Characterization of the Transcriptional Regulator YY1

THE BIPARTITE TRANSACTIVATION DOMAIN IS INDEPENDENT OF INTERACTION WITH THE TATA BOX-BINDING PROTEIN, TRANSCRIPTION FACTOR IIB, TAF$_{II}$, OR AMP-RESPONSIVE ELEMENT-BINDING PROTEIN (CBP)-BINDING PROTEIN

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YY1 is a multifunctional transcription factor implicated in both positive and negative regulation of gene expression as well as in initiation of transcription. We show that YY1 is ubiquitously expressed in growing, differentiated, and growth-arrested cells. The protein is phosphorylated and has a half-life of 3.5 h. To define functional domains, we have generated a large panel of YY1 mutant proteins. These were used to define precisely the DNA-binding domain, the region responsible for nuclear localization, and the transactivation domain. The two acidic domains at the N terminus each provide about half of the transcriptional activating activity. Furthermore, the spacer region between the Gly/Ala-rich and zinc finger domains has accessory function in transactivation. YY1 has been shown previously to bind to TAF$_{II}$, TATA box-binding protein, transcription factor IIB, and p300. In addition, we identified cAMP-responsive element-binding protein (CBP)-binding protein as a YY1 binding partner. Surprisingly, these proteins did not bind to the domains involved in transactivation, but rather to the zinc finger and Gly/Ala-rich domains of YY1. Thus, these proteins do not explain the transcriptional activating activity of YY1, but rather may be involved in repression or in initiation.

Different mechanisms have been implicated in the regulation of gene transcription by YY1. Depending on the context, YY1 was shown to either stimulate or repress gene expression (for review, see Refs. 1 and 2). The mechanistic basis of these two different activities has not been characterized. However, recent evidence indicates that the interaction of YY1 with the coactivator p300 may be relevant in determining whether YY1 functions as an activator or repressor (3). Furthermore, YY1 has been described as an initiator-binding protein (4). This has been supported by the finding that YY1 can stimulate basal transcription in vitro in combination with TFIIIB and RNA polymerase II, notably in the absence of the TATA box-binding protein (TBP) (5). In addition, YY1 has been recently identified as a component of a large RNA polymerase II complex that contains YY1 in stoichiometric amounts with RNA polymerase II and several general transcription factors as well as DNA repair proteins (6). Yet another aspect of YY1 function has been uncovered by demonstrating its identity to the nuclear matrix protein NMP-1 (7). These data imply that YY1 may also be involved in aspects of chromatin organization possibly by tethering DNA to the nuclear matrix. Together, these findings suggest that YY1 participates in a number of different processes associated with regulation of gene transcription.

Interestingly, YY1 function and regulation have been linked to the adenovirus protein E1A and the proto-oncoprotein c-Myc (3, 4, 8–10). Originally, it was found that E1A-mediated activation of the adeno-associated virus (AAV) P5 promoter results from relief of YY1 repression (4). This seems not to be due to a direct interaction of E1A with YY1, but rather the effect of binding of E1A to the coactivator p300 in a p300 YY1 complex (3). Thus, in this complex, p300 appears to acquire a new quality as mediator of repression, whereas it supports activation of all other studied transcriptional regulators including CREB and c-Myb (11, 12). In contrast to E1A, c-Myc directly interacts with and alters the function of YY1 (10). In addition, YY1 can also transactivate the mouse c-Myc promoter (9). Since both E1A and c-Myc are potent cell growth regulators (for review, see Refs. 13 and 14), their interaction with YY1 suggests a role for this protein in cell growth control.

YY1 is a zinc finger-containing transcriptional regulator with homology to the GLI-Krüppel family of proteins (4, 15–17). The analysis of YY1 deletion mutants, mainly in the context of Gal4 fusion proteins, has indicated that the zinc finger region is responsible for DNA binding and that the N-terminal region contains a transactivation domain (8, 16, 18–20). The repression function of YY1 has been mapped to the very C terminus, a region also essential for DNA binding (4, 8, 19).

Here we report that YY1 is a rather stable phosphorylated protein expressed at comparable levels in both growing and differentiating cells. In addition, using a panel of YY1 mutant proteins, we show that all four zinc fingers are required for specific DNA binding. We have mapped a region, including fingers 2 and 3, essential for efficient nuclear targeting. Furthermore, the transactivation domain is bipartite, with each of the two acidic domains at the N terminus contributing about half of the transactivating potential, whereas the spacer region between the Gly/Ala-rich and zinc finger domains has accessory function for transactivation. In addition to binding to p300 (3), we demonstrate that YY1 can also interact with the CREB-binding protein (CBP). However, binding to CBP as well as to the previously described interaction partners TFIIIB, TBP, and

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1 The abbreviations used are: TFIIIB, transcription factor IIB; TBP, TATA box-binding protein; AAV, adeno-associated virus; CREB, cAMP-responsive element-binding protein; CBP, CREB-binding protein; HA, hemagglutinin; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis.

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TAF255 (5, 21) does not require the transactivation domains, but instead the Gly/Ala-rich and zinc finger domains. These findings connect the binding of YY1 to CBP, TFIIH, TBP, and/or TAF255 to repression or initiation rather than transactivation.

**EXPERIMENTAL PROCEDURES**

**Cells and Transfections—**RK13 cells, a rabbit kidney epithelium-derived cell line, were maintained in minimum Eagle’s medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. CV1, HeLa, F9 teratocarcinoma, NIH3T3, PC12, and primary rat embryo fibroblasts were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum and 1% penicillin/streptomycin. 70Z, U937, Jurkat, Ramos, and Manca cells were grown in RPMI 1640 medium containing 10% fetal calf serum and 1% penicillin/streptomycin. The medium for 70Z cells also contained 0.2 mM b-mercaptoethanol. For differentiation, F9 cells were treated with 0.5 mM retinoic acid and 1 mM dibutyryl cAMP. 70Z cells with 0.4 mg/ml bacterial lipopolysaccharide, and U937 cells with 10 mM 12-O-tetradecanoylphorbol-13-acetate, 1 mM retinoic acid, and 10 ng/ml vitamin D3. Transient transfections were performed using a standard calcium phosphate transfection protocol as described previously (22). Briefly, cells were plated at a density of 1.5 × 105 cells/plate. Each 6-cm plate received 2 μg of reporter plasmid, 2 μg of pBSVlacZ as an internal control, and the effectors plasmids indicated. All transfections were done in duplicates or triplicates, and all experiments were performed at least four times. Cells were harvested after 36–48 h, and luciferase and b-galactosidase activities were determined.

**Plasmids—**The pCB6-based YY1 expression vector (pCMVYY1) was a gift of M. Atchison (17). A BgIII-ClaI fragment from this construct was inserted into pBluescript KS+ (Stratagene), and the resulting pBS-YY1 plasmid was used for mutagenesis. Deletions were made either by exploiting existing restriction sites or by introducing new sites by polymerase chain reaction. All junctions and all polymerase chain reaction-derived sequences were verified by sequencing. None of the deletion mutants contains additional amino acids at the junctions. The YY1 deletion mutants were then cloned into the EcoRI site of pCB6. pCMVHAYY1-pCMVHAYY1 (gift of T. Shenk (4)) was bacterially expressed and purified His-tagged YY1. pDS56HisYY1 was a gift of R. Janknecht (24).

**DETECTION OF INDUCIBLE PROTEINS—**For detection of inducible proteins, cells were transfected with reporter plasmid, 2 μg of pBSVlacZ, or combination of effector plasmids (see RESULTS). Cells were harvested after 36–48 h, and luciferase and b-galactosidase activities were determined. For Western blots, cells were transfected as for reporter gene assays. The cells were then lysed in antibody buffer (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5% deoxycholate, 0.5% SDS, and 0.5% aprotinin) (26), standardized for b-galactosidase activity, and separated by SDS-polyacrylamide gel electrophoresis (PAGE). Staining was carried out overnight in an enhanced chemiluminescence system (Amersham Corp.) according to the instructions of the manufacturer.

**RESULTS**

YY1 is a widely expressed, stable protein—To study the YY1 protein, we developed an antisera against full-length bacterially expressed YY1. This serum (265) reacted specifically with a protein of 68 kDa in all cell lines analyzed as well as with bacterially expressed His-tagged YY1 (Fig. 1 and data not shown). The specificity of the serum was established by performing immunoprecipitation/Western blotting and blocking experiments in combination with a commercially available antiserum (Fig. 1A). YY1 was used with fibroblastin (NIH3T3, CV1, and RK13), in primary rat embryo fibroblasts, in cells of hematopoietic origin (Jurkat, 70Z/3, Manca, Ramos, and U937), in PC12 pheochromocytoma cells, in HeLa epithelium-like cells, and in the F9 embryonal carcinoma cell line by metabolic labeling with [35S]methionine and immunoprecipitation as well as by immunoblotting (Fig. 1B–D) and data not shown.
Functional Domains in YY1—To define functional domains in YY1, a series of deletion mutants were generated (Fig. 2). All these proteins were expressed efficiently in COS-7 and RK13 cells (Fig. 3 and data not shown). The DNA binding capacity of YY1 and YY1 mutant proteins overexpressed in COS-7 cells was analyzed in electrophoretic mobility shift assay experiments. As probe, the P5+1 sequence from the AAV P5 promoter (4) was used, which was bound by endogenous YY1 in COS-7 and F9 cells as well as by bacterially expressed His-tagged YY1 (Fig. 4). The specificity of the complex was demonstrated by the ability of purified YY1 antibodies to inhibit binding, whereas unrelated antibodies had no effect (Fig. 4). Furthermore, binding to P5+1 was competed by specific (but not by nonspecific) oligonucleotides (data not shown). All the mutant YY1 proteins with deletions in the zinc finger region were unable to bind to the P5+1 oligonucleotide (Fig. 4). These findings show that all four zinc fingers are essential for the specific binding of YY1 to DNA.

Immunofluorescent staining of control and transiently transfected RK13 cells was used to determine the subcellular localization of YY1 and YY1 mutant proteins. Endogenous YY1 was detected exclusively in the cell nucleus using affinity-purified 263-7 antibodies (Fig. 5a). Exogenously expressed YY1 was stained with a commercially available anti-peptide serum recognizing the C terminus of YY1 since it recognizes a defined epitope and its reactivity was too low to stain the endogenous protein under the conditions employed. Mutants with deletions of the C terminus (YY1Δ399-414 and YY1Δ334-414) were tagged with an HA epitope and detected with a monoclonal antibody against HA. All the mutant proteins either with deletions of N-terminal regions or with deletions affecting either the first or fourth zinc finger showed nuclear localization (Fig. 5b). The Western blot was developed with purified YY1 antibodies (serum 263-7). c, F9 embryonal teratocarcinoma cells were differentiated in the presence of retinoic acid and dibutryl cAMP for the times indicated. Whole cell lysates of equal numbers of cells were analyzed by Western blotting using purified YY1 antibodies (serum 263-7). For comparison, three different amounts of control lysate were loaded. d, Jurkat or undifferentiated or differentiated F9 embryonal carcinoma (EC) cells were labeled for 15 min with [35S]methionine. The labeled cells were then chased in the presence of excess unlabeled methionine. The cells were harvested at the times indicated, lysed in antibody buffer, and immunoprecipitated using purified YY1 antibodies (serum 263-7). For blocking, the antibodies were preincubated with GST-YY1 prior to the addition of lysate (0/bl). The immunoprecipitates were separated by SDS-PAGE, and the proteins were detected by fluorography. The radioactivity of the different bands were quantified with a phosphorimager.

FIG. 1. YY1 is a constitutively expressed protein. a, to establish the specificity of our YY1 antiserum (263) generated against bacterially expressed and purified His-tagged YY1, we performed immunoprecipitation/Western blot analysis. HeLa whole cell lysates were immunoprecipitated using affinity-purified YY1 antibodies (serum 263-7, a-YY1), unrelated affinity-purified antibodies (control AB), 263 preimmune serum (a-YY1 PI), or purified YY1 antibodies preincubated with GST-YY1 (a-YY1 block). The immunoprecipitates were separated by SDS-PAGE, blotted onto nitrocellulose, and stained with anti-YY1 C20. The positions of the Ig heavy chains (IgH), YY1, and GST-YY1 are indicated. b, whole cell lysates of the different cell types indicated were prepared in antibody buffer, and equal amounts of protein (~10% of a subconfluent 10-cm tissue culture plate) were separated by SDS-PAGE and blotted onto nitrocellulose. The Western blot was developed with purified YY1 antibodies (serum 263-7). Comparable levels of YY1 were expressed in all cell lines analyzed. To determine the stability of YY1, we performed pulse-chase experiments. Jurkat or F9 cells were pulse-labeled for 15 min with [35S]methionine and chased in excess unlabeled methionine for the times indicated (Fig. 1d). YY1 appeared to be a rather stable protein with a half-life of 3.5–4 h as revealed by quantification of immunoprecipitated YY1 using a phosphorimager. A similar half-life of YY1 was determined in NIH3T3 cells and in rat embryo fibroblasts (data not shown).
A deletion of the first zinc finger and the two Cys residues involved in coordinating Zn\textsuperscript{2+} of the second zinc finger (YY1\textsubscript{D296–331}) distributed mainly to the nucleus (Fig. 5\textsuperscript{b}). Deletion of the entire C terminus including part of the second, third, and fourth zinc fingers resulted in a protein (YY1\textsubscript{D334–414}) with predominant cytoplasmic staining (Fig. 5\textsuperscript{c}). These data suggest that the nuclear localization signal of YY1 is contained within the region encoding the second and third zinc fingers as summarized in Fig. 2.

**YY1 Shows a Bipartite Transactivation Domain**—YY1 has been implicated in both positive and negative regulation of gene transcription. To analyze the domains in YY1 responsible for these functions, the gene regulatory activities of the YY1 mutants were tested. Reporter constructs were made containing a minimal thymidine kinase promoter and the luciferase gene with or without the P5\textsuperscript{1} YY1-binding site (4). First, the role of the P5\textsuperscript{1} binding site was determined in three different cell lines. Whereas in CV1 and RK13 cells the presence of a P5\textsuperscript{1} site led to an increase in reporter activity, a slight decrease was observed in NIH3T3 cells (Fig. 6\textsuperscript{a}). Expression of exogenous YY1 resulted in a binding site-dependent activation of the reporter construct in all three cell lines. In addition, the activation was dose-dependent in the range of 1 ng to 1 \(\mu\)g of pCMVYY1 (Fig. 6\textsuperscript{b} and data not shown). Under these conditions, we have not observed any repression. However, reduced activation was seen when pCMVYY1 concentrations of 2 \(\mu\)g or higher were used, most likely due to squelching as a result of highly overexpressed YY1.

Next we were interested to determine the transactivating potential of the different YY1 mutant proteins. These analyses revealed that the YY1 mutant proteins can be divided into three classes. Deletion of the His cluster (YY1\textsubscript{D69–85}) or the Gly/Ala-rich region (YY1\textsubscript{D154–199}) did not affect the transactivating activity of the resulting mutant proteins compared with wild-type YY1 (Fig. 7). Proteins with deletions of either of the two acidic regions (YY1\textsubscript{D2–62} and YY1\textsubscript{D92–153}) or of the spacer region between the Gly/Ala-rich and DNA-binding do-

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**Fig. 2. Summary of the YY1 mutant proteins and their functional properties.** Expression plasmids for the different YY1 mutant proteins were generated as described under “Experimental Procedures.” The structural elements of YY1 are indicated on top, and the amino acids deleted for the individual mutants are indicated on the left. DNA binding, nuclear localization, and transactivation of the different proteins are summarized on the right (for detailed functional analysis, see Figs. 4, 5, and 7).

**Fig. 3. Expression of the YY1 mutant proteins.** RK13 cells were cotransfected with constructs expressing wild-type YY1 (wt), the indicated mutants, or control vector and a construct expressing \(\beta\)-galactosidase and P5\textsuperscript{1}-tk-luc, identical as for the reporter gene assays. Whole cell lysates were prepared in antibody buffer, and the expression of the different proteins was analyzed by Western blotting. The blot on the left was developed using the YY1 C20 antibodies, and the one on the right using purified YY1 antibodies (serum 263-7). Wild-type YY1 is indicated as well as a nonspecific band cross-reacting with YY1 C20 antibodies (*).
observed that bacterially expressed YY1 was able to interact efficiently with GST-TFIIB and GST-TBP and to a lower degree with GST-TAF1155, whereas no binding to GST alone was observed (Fig. 8a). Since YY1 interaction with p300 has been shown, we tested whether YY1 can also bind to CBP. Binding was detected to GST-CBP(451–721), the CREB-binding domain, and to GST-CBP(1891–2175) (Fig. 8b). The interaction with CBP(451–721) was weaker than with GST-TFIIB or GST-TBP (Fig. 8a).

To define interaction domains, YY1 and YY1 mutant proteins were synthesized in vitro (Fig. 8c, INPUT), and their binding to GST-TBP, GST-TFIIB, GST-TAF1155, and GST-CBP(451–721) was determined (Fig. 8c, BOUND). Whereas wild-type YY1 and several of the mutant proteins bound to all four GST fusion proteins, but not to GST alone, deletion of part of the zinc finger domain (YY1Δ296–331 and YY1Δ334–414) reduced or abolished binding, respectively. In addition, YY1Δ154–199, in which the Gly/Ala-rich domain is removed, bound consistently less well to all four fusion proteins. These findings indicate that the DNA-binding and Gly/Ala-rich domains are important for four different protein-protein interactions analyzed. TBP, TFIIB, TAF1155, and CBP did not require the two acidic transactivation domains for interaction.

**DISCUSSION**

Several lines of evidence suggest that YY1 is a multifunctional transcriptional regulator, activating or repressing transcription depending on both the promoter and the cellular context. YY1 has been detected in a number of different tissues and cell types. Our analyses further support the concept that YY1 is a ubiquitously expressed protein. In all cell lines tested, comparable levels of YY1 were detected as determined by Western blotting (Fig. 1 and data not shown). In addition, no changes in the level of expression were observed in differentiating F9, 70Z/3, or U937 cells (Fig. 1 and data not shown). The finding in F9 cells is in agreement with previously published data showing constitutive YY1 mRNA expression during retinoic acid-induced F9 differentiation (15). Although YY1 is expressed constitutively during F9 differentiation, indirect regulation of YY1 activity has been suggested to occur through CpG methylation of YY1-binding sites (20, 27). The accessibility of YY1 to its cognate binding site appears also to be regulated in the context of the κ3' enhancer (28). Early in B cell development until the activated B cell stage, the YY1-binding site in the κ3' enhancer is covered by a nucleosome. However, the YY1 site becomes accessible in plasma cells paralleling increased expression from the κ locus. Interestingly, the sequence of a YY1 footprint in the κ3' enhancer suggests, in contrast to an earlier study (17), a positive role for YY1 in κ-chain expression (28).

While in differentiating F9 cells little difference in the DNA binding capacity of YY1 was seen, a decrease in YY1 binding activity was observed during differentiation of chicken embryonic myoblasts (29). Presently, it is unclear whether this reflects a down-regulation of the protein, modulation of the DNA binding activity, or altered association with the nuclear matrix that may result in differential extractability. Further work will be required to determine whether YY1-DNA binding is regulated in other differentiation systems. In addition to the data described above, we could not observe any difference in YY1 protein expression in quiescent fibroblasts compared with serum-stimulated cells or exponentially growing cells (data not shown). This is in contrast to a recent study showing reduced YY1 mRNA expression in quiescent NIH3T3 cells as compared
with growing cells (30). Since in this latter study protein expression was not analyzed, direct comparison with our findings is currently not possible. In summary, constitutive expression of YY1 was observed under most cellular conditions. Therefore, one could consider YY1 as a permanently present “basal” transcription factor whose activity may be controlled exclusively by secondary events such as competition with other transcription factors (31, 32), effects on the binding site (20, 27, 28), or binding by cell cycle- or differentiation-regulated factors such as p300 or CBP (Ref. 3 and this study).

Using lysates of [32P]orthophosphate-labeled cells and specific immunoprecipitation, we found YY1 to be phosphorylated (data not shown), as are many other transcription factors (33). Since altered phosphorylation is frequently associated with functional changes in the activities of transcription factors, we analyzed YY1 phosphorylation under different cellular conditions. At present, we have not found any differences in the phosphorylation pattern of YY1 during growth or differentiation by peptide mapping.2

To transport proteins into the cell nucleus, at least two potential mechanisms can be envisaged (34). First, the protein contains a nuclear localization signal and by this interacts directly with the nuclear import machinery. Second, the protein is cotransported with a nuclear localization signal-containing protein. Both possibilities appear conceivable for YY1. Whereas no obvious nuclear localization signal is present within the region of the second and third zinc fingers, which are important for nuclear localization (Fig. 5), a number of basic residues have been noted that may function not only in DNA binding, but also in nuclear targeting. Alternatively, this region may interact with B23, which has been identified as a YY1-interacting protein in a yeast two-hybrid screen (35). Since B23 is a protein shuttling between the nuclear and cytoplasmic compartments, possibly transporting proteins across

**FIG. 5.** YY1 is nuclear-localized. To determine the subcellular localization of YY1 and YY1 mutant proteins, untreated or transiently transfected RK13 cells were fixed in paraformaldehyde, permeabilized with Triton X-100, and stained as outlined below. a, RK13 cells were stained with affinity-purified YY1 antibodies (serum 263-7) or with control antibodies (Ab) as indicated (left panels). The DNA was stained using Hoechst 33528 (right panels). b, RK13 cells were transfected with plasmids expressing the indicated YY1 or YY1 mutant proteins and stained with YY1 C20 antibodies (left panels). The DNA was stained using Hoechst 33528 (right panels). c, RK13 cells were transfected with plasmids expressing the indicated YY1 or YY1 mutant proteins and stained with YY1 C20 antibodies (left panel) or with HA-tagged antibodies (right panels). The DNA was stained using Hoechst 33528 (middle panels). wt, wild-type YY1.
the nuclear envelope (36), it may be involved in the accumulation of YY1 in the nucleus.

In a previous study, placement of the YY1-binding site from the initiation site of the AAVP5 promoter (P5; see Ref. 4) in front of a minimal promoter resulted in a repression of transcription. Using a similar construct, we also observed a small repressive effect in NIH3T3 cells (Fig. 6). However, in CV1 and RK13 cells, the addition of the P5 + 1-kb fragment resulted in a significant activation of the minimal thymidine kinase promoter, although equal amounts of endogenous YY1 are present in all three cell lines (Fig. 1). Cotransfection of YY1 expression plasmids in the range of 1 ng to 1 μg of DNA activated the P5 + 1-kb luciferase reporter gene in all three cell lines, indicating that YY1 by itself is an activator of transcription. This is supported by findings from other investigators who have observed an activating effect of YY1 overexpression in a variety of systems (9, 18–20, 37, 38). The repressive effect of YY1 on transcription in NIH3T3 cells could then be caused by a protein different from YY1, although it is the predominant protein observed in in vitro band shift reactions.

To characterize the protein further, we constructed an extensive panel of YY1 deletion mutants (Fig. 2). Previous studies involving large deletions have shown that zinc fingers 2, 3, and 4 are required for DNA binding (16). We extend this observation by showing that a mutation that disrupts zinc finger 1 also abolishes binding to DNA in a band shift assay (Fig. 4), demonstrating a requirement for all four zinc fingers for specific DNA binding.

Our data define three regions of YY1 important in the regulation of specific transactivation in addition to the zinc finger domain (Fig. 7). Whereas the two acidic domains (YY1D2–62 and YY1D92–153) each contribute about half of the transactivating potential, the spacer region is also important for full activity, but does not have transactivating activity on its own. The notion that the N-terminal region of YY1 is involved in transactivation has been suggested by the analysis of YY1 deletion mutants on the c-myc promoter (19). These findings were further confirmed by the analysis of Gal4-YY1 fusion proteins, implicating the N-terminal region of YY1 in transactivation (8, 18–20). Detailed analysis of such Gal4-YY1 fusion proteins revealed an important role for the first acidic domain in transcriptional activity, but showed little significance of the second acidic domain (18). This is in contrast to our findings that demonstrated equal importance of both acidic domains. In addition, no specific function for the spacer region could be determined using Gal4 fusion proteins. This region of YY1 may be required for correct folding and presentation of the two transactivation domains. Together, the mutants analyzed here allow us to delineate a more detailed map of functional domains of YY1 in a context not relying on fusion proteins.

A number of proteins involved in gene transcription that are frequently targeted by transactivation domains have been shown previously to interact with YY1, namely TFIIB, TBP, TAF₁₅₅, and the coactivator p300 (3, 5, 21). Therefore, we asked whether one or more of these factors could bind directly
to the domains in YY1 that we have identified as important for transactivation. First, we confirmed the direct binding of YY1 to TFIIB and TAF155 and demonstrated an interaction with TBP (Fig. 8) as has been suggested previously (4). Second, we showed that a C-terminal domain of CBP, a p300-related protein (39, 40), also interacted with YY1. However, we observed an even stronger interaction of YY1 with the CREB-binding domain of CBP (Fig. 8). The corresponding domain in p300 may have been disrupted in GST-p300 fusion proteins used previously, possibly explaining the lack of binding to this region (3). Surprisingly, none of these interaction partners bound to a domain involved in transactivation (Fig. 8). Instead, all displayed similar patterns of binding, requiring the core of the YY1 DNA-binding domain and the Gly/Ala-rich domain. It is possible that the interactions with TFIIB, TBP, TAF155, or CBP/p300 may be relevant for repression rather than activation by YY1. In addition, the interaction with TFIIB may be important for the function of YY1 as an initiator-binding protein (5). Thus, it remains open which protein(s) is contacted by the transactivation domains of YY1.

The picture that is emerging of YY1 in transcriptional regulation is quite complex. It can bind to enhancer and initiator sequences, can contact several different components involved in RNA polymerase II transcription, possesses two transactivation domains of unknown specificity, and can be part of a large RNA polymerase II complex. Recent evidence suggests that transcriptional regulators may recruit RNA polymerase II holoenzyme, which has been estimated to consist of at least 50 polypeptides (41). Since a single contact of a transcriptional activator with a component of the holoenzyme appears to be sufficient for activation of gene expression (42), multiple possibilities exist for interaction, and it will now be important to define the contact(s) of YY1 relevant for activation. Also, the contribution of this protein to the other proposed functions and the role of the identified interaction partner awaits further detailed analysis.

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Functional Domains in YY1

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