Multiple Mechanisms of Transcriptional Repression by YY1

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Received 23 December 1996/Returned for modification 11 February 1997/Accepted 21 April 1997

The four C-terminal GLI-Krüppel type zinc fingers of YY1 have been identified as a transcriptional repression domain. Previous reports have proposed DNA-bending and activator-quenching mechanisms for this zinc finger-mediated repression. In addition, previous work indicated that p300 and CBP might be involved in YY1-mediated repression. We have analyzed these possible models for the zinc finger-mediated repression. The role of each zinc finger in the repression and DNA-binding functions was determined by using a structure-and-function approach. We show that zinc finger 2 of YY1 plays a central role in both DNA binding and transcriptional repression. However, a survey of a panel of YY1 mutants indicates that these two functions can be separated, which argues against the DNA-bending model for repression. We show that the physical interaction between YY1 and p300, a coactivator for CREB, is not sufficient for repression of CREB-mediated transcription. Our studies indicate that YY1 functions as an activator-specific repressor. Repression of CTF-1-directed transcription may be accomplished through direct physical interaction between YY1 and this activator. In contrast, physical interaction is not necessary for YY1 to repress Sp1- and CREB-mediated transcription. Rather, the repression likely reflects an ability of YY1 to interfere with communication between these activators and their targets within the general transcription machinery. Taken together, our results suggest that YY1 employs multiple mechanisms to achieve activator-specific repression.

YY1 is a DNA-binding zinc finger transcription factor that is highly conserved from xenopus through humans. YY1 has been shown to function as an activator, a repressor, and an initiator of transcription (reviewed in references 43 and 44). The observation that YY1 can simultaneously activate and repress different genes in the same cell illustrates the role of promoter-specific factors in regulating the activities of YY1 (3, 25, 26, 38). While the identification of separate activation and repression domains in YY1 has shed some light on its multifunctionality (4, 23, 26), the mechanisms by which YY1 carries out and regulates its transcriptional activities remain unclear. Delineating the mechanisms of each individual function may lead to a better understanding of the global role of YY1 in transcriptional regulation. In this study, we have examined possible mechanisms of repression by YY1.

For several well-characterized promoters, one level of repression by YY1 results from its ability to displace activators with overlapping binding sites (reviewed in references 43 and 44). This passively accomplished repression may be augmented by active repression once YY1 is promoter bound. Active repression is demonstrated when elimination of YY1 binding by mutation of its recognition site results in a higher level of promoter activity than that resulting from the simple lack of activator binding (for an example, see reference 29). In formulating mechanistic models for this active repression, the influence of promoter-specific factors on the activities of YY1 must be considered.

Three models of active repression have been defined (reviewed in references 8, 13, 17, and 27). These are direct repression, quenching by targeting an activator (which will be called type I quenching), and quenching by targeting a general factor (type II quenching). A direct repressor interferes with the assembly or action of the transcriptional preinitiation complex through physical interactions with its components. This model predicts repression that is independent of the specific activators functioning on the promoter. In contrast, both quenching models predict activator-specific repression, because a quenching repressor specifically interferes with productive interactions between a particular activator and the preinitiation complex. A protein that represses transcription by interacting with the activator itself is defined as a type I quenching repressor. In contrast, a type II quenching repressor, by definition, interferes with targets of the activator; i.e., it may interact with a general factor (for example, TFIID) in a way that specifically blocks the response to an activator.

Deletion analysis has indicated that the four C-terminal GLI-Krüppel type zinc fingers of YY1 constitute a strong repression domain (4, 23, 26, 28). Several models have been proposed for the mechanism of repression by this domain. A study of YY1-mediated repression of the c-fos promoter suggested a type I quenching mechanism (51). The repression requires an ATF/CREB binding site located adjacent to the −54 YY1 binding site. The demonstration of protein-protein interactions between the zinc fingers of YY1 and several ATF/CREB family members suggests that YY1 may target these factors to block transcriptional activation. Interestingly, a previous study of the same promoter proposed a distinctive mechanism of repression involving the DNA-binding function of the zinc fingers. It was suggested that YY1 binding bends the c-fos promoter so as to disrupt interactions between the ATF/CREB factors and their activation targets on the preinitiation complex (33).

Previous studies in our laboratory raised the additional possibility that p300, which has been shown to interact with both YY1 and CREB, as well as with other activators, might be involved in repression by YY1 (21, 31). Originally described as an adenovirus E1A-associated protein (14, 50), p300 has been identified as a transcriptional coactivator that is closely related to, and largely functionally interchangeable with, the CREB-binding protein CBP (1, 6, 11, 20, 31). We have shown that E1A relieves YY1-mediated repression by binding to p300,
which is complexed with YY1 (21). The function of the physical interaction between YY1 and p300 in the absence of E1A remains unclear. As a widely utilized coactivator, p300 could be a target for quenching by YY1 or, alternatively, might function as a YY1-bound cofactor.

The importance of promoter-specific factors in repression by YY1 points to a possible quenching mechanism for repression, involving either activators or general factors as targets of YY1. YY1 has been reported to physically interact with several proteins of each class, including the general factors TFIIIB, TBP and the specific activators c-myc, C/EBPβ, Sp1, and members of the ATF/CREB family (2, 5, 22, 40, 45–47, 51).

Given the variety and complexity of the possible means by which YY1 may repress transcription, we decided to systematically analyze the biochemical requirements for repression through a structure-and-function study of YY1’s zinc finger repression domain. Specifically, we sought to determine the role of DNA binding (bending), interaction with p300, and interaction with specific transcription factors in repression by YY1. Using a panel of chimeric YY1 proteins, we have determined that the ability to specifically bind DNA does not correlate with repression activity, arguing against the DNA-bending model for repression. We have also shown that while physical interaction with p300 mediates the regulation of YY1 by E1A, in the promoter contexts tested, this interaction is not sufficient for repression. Experiments testing quenching models of repression indicated that YY1 is an activator-specific repressor that may employ both type I and type II quenching mechanisms.

**MATERIALS AND METHODS**

**Plasmids.** The GAL4-TK CAT and GAL4-E1B CAT (gift from A. Levine, Princeton University, Princeton, N.J.) reporter plasmids have been described previously (23). L655-EB1 CAT has six binding sites for LexA inserted upstream of the five GAL4 sites in GAL4-E1B CAT, and was a gift from C. D. Southgate and M. R. Green (University of Massachusetts, Worcester, Mass.). To construct the chimeric cDNA Y/GFI, a silent mutation producing a unique SacII site was introduced into the YY1 coding sequence at amino acids (aa) 293 and 294 (Pro→Arg). Fingers 1 to 4 of GF1 were then PCR amplified from a cDNA clone (provided by H. L. Grimes and P. N. Tsichlis, Fox Chase Cancer Center, Philadelphia, Pa.) (12) by using primers to add a SacII site and a stop codon in the position analogous to that of GF1, as well as a 3′ KpnI site. The SacII-KpnI fragment was fused to the YY1 coding sequence cut with SacII and the chimeric coding sequence of Y/GFI was cloned in frame with GAL4(1–147) into pSG424 (39) and in frame with glutathione S-transferase (GST) into pGEX2TK (18).

Finger swaps between YY1 and Y/GFI were accomplished by using chimeric primers covering 15 nucleotides on each side of a desired junction to PCR amplify sequences between the junction and the SacII site on one template and sequences between the junction and the stop codon on the other. The annealed products were reamplified with the external primers, and this second PCR product was fused to YY1 at aa 1 to 293 at the SacII site in the GAL4 and GST fusion constructs. Sequential rounds of this process with previously generated chimeras as templates allowed the introduction of multiple junctions.

The CMV–LeuA (DNA-binding domain) expression vector was a gift from C. D. Southgate and M. R. Green. The full-length YY1 coding sequence and the chimeric Y/GFI cDNA were cloned as in-frame fusions to LeuA (aa 1 to 202). The GST-p52A-63 and SP6-p2A-36 plasmids were provided by K. S. Campbell and T. M. Roberts (Dana-Farber Cancer Institute, Boston, Mass.). GST–E1S E1A was a gift from J. Nevin (Duke University, Durham, N.C.). Plasmids for in vitro translation of proteins were provided as follows: for TBP, D. Reinberg, W. R. Johnson Medical School, New Brunswick, N.J.; for Sp1, R. Tjian, University of California, Berkeley, Calif.; for CREB, T. Maniatis, Harvard University, Cambridge, Mass.; and for p300, R. Eckner and D. Livingston, Dana-Farber Cancer Institute. CREB was constructed by cloning the EcoRI-XbaI fragment from GAL4-CREB (42) into pBSK.

The GST vector and CMV–E1S E1A were gifts from J. Nevin. The CMV–E1S (RG2) expression construct has been described previously (21, 48). All GST–E1S activator constructs are in simian virus 40-driven fusion vectors. GAL4–Sp1(1–262), GAL4–Sp1(138–262), and GAL4–Sp1(338–542) were constructed by PCR amplifying the appropriate fragment from an Sp1 cDNA clone, adding initiator methionine and stop codons as required, and cloning the products into frame with GAL4(1–147) into pSG424. GAL4–Sp1(1–262) and GAL4–Sp1(263–542) were gifts from G. Gill (Harvard Medical School, Boston, Mass.). GAL4–CTF–1 was constructed by excising the NotI–EcoRI fragment from p17–CTF–1 (32) and cloning the full-length CTF–1 coding sequence into pSG424. GAL4–CREB and GAL4–CREBΔL2 (42) were gifts from M. E. Greenberg (Harvard Medical School). The GAL4–c-myb activation domain (aa 224 to 400; from chicken E1A, the GAL4–P53 activation domain (aa 412 to 442) were gifts from J. Licht (Mt. Sinai School of Medicine, New York, N.Y.). The GAL4–P53 activation domain (aa 1 to 92) was a gift from A. J. Berk (University of California, Los Angeles, Calif.).

All constructs used for this work were confirmed by sequence analysis of all junctions and PCR-amplified portions.

**Transfections and CAT assays.** Transfections and chloramphenicol acetyltransferase (CAT) assays were performed essentially as previously described (25) in HeLa cells. Cells were transfected with a total of 20 μg of DNA (including pSP72 as carrier) by the calcium phosphate method, the media was changed at 12 to 16 h, and cells were harvested at 48 h. For the repression assay, 10 μg of GAL4–TK CAT reporter was cotransfected with 1 μg of pSG424 either alone or with chimeric YY1 variants. For the E1A response assay, 10 μg of GAL4–E1B CAT was cotransfected with 5 μg of the pSG424 construct and 2 μg of pCMV or the CMV–E1A expression vectors. For the activator specificity assays, 10 μg of L655–E1B CAT or GAL4–E1B CAT was cotransfected with 0.1 to 3.5 μg of GAL4–activator constructs (activation was titrated and tested at the minimally saturating level for all activators except VP16, which was subsaturating) and increasing amounts of LeuA fusion constructs, as indicated on the graphs. GAL4–CREB, GAL4–CREBΔL2, and GAL4–CTF–1 were assayed in the presence of 5 μg of the murine sarcoma virus-delta kinase catalytic subunit, a gift from R. Goodman, Vollum Institute, Portland, Ore.), which increased the level of activation.

**Electrophoretic mobility shift assay (EMSA).** GST fusion proteins were expressed in bacteria, purified on glutathione agarose beads, and eluted essentially as described previously (18), except that the beads were washed twice in NETN and twice in phosphate-buffered saline and 1 mM ZnCl2, was added to the elution buffer. The eluted proteins were then used in EMSA. Given the variety and complexity of the possible means by which YY1 may repress transcription, we decided to systematically analyze the biochemical requirements for repression through a structure-and-function study of YY1’s zinc finger repression domain. Specifically, we sought to determine the role of DNA binding (bending), interaction with p300, and interaction with specific transcription factors in repression by YY1. Using a panel of chimeric YY1 proteins, we have determined that the ability to specifically bind DNA does not correlate with repression activity, arguing against the DNA-bending model for repression. We have also shown that while physical interaction with p300 mediates the regulation of YY1 by E1A, in the promoter contexts tested, this interaction is not sufficient for repression. Experiments testing quenching models of repression indicated that YY1 is an activator-specific repressor that may employ both type I and type II quenching mechanisms.

**In vitro protein binding.** GST fusion proteins were expressed in bacteria and purified on glutathione agarose beads (Sigma) essentially as described previously (18), except that the IPTG (isopropyl-D-1-thiogalactopyranoside) induction was at 0°C, no milk was added to the NETN buffer, and an aliquot of the beads was run on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel for Coomassie staining, the beads were washed twice and stored in the incubation buffer. In vitro translation incorporating [35S]methionine was performed using the TNT kit (Promega) according to the manufacturer’s instructions. In vitro translation products and beads were mixed, followed by incubation buffer and rotated at 4°C for 1 to 2 h. The beads were washed twice to six times, depending on background levels, with washing buffer. The washed beads were boiled in SDS-PAGE sample buffer and loaded on SDS-PAGE gels, which were subsequently Coomassie stained to confirm that equal amounts of GST–YY1 and Y/GFI were used, fixed, incubated with Amplify (Amersham), dried, and exposed at –80°C. To study the interaction with the large subunit of RNAP II, the GST beads were incubated with HeLa cell nuclear lysate, prepared as described previously (10) from one 10-cm diameter plate of cells for each lane, diluted 1:3 with incubation buffer, and then washed and electrophoresed as described above. The gel was blotted onto nitrocellulose and probed with an antibody to the C-terminal domain (provided by R. Young, Massachusetts Institute of Technology, Cambridge, Mass.).

**RESULTS**

**Finger 2 of YY1 is required for repression.** Structural studies of GLI-Krüppel type zinc finger proteins have demonstrated that these zinc fingers share a rather constant general structure and that sequential zinc fingers within a protein are quite structurally independent. Several conserved, structurally important residues found within each finger have been identified (19, 35, 37). The zinc fingers of YY1 contain none of this general structure (15). Given these characteristics, we chose to mutate zinc finger YY1 by replacing its zinc fingers with structurally similar heterologous zinc fingers to form chimeric constructs, with the goal of maintaining the basic structure of YY1 while eliminating its specific functions. The ideal chimera partner for
YY1 would have the conserved, structurally important zinc finger residues spaced exactly as those in YY1 but minimal homology at nonconserved positions. Of the available proteins, the first four (of six) zinc fingers of GFI-1 (12) best met these criteria and were used to replace the four zinc fingers of YY1. As predicted by previous results indicating that the zinc fingers of YY1 are responsible for the DNA-binding and repression activities (4, 23, 26, 28), this YY1-GFI chimera, called Y/GFI, was defective for both functions (Fig. 1A). Seventeen additional chimeras were generated by swapping intact zinc fingers between YY1 and Y/GFI (Fig. 1B). The linkers between fingers (defined in the legend to Fig. 1B) were generally swapped with the adjacent C-terminal finger. The linker between fingers 1 and 2 of YY1 is quite divergent from the GLI-Krüppel type linker consensus, so those fingers were swapped with both that linker and the heterologous one to test its importance.

The chimeras were fused in frame to the DNA-binding domain of GAL4 (residues 1 to 147) in pSG424 and assayed for repression of the GAL4-TK CAT reporter (Fig. 2A). Cotransfection of 10 μg of GAL4-TK CAT with 1 μg of GAL4-YY1 resulted in a fourfold repression (relative to the GAL4 DNA-binding domain) of CAT activity. This repression was largely binding site dependent, as repression of pBLCAT2, which lacks the GAL4 sites of GAL4-TK CAT, was minimal. Transfection of larger amounts of GAL4-YY1 resulted in further repression which was not entirely binding site dependent (data not shown) (38). GAL4-Y/GFI activated the GAL4-TK CAT reporter slightly, and the transfection of the finger-swapped chimeric constructs resulted in CAT activities that spanned the range of those for YY1 and Y/GFI. Approximately equal expression of the chimeras was confirmed by EMSA analysis of transfected cell lysates with an oligonucleotide containing a GAL4 DNA-binding site (Fig. 2B).

A comparison of the chimeras that repressed CAT activity with those that did not (Fig. 2B) revealed a major role for finger 2 in repression. The lack of repression by chimera 17, which has only zinc finger 2 of YY1 replaced, demonstrated that finger 2 is necessary for repression. The intermediate activity of chimera 7, with all zinc fingers except 2 replaced, showed that finger 2 is not sufficient to confer the full repression activity of YY1 on a heterologous zinc finger protein (Fig. 2A). In fact, finger 2 alone confers repression activity that is comparable to that of fingers 1 and 4 (compare chimera 7 with chimeras 1 and 6; finger 3 alone confers no repression activity). No other YY1 finger is required for repression (chimeras 3, 4, and 11 repressed transcription comparably to wild-type YY1). However, the other YY1 fingers enhance the repression activity of finger 2, as chimeras 2, 9, and 13, which each contain YY1 finger 2 and one additional YY1 finger, all repressed transcription of GAL4-TK CAT to a greater extent than chimera 7.

Repression and DNA binding are separable functions. Use of GAL4 fusion proteins in the repression assay allowed promotor targeting of all the chimeric mutants, independent of the ability to specifically recognize a YY1-binding site. A separate analysis of the specific DNA-binding and repression activities was necessary to determine a possible correlation between these functions. The chimeric cDNAs were fused to GST in pGEX2TK (18), expressed in bacteria, purified, and tested by EMSA for binding to a 32P-labeled oligonucleotide containing a YY1 site corresponding to the AAV P5 promoter recognition sequence (Fig. 3A).

As shown in Fig. 3B, several of the chimeric proteins had DNA-binding activity comparable to that of wild-type YY1. All of these proteins contain at least three YY1 zinc fingers, including finger 2. The inability of chimera 17 to bind the probe demonstrates that finger 2 of YY1 is absolutely required for DNA binding. Chimera 9 was the only protein containing two YY1 zinc fingers with detectable DNA-binding activity, suggesting that fingers 2 and 3 of YY1 constitute a minimal specific DNA-recognition domain. DNA binding by chimera 11 shows that finger 3 is not required when fingers 1 and 4 are present. Interestingly, the cocrystal structure of the YY1 zinc fingers bound to the AAV P5 +1 site shows that residues which make both DNA base and phosphate backbone contacts are found only in finger 2, perhaps explaining its unique sensitivity to mutation (15). Experiments with two additional YY1-binding sites showed a similar pattern of binding by the chimeras,
and a control experiment determined that DNA-binding activity was not affected by the presence of the GST moiety (data not shown).

A comparison of the repression and DNA-binding results shows that while finger 2 plays a central role in both functions, these functions are separable. Specifically, chimera 2 which contains fingers 1 and 2 of YY1 repressed transcription comparably to wild-type YY1 (Fig. 2A) but was completely defective for binding to a YY1 site (Fig. 3A). To confirm that the bacterially expressed chimera 2 protein was functional, we examined its ability to interact with CREB, which binds to the zinc fingers of wild-type YY1 (see Fig. 7A). Fig. 3C shows that the same bacterially expressed chimera 2 protein used in the DNA-binding assay was capable of interaction with CREB, suggesting that the zinc fingers are folded correctly.

The minimal DNA-binding domain of YY1 is composed of fingers 2 and 3, as shown by the ability of chimera 9 to bind a YY1 site (Fig. 3D). Therefore, finger 3 clearly contributes significantly to DNA binding when present in combination with finger 2, while its role in repression is not greater than that of fingers 1 and 4 (Fig. 2A; chimera 9 is not a stronger repressor than chimera 2, which contains YY1 fingers 2 and 1, or chimera 13, which contains YY1 fingers 2 and 4). Taken together with the divergent phenotypes of chimera 2, the results indicate that the structural requirements for the repression and DNA-binding functions are different.

Y/GFI binds p300 and responds to E1A. To determine whether the interaction between YY1 and p300 is important for repression in the GAL4-TK CAT assay, the repression-defective Y/GFI chimera was tested for its ability to bind to p300. GST-YY1 and GST-Y/GFI as well as negative controls GST and GST-pp2A-63 were expressed in bacteria. Glutathione agarose beads loaded with the purified fusion proteins were incubated with in vitro-translated, 35S-labeled p300. As shown in Fig. 4A, the GST-Y/GFI beads retained at least as much p300 as GST-YY1, while the negative control proteins did not retain detectable amounts of p300.

The ability of Y/GFI to interact with p300 led to the prediction that it would respond to cotransfected E1A. The response of Y/GFI to E1A was tested as previously described for wild-type YY1 (23). Expression plasmids for the GAL4 DNA-binding domain (aa 1 to 147), GAL4-YY1, or GAL4-Y/GFI were transfected into HeLa cells with the reporter GAL4-E1B CAT. Cotransfection of either GAL4-YY1 or GAL4-Y/GFI and a cytomegalovirus (CMV)-driven vector expressing wild-type 13S E1A resulted in a 6.6-fold increase in CAT activity relative to that with the CMV vector alone (Fig. 4B). In contrast, cotransfection of the 13S E1A RG2 mutant, which is defective for interaction with p300 (48) and does not activate transcription through YY1 (21), also failed to activate through Y/GFI. This control strongly suggested that the mechanism by which E1A activates transcription through Y/GFI is the same mechanism involving p300 that was previously determined for YY1. These results separate the ability of YY1 to repress GAL4-TK CAT from its ability to bind to p300 and to respond to E1A.

YY1 is an activator-specific repressor. Repression by quenching mechanisms can be distinguished from direct repression by evidence of activator specificity (13, 17). The ability of YY1 to repress transcription directed by different isolated activators was tested on the L6G5-E1B CAT reporter, which has six LexA binding sites upstream of the five GAL4 binding sites in GAL4-E1B CAT (Fig. 5A). GAL4 fusions of the activators were cotransfected with the reporters, and the resulting
The level of CAT activity was assigned a value of 1. The graphs in Fig. 5B show the effect of cotransfection of increasing amounts of plasmids encoding the LexA-YY1 fusion proteins on transcription directed by each activator. The requirement for the LexA binding sites for repression was examined in parallel experiments using GAL4-E1B CAT as the reporter.

An initial survey of activation domains from three different proteins suggested that YY1 is an activator-specific repressor. As shown in Fig. 5B, YY1 did not repress transcription directed by a GAL4 fusion of the activation domain of VP16. Binding-site-dependent repression was observed with the activation domain of c-myb. In contrast, repression of the activation domain of p53 was binding site independent (the lines for YY1 with L6G5-E1B CAT and GAL4-E1B CAT overlap; Fig. 5B). The lack of repression by LexA-Y/GFI suggests that the repression seen with wild-type YY1 does not result from steric hindrance of activator binding.

**Physical interaction between YY1 and activator proteins is not always required for repression.** To investigate the role of physical interactions between YY1 and activator proteins in repression, further experiments were conducted with the activators Sp1, CREB, and CTF-1. Our previous analysis of the interaction between YY1 and Sp1 demonstrated that the zinc fingers of YY1 interact with a region at the C terminus of Sp1 that includes its three zinc fingers as well as domain D. Previous results from our laboratory and others are summarized in Fig. 6A (7, 22). Four GAL4-Sp1 fusion constructs were tested in the activator specificity assay. YY1 repressed transcription directed by GAL4-Sp1(full-length) and GAL4-Sp1(338-542), which encodes the glutamine-rich segment of domain B, but not that directed by GAL4-Sp1(1-262), which encodes both the serine/threonine-rich and the glutamine-rich segments of domain A (Fig. 6B). The difference in the results with the latter two constructs is not attributable to physical interactions with YY1, as neither construct contains the YY1 interaction domain of Sp1. The difference also appears to be unrelated to the presence or absence of the Ser/Thr-rich segment, as shown by analysis of GAL4-Sp1(263-542), which contains both the serine/threonine-rich and the glutamine-rich segments of domain B. Results with this construct were similar to those with GAL4-Sp1(338-542) (Fig. 6B). The results point to the possibility of a difference in the mechanisms of activation by domains A and B that is revealed by the ability of YY1 to repress only domain B. More importantly for the present study, the ability of YY1 to physically interact with Sp1 did not appear to underlie its ability to repress Sp1-directed transcription.

The importance of physical interactions between YY1 and specific activators was further examined by using CREB as a model. Physical interaction between YY1 and CREB has been previously reported (51). As implied by the previous observation that the YY1-ATF2 interaction is mediated by the zinc fingers of YY1 and the leucine zipper of ATF2 (51), Y/GFI interacted poorly with CREB, and deletion of the leucine zipper of CREB abolished its interaction with wild-type YY1 (Fig. 7A). The weak interaction between Y/GFI and CREB is likely not due to incorrect folding of bacterially expressed Y/GFI protein, as this protein was indistinguishable from wild-type YY1 in interactions with p300 (Fig. 4A), RNAP II large subunit, and TBP (Fig. 7A).

As shown in Fig. 7B, results of the activator specificity assay...
using wild-type CREB and the deletion mutant were similar. These results demonstrate that the physical interaction between YY1 and CREB is not required for repression of CREB-mediated transcription. Therefore, the weak repression by Y/GFI (Y/GFI repressed about 2-fold when 2 μg of expression plasmid was transfected, while wild-type YY1 repressed 10-fold at 0.5 μg; Fig. 7B) is not attributable to its lack of binding to CREB but rather reveals a different zinc finger-encoded function. Together, the studies of Sp1 and CREB deletion mutants suggest a type II quenching mechanism for repression by YY1; i.e., YY1 may be interfering with the interactions of these activators with their targets in the general transcription machinery.

The final activator studied was CTF-1. Unlike transcription mediated by the other activators, which was not repressed or marginally repressed by Y/GFI, CTF-1 was strongly repressed by this chimera (Fig. 8A). The degree of repression by Y/GFI is weaker than that seen with wild-type YY1 at very low levels of transfected LexA fusion plasmid but is comparable when LexA fusion plasmid is transfected at levels of 0.5 μg or more. A much larger difference was seen in binding-site-independent repression by YY1 and Y/GFI, suggesting that while both YY1 and Y/GFI repressed CTF-1-mediated transcription when ef-
ficiently recruited to the promoter via LexA DNA binding, only wild-type YY1 was also recruited by protein-protein interactions. This result possibly reflects a direct role for the YY1-CTF-1 physical interaction in the recruitment, as the interaction between Y/GFI and CTF-1 is significant but diminished relative to the YY1-CTF-1 interaction (Fig. 8B).

As a step toward determining whether the interaction between Y/GFI and CTF-1 underlies its ability to specifically repress this activator when recruited to the promoter by the LexA sites, we mapped the interaction on Y/GFI. Figure 8B shows that the four GFI zinc fingers present in Y/GFI, and not the non-zinc finger portion of YY1, were responsible for the physical interaction.

**DISCUSSION**

We have used structure-and-function analysis of the zinc finger repression domain to test several models for the mechanism of transcriptional repression by YY1. Previous reports have suggested that YY1 may repress transcription by bending promoter DNA (33) or through physical interactions with activator proteins (51). Our previous work on the mechanism of relief of YY1-mediated repression by adenovirus E1A suggested a possible role for the E1A-associated cellular protein p300 in repression by YY1 (21). Additional studies identified a variety of interactions with general and specific transcription factors that could be involved in repression (2, 5, 22, 40, 46, 47). Our analysis of these models suggested that YY1 is an activator-specific repressor which functions by quenching the activity of specific activators.

Zinc finger 2 of YY1 plays central roles in repression and DNA binding, but these functions are separable. This study for

**FIG. 6.** YY1 repression of Sp1-mediated transcription does not require the YY1-binding domain of Sp1. (A) Schematic representation of Sp1, showing previously identified activation domains and the region required for interaction with YY1 (7, 22). (B) Repression assayed as described in the legend for Fig. 5B.

**FIG. 7.** YY1 repression of CREB-mediated transcription does not require the YY1-binding domain of CREB. (A) The leucine zipper region of CREB interacts with the zinc fingers of YY1. Deletion of the C-terminal 29 aa of CREB, constituting the leucine zipper, abolishes the interaction with YY1. The Y/GFI chimera is severely compromised in the ability to interact with wild-type CREB. Equal amounts of RNAP II (large subunit) and TBP are retained by YY1 and Y/GFI (shown as controls). (B) Repression assayed as described in the legend for Fig. 5B.
the first time defines the individual roles of the four zinc fingers of YY1 in two functions, repression and DNA binding. Structure-and-function analysis demonstrated that while both functions are carried out by the zinc fingers, they are genetically separable. This finding argues against DNA bending as a general mechanism of repression by YY1, consistent with the observation that repression by YY1 is not generally dependent on the orientation of the DNA-binding site (16). However, this interpretation is based on the definitions of the two functions used in this study. Specifically, the mutants were characterized for repression activity on a particular promoter, which is activated by cellular activators binding to a CCAAT motif and two Sp1 sites (30). Given the activator-specific nature of YY1’s repression activity, the mutants might not show the same pattern of repression phenotypes on all promoters. Further, it is possible that once the proteins are tethered to the GAL4-TK CAT reporter by the GAL4 DNA-binding domain, the zinc fingers can bind to and bend promoter DNA in a relatively sequence-independent manner. Such a phenomenon would not be revealed by our analysis of DNA binding to three specific sites in solution. However, assuming all the mutants would be equally capable of nonspecific DNA binding, this activity appears not to underlie repression. The conclusion that repression by YY1 is mediated through mechanisms other than DNA bending is further supported by observations of binding-site-independent repression (for an example, see Fig. 5B). Finally, the cocrystal structure of the YY1 zinc fingers binding to a specific oligonucleotide reveals no evidence of DNA bending (15).

The ability to interact with p300 and respond to E1A is separable from repression activity. The finding reported here that a repression-defective mutant, Y/GFI, is capable of interacting with p300 and responding to E1A suggests that the p300 interaction is not sufficient for repression. This interpretation must also be considered in light of YY1’s activator specificity. Y/GFI was originally defined as repression defective on the GAL4-TK CAT reporter by the GAL4 DNA-binding domain, the zinc fingers can bind to and bend promoter DNA in a relatively sequence-independent manner. Such a phenomenon would not be revealed by our analysis of DNA binding to three specific sites in solution. However, assuming all the mutants would be equally capable of nonspecific DNA binding, this activity appears not to underlie repression. The conclusion that repression by YY1 is mediated through mechanisms other than DNA bending is further supported by observations of binding-site-independent repression (for an example, see Fig. 5B). Finally, the cocrystal structure of the YY1 zinc fingers binding to a specific oligonucleotide reveals no evidence of DNA bending (15).

**YY1 is an activator-specific repressor.** Our demonstration that YY1 is an activator-specific repressor complements evidence from studies of natural promoters indicating that YY1 functions in a promoter-specific manner. We found that YY1 did not repress transcription directed by the activation domain of VP16 or the A domain of Sp1, which represent the most and least potent activators, respectively, analyzed in this study. YY1 did repress CREB, full-length Sp1 and its B domain, c-myb, and p53, with varying degrees of binding-site dependence. Transcription mediated by CTF-1 was unique in that it was repressed by both YY1 and Y/GFI.

The observation of activator specificity argues against the direct repression model and in favor of the quenching models. In order to determine whether quenching is mediated by direct contact with activator proteins or by interference with functional interactions between activators and their targets, we examined whether repressor-activator physical interaction was necessary for repression. Our results with deletion mutants of CREB and Sp1 show that while YY1 can bind to these activators, the physical interaction is not required for repression under our assay conditions. The equivalent repression by YY1 of wild-type CREB and a CREB deletion mutant defective for interaction with YY1 indicates that the inability of Y/GFI to repress CREB-mediated transcription cannot be attributed to its inability to interact with the activator. Instead, the results suggest that the Y/GFI chimera is defective in an as-yet-unidentified function which is mediated by the zinc fingers of wild-type YY1 and which is critical for repression of CREB-mediated transcription. Since other results shown here suggest
that this function is neither DNA binding nor interaction with p300, it appears that YY1 represses CREB (and Sp1) by a type II quenching mechanism, i.e., by interfering with interactions between these activators and their targets.

The strong repression by Y/GFI that is uniquely seen with CTF-1 indicates that CTF-1-mediated activation is repressed by a distinctive mechanism. Direct physical interaction between YY1 and the activator may mediate repression in this case. Two lines of evidence support this hypothesis. The first is based on the relative strengths of repression by YY1 and Y/GFI in the presence and absence of promoter binding sites. Y/GFI is a slightly weaker repressor than YY1 in the presence of LexA binding sites, possibly owing to the weaker interaction with CTF-1. However, in the absence ofLexA sites, where recruitment through protein-protein interaction is critical, the Y/GFI protein does not repress transcription. These observations are consistent with the idea that the weaker interaction seen with Y/GFI suffices for repression only when the protein is independently tethered to the promoter via DNA binding.

Second, the interaction between Y/GFI and CTF-1 is mediated by the GFI zinc fingers (Fig. 8B). The addition of the GFI zinc fingers fortuitously restored the interaction to the chimera, since the non-zinc finger portion of YY1 did not interact with CTF-1. It is possible that in the process, the GFI fingers also restored the repression activity of Y/GFI that is uniquely seen with CTF-1. Overall, the results with CTF-1 seem most consistent with type I quenching; i.e., repression is mediated by direct contact between the activator and the repressor. The different models for repression of Sp1 and CREB versus repression of CTF-1 predicted by our results are illustrated in Fig. 9.

**Multiple mechanisms of repression by YY1.** It has become clear through recent work in several laboratories that YY1 is a multifunctional repressor. The simplest mechanism of repression is activator displacement, which has been well documented for YY1 on several natural promoters (reviewed in references 43 and 44). Our results do not support DNA binding as a general mechanism, but it remains a possibility which may apply to specific promoters. These two mechanisms, in addition to the two forms of quenching repression suggested by the present study, all apply to the zinc finger repression domain of YY1. A fifth mechanism, recruitment of the putative corepressor RPD3, has been attributed to a second, glycine-rich repression domain of YY1, encompassing aa 170 to 200 (49).

The results presented here suggest that the mechanism of repression is dictated by the particular activators directing transcription. Specifically, this work suggests that YY1 may interfere with functional interactions between the activators Sp1 and CREB and their targets on the preinitiation complex, indicating a direction for future studies.

A major remaining question relates to the regulation of the repertoire of activities carried out by YY1, which include activation and inhibition in addition to the multiple modes of repression. As an example, it is presently unclear what causes YY1 to repress Sp1-mediated transcriptional activation on one promoter (shown here) but to cooperate with Sp1 to activate transcription when functioning as an initiator protein (41). The identified protein-protein interactions that appear not to be required for repression are now candidates for involvement in this regulatory role.

**ACKNOWLEDGMENTS**

We thank Ceri Batchelder and Keith Blackwell for discussion and critical reading of the manuscript, as well as for help with EMSA assays. We thank H. L. Grimes and P. N. Tsichlis for the GFI-1 cDNA clone. We are grateful for reagents provided by the numerous individuals mentioned in the Materials and Methods section. We appreciate the support and helpful advice of members of the Department of Pathology.

This work was supported by a grant to Y.S. from the NIH (GM53874). Y.S. is the recipient of a Junior Faculty Research Award from the American Cancer Society.

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