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Functional interactions between YY1 and adenovirus E1A

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ABSTRACT

YY1 is a C_2H_2-type zinc finger transcription factor that is a member of the human GLI–Kruppel family of proteins. YY1 represses transcription when bound upstream of transcription initiation sites. The repression can be relieved by adenovirus E1A and activation of target genes occurs. We have mapped the repression domain of YY1 to the C-terminal region, overlapping its DNA binding domain. We have also identified an activation domain within the first 69 amino acids of YY1. The YY1 C-terminal region is involved in physical interactions with E1A and is functionally necessary for YY1 to respond to E1A. This suggests that relief of YY1 repression by E1A involves YY1–E1A physical interactions. Although not involved in interactions with E1A, the N-terminal activation domain is also necessary for YY1 to respond to E1A. Presumably, under repressing conditions, the activation domain is masked by the conformation of YY1, but is released upon binding of E1A and is required to subsequently activate transcription. Consistent with this hypothesis, an ATF-2–YY1 chimeric protein containing the activation domain of ATF-2 and the C-terminal two-thirds of YY1 is still a potent repressor. Unlike the mutant YY1 lacking its own N-terminal activation domain, the chimeric protein is fully responsive to E1A.

INTRODUCTION

The mechanisms by which adenovirus E1A activates transcription remain to be fully elucidated. However, a consensus is emerging that E1A may affect transcription of its target genes through interactions with various components of the transcriptional machinery. E1A has been shown to physically interact with the general transcription factor TBP (1) and with a number of sequence-specific DNA binding transcription factors (2–4). In other cases, E1A modulates transcription by disrupting protein complexes involving specific transcription factors (5).

One of the targets of E1A is the transcription factor YY1 (Yin Yang 1) (6) [NF-E1 (7), δ (8), UCRBP (9)]. YY1 is a zinc finger-containing protein which can either repress or activate transcription dependent on the promoter context (reviewed in 10). The dual functionality of YY1 has been demonstrated in many different systems. As a negative regulator, YY1 has been suggested to repress transcription of cellular genes encoding α-skeletal actin (11,12), β-casein (13) and ε-globin (14). It binds to the immunoglobulin κ 3′ enhancer and negatively regulates its activity (7). YY1 also represses viral genes, such as the human papillomavirus promoters (15,16), the BZLF1 promoter of Epstein Barr virus (17), the long terminal repeat of the Moloney murine leukemia virus (9) and the type 1 human immunodeficiency virus (18). As a positive regulator, YY1 has been shown to activate the c-*myc* (19) and certain ribosomal protein genes (8). In addition, YY1 has been shown to direct and initiate transcription when positioned at transcription initiation sites (20,21).

Transcriptional repression mediated by YY1 can be relieved by E1A and further activation of target gene expression occurs (6,22). In essence, E1A is able to convert YY1 from a repressor to an activator. In this paper we present evidence that YY1 contains multiple transcriptional domains. We map the repression domain to the C-terminal and the activation domain to the N-terminal regions of YY1. Both the repression and activation domains of YY1 were found to be required for YY1 to fully respond to E1A. We demonstrate physical interactions between E1A and YY1 and map the E1A interacting domain to the C-terminal half of YY1, which contains the repression domain. Although not required for YY1 interactions with E1A, the N-terminal activation domain of YY1 is indispensable for E1A to relieve YY1 repression. Deletion of the activation domain of YY1 abrogated its response to E1A. Interestingly, appending the activation domain of ATF-2 to an N-terminal deletion mutant of YY1 lacking its own activation domain restored its ability to respond to E1A in transient transfection assays.

MATERIALS AND METHODS

Plasmids

pGAL4-TKCAT contains five GAL4 binding sites inserted 5′ of the HSV TK promoter in plasmid pBLCAT2 (6). pGAL4-E1BCAT contains five GAL4 binding sites inserted

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upstream of the minimal E1B promoter that is linked to the CAT gene (gift of A. Levine, Princeton University). pGAL4-YY1 was described previously (6). N- and C-terminal deletions of YY1 were generated by exonuclease III digestion and fused in-frame to GAL4 DNA binding domain amino acids 1–147. pCMV-13S E1A expression vectors were provided by J. Nevins (Duke University). The pGAL4-ATF2/YY1 plasmid was constructed by fusing YY1 amino acids 116–414 in-frame to pGAL4-ATF2 amino acids 1–109 (gift of M. Green, University of Massachusetts). All the above recombinants were verified by sequence analysis.

Cells and transfections

HeLa cells were grown on 10 cm dishes in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated calf serum. 293 cells were grown in a similar way except that fetal calf serum was used. The cells were transfected by the calcium phosphate precipitation method as described (6). The total amount of DNA was adjusted with the plasmid pSP72 to be identical for each transfection. Cells were harvested 48 h after addition of the precipitate. All transfection assays were carried out with at least two independent DNA preparations and were repeated at least three times.

CAT assays

Whole cell extracts were prepared from the transfected cells. CAT activity was assayed as described (6) and quantitated with a Beckman LS 6500 scintillation counter. Proper amounts of cell extracts were used to measure CAT activity to ensure that the assays were performed within the linear range. The data presented were derived from at least three independent transfections and CAT assays.

Expression of GAL4–YY1 wild-type and deletion mutants in transfected cells by Western blot analysis

GAL4–YY1 wild-type and various deletion mutants were transfected into HeLa cells as described in the previous section. Forty eight hours after transfection, the cells were harvested and nuclear extracts were prepared (23). Following SDS–PAGE, proteins were transferred to a nitrocellulose filter and probed with α-YY1 polyclonal antibodies. The blot was developed with NBT/BCIP. The filter was then stripped, reprobed with polyclonal antibodies against GAL4 amino acids 1–147 (24) and developed by a chemiluminescence method (Bio-Rad Immun-Lite II kit).

Analysis of YY1 and E1A interactions with GST fusion proteins

pGST-YY1 and various deletion derivatives have been described before (25). Fusion proteins were induced and purified as described (26). 293 cells were lysed in lysis buffer (25 mM HEPES, pH 7.0, 0.25 M NaCl, 2.5 mM EDTA, 0.5 mM DTT, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 μg/ml chymostatin, 10 μg/ml aprotonin, 2 mM phenylmethylsulphonyl fluroide, 0.1% NP-40) for 30 min on ice. Extracts prepared from 293 cells (3×10^7 cells/reaction) were incubated for 4 h with various GST–YY1 fusion proteins coupled to glutathione–agarose beads (Sigma). The beads were washed with the lysis buffer and protein complexes were eluted with Laemmli sample buffer. Following SDS–PAGE, proteins were transferred to nitrocellulose filters and probed with M73, a monoclonal antibody against adenovirus E1A protein (27).

RESULTS

Identification of repression and activation domains within the YY1 protein

Previous data have suggested that the C-terminal region of YY1 may be important for its repressor function (6). To systematically analyze the domains involved in repression, GAL4–YY1 plasmids with various N- and C-terminally deleted YY1 fragments linked to the GAL4 DNA binding domain were constructed (Fig. 1A). These GAL4–YY1 deletion mutants were co-transfected with the reporter plasmids pGAL4-TKCAT (6) or pGAL4-E1BCAT (28) into HeLa cells. The TK promoter directs high levels of CAT expression and was used to observe the repressor activity of YY1. The E1B minimal promoter has very low intrinsic activity and was used for detection of the activator activity of YY1 deletion mutants described below. While neither CAT reporter construct was affected by co-transfection with the GAL4 DNA binding domain expression plasmid alone (Fig. 1A, lane 1), GAL4–YY1 repressed the CAT activity by about 6-fold (Fig. 1A, lane 2, GAL4–TKCAT column). Repression of GAL4–TKCAT by GAL4–YY1 was dependent on the GAL4 sites in the promoter, since TKCAT which does not contain GAL4 sites was not affected (6; data not shown). Since the CAT conversion directed by the E1B promoter is minimal, repression by full-length GAL4–YY1 was not seen with this promoter (Fig. 1A, lane 2, E1BCAT column). Consistent with the notion that the primary repression domain is located in the C-terminal portion of the protein, deletion from the N-terminus of YY1 up to amino acid 297 had no effect on its repression function. The C-terminal 83 amino acids (GAL4–YY1 amino acids 332–414) are sufficient to repress transcription (Fig. 1A, lane 8). This is in line with the observation from the analysis of the C-terminal deletion mutants shown below that the last 83 amino acids are necessary for YY1 to repress transcription. However, this mutant is 10-fold less potent as a repressor compared with the 298–414 mutant (Fig. 1A, GAL4–TKCAT column, compare lane 8 with 7), suggesting that sequences N-terminal to the last 83 amino acids are important for the full repressor activity of YY1. Taken together, the data suggest that the repression domain is located within amino acids 298–414 of YY1. Interestingly, this region also encompasses the DNA binding domain. Thus, the repression domain and the DNA binding domain of YY1 overlap.

To complement the results from the analysis of the N-terminal deletion mutants, the activities of a number of C-terminally deleted GAL4–YY1 fusion proteins were examined. Consistent with the above results, deletion of the C-terminal 83 amino acids abolished the ability of YY1 to repress transcription (Fig. 1A, lane 10). In fact, this mutant was able to activate CAT expression when assayed with two different target promoters, the TK and the minimal E1B promoters (Fig. 1A, lane 10; 6.38-fold activation with the TK promoter, 5.93-fold activation with the minimal E1B promoter). Finer mapping suggests that removal of as few as 17 amino acids from the C-terminus of YY1 practically eliminated the repressor activity of YY1 (Fig. 1A, lane 9, GAL4–TKCAT column). As shown in Figure 1A, removal of the 17 amino acids disrupts the last of the four zinc fingers located at the very

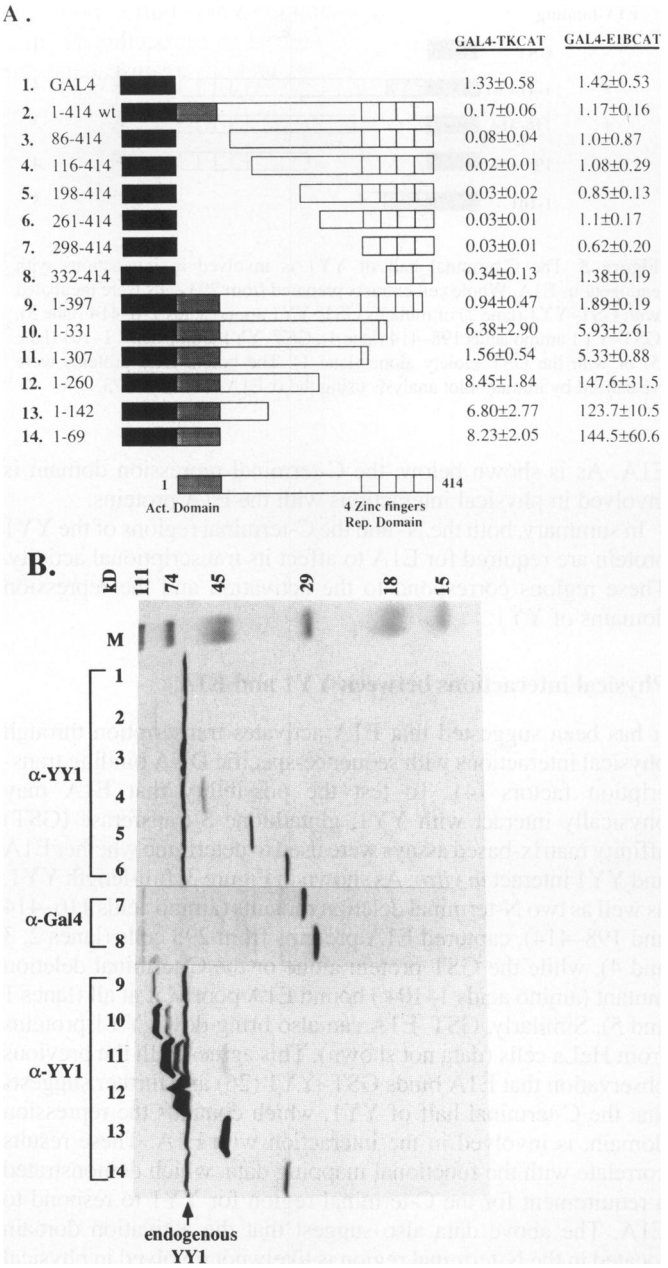

Figure 1. YY1 contains both transcriptional repression and activation domains. (**A**) Identification of functional domains within YY1. N- and C-terminally deleted YY1 fused to the DNA binding domain of GAL4 (dark rectangle) are shown with the end-point of each deletion construct indicated. These deletion plasmids were transfected into HeLa cells together with the reporter plasmid pGAL4-TKCAT or pGAL4E1BCAT. The extent of acetylation was determined relative to that for the reporter plasmid transfected alone. The relative CAT activity reported as 1 represents an average CAT conversion of 50% for most of the assays using pGAL4-TKCAT, but 0.3% for pGAL4-E1BCAT. In analyzing the activating activity of GAL4–YY1 deletion constructs, CAT conversion for GAL4–TKCAT alone was 7%, to ensure that the assays were within the linear range. CAT assay results are presented as mean ± SD from three independent transfections. Each transfection contained 10 μg reporter plasmid and 5 μg effector plasmid. A schematic diagram of the YY1 protein depicting the location of its four zinc fingers and the activation (shaded region) and the repression domains is shown. (**B**) Expression of wild-type and various GAL4–YY1 deletion mutants in HeLa cells. The same set of GAL4–YY1 fusion plasmids used in (A) was transfected into HeLa cells and nuclear extracts were prepared for Western blot analysis using polyclonal antibodies against YY1 or GAL4 amino acids 1–147. The order of the mutants is the same as shown in (A).

C-terminus of YY1, delimiting the C-terminal boundary of the repression domain to the last zinc finger. By and large, deletion of the C-terminal sequence of YY1 abrogated its repressor function, as evidenced by the inability of the C-terminal deletion mutants to repress transcription. This is consistent with the hypothesis that the primary repression domain of YY1 is located at its C-terminus.

Analysis of the C-terminal deletion mutants also revealed a potent activation domain, which was mapped to the first 69 amino acids at the N-terminus. This region, when fused to the DNA binding domain of GAL4, is a potent transcriptional activator, since it was able to activate CAT expression directed by both the TK and the minimal E1B promoters (Fig. 1A, lane 14; 8.23-fold activation for the TK promoter, 144-fold for the E1B promoter). Taking these data together, we assign the first 69 amino acids as the activation domain of YY1. This domain also plays an important role in the YY1 response to adenovirus E1A, as described in the following sections. The N-terminal region (amino acids 1–69) of YY1 is enriched for acidic amino acids, which is a well-characterized feature of transcriptional activation domains (29).

To ensure that GAL4–YY1 fusion proteins were stably expressed, the same set of GAL4–YY1 fusion plasmids was transfected into HeLa cells and nuclear extracts were prepared for Western blotting analysis with α-YY1 or α-GAL4 antibodies. As shown in Figure 1B, all the deletion mutants of GAL4–YY1 fusion proteins used in the functional assays were detectable. One of the fusion proteins has a molecular weight similar to the native YY1, resulting in a band migrating close to the endogenous YY1 (Fig. 1B, lane 3). The two smallest N-terminally deleted GAL4–YY1 fusion proteins were not detected by α-YY1 antibodies, but were readily visible with α-GAL4 antibodies (Fig. 1B, lanes 7 and 8).

In sum, YY1 contains discrete domains that are capable of repressing and activating transcription. The main repression domain is located in the C-terminal part of the YY1 protein (amino acids 298–414). The activation domain of YY1 is highly acidic and is mapped to the N-terminal 69 amino acids. The finding that YY1 contains both repression and activation domains is consistent with the observation that YY1 can either repress or activate transcription.

Identification of functional domains within YY1 that are required for its response to adenovirus E1A

Previous data has indicated that transcriptional repression mediated by YY1 can be relieved by adenovirus E1A proteins (6). We were interested in identifying the domains of YY1 that are involved in its response to E1A. To address this question, the abilities of the N- and C-terminal deletion mutants of YY1 to respond to E1A were analyzed. In Figure 2, the first column (E1A.FS) represents data (average of three independent experiments) collected from transfections of the reporter plasmid pGAL4-TKCAT and various deletion mutants of GAL4–YY1 together with a frame-shift mutant of E1A. As expected, the frame-shift mutant had no effect on the transcriptional activity of YY1. Thus, the data in this column reflect the activities of various deletion mutants of YY1, e.g. wild-type and N-terminal deletion mutants of YY1 repressed CAT activity, while C-terminal deletion mutants either failed to repress or activated CAT activity. In the presence of E1A, repression mediated by full-length YY1 was relieved, as expected (Fig. 2,

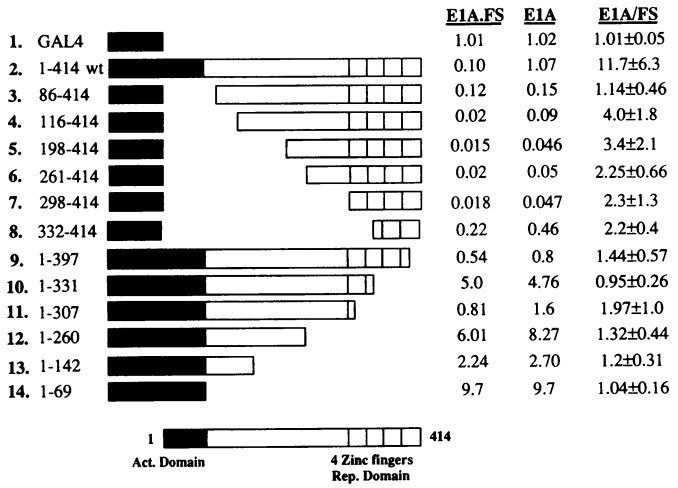

Figure 2. Both the repression and the activation domains of YY1 are required for its full response to adenovirus E1A. The plasmid pGAL4-TKCAT was transfected into HeLa cells together with pGAL4-YY1 or its various N- and C-terminal deletion derivatives in the presence of a plasmid encoding the 13S E1A protein or an E1A frame-shift mutant. Determination of the relative CAT activity is described in Figure 1. CAT assay results are presented as mean values (E1A.FS and E1A columns) or mean ± SD from three independent transfections. Each transfection contained 10 µg reporter plasmid, 5 µg effector plasmid and 2 µg pCMV13S E1A or the frame-shift mutant of E1A.

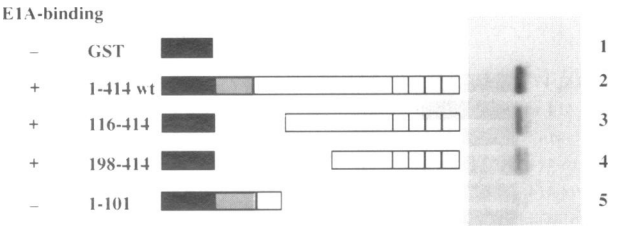

Figure 3. The C-terminal half of YY1 is involved in interactions with endogenous E1A. Whole cell extracts prepared from 293 cells were incubated with GST–YY1 (lane 2) or mutants GST–YY1 amino acids 116–414 (lane 3), GST–YY1 amino acids 198–414 (lane 4), GST–YY1 amino acids 1–101 (lane 5) or with the GST moiety alone (lane 1). The bound E1A proteins were visualized by immunoblot analysis using the α-E1A antibody M73.

E1A column, lane 2). This effect of E1A is dependent on the presence of YY1, since in its absence the activity of the GAL4–TK promoter is virtually unaffected (6; Fig. 2, lane 1). Interestingly, none of the deletion mutants of YY1 responded to E1A to the same extent as did full-length YY1 (Fig. 2, compare E1A column with E1A.FS column, lanes 3–14). The response of the YY1 mutants to E1A is best demonstrated in column 3 (E1A.FS). The effect of E1A is expressed as its ability to raise the CAT activity (fold increase) from a repressed state (when either wild-type or mutant YY1 is present, together with the E1A.FS mutant) to an elevated level. As shown in Figure 2, the response of full-length YY1 to E1A is about 12-fold (E1A.FS column, lane 2), while that of the N-terminal deletion mutants of YY1 ranged between 2- and 4-fold (lanes 3–8). This suggests the importance of the N-terminal region of YY1 in its response to E1A. Our analysis mapped this region to the first 86 amino acids, which overlaps the activation domain of YY1. We next analyzed the effect of E1A through the YY1 deletion mutants on a different promoter, the E1B minimal promoter. Consistent with the findings obtained using the TK promoter, full-length GAL4–YY1 activated CAT expression 5.5-fold in the presence of E1A (data not shown; see also Fig. 4, lanes 11 and 12), while the N-terminal deletion mutants, such as GAL4–YY1 (amino acids 86–414) did not (data not shown). Taken together, our data strongly imply the involvement of the N-terminal region of YY1 in activating transcription induced by E1A.

The effect of E1A on C-terminally deleted YY1 was next examined. As shown above, deletion of the very C-terminal 17 amino acids abolished the ability of YY1 to repress transcription. This mutant also failed to respond to E1A (Fig. 2, E1A column, compare lane 9 with lane 2). The inability to respond to E1A was observed for all the C-terminally deleted YY1, as shown in Figure 2 (lanes 9–14). This suggests that the C-terminal region, important for repression, is also critical for YY1 to respond to E1A. As is shown below, the C-terminal repression domain is involved in physical interactions with the E1A proteins.

In summary, both the N- and the C-terminal regions of the YY1 protein are required for E1A to affect its transcriptional activity. These regions correspond to the activation and the repression domains of YY1.

Physical interactions between YY1 and E1A

It has been suggested that E1A activates transcription through physical interactions with sequence-specific DNA binding transcription factors (4). To test the possibility that E1A may physically interact with YY1, glutathione S-transferase (GST) affinity matrix-based assays were used to determine whether E1A and YY1 interact *in vitro*. As shown in Figure 3, full-length YY1, as well as two N-terminal deletion mutants (amino acids 116–414 and 198–414), captured E1A proteins from 293 cells (lanes 2, 3 and 4), while the GST protein alone or the C-terminal deletion mutant (amino acids 1–104) bound E1A poorly, if at all (lanes 1 and 5). Similarly, GST–E1A can also bring down YY1 proteins from HeLa cells (data not shown). This agrees with the previous observation that E1A binds GST–YY1 (26) and further suggests that the C-terminal half of YY1, which contains the repression domain, is involved in the interaction with E1A. These results correlate with the functional mapping data, which demonstrated a requirement for the C-terminal region for YY1 to respond to E1A. The above data also suggest that the activation domain located in the N-terminal region is likely not involved in physical interactions with E1A.

The activation domain of ATF-2 can restore the ability of an N-terminal deletion mutant of YY1 to respond to E1A

As described above, the N-terminal activation domain of YY1 is dispensable for physical interactions with E1A. However, it is essential for YY1 to respond to E1A (Fig. 2). Interestingly, a similar behavior has been reported for transcription factor ATF-2. E1A activates AT-F2-mediated transcription by binding to the DNA binding domain of ATF-2 (4). Meanwhile, the activation process also requires the N-terminal activation domain, which is not involved in physical interactions with E1A. It is thought that the activation domain may be required for subsequent transcriptional activation upon binding of E1A to ATF-2.

To determine whether the activation domain of ATF-2 can restore the ability of the N-terminal deletion mutant of YY1

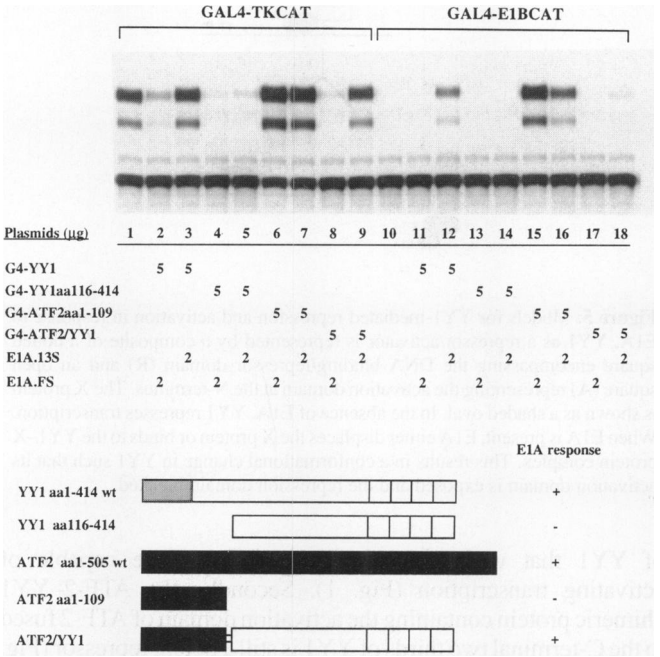

Figure 4. Appending the ATF-2 activation domain to a YY1 N-terminal deletion mutant restores its ability to fully respond to E1A. The ATF-2–YY1 hybrid was constructed by fusing GAL4–ATF-2 amino acids 1–109 in-frame to the N-terminus of the YY1 deletion mutant (YY1 amino acids 116–414). The response of the hybrid protein to E1A was examined by transfecting the plasmid into HeLa cells together with the reporter plasmids pGAL4-TKCAT or pGAL4-E1BCAT in the presence of a plasmid encoding the 13S E1A protein or a E1A frame-shift mutant (lanes 8, 9, 17 and 18). Also shown are E1A responses of full-length YY1 (lanes 2, 3, 11 and 12), the N-terminal deletion mutant YY1 amino acids 116–414 (lanes 4, 5, 13 and 14) and ATF-2 amino acids 1–109 alone (lanes 6, 7, 15 and 16). The amount of each plasmid used in the transfection (μg) is indicated. The data indicating the response of ATF-2 to E1A is taken from Liu and Green (3,4).

lacking its own activation domain to respond to E1A, a chimeric protein was made that consists of the N-terminal activation domain of ATF-2 (amino acids 1–109) linked in-frame to YY1 deleted of its first 115 amino acids (amino acids 116–414). Similar to wild-type GAL4–YY1, the GAL4–ATF-2–YY1 chimeric protein repressed the activities of GAL4–TKCAT. Furthermore, ATF-2–YY1 was fully responsive to E1A, suggesting that addition of the ATF-2 activation domain restored the ability of the mutant YY1 to respond to E1A (Fig. 4, compare lane 9 with 8). In contrast, the N-terminal deletion mutant YY1 (amino acids 116–414) and the ATF-2 N-terminal region alone did not respond to E1A (Fig. 4, lanes 4–7). Essentially the same results were obtained using the GAL4–E1BCAT reporter, as shown in Fig. 4 (lanes 10–18). These observations support the hypothesis that the N-terminal region of YY1 is masked in the repressing conformation and that E1A converts YY1 to an activator by unmasking the activation domain located at the N-terminus.

DISCUSSION

Functional domains within YY1

YY1 is a multifunctional protein that can either repress or activate transcription. When bound upstream of transcription initiation sites, YY1 has been found to act as a repressor in most reported cases (6,7,9,14,15,17,30), with the exception of the c-*myc* promoter (19). The repressor activity of YY1 was localized to its C-terminal 116 amino acids, where the DNA binding domain of YY1 resides (Fig. 1A). Deletion analyses suggest that the C-terminal 116 amino acids of YY1 are necessary and sufficient to repress transcription of target genes. Consistent with the notion that YY1 can also activate transcription, a potent transcriptional activation domain was identified within the first 69 amino acids of YY1. This region is enriched for acidic amino acids and, when fused to the DNA binding domain of GAL4 (amino acids 1–147), activated the minimal E1B promoter up to 144.5-fold (Fig. 1A). Deletions of the C-terminus of YY1 abrogated its repressor function. By and large, the C-terminally deleted YY1 proteins behaved as transcriptional activators. This suggests that removal of the repressor domain may have caused a conformational change in the protein which leads to unmasking of the N-terminal activation domain. It is worth noting that the abilities of the C-terminally deleted YY1 mutants to activate transcription vary. For instance, amino acids 1–260 activated E1BCAT 147-fold, while amino acids 1–307 activated the same promoter only 5-fold. At present, the possibility that an inhibitory domain is located within amino acids 260–307 of YY1 cannot be excluded.

We have noticed variabilities in the amounts of fusion proteins in transfected cells, although equal amounts of plasmid DNAs were used. However, we do not believe that the variabilities should affect the conclusion that the primary repression and activation domains of YY1 are located in its C- and N-terminal regions. We view these results as qualitative, which help direct identification of the functional domains in YY1. The level of GAL4 fusion proteins did not correlate with the strength of the transcriptional activity of YY1. For instance, the mutant amino acids 1–307 expresses more proteins than the mutant amino acids 1–69 (Fig. 1B, lanes 11 and 14), but its activation activity is 27-fold less than that of the latter (Fig. 1A, lanes 11 and 14, GAL4–E1BCAT column). In addition, the protein levels of the mutants amino acids 1–260, 1–142 and 1–69 appear to vary by several folds, but their activities are essentially the same (Fig. 1A and B, lanes 12–14). The lack of correlation may be due to the five GAL4 sites in the reporter plasmid being saturated. As a result, more protein above the saturating level does not further increase (or decrease) the CAT activity of the reporter plasmids. Consistent with this idea, it has been previously shown that GAL4 proteins bind cooperatively to its recognition sites *in vivo* (31). Finally, our mapping data are in agreement with the published data of others (32).

In sum, YY1 contains discrete domains that are capable of repressing and activating transcription. The main repression domain is located in the C-terminal part of YY1 (amino acids 298–414). There is a possible inhibitory domain between amino acids 260 and 307. The activation domain of YY1 is located within the first 69 amino acids and is highly acidic. Identification of both the repression and the activation domains within YY1 provides a structural basis for its dual functionality.

Previously, the transcriptional activity of YY1 has been attributed to its DNA bending activity and the orientation of YY1 binding sites has been suggested to determine the activation or repression activities of YY1 in the regulation of the c-*fos* promoter (33). The finding that GAL4–YY1 represses transcription and some of its deletion derivatives activate transcription via the GAL4 sites suggests that the transcriptional activity of YY1 cannot be solely accounted for by its DNA bending property, as

these assays were not dependent on the DNA binding activity of YY1. The fact that YY1 contains distinct repression and activation domains strongly suggests that repression/activation activities are intrinsic properties of YY1. Since the activation domain of YY1 is highly acidic, it is possible that it functions in a similar way to other well-characterized acidic activation domains. Although the repressor domain of YY1 coincides with its DNA binding domain, preliminary data in the laboratory suggest that these two properties can be separated by subtle mutations in this region (K. M. Galvin and Y. Shi., unpublished results). Finally, E1A relieves transcriptional repression by GAL4–YY1, as it does for the repressor activity of endogenous YY1. Since E1A does not bind to specific DNA sequences and the relief of repression occurs even when YY1 is targeted to DNA through GAL4, it is unlikely that the effect results solely from differential bending of the DNA template. Therefore, we believe YY1 may modulate transcription by more than one mechanism.

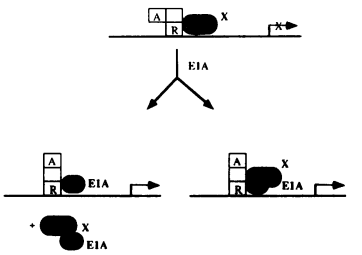

Figure 5. Models for YY1-mediated repression and activation in response to E1A. YY1 as a repressor/activator is represented by a composite of a dotted square encompassing the DNA binding/repressor domain (R) and an open square (A) representing the activation domain at the N-terminus. The X protein is shown as a shaded oval. In the absence of E1A, YY1 represses transcription. When E1A is present, E1A either displaces the X protein or binds to the YY1–X protein complex. This results in a conformational change in YY1 such that its activation domain is exposed and the repression domain blocked.

Physical interactions between YY1 and E1A

It has been postulated that E1A activates transcription via interactions with sequence-specific DNA binding proteins such as ATF-2 (4). Our finding that YY1 and E1A physically interact is consistent with this hypothesis, although we believe that the interaction is likely to be indirect. Studies of the domains of E1A necessary for relief of YY1-mediated transcriptional repression have implicated the involvement of the E1A-associated protein p300 and a physical complex involving YY1 and p300 has been detected *in vivo* (detailed analysis of YY1–p300 interactions will be reported elsewhere). The same region of YY1 necessary for capturing E1A proteins from 293 cells is also involved in binding to p300 proteins translated *in vitro*. Furthermore, the slightest deletion from the C-terminus of YY1 (amino acids 1–397) abrogated its ability to respond to E1A (Fig. 2, lane 9), as well as its ability to interact with p300 in a two hybrid assay in HeLa cells (J.- S. Lee and Y. Shi, unpublished data). These data suggest a correlation between YY1–p300 interactions and the ability of YY1 to respond to E1A. Therefore, we favor the hypothesis that functionally significant physical interactions between E1A and YY1 *in vivo* are mediated by cellular proteins such as p300. However, a direct interaction between the two proteins cannot be completely excluded. It has been demonstrated that GST–YY1 can bind to *in vitro*-translated E1A proteins (26). We have also observed a weak interaction (2- to 3-fold above background) between YY1 and E1A in yeast using the yeast interaction trap assay (34,35). This suggests that either certain yeast proteins are capable of mediating YY1–E1A interactions or that there may be a weak direct interaction between the two proteins.

Mechanisms underlying the response of YY1 to E1A

As discussed earlier, YY1 contains both repression and activation domains and the former is involved in physical interactions with E1A (Fig. 3). Although it contains a strong activation domain, YY1 acts as a repressor under our assay conditions. Therefore, it can be envisioned that under repressing conditions the conformation of YY1 dictates that its N-terminal activation domain be masked, so that it acts as a repressor. E1A binds to the C-terminal region of YY1, causing conformational changes that lead to unmasking of the activation domain. This process converts YY1 from a repressor to an activator. Two lines of observation are consistent with this hypothesis. First, C-terminal deletion mutants of YY1 that were defective for repression are capable of activating transcription (Fig. 1). Secondly, the ATF-2–YY1 chimeric protein containing the activation domain of ATF-2 fused to the C-terminal two thirds of YY1 is still a potent repressor (Fig. 4), supporting the notion that the N-terminal region of YY1 is masked due to its conformation. E1A relieved repression mediated by ATF-2–YY1, presumably by unmasking the activation domain located at the N-terminus. The above hypothesis is summarized in Figure 5. YY1 is complexed with cellular proteins (represented by X) at the promoter. Such a configuration is favorable for repression. E1A is targeted to the promoter via its interactions with one of the X proteins. This is predicted to cause a conformational change in YY1 that leads to unmasking of its activation domain at the N-terminus. As discussed earlier, a likely candidate for the protein that mediates YY1–E1A interactions is the E1A-associated protein p300.

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