Synergistic activation of transcription by nuclear factor Y and sterol regulatory element binding protein

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Abstract The current studies define the role of three distinct cis-elements in the proximal promoter of the rat farnesyl diphosphate (FPP) synthase gene. The three cis-elements, a sterol regulatory element (SRE-3) flanked by an ATTGG motif (inverted CCAAT box), and a CCAAT box, form a sterol regulatory unit that is necessary and sufficient for sterol-regulated expression of FPP synthase promoter-reporter genes. FPP synthase promoter-reporter genes, that contain promoters with either wild-type nucleotide sequences or mutations in one or more of the three cis-elements, were transiently transfected into CV-1 cells. The activity of the wild-type promoter-reporter gene increased when the cells were incubated in sterol-depleted media or when the cells were co-transfected with a plasmid encoding the mature form of SRE binding protein (SREBP-1a). The results with the mutant promoter-reporter genes demonstrated that all three cis-elements were necessary for normal expression/regulation of the reporter gene by either sterols or by co-expressed SREBP-1a. Gel mobility shift assays demonstrated that the synergistic binding of SREBP-1a to SRE-3 was dependent on the binding of recombinant nuclear factor Y (NF-Y) to the DNA, consistent with the in vivo regulation studies. —Jackson, S. M., J. Ericsson, R. Mantovani, and P. A. Edwards. Synergistic activation of transcription by nuclear factor Y and sterol regulatory element binding protein. J. Lipid Res. 1998. 39: 767–776.

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In previous studies we identified a 61 bp sequence in the proximal promoter of FPP synthase (nucleotides 229 to 233) that was necessary and sufficient to impart sterol-regulated expression to a reporter gene (1). These studies also identified four domains (defined by mutants 2, A, B and C in ref. 1) within this 61 bp sequence that, when mutated, impaired sterol-regulated expression of reporter genes. Mutant 2, the most 5’ domain, identified a 5 bp motif (ATTGG) termed an inverted CCAAT box (1–3). The high expression of FPP synthase promoter-reporter genes observed in cells incubated in sterol-depleted media was attenuated by either mutation of the ATTGG motif within the FPP synthase promoter or by co-expression of a dominant negative form of NF-YA (2). Based on these studies, we proposed that the induced expression of FPP synthase was dependent upon the binding of the heterotrimeric transcription factor NF-Y to the inverted CCAAT box motif (2).

Mutants A and B defined a novel motif termed SRE-3 (CTCACACGAG) that was shown to function as a binding site for SREBP-1a (1, 4). SRE-3 has 60% identity with SRE-1 (ATCACCCCAC), an element originally identified as an SREBP-binding site in the promoters of the LDL receptor and HMG-CoA synthase genes (5, 6). SRE-3 also has 50% identity with the nucleotides surrounding the E-box motif in the promoter of the fatty acid synthase gene (7). This latter motif was originally shown to bind ADD1, the rat homologue of SREBP-1 (7, 8). More recent studies have demonstrated that the fatty acid synthase promoter contains four half-sites that overlap the E-box motif and appear to function as two distinct binding sites for SREBP (9).

Electromobility shift assays that utilized oligonucleotides containing the SRE-3 and the 5’ inverted CCAAT box of the FPP synthase promoter demonstrated that binding of SREBP-1a to SRE-3 in vitro was enhanced over 20-fold when partially purified NF-Y bound to the inverted CCAAT box, located 20 bp 5’ of SRE-3 (4). A different but somewhat analogous situation has been reported in studies utilizing the promoter of the LDL receptor gene (10, 11). In those studies, the binding of SREBP to SRE-1 was shown to stimulate the binding

Abbreviations: FPP, farnesyl diphosphate; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein; NF-Y, nuclear factor Y; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; CAT, chloramphenicol acetyltransferase; LDL, low density lipoprotein; bp, base pair; CHO, Chinese hamster ovary.

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of Sp1 to an adjacent low affinity Sp1-binding motif. Thus, the requirement for an additional transcription factor(s), in addition to SREBP, appears to be a common theme in achieving optimal regulation of sterol-sensitive genes; studies with the LDL receptor (10, 11), fatty acid synthase (9, 12), and acetyl CoA carboxylase (13) promoters indicate that Sp1 fulfills this function in these promoters. Other transcription factors (NF-1, AP1, or Oct1) were unable to substitute fully for Sp1 in the sterol-regulated expression of LDL receptor promoter-reporter constructs (10). In contrast, high expression of reporter genes under the control of promoters derived from the FPP synthase (1, 2), HMG-CoA synthase (2), SREBP-2 (14), SREBP-1a (15), and possibly squalene synthase (16, 17) genes requires NF-Y, in addition to SREBP.

SREBP (5, 18), Sp1 (11, 19), and NF-Y (20) all contain transcriptional activation domains. Yieh, Sanchez, and Osborne (11) demonstrated that two distinct domains of Sp1 are involved both in transcriptional activation and SREBP-dependent DNA binding to the LDL receptor gene/promoter. However, the mechanism by which synergistic stimulation of transcription occurs when SREBP and either NF-Y or Sp1 bind to DNA is poorly understood.

A fourth mutation, termed mutant C, defined a 12 bp sequence in the FPP synthase promoter that was necessary for the increased expression observed in cells incubated in sterol-depleted media (1). These changes in activity and regulation of the mutant construct were similar to those exhibited by FPP synthase promoter-reporter genes that contained a mutation in SRE-3 (1, 4). The results are consistent with the presence of important regulatory sequences in the 12 bp defined by mutant C. The wild-type nucleotides in this region contained a CCAAT box motif located 10 bp 3' of the SRE-3 motif (1).

Several proteins have been identified that bind to CCAAT or related sequences (21–23). NF-Y, a heterotrimeric protein that binds to a CCAAT sequence, has been given many names including CBF, a-CP1, EF1, and CBPtk (reviewed in ref. 24). All three NF-Y subunits (A, B, C) are required for DNA binding (25), which results in distortion of the DNA (24). The mechanism by which NF-Y regulates transcription of different genes is unknown. It may depend on the glutamine- and serine/threonine-rich domains of NF-YA (20), the recruitment of other transcription factors to adjacent sites on the DNA (4), or recruitment of other non-DNA binding proteins.

The current studies were performed to determine the role of the nucleotides within the region defined by mutant C in the rat FPP synthase promoter (1). First, we demonstrate that NF-Y binds to the CCAAT box motif defined by mutant C. Second, we utilized recombinant NF-YA, NF-YB, and NF-YC to demonstrate that the enhanced binding of recombinant SREBP-1a to SRE-3 within the FPP synthase promoter requires that the heterotrimeric NF-Y binds to the adjacent ATTGG and/or CCAAT motifs. This NF-Y-dependent binding of SREBP-1a to DNA occurs in the absence of other nuclear proteins. Surprisingly, the increased formation of the SREBP-1a:DNA complex did not require that NF-Y remain bound to the DNA.

We conclude that the normal regulation of expression of FPP synthase promoter-reporter genes, either in response to cellular sterol deprivation or after co-expression of mature SREBP, requires the binding of NF-Y to two motifs; one motif (CCAAT) is present within the sequence defined by mutant C (10 bp 3' of the SRE-3) and the second (ATTGG) lies 20 bp 5' of the SRE-3.

**EXPERIMENTAL PROCEDURES**

**Materials**

DNA restriction and modification enzymes were obtained from Gibco BRL. 32P-labeled nucleotide triphosphates were obtained from Amersham Corp. pRSETB (Invitrogen) containing both a partial sequence of SREBP-1a (amino acids 1–490) and 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, UC Irvine). Lipoprotein-deficient fetal calf serum was purchased from PerImmune. Antibodies to CBF-A, that also recognizes human NF-YB, were a generous gift from Dr. B. de Crombrugge (University of Texas). Antibodies to CBP-HSP were a generous gift from Dr. B. Wu (Northwestern University) (26). The T7 antibody was from Invitrogen. The sources of all other reagents and plasmids have been given (1, 2, 27).

**Cell culture, transient transfections, and reporter gene assays**

CV-1 cells were cultured as previously described (4). Cells were transiently transfected with promoter-reporter genes, a plasmid encoding β-galactosidase and, where indicated, pCMV-CSA10 (encoding mature SREBP-1a) as previously described (1, 2). After transfection, the cells were incubated for 20 h in media supplemented with either 10% lipoprotein-deficient calf serum in the absence (inducing media) or presence (repressing media) of sterols (10 μg/ml cholesterol and 1 μg/ml 25-hydroxycholesterol) (1, 2) or 10% fetal bovine serum, as indicated in the legends. Cells were then lysed and the CAT and β-galactosidase activities
were determined (1, 2). The β-galactosidase activity was used to normalize for transfection efficiencies (1).

**FPP synthase promoter-reporter gene constructs**

The wild type (wt) pTKCIII-0.061 promoter-reporter plasmid used in these studies is a promoter-chloramphenicol acetyltransferase (CAT) reporter construct containing 61 bp of the FPP synthase proximal promoter (−293 to −233) (see Fig. 1). This 61 bp fragment was flanked 5’ by 24 bp containing a HindIII restriction site and 3’ by 9 bp containing a BamHI site. The double digested fragment was cloned into the HindIII/BamHI sites of pTKCIII (1). The mutant promoter-reporter plasmids were constructed as follows. The Sculptor in vitro mutagenesis kit (Amersham) was used to produce a 3 bp transversion (nucleotides −242 to −239; CCAAT to CACC) in pFPPS-0.247. A 61 bp fragment (−293 to −233) was then generated utilizing the mutated pFPPS-0.247 and polymerase chain reaction (PCR) utilizing primers containing restriction sites (HindIII/BamHI) compatible with pTKCIII to produce the Mut w construct utilized in this study. Mut 2-5 (ATTGG to ATTTG; a mutation in the inverted CCAAT box motif) and Mut q (CAC to ACA; a mutation in the SRE-3 motif) (Fig. 1) were generated in a similar manner and the 61 bp fragments (−293 to −233) were subcloned into pTKCIII. Mut 2-5:w was generated by digesting gel-purified HindIII/BamHI fragments of Mut 2-5 with MaelII. The gel-purified upstream fragment derived from the digestion of Mut 2-5 was subsequently religated with the downstream fragment derived from Mut w and subcloned into pTKCIII to produce the double mutant construct. All PCR products were sequenced after subcloning in order to verify the sequence.

**Purification of recombinant SREBP-1a, NF-YA, NF-YB, and NF-YC**

Recombinant SREBP-1a, containing the N-terminal T7 and polyhistidine (His6) tags was purified to homogeneity from E. coli extracts by nickel affinity chromatography as described by Sanchez, Yeh, and Osborne (10). Nucleotides corresponding to amino acids 262 to 317 of mouse NF-YA (28), 51 to 140 of mouse NF-YB (CBF-A) (29), and 37 to 120 of human NF-YC (30), respectively, were generated by polymerase chain reaction and cloned into parent plasmids to produce PET15YA9, PET29YB4, and PET29YC5. The polyhistidine-tagged proteins were purified from E. coli lysates by nickel affinity chromatography, as described above.

**Oligonucleotides**

Oligonucleotides used in gel mobility shift assays were synthesized by Gibco BRL. They include a 27 bp wild-type oligonucleotide (−298 to −272) (27) containing the ATTGG motif of the FPP synthase promoter, a 21 bp oligonucleotide (−250 to −230) containing the CCAAT motif and a 27 bp oligonucleotide (−298 to −272) containing a mutated ATTGG motif (CGTGT; Mut 2-0).

**Electroblotting shift assay**

CHO nuclear extracts were isolated as described (1, 4). Complementary single-stranded DNA, corresponding to the indicated nucleotides of the FPP synthase promoter (Fig. 1) and containing either the wild-type sequence or the indicated mutations, were annealed, and the double-stranded probe was isolated from acrylamide gels (31). Radiolabeled probes were generated in the same manner after an initial radiolabeling of a single-stranded oligonucleotide with 32P (31). Double-stranded probes were also generated by HindIII/BamHI restriction enzyme digestion of the wt and mutant pTKCIII constructs. The fragments, containing nucleotides −293 to −233 of the FPP synthase promoter and HindIII/BamHI flanking sequences, were gel-purified and end-radiolabeled with 32P. The radiolabeled probes (20,000 cpm; 1.5 fmol) were used in gel mobility shift assays in the presence of 2 or 5 μg of CHO nuclear extract and non-fat milk (2.5 mg/ml) or in the presence of recombinant purified histidine-tagged NF-YA, NF-YB, NF-YC, and/or SREBP-1a in the absence of non-fat milk. The concentration of the recombinant proteins, based on silver-stained gels, was less than 50 ng/μl. Where indicated, antibody (0.2 μg) to NF-YB or CBF-HSP was added to the incubations on ice for 30 min before gel electrophoresis was performed (1, 2).

**RESULTS**

**Mutational analysis of the FPP synthase promoter identifies three cis-elements required for sterol-regulated expression**

**Figure 1** shows the nucleotide sequence of the rat FPP synthase proximal promoter between nucleotides −293 and −233 (1). The ATTGG (inverted CCAAT box), SRE-3, and CCAAT box motifs are indicated, as are the mutations that were introduced to produce the mutant promoters (Fig. 1). We have previously demonstrated that the expression of a reporter gene under the control of nucleotides −293 to −233 from the FPP synthase promoter was regulated by sterols and by co-expressed SREBP-1a (1). We concluded that these 61 bp of the FPP synthase promoter were sufficient to confer sterol-regulated expression to an otherwise sterol-unresponsive reporter gene (1).
A series of reporter genes were constructed in which the wild-type 61 bp FPP synthase promoter sequence (−293 to −233) or the same sequence containing the indicated mutations (Mut 2-5, Mut q, Mut w, and Mut 2-5:w, Fig. 1) was placed 5' of a minimal thymidine kinase promoter-CAT gene. CV-1 cells were transiently transfected with these constructs and the cells were then incubated for 20 h in media either depleted of sterols (inducing media) or supplemented with cholesterol and 25-hydroxycholesterol (repressing media). As expected, the expression of the reporter gene under the control of the wild-type FPP synthase promoter was induced approximately 14-fold when the cells were deprived of sterols (Fig. 2). The expression of a reporter gene that contained a 3 bp mutation in the CCAAT box motif (Mut w) was severely crippled; the reporter gene activity was low and was induced less than 1.5-fold after incubation of cells in the inducing media (Fig. 2). Similar results were observed when the promoter contained mutations in both the CCAAT box and the ATTGG motif (Mut w:2-5) (Fig. 2).

Reporter genes that contained a 3 bp mutation in the SRE-3 motif (Mut q) were expressed at levels that approximated the repressed levels of the wild-type construct (Fig. 2). As expected, the activity of this mutant reporter gene was not induced when the cells were incubated in sterol-deficient medium (Fig. 2), consistent with the inability of endogenous SREBP to bind to the mutated SRE-3 (1). Finally, reporter genes that contained a point mutation in the ATTGG motif (Mut 2-5) also exhibited impaired regulation in response to sterol deprivation; reporter gene activity was regulated 3.6-
fold versus 14.1-fold in controls (Fig. 2). The data in Fig. 2 also demonstrate that, under inducing conditions, the absolute activity of the wild-type reporter construct exceeds the additive activities of the Mut q (two functional NF-Y binding sites) and Mut 2-5:w (functional SREBP binding site). Thus, synergistic activation of the FPP synthase promoter-reporter gene requires the presence of three motifs (ATTGG, SRE-3, and CCAAT).

Figure 3 shows that co-expression of low levels of a plasmid encoding mature SREBP-1a, together with the wild-type FPP synthase promoter-reporter gene, resulted in a 6.7-fold increase in CAT activity. In the absence of co-expressed SREBP-1a, the FPP synthase promoter-reporter genes, containing mutations in the ATTGG, CCAAT, or SRE-3 motifs, were expressed at lower levels than the control (Fig. 3). In the presence of co-expressed SREBP-1a, there was a variable increase in reporter gene activity, although the maximal levels obtained were significantly less than the control (Fig. 3). Figure 3 demonstrates that co-expressed SREBP-1a does stimulate reporter genes containing an intact SRE-3 and either the wild-type ATTGG or CCAAT motif. However, mutation of both of these motifs (Mut 2-5:w) results in a reporter gene that is the least responsive to the co-expressed SREBP-1a (Fig. 3). The small stimulation of the Mutant q reporter gene by co-expressed SREBP-1a (Fig. 3) is likely the result of the relatively high levels of nuclear SREBP-1a that persist under these conditions. Gel mobility shift assays have shown that SREBP-1a binds weakly to DNA containing the q mutation, under specific conditions that include high concentration of the recombinant protein (data not shown). The observation that the maximal reporter gene activity obtained with the wild-type construct was greater than the added activities obtained with Mut q and Mut 2-5:w (Fig. 3) is consistent with synergistic activation of the reporter gene under conditions where nuclear proteins bind to all three motifs.

Taken together, the data in Figs. 2 and 3 identify a third motif (CCAAT), in addition to the previously defined ATTGG and SRE-3 motifs (1, 2, 27), that is important for sterol- and SREBP-1a-regulated expression of FPP synthase promoter-reporter genes.

Role of CCAAT- and ATTGG-binding proteins in the synergistic binding of SREBP-1a to SRE-3

Electromobility shift assays were carried out with oligonucleotides corresponding to different motifs within the FPP synthase promoter; a 27 bp 5′ probe (−298 to −272) containing either the wild-type ATTGG or CCAAT sequence or 5 bp transversion (CGGTT; Mut 2-0) and a 21 bp 3′ probe (−250 to −230) containing the CCAAT motif (Fig. 1).

Incubation of the 32P end-labeled 5′ probe with nuclear extract derived from CHO cells resulted in the formation of one major complex (Fig. 4A, lanes 2 and 7) that was competed by unlabeled competitor DNA.
corresponding to either the 5′ probe (Fig. 4A, lanes 3–6) or the 3′ oligonucleotide containing the CCAAT motif (lanes 12–15). The competition by these two probes was similar at equivalent molar concentrations (Fig. 4A, lanes 3–6 vs. 12–15). In contrast, the formation of the shifted complex was unaffected by inclusion of the 5′ mutant oligonucleotide over the same molar concentration range (Fig. 4A, lanes 8–11).

Incubation of radiolabeled 5′ ATTGG- or 3′ CCAAT-containing probes with CHO nuclear extract produced a supershifted complex when antibody to NF-YA was also included in the assay (Fig. 4B, lanes 4 and 12 vs. lanes 2 and 10, respectively). This latter effect was specific, as inclusion of antibody to CBF-HSP, a distinct CCAAT box binding protein (26), did not affect the formation or migration of the single complex (Fig. 4B, lanes 3 and 11). The mutant probe (Mut 2-0) formed a non-specific shifted complex that was unaffected when antibodies to either CBF-HSP or NF-YA were included in the assay (Fig. 4B, lanes 6–8).

We previously utilized nuclear extracts, enriched in NF-Y, to demonstrate that the binding of NF-Y to the 5′ ATTGG motif within the FPP synthase promoter, stimulated by more than 20-fold the binding of SREBP-1a to the adjacent SRE-3 motif (4). Immuno-depletion of this nuclear fraction with anti-NF-Y antibodies provided direct evidence of the importance of NF-Y in enhancing the binding of SREBP-1a to SRE-3 (4). However, we were unable to assess whether other nuclear proteins, in addition to NF-Y, played a role in the stimulated binding of SREBP-1a to SRE-3.

The data shown in Figs. 2 and 3 suggested that the binding of NF-Y to the 3′ CCAAT motif, in addition to binding to the 5′ ATTGG motif, might influence the binding of SREBP-1a to the adjacent SRE-3 (Figs. 2 and 3). The availability of recombinant NF-YA, NF-YB, NF-YC, and SREBP-1a permitted us to determine more exactly the role of the three NF-Y proteins per se in stimulating the binding of SREBP-1a to SRE-3 and to determine the function of the 3′ CCAAT motif in this latter process.

Recombinant truncated NF-YA, NF-YB, and NF-YC, containing N-terminal T7 and polyhistidine tags, were purified from Escherichia coli extracts by nickel affinity chromatography. Each of the proteins migrated as a single major band when analyzed by polyacrylamide gel electrophoresis (data not shown).

Figure 5A demonstrates that, under these conditions, recombinant trimeric NF-Y (lane 2) and recombinant SREBP-1a (lane 3) bound poorly to the wild-type probe containing the ATTGG, SRE-3, and CCAAT motifs. Addition of increasing amounts of the three recombinant
subunits of NF-Y together with SREBP-1a resulted in a large increase in the formation of both an SREBP-1a:DNA complex and an NF-Y:DNA complex (Fig. 5A, lanes 4–7). Under these conditions, an NF-Y:SREBP-1a:DNA complex was not observed. A slower migrating complex (indicated by * in Fig. 5A and B) corresponds to multimeric SREBP-1a bound to DNA. This complex was observed in some, but not all, experiments in which NF-Y was included and in other gel mobility shift assays where high levels of SREBP-1a and non-fat milk were used in the absence of NF-Y (data not shown).

Formation of the SREBP-1a:DNA complex required...
that the DNA contain binding sites for the added recombinant NF-Y; a probe that contained mutations in both the ATTGG and CCAAT motifs bound neither SREBP-1a nor NF-Y (Fig. 5A, lanes 9–14). Figure 5B shows that recombinant NF-Y also stimulated, in a dose-dependent manner, the formation of an SREBP:DNA complex when the radiolabeled probe contained a mutation in either the 5' ATTGG (compare lane 3 with lanes 4–7) or 3' CCAAT (compare lane 10 with lanes 11–14) motifs. Figure 5B also demonstrates that the heterotrimeric NF-Y forms a more stable complex with the DNA when bound to the 5' ATTGG motif as compared to the 3' CCAAT motif (compare lanes 7 and 14). However, when the probe contained both the ATTGG and CCAAT motifs, the NF-Y:DNA complex was observed at lower concentrations of NF-Y than when the probe contained only one of these motifs (compare Fig. 5A with 5B). This enhanced binding of NF-Y to the DNA may be important for the subsequent binding of SREBP-1a to the SRE-3 motif.

Previous studies have demonstrated that the ATTGG/CCAAT nucleotides are absolutely required for the binding of the heterotrimeric NF-Y to DNA but that there is a strong preference for additional flanking nucleotides (21, 23, 32). We hypothesize that, under the DNA binding and electrophoretic conditions used in these studies, the stability and/or affinity of truncated recombinant NF-YA, B, and C for the DNA is reduced, when compared to non-truncated NF-Y, and results in impaired formation of stable SREBP:DNA:DNA:DNA complexes. Nonetheless, the results demonstrate that the formation of a stable SREBP-1a:DNA complex is markedly enhanced by NF-Y by a process that requires either the ATTGG and/or CCAAT motifs.

Figure 5C demonstrates that the formation of the SREBP-1a:DNA complex is dependent on the presence of all three NF-Y subunits (compare lane 4 with lanes 3, 5, 6, and 7).

DISCUSSION

In the current studies we demonstrate that the regulated expression of FPP synthase promoter-reporter genes by sterols is dependent on three cis-elements in the proximal promoter. We have termed this 61 bp fragment a sterol regulatory unit because mutation in any of the three elements interferes with the regulation of reporter genes by sterols. The stimulated expression of FPP synthase promoter-reporter genes that occurs when cells are incubated either under conditions of sterol deprivation or when the cells are co-transfected with a plasmid encoding mature SREBP-1a, is dependent on the binding of SREBP-1a to SRE-3. However, the binding of SREBP-1a to SRE-3 is itself dependent on the binding of NF-Y to the ATTGG and CCAAT motifs. These latter two motifs lie 20 bp and 10 bp 5' and 3', respectively, of SRE-3. The current studies demonstrate that the CCAAT motif, 3' of SRE-3, is also critical for the sterol- and SREBP-1a-regulated expression of FPP synthase promoter-reporter genes (Figs. 2 and 3), presumably because of its role in stimulating the binding of SREBP to SRE-3 (Fig. 5). The in vitro gel mobility shift studies utilized truncated recombinant NF-YA, B, and C proteins that are devoid of activation domains but are able to form a heterotrimeric core complex that binds DNA. The binding of this heterotrimeric NF-Y core to either the ATTGG or CCAAT motifs in the FPP synthase promoter is required and sufficient to stimulate the binding of SREBP-1a to its cognate DNA binding site (Fig. 5). Other nuclear proteins are not necessary for the observed synergism. We hypothesize that the NF-Y-dependent increase in SREBP-1a:DNA complex formation is a result of altered conformation of the SREBP-1a bound to the DNA. This hypothesis is based on previous studies in which both the heterotrimeric full length and truncated NF-Y subunits were shown to bend DNA (24) (R. Mantovani, unpublished results). Further experiments will be necessary to determine whether the synergistic binding of SREBP-1a to the SRE-3 motif is dependent on this NF-Y-induced bending of the DNA.

Studies with other transcriptional factors, including Ets-1 (33), p53 (34), and the TATA binding protein (35) demonstrate that truncated forms of these proteins exhibit increased DNA binding activity relative to the full-length proteins. Such results are consistent with the presence of inhibitory protein domains that are deleted from the truncated proteins. In more recent studies, BamHI endonuclease was shown to undergo a conformational change upon binding to DNA that disordered an inhibitory domain of the protein (36). The protein:DNA interactions in these latter studies did not require the presence of a second DNA binding protein. In contrast, the current studies demonstrate that SREBP-1a conformation/affinity for DNA is altered in an NF-Y:DNA-dependent manner. Further studies will be required to determine whether SREBP-1a also contains an inhibitory domain that modulates the interaction with DNA.

Induced expression of reporter genes in sterol-deprived cells has been observed for a number of different constructs; reporter genes containing promoters derived from the genes encoding either FPP synthase, HMG-CoA synthase (2, 37), SREBP-2 (14), squalene synthase (17), or glycerol-3-phosphate acyltransferase (15) are dependent on the presence of binding sites...
for both SREBP and NF-Y. Based on the current studies, we hypothesize that SREBP and NF-Y will bind synergistically to the promoters of the latter four genes and result in transcriptional activation.

In contrast, under similar conditions of cellular sterol deprivation, high levels of expression of reporter genes under the control of promoters derived from either the LDL receptor (10, 11, 38, 39), fatty acid synthase (12) or acetyl CoA carboxylase (13) genes, require that the promoters contain binding sites for SREBP and Sp1.

It is possible that transcriptional activation of the FPP synthase gene observed in sterol-depleted cells is dependent in part on the binding of DNA that has been shown to occur when NF-Y binds to its cognate site (24) and in part on the interaction of SREBP with other nuclear proteins, including CBP (40). Further studies should determine whether CBP and/or other associated proteins are involved in the NF-Y- and SREBP-dependent expression of genes encoding FPP synthase, HMG-CoA synthase, squalene synthase, SREBP-2, and glycerol-3-phosphate acyltransferase.

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