
26. Courey, F., Maity, S. N., Sinha, S., and De Crombrugghe, B. (1996) J. Biol. Chem. 271, 14485–14491
27. Sinha, S., Kim, I.-S., Sohn, R.-Y., De Crombrugghe, B., and Maity, S. N. (1996) Mol. Cell. Biol. 16, 328–337
28. Molkentin, J. D., Black, B. L., Martin, J. F., and Olsen, E. N. (1995) Cell 83, 1125–1136
29. Groisman, R., Masutani, H., Leibovitch, M.-P., Robin, P., Soudant, I., Trouche, D., and Harel-Bellan, A. (1996) J. Biol. Chem. 271, 5258–5264
30. Hua, X., Yokoyama, C., Wu, J., Briggs, M. R., Brown, M. S., Goldstein, J. L., and Wang, X. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11603–11607
31. Mitchell, P. F., and Tjian, R. (1989) Science 245, 371–378
32. Courey, A. J., Holtzman, D. A., Jackson, S. P., and Tjian, R. (1989) Cell 59, 827–836
33. Li, Y.-X., Hoof van Huisjduijnen, R., Mantovani, R., Benoist, C., and Mathis, D. (1992) J. Biol. Chem. 267, 8984–8990
34. Gill, G., Pascal, E., Tseng, Z. H., and Tjian, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 192–196