YY1 Is a Negative Regulator of Transcription of Three Sterol Regulatory Element-binding Protein-responsive Genes*

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Ying Yang 1 (YY1) is shown to bind to the proximal promoters of the genes encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, farnesyl diphosphate (FPP) synthase, and the low density lipoprotein (LDL) receptor. To investigate the potential effect of YY1 on the expression of SREBP-responsive genes, HepG2 cells were transiently transfected with luciferase reporter constructs under the control of promoters derived from either HMG-CoA synthase, FPP synthase, or the LDL receptor genes. The luciferase activity of each construct increased when HepG2 cells were incubated in lipid-depleted media or when the cells were cotransfected with a plasmid encoding mature sterol regulatory element-binding protein (SREBP)-1a. In each case, the increase in luciferase activity was attenuated by coexpression of wild-type YY1 but not by coexpression of mutant YY1 proteins that are known to be defective in either DNA binding or in modulating transcription of other known YY1-responsive genes. In contrast, incubation of cells in lipid-depleted media resulted in induction of an HMG-CoA reductase promoter-luciferase construct by a process that was unaffected by coexpression of wild-type YY1.

Electromobility shift assays were used to demonstrate that the proximal promoters of the HMG-CoA synthase, FPP synthase, and the LDL receptor contain YY1 binding sites and that YY1 displaced nuclear factor Y from the promoter of the HMG-CoA synthase gene. We conclude that YY1 inhibits the transcription of specific SREBP-dependent genes and that, in the case of the HMG-CoA synthase gene, this involves displacement of nuclear factor Y from the promoter. We hypothesize that YY1 plays a regulatory role in the transcriptional regulation of specific SREBP-responsive genes.

Three sterol regulatory element-binding proteins (1) termed SREBP-1a, SREBP-1c, also called ADD-1 (2), and SREBP-2 constitute a unique family of transcription factors that are synthesized as 125-kDa precursor proteins that are localized to the endoplasmic reticulum in sterol-loaded cells. Cellular sterol deprivation results in two sequential proteolytic cleavages of SREBP and the release of a mature 68-kDa amino-terminal domain of the protein from the endoplasmic reticulum (1, 3). Mature SREBP enters the nucleus and activates transcription of target genes by a process that is dependent upon the binding of SREBP and either NF-Y and/or Sp1 to the proximal promoters of these genes (1, 4–8). In vitro studies have demonstrated that SREBP and either NF-Y or Sp1 bind synergistically to DNA derived from the proximal promoters of the FPP synthase (5, 9) or LDL receptor (6) genes, respectively. This synergy is thought to account for the increased transcription of SREBP-responsive genes that include, in addition to the SREBP-2 gene itself (10), those that control cholesterol homeostasis (the LDL receptor, HMG-CoA synthase, HMG-CoA reductase, FPP synthase, and squalene synthase) (1, 7, 11), fatty acid synthesis (fatty acid synthase and acetyl CoA carboxylase) (12, 13), fatty acid desaturation (stearoyl-CoA desaturase 2) (14), and triacylglyceride synthesis (glycerol-3-phosphate acyltransferase) (15).

In our continued efforts to elucidate the role of the transcriptional coactivator CREB-binding protein (CBP) in sterol-regulated transcription, we noted that overexpression of the most amino-terminal domain of CBP (amino acids 1–451), as a Gal4 fusion protein, increased the expression of certain sterol-regulated reporter genes under conditions where the cells were incubated in the presence of excess sterols.3 A potential explanation for these results could be that overexpression of this domain of CBP interfered with the function of a transcriptional repressor. This prompted us to search for a CBP-dependent repressor that may regulate the expression of specific sterol-regulated genes. One potential candidate was the transcription factor Ying Yang 1 (YY1), which is known to have repressor functions and to interact with the amino-terminal domain of CBP (16). A visual inspection of a number of promoters of sterol-regulated genes indicated that several of these contained potential YY1 binding sites (CCAT or ACAT), either overlapping or adjacent to binding sites for NF-Y, Sp1, or SREBP. Earlier studies show (4) that mutation of three of the four nucleotides in a CCAT motif in the FPP synthase promoter resulted in increased expression of an FPP synthase promoter-reporter gene. Because this CCAT motif was adjacent to SRE-3, the SREBP binding site that is important for sterol-regulated transcription of this gene (4), the result was consistent with the hypothesis that mutation of the CCAT sequence disrupted a cis element that normally functions to inhibit transcription of the FPP synthase gene. These observations prompted us to analyze cAMP response element-binding protein (CREB) binding protein; PMSF, phenylmethylsulfonyl fluoride.

1 The abbreviations used are: SREBP, sterol regulatory element-binding protein; FPP, farnesyl diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SRE, sterol regulatory element; YY1, Ying Yang 1; NF-Y, nuclear factor Y; LDL, low density lipoprotein; CBP, constituting a unique family of transcription factors that are synthesized as 125-kDa precursor proteins that are localized to the endoplasmic reticulum in sterol-loaded cells. Cellular sterol deprivation results in two sequential proteolytic cleavages of SREBP and the release of a mature 68-kDa amino-terminal domain of the protein from the endoplasmic reticulum (1, 3). Mature SREBP enters the nucleus and activates transcription of target genes by a process that is dependent upon the binding of SREBP and either NF-Y and/or Sp1 to the proximal promoters of these genes (1, 4–8). In vitro studies have demonstrated that SREBP and either NF-Y or Sp1 bind synergistically to DNA derived from the proximal promoters of the FPP synthase (5, 9) or LDL receptor (6) genes, respectively. This synergy is thought to account for the increased transcription of SREBP-responsive genes that include, in addition to the SREBP-2 gene itself (10), those that control cholesterol homeostasis (the LDL receptor, HMG-CoA synthase, HMG-CoA reductase, FPP synthase, and squalene synthase) (1, 7, 11), fatty acid synthesis (fatty acid synthase and acetyl CoA carboxylase) (12, 13), fatty acid desaturation (stearoyl-CoA desaturase 2) (14), and triacylglyceride synthesis (glycerol-3-phosphate acyltransferase) (15).

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YY1, also known as δ, CF-1, UCRBP, NF-E1, FACT-1, CBP, LBF, NMP, and MAPF-1 (17–19), binds to a core DNA motif (5’-CCAT-3’ or 5’-ACAT-3’) that shows considerable heterogeneity in the flanking nucleotides (17, 18, 20). YY1 contains distinct domains that regulate, either by transactivation (17–19, 21, 22) or transpression (17–19, 24–31), the expression of a number of genes. YY1 may also function as an initiator-binding protein that activates transcription in the absence of the TATA box-binding protein (17–19, 32). YY1 is identical to NMP-1, a nuclear matrix protein (33) that is thought to regulate histone H4 gene transcription.

YY1 is expressed constitutively in many growing, differentiated, and growth-arrested cells (16). However, recent studies demonstrated that YY1 protein levels decreased during skeletal and cardiac myocyte differentiation (24), consistent with an earlier proposal that down-regulation of YY1 was essential for the expression of the sarcomeric α-actin genes (25, 26). Earlier studies had demonstrated that YY1 inhibited muscle-restricted expression of the skeletal α-actin gene by excluding serum response factor from the serum response element in the proximal promoter of the α-actin gene (26). Thus, differential expression of the YY1 protein in myocytes is thought to affect both transcription of specific genes and cell differentiation.

The current studies demonstrate that co-expression of YY1 results in transcriptional repression of specific SREBP-responsive genes, that the promoters of these genes contain binding motifs that are recognized by YY1, and that YY1 and NF-Y bind cooperatively to overlapping motifs in the promoter of the HMG-CoA synthase gene. These results are consistent with the hypothesis that transcription of specific sterol-regulated genes, including HMG-CoA synthase, FPP synthase, and the LDL receptor, is affected by YY1.

**EXPERIMENTAL PROCEDURES**

**Materials—DNA restriction and modification enzymes were obtained from Life Technologies, Inc.** 7P-Labeled nucleotide triphosphates were obtained from Amersham Pharmacia Biotech. 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, University of California, Irvine, CA). Constructs encoding wild-type YY1 or mutant YY1 proteins under control of the cytomegalovirus promoter, were generous gifts from Dr. L. Lissher and M. Austein (Institut fur Molekularbiology, Hannover, Germany) (16). Antibodies to YY1 protein and oligonucleotides containing a consensus YY1 binding site (5’-CGTCGCGCCGCATCATTTGGCGCCTGTTG-3’) were from Santa Cruz Biotechnology, Inc. Lipoprotein-deficient fetal calf serum was purchased from PerImmune. The sources of all other reagents and plasmids have been given (4, 5, 7, 9).

**Cell Culture, Transient Transfections, and Reporter Gene Assays—** HepG2 cells were cultured as described (4). Plasmids were transiently transfected into HepG2 cells using the MBS Transfection Kit (Stratagene) with minor modifications (4, 14). The luciferase reporter constructs (1 µg/60-mm dish) under the control of promoters derived from genes encoding FPP synthase (pPPPS-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) have been previously described (7). Plasmids encoding SREBP-1a (pCMV-CSA10), YY1, or YY1 mutants (Δ296–331, Δ399–414, Δ334–414, Δ154–199) were transfected into cells as indicated in the specific legends. After transfection, cells were incubated for 24 h in media supplemented with either 10% lipoprotein-deficient calf serum in the absence (inducing media) or presence (repressing media) of sterols (10 mM cholesterol and 1 µg/ml 25-hydroxycholesterol) (4) as indicated in the legends. Cells were then lysed, and the luciferase and β-galactosidase activities were determined (4). The β-galactosidase activity was used to normalize for any variations in transfection efficiencies (4). Each experimental point was performed in duplicate (variation <10%), and each experiment was repeated two or more times with similar results.

**RESULTS**

YY1 Inhibits the Expression of Three Sterol-regulated Genes—The experiments of Fig. 1 demonstrate that incubation of cells in sterol-depleted media resulted in increased expression of an HMG-CoA synthase promoter-reporter gene (pSYNSRE) (lane 1 versus 2). The induction was attenuated approximately 80% when cells were cotransfected with low levels of a plasmid encoding wild-type YY1 (Fig. 1A, lane 1 versus 3, 5, and 7). In contrast, the induction of pSYNSRE, in response to cellular sterol depletion, was relatively unaffected when cells were cotransfected with plasmids encoding mutant YY1 proteins YY1(Δ296–331) (Fig. 1B) or YY1(Δ399–414) (Fig. 1C). YY1(Δ296–331) and YY1(Δ399–414), unlike wild-type YY1, are unable to bind DNA in vitro or to transactivate other reporter genes in vivo (16). In agreement with the report by Austein et al. (16), Western blot assays, utilizing antibody to YY1 and extracts obtained from cells transiently transfected with either the wild-type or mutant YY1 constructs, demonstrated that similar levels of YY1 protein were expressed from these different constructs (Fig. 1D).

The experiments of Fig. 2 demonstrate that the induction of two other reporter genes (pPPPS and pLDLr), in response to cellular sterol depletion, was attenuated by coexpression of wild-type YY1 (Fig. 2, A–B, lanes 3 versus 1). The inhibitory effects of YY1 were specific because induction of the HMG-CoA reductase promoter-reporter construct (pRED) was unaffected by...
coexpressed YY1; indeed, coexpression of YY1 often resulted in a small increase in the expression of this promoter-reporter gene (Fig. 2C, lane 3 versus 1).

The increased expression of pFPPS, pSYNSRE, and pLDDLr that occurred when cells were incubated in sterol-depleted media was unaffected following cotransfection of the cells with the YY1 mutant constructs YY1(Δ296–331), YY1(Δ399–414) (Figs. 3A and B), YY1(Δ334–414) (Fig. 3C), or YY1(Δ154–199) (data not shown). Deletion of amino acids 334–414 or 154–199 from YY1 prevents nuclear localization, DNA binding, and transactivation or interaction with CBP (16), respectively.

Sterol-regulated induction of the reporter genes utilized in the current study requires nuclear localization of mature SREBP, a process that is normally dependent on cellular cholesterol depletion (1). In the experiment of Fig. 4, cells were incubated in the presence of excess sterols to repress the proteolytic cleavage and release of mature endogenous SREBP from the endoplasmic reticulum. Consequently, luciferase activity measured in cells transiently transfected with either an HMG-CoA synthase (Fig. 4A) or HMG-CoA reductase (Fig. 4B) promoter-reporter gene was low but increased dramatically when sterol-treated cells were cotransfected with a plasmid encoding mature SREBP-1a (Fig. 4, lanes 4 versus 1). Fig. 4A shows that the SREBP-1a-dependent induction of the HMG-CoA synthase promoter-reporter gene was attenuated by coexpression of wild-type YY1 (lane 4 versus 2) but not by coexpression of mutant YY1(Δ296–331) (lane 6 versus 2) or YY1(Δ399–414) (lanes 8 versus 2) proteins. In contrast, induction of the HMG-CoA reductase promoter-reporter gene by coexpressed SREBP-1a was unaffected by coexpressed YY1 (Fig. 4B, lane 4 versus 2). Under sterol-repressed conditions, the expression of pFPPS, pLDDLr, pSYNSRE, and pRED was unaffected by coexpressed wild-type or mutant YY1 (Figs. 1–4; data not shown).

Taken together, the studies described in Figs. 1–4 demonstrate that wild-type YY1 represses the transcriptional induction of promoter-reporter constructs derived from three genes (FPP synthase, HMG-CoA synthase, and the LDL receptor. The increased expression of pFPPS, pSYNSRE, and pLDDLr that occurred when cells were incubated in sterol-depleted media was unaffected following cotransfection of the cells with the YY1 mutant constructs YY1(Δ296–331), YY1(Δ399–414) (Figs. 3A and B), YY1(Δ334–414) (Fig. 3C), or YY1(Δ154–199) (data not shown). Deletion of amino acids 334–414 or 154–199 from YY1 prevents nuclear localization, DNA binding, and transactivation or interaction with CBP (16), respectively.

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Taken together, the studies described in Figs. 1–4 demonstrate that wild-type YY1 represses the transcriptional induction of promoter-reporter constructs derived from three genes (FPP synthase, HMG-CoA synthase, and the LDL receptor.
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YY1 binds to the promoters of the FPP synthase, HMG-CoA synthase, and the LDL receptor genes and displaces NF-Y from an Inverted CCAAT Box in the HMG-CoASynthase Promoter—The proximal promoters of HMG-CoA reductase, FPP synthase, HMG-CoA synthase, and the LDL receptor genes that were used in the current study are illustrated in Fig. 5A. Transcriptional activation of the latter three genes in response to cellular sterol depletion is dependent on the binding of SREBP and either NF-Y or Sp1 to the proximal promoters (Fig. 5A; 1, 5–8, 34, 36). Transcriptional activation of the HMG-CoA reductase gene is less well understood, although such activation does require SREBP (1, 37). The proximal promoters of all four SREBP-responsive genes contain putative binding sites (CCAT or ACAT) for YY1 that are adjacent to or overlap with binding sites for SREBP or NF-Y (Fig. 5A). We postulated that the inhibitory effect of YY1 (Figs. 1–4) might result from YY1-mediated displacement of SREBP, NF-Y, or Sp1 from the promoters of pFPPS, pSYNSRE, and pLDLr. Alternatively, YY1 might bind to DNA and sterols (10 μg/ml cholesterol and 1 μg/ml 25-hydroxycholesterol). The relative luciferase activities are the means of three experiments, each performed in duplicate.

To test these postulates, we utilized 32P-end-labeled DNA probes corresponding to the proximal promoters of these genes together with recombinant YY1 and YY1 proteins in electromobility shift assays (Figs. 5, B and D) and DNase I footprint assays (Figs. 5C). Fig. 5B shows the results obtained in EMSAs that utilized recombinant YY1 and 32P-end-labeled DNA, corresponding to the proximal promoters of either HMG-CoA synthase (−324 to −225), FPP synthase (−365 to −47) or the LDL receptor (−225 to −116) genes. The results indicate that recombinant YY1 binds to all three DNA fragments and results in the formation of multiple-shifted DNA-protein complexes (Fig. 5B). The shifted DNA-YY1 complexes were specific because inclusion of excess unlabeled DNA containing a consensus YY1 binding site competed for binding, whereas the addition of excess unlabeled DNA containing a mutated YY1 binding site did not affect the formation of the 32P-DNA:YY1 complex (data not shown). YY1 also bound to a 117-base pair fragment of the FPP synthase promoter (−293 to −177) that includes the binding sites for NF-Y and SREBP (Fig. 5A) (data not shown). The formation of multiple-shifted complexes was also observed when a radiolabeled DNA fragment containing a single consensus YY1 binding motif was incubated with recombinant YY1 protein (data not shown). The formation of multiple specific complexes between DNA and recombinant YY1 has been noted previously (19).

Fig. 5C shows that recombinant YY1 protein bound to the HMG-CoA synthase promoter and protected nucleotides −306 to −290 from DNase I digestion. Analysis of the complementary (upper) DNA strand sequence indicates that the protected DNA includes a classical core YY1 binding site (CCAT, −297 to −294) (Fig. 5C, open circle) that overlaps an NF-Y binding site (ATTTG, −295 to −291) (Fig. 5C, open rectangle). The binding of NF-Y to this ATTTG sequence has been shown to be critical and necessary for elevated transcription of the HMG-CoA synthase promoter-reporter gene in response to cellular sterol deprivation (7, 8). Additional EMSAs demonstrated that a 100-base pair fragment of the HMG-CoA synthase promoter (−324 to −225) bound either recombinant YY1 (Fig. 5D, lane 2) or NF-Y (Fig. 5D, lane 3). However, the addition of excess YY1 displaced NF-Y from the DNA, and no DNA:YY1: NF-Y complex was observed (Fig. 5D, lane 4). In addition, when the protein ratio of YY1: NF-Y was <4:1, the radiolabeled DNA probe was bound by either YY1 or NF-Y but not by the two proteins at once (data not shown), consistent with the hypothesis that the two proteins are unable to bind to the probe at the same time. Incubation of the FPP synthase probe with YY1 and either NF-Y or SREBP resulted in the formation of multiple complexes (data not shown). In contrast to the studies with the HMG-CoA synthase probe, we obtained no evidence that YY1 displaced either NF-Y or SREBP from the FPP synthase probe (data not shown).

DISCUSSION

The current studies have led to the identification of YY1 as a transcriptional repressor of some, but not all, SREBP/ADD1-regulated genes. We identified cis elements that bind recombinant YY1 protein in the proximal promoters of FPP synthase, HMG-CoA synthase, the LDL receptor, and HMG-CoA reductase genes (Fig. 5B and data not shown). However, coexpression of wild-type YY1 inhibited the sterol-dependent regulation of pFPPS, pSYNSRE, and pLDLr but not pRED (Figs. 1, 2, and 4), consistent with an inhibitory effect of YY1 on the transcription of some, but not all, SREBP/ADD1-regulated genes. Detailed studies demonstrate that YY1 and NF-Y bind to overlapping sites on the HMG-CoA synthase promoter and that NF-Y is displaced from the DNA by excess YY1 (Figs. 5A′ and D′). Previous studies have demonstrated that elevated transcription of the HMG-CoA synthase gene is dependent, in part, on the binding of NF-Y to ATTTG (an inverted CCAAT box) (7–9), a motif that overloads the YY1 binding site (Fig. 5C). Thus, it appears likely that the displacement of NF-Y from the inverted CCAAT box by YY1 (Fig. 5D) accounts for the attenuated transcription of the HMG-CoA synthase gene by cotransfected
We have been unable to demonstrate YY1-dependent displacement of either NF-Y or SREBP from the FPP synthase or LDL receptor proximal promoters (data not shown). These results are perhaps not surprising because the putative YY1 binding sites do not overlap with the binding sites for NF-Y, Sp1, or SREBP (Fig. 5A). Further studies will be necessary to determine the mechanism by which YY1 represses the transcription of the FPP synthase and LDL receptor genes. Repression may result from inappropriate bending of DNA by YY1 (18, 38) or quenching, as the result of the sequestration of, or interference with, coactivator proteins such as CBP (16, 18, 19). CBP is known to interact with both SREBP (39, 40) and YY1 (16) and to be required for SREBP-dependent transcriptional induction of FPP synthase and the LDL receptor genes (39, 40).

Alternatively, YY1 may bind to the promoters and recruit histone deacetylases such as histone deacetylase 1–3 that are known to interact with YY1 (41). These histone deacetylases repress transcription when tethered to promoters through interaction with YY1 (41). Further studies are needed to determine whether histone deacetylation plays a role in sterol-regulated transcription.

Regardless of the exact mechanism, we hypothesize that the role of YY1 is to maintain SREBP-regulated genes in a transcriptionally repressed state in the absence of nuclear SREBP. When the nuclear levels of SREBP increase, following either cellular cholesterol starvation (1) or adipocyte differentiation (2, 15, 42), we propose that the YY1-mediated repression is overcome through synergistic interactions between SREBP and either NF-Y or Sp1 on the DNA. The finding that the nuclear levels of YY1 remain constant would ensure that the expression of these genes is turned off in a timely manner as the nuclear levels of SREBP decrease. Future studies will be directed at understanding the factors that modulate the activity of YY1.

Transcription of each SREBP-regulated gene is likely to be complex and to depend not only on the nuclear localization of YY1 (Figs. 1 and 4).
both positive and negative transcription factors but also on the relative affinity of each factor for specific cis elements, the number of these elements in each promoter, and the transcriptional strength of the final complex. The observation that transcriptional induction of different genes in response to SREBP is markedly different in mice that overexpress SREBPs (35) is but one example of the complexity of this process.

In preliminary studies, an HMG-CoA synthase promoter-reporter gene was transiently transfected into HepG2 cells, and the cells were then grown in media supplemented with sterols to repress the proteolytic release of SREBPs from the endoplasmic reticulum and the transcription of the reporter gene. A partial relief of this repression was observed in cells cotransfected with a plasmid encoding the adenoviral E1A protein (data not shown). A further increase in the expression of the reporter gene was observed in cells cotransfected with plasmids encoding both E1A and CBP (data not shown). This result is consistent with earlier observations demonstrating that E1A can relieve YY1 transcriptional repression in a CBP/p300-dependent fashion (29). These additional results with the HMG-CoA synthase promoter-reporter gene provide further support for the proposal that endogenous YY1 affects the transcription of specificsterol- and SREBP-regulated genes.

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