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YY1 and NF1 Both Activate the Human p53 Promoter by Alternatively Binding to a Composite Element, and YY1 and E1A Cooperate To Amplify p53 Promoter Activity

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A novel transcription factor binding element in the human p53 gene promoter has been characterized. It lies about 100 bp upstream of the major reported start site for human p53 gene transcription. On the basis of DNase I footprinting studies, electromobility shift assay patterns, sequence specificity of binding, the binding pattern of purified transcription factors, effects of specific antibodies, and methylation interference analysis we have identified the site as a composite element which can bind both YY1 and NF1 in an independent and mutually exclusive manner. The site is conserved in the human, rat, and mouse p53 promoters. The occupancy of the site varies in a tissue-specific manner. It binds principally YY1 in nuclear extracts of rat testis and spleen and NF1 in extracts of liver and prostate. This may facilitate tissue-specific control of p53 gene expression. When HeLa cells were transiently transfected with human p53 promoter-chloramphenicol acetyltransferase reporter constructs, a mutation in this composite element which disabled YY1 and NF1 binding caused a mean 64% reduction in basal p53 promoter activity. From mutations which selectively impaired YY1 or NF1 binding and the overexpression of YY1 or NF1 in HeLa cells we concluded that both YY1 and NF1 function as activators when bound to this site. In transient cotransfections E1A could induce the activity of the p53 promoter to a high level; 12S E1A was threefold as efficient as 13S E1A in this activity, and YY1 bound to the composite element was shown to mediate 55% of this induction. Overexpressed YY1 was shown to be able to synergistically activate the p53 promoter with E1A when not specifically bound to DNA. Deletion of an N-terminal domain of E1A, known to be required for direct E1A-YY1 interaction and E1A effects mediated through transcriptional activator p300, blocked the E1A induction of p53 promoter activity.

p53 is a phosphoprotein which has been shown to mediate a number of key cellular effects. It induces growth arrest via the WAF1/p21 and the retinoblastoma (Rb) pathway (29). p53 is involved in DNA repair, in which it is thought to screen genomic DNA for mutations at the G_1/S checkpoint (31, 37). p53 can also induce cell death by apoptosis in certain cell types (21, 67). Many of these functions are mediated by p53 acting as a transcription factor (15). Genes whose promoters contain a specific p53 DNA binding element are transcriptionally upregulated by p53; these include *mdm2* (5), *WAF1/p21* (12), and the p53 gene itself (10). Gene promoters that do not contain a p53 DNA binding element can be transcriptionally repressed by p53 via effects at TATA box elements; such genes include the c-*fos* (28) and the Rb genes (58). On the basis of its cellular actions, p53 is classified as a tumor suppressor protein, and the presence of high constitutive levels of stable afunctional and functional mutated forms of p53 is common in a wide variety of tumor cells (11, 27).

A number of posttranscriptional mechanisms for regulating the life span of cellular p53 have been described (27). However, there is also clear evidence that regulation at a transcriptional level may contribute significantly to controlling cellular p53 levels and cell fate in both normal and tumor cells. In primary human diffuse astrocytomas Pax 5 binding to a site downstream of the transcriptional start site has been shown to limit cellular p53 levels by suppressing the p53 promoter (60). p53 promoter activity is also repressed through interaction of the human T-cell leukemia virus type 1 Tax protein with factors such as c-Myc occupying a promoter-proximal basic helix-loop-helix (b-HLH) binding element, a function which has been implicated in Tax-induced tumorigenicity (63). Furthermore, the levels of expression of mutant forms of p53 and p53 mRNA levels in some B-lymphoid cell lines correlate with cellular c-Myc protein levels; c-Myc–Max heterodimers bind to the b-HLH site in the p53 promoter and activate it (55). In nontransformed cells a novel transcription factor has been characterized which mediates the rapid activation of the p53 promoter in response to UV irradiation by binding to the so-called genotoxic stress site proximal to the transcription start site; this stimulus would provide additional p53 protein to coordinate the cell stress response (61). In Fanconi anemia cell lines, in contrast, UV irradiation does not induce p53 accumulation, and the failure of the cells to trigger p53-dependent apoptosis in response to certain stresses may contribute to this disease phenotype (53). Lastly, studies with transgenic mice have demonstrated high testicular specific p53 promoter activity, while, in contrast, some p53-null mice exhibit testicular giant-cell degenerative syndrome which may arise because of deficiencies in p53-regulated DNA repair (54).

Thus, a series of tumor initiation or progression events have been associated with reduced cellular p53 levels or overproduction of mutant p53, and there is evidence to link changes in p53 promoter activity to these events. It is important, therefore, that we begin to understand the nature of the p53 promoter and identify the key mechanisms controlling its activity. The human p53 gene promoter is unusual in that it does not contain a TATA box (62) or a sequence that fits any known initiator consensus. It has an untranslated exon 1 (62), which contains some regulatory binding elements, e.g., the Pax 5

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binding element discussed above (60). Within the first 100 bp upstream of the start site, a number of elements have been shown to cause strong induction of the promoter: the UV-responsive element (61), a stress-responsive element which binds NF-κB (66), an autoregulatory p53-binding element (10), and the b-HLH element which binds Myc/Max heterodimers (55) and USF (49). The p53 promoter sequence is highly conserved between humans, rats, and mice (7). However, interestingly, the major transcription start site of the mouse p53 promoter (7) has been mapped to ~100 bp upstream from the human start site (62).

We have studied a composite element within the proximal promoter of the human p53 gene which can bind both YY1 and NF1. NF1 (nuclear factor 1) proteins are a heterogeneous family of proteins which play roles in the cell-specific expression and function-specific expression of genes; e.g., a liver-specific enhancer in the serum albumin gene promoter depends on both NF1/CTF and hepatocyte nuclear factor 3 (HNF3) for transcriptional activity during hepatocyte differentiation (25). In the mammary gland NF1 plays a critical role in activating milk protein gene promoters during lactation (64). NF1 has a highly conserved N-terminal DNA binding and dimerization domain which enables all such factors to bind to the inverted repeat, 5′-TGG(A/C)N_5GCCAA-3′ (22). NF1 binds to DNA as a dimer through an unusual DNA binding domain which contains four cysteines which are essential for binding (45). Some NF1's can also bind to "half-sites," where a promoter contains only one copy of 5′-TGGCA-3′ (8). Their C-terminal proline-rich transactivating domains are highly divergent and are generated by alternate RNA splicing of one of the four gene products (NF1-A, -B, -C, and -X). For example, the NF1-X gene gives rise to two differentially spliced forms, NF1-X1 and NF1-X2 (69). This diversity allows possible heterodimer formation and a high degree of selective control through NF1 binding sites, indicating the complexity of the NF1's as a family of transcription factors. Further control of these transcriptional regulators occurs by posttranslational modification; e.g., cdc2 kinase can phosphorylate NF1 (26).

YY1 (Yin-Yang 1) is a zinc finger transcription factor of the C_2H_2 type which contains structural similarity to the drosophila *GLI*-krüppel protein (47), so called because of its ability to both activate and repress transcription. Promoters whose activity is increased by YY1 include the c-*myc* 1 and 2 (50) and dihydrofolate reductase (14) promoters and the immunoglobulin heavy chain (H) μ enhancer (13). Cellular genes which are repressed by YY1 include the c-*fos* (19), serum amyloid (36), and β-casein (39) promoters. A number of mechanisms of YY1-mediated repression have been proposed: YY1 may induce DNA bending and so block other transcription factors binding to their sites on the promoter (44). By binding to certain DNA binding elements, YY1 can compete for occupation of the site by a second factor. A number of such composite binding elements have been described, e.g., YY1/MGF (39), YY1/SRF (34), and YY1/NF-κB binding sites (36). Hyde-DeRuyscher et al. suggest that YY1 may contain a repressor domain in its C terminus (24). The context in which YY1 binds may also effect its function (44). A switch element upstream of the YY1 element in the human papillomavirus type 18 upstream regulatory region binds a factor which determines whether bound YY1 acts as an activator or a repressor (6). In some promoters YY1 has been shown to activate transcription at initiator elements (56). The activity of YY1 can be modulated by the adenovirus oncoprotein E1A (17, 32, 57), which has been shown to relieve YY1-induced transcriptional repression. This may be due to the formation of a triprotein complex composed of YY1, E1A, and p300 (32) or through a direct protein-protein interaction between YY1 and E1A (33, 35). E1A has been shown to increase cellular p53 levels, an effect which has been observed as an increase in the p53 protein level and an increase in p53 mRNA level (9).

In this report, we demonstrate that there is a composite site within the immediate human p53 promoter which binds both YY1 and NF1. This site is conserved in the human, rat, and mouse promoters. Both factors bind to the site in an independent and mutually exclusive manner. There is tissue-specific occupancy of this site; in testis (and spleen) nuclear extracts YY1 binding predominates, while in the liver (and prostate) extracts the site is occupied predominately by NF1. Transfection studies show that this site is important for p53 promoter function and that both factors can contribute to basal transcriptional activity. YY1 functions as an activator to increase p53 promoter activity, which is unusual for a composite YY1 binding site. We show that E1A can amplify p53 promoter activity in HeLa cells. Mutation of the composite element reduces the amplification by 55%. We demonstrate that YY1 can contribute to the E1A effect. Lastly, an E1A amino-terminal mutant which cannot bind to p300 or YY1 caused no induction of p53 promoter activity.

MATERIALS AND METHODS

Plasmid constructs and PCR-based site-directed mutagenesis. The human p53 promoter-chloramphenicol acetyltransferase (CAT) construct, p5DU356, which contains the gene fragment −458 to −104 (62), a gift from S. Tuck (Princeton, N.J.), was used as the wild-type (WT) promoter-reporter construct in this study. It was also used as a template to create promoters containing the mutations M1 to M4 (see Fig. 2A) by PCR-based site-directed mutagenesis using the procedure of Kuipers et al. (30). Vent DNA polymerase (New England Biolabs) was used in the PCRs. The mutated fragments were subcloned into pBS KS− and verified by sequencing. The fragments were subsequently released from pBS KS− by *Bam*HI-*Xba*I restriction and were subcloned back into p5DU356 by using the promoter internal *Bam*HI and *Xba*I sites.

Expression vectors for the E1A 12 and 13S variants, pCMV 12S and 13S E1A, were provided by J. Nevins (23). The pCMV 12S dl2-36 expression vector, which encodes the N-terminal deletion mutant dl2-36 of E1A 12S (23), was a gift from M. Mathews (Cold Spring Harbor Laboratory). The mammalian expression vector for YY1 pSV-E1, and pSV-rE1, in which the YY1 cDNA is cloned in reverse orientation, were obtained from M. Atchison (Philadelphia, Pa.) (46). The mammalian expression vector for NF1, pRSV CTF1 (3), was a gift from N. Mermod (Lausanne, Switzerland).

Bacterial expression of YY1, preparation of tissue extracts, and gel retardation analysis. A bacterial expression vector encoding his-YY1 was kindly supplied by T. Shenk (Princeton University). Bacterial expression and purification of his-YY1 was carried out as described by Shi et al. (57). Baculovirus-expressed and -purified NF1 was prepared as described previously (48). Nuclear extracts from rat liver, ventral prostate, testis, mouse mammary gland, and HeLa cells were prepared essentially as described by Andrews and Faller (2) in the presence of a cocktail of protease inhibitors (51). Electromobility shift analysis (EMSA) was performed as described by Frain et al. (16). The double-stranded oligonucleotides (Fig. 2A) were [^{32}P]phosphate end labeled. Specific complex formation on the oligonucleotide was blocked by preincubating the nuclear extracts (10 μg of protein) for 30 min on ice with a monoclonal anti-YY1 antibody (32) (a gift from Y. Shi [Boston, Mass.]) or a polyclonal anti-NF1 antibody (42) (a gift from J. Dekkar, Utrecht, The Netherlands). Either Dulbecco modified Eagle medium–10% fetal calf serum or normal rabbit serum was used in place of the antibodies as a negative control.

DNase I footprinting and methylation interference analysis. The human p53 promoter and the mutated promoter fragments were ^{32}P end labeled on the coding strand by using the internal *Bam*HI site (at −458) (62) and on the noncoding strand by using the internal *Xba*I site (at −104) and released at the unlabeled end to yield a 354-bp restriction fragment. Nuclear extract (50 μg) was preincubated with 300 ng of poly(dI-dC) in a buffer containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.9), 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, 60 mM KCl, 0.25 mM Na_2-EDTA, and 20% (vol/vol) glycerol on ice for 10 min. Purified labeled fragment (1.5×10^4 cpm) was then added, and the reaction mixture was incubated on ice for 15 min. DNase I digestion was carried out at room temperature for 1 min with a predetermined DNase I dilution from the stock. The stock DNase I (Worthington) was stored at 1 mg/ml and contained approximately 2.62 U/μl. The reactions were terminated by adding twice the reaction volume of 50 mM EDTA, 0.1% sodium dodecyl sulfate, and 200 μg of proteinase K per ml, and the mixtures were then incubated at 44°C for 30 min. The products were precipitated with ethanol and separated on 8% polyacrylamide gels containing 8 M urea. Maxam and Gilbert

G and G+A reactions for the appropriate fragment were run in adjoining lanes. The procedures used were essentially those described in reference 4.

For methylation interference analysis, a double-stranded 21-bp oligonucleotide containing the sequence of the wild-type composite element (Fig. 2A) was subcloned into the *Sma*I site of pBS KS1 in both the sense and the antisense directions. Following restriction with *Not*I, each construct was labeled with the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP. The labeled fragment was then released from the plasmid by digesting with *Xho*I, gel purified, precipitated, and resuspended in 100 μl of 10 mM Tris (pH 8.0)–1 mM EDTA. This fragment (2×10^6 cpm) was then partially methylated by incubating in 200 μl of 50 mM Na cacodylate (pH 8.0)–10 mM $MgCl_2$–0.1 mM EDTA–1 μl of dimethyl sulfate (DMS) at room temperature for 3 min. This reaction was stopped and the DNA was precipitated as described in reference 4. The pellet was resuspended in water and used as probe for EMSA. Both the retarded complex and the free probe were excised from the gel, eluted, cleaved with piperidine, and analyzed on a 10% polyacrylamide gel containing 8 M urea. Procedures used were again essentially the same as those described in reference 4.

Transfections and CAT assays. HeLa cells were maintained in Dulbecco modified Eagle medium–10% fetal calf serum. Transient transfections were carried out with calcium phosphate as described by Wigler et al. (65). The cells were harvested 48 h after transfection and standardized by cotransfecting pRSV βgal. CAT activity was assayed as described in reference 59, and β-galactosidase activity was assayed as described in reference 52. CAT activity was quantitated by cutting out the appropriate sections of the thin-layer chromatography plates and counting their ^{14}C contents in a Beckman scintillation counter. For all the data presented, at least three independent transfections and CAT assays have been performed.

RESULTS

DNase I footprinting analysis of promoter sequences in the human p53 gene. A 354-bp fragment covering the transcriptional start site of the human p53 promoter (as mapped by Tuck and Crawford [62]) with 344 bp of upstream sequence was strand specifically labeled and subjected to DNase I footprinting analysis in the presence of nuclear extracts prepared from rat ventral prostate, spleen, liver, and testis cells. Autoradiographs of the DNase I fragment ladders (Fig. 1A) show a predominant single footprint which on closer examination contains subtle tissue-specific differences. For extracts from prostate and liver (lanes 4 and 6), the footprint maps to −197 to −224 on the noncoding strand and −225 to −194 on the coding strand (start site for transcription, −114 [62]). Note, however, that the footprint for spleen and testis extracts maps to −204 to −227 on the noncoding strand and to −225 to −205 on the coding strand, suggesting that there is tissue-to-tissue variation in the occupancy of this site by transcription factors. The sequences contained within the footprint boundaries are shown in Fig. 1B. Consideration of the DNA sequence of the footprinted region revealed it to contain two overlapping transcription factor binding elements, 5′-GTCATGGCGA-3′ (24) and 5′TGGNNNNNNCCA-3′ (22), which bind YY1 and NF1, respectively. This composite binding element is conserved in the human, rat, and mouse promoters (Fig. 1B), suggesting a significant role for both YY1 and NF1 in the regulation of p53 promoter function. It was of interest, therefore, to test the hypothesis that YY1 and NF1 might alternatively occupy this site, in a tissue- and/or function-specific manner, that alternate occupation determines the different footprints detected, and that alternate occupation of the site might influence basal and/or enhanced p53 promoter activity. The position of this composite element in relation to the transcriptional start site and to other previously reported binding elements is shown as a schematic diagram in Fig. 1B. We could also detect footprints over the previously described USF and NF-κB sites with extracts of prostate and testis cells on both strands (Fig. 1A). A footprint which maps over a predicted C/EBP site could also be detected with the spleen extract (Fig. 1A).

YY1 and NF1 bind to the composite element in vitro. To facilitate EMSA on the composite element, a series of double-stranded synthetic oligonucleotide probes were prepared. These potentially allow binding of both factors (WT), neither factor (M1), YY1 only (M2), predominantly NF1 (M3), and only NF1 (M4) (Fig. 2A). The binding of NF1 to the element was confirmed with a purified recombinant 36-kDa NF1 produced in a baculovirus expression system and previously characterized by Rein et al. (48): Fig. 2B, left-hand panel, shows the formation of a single retarded complex on the WT probe (lane 1) and that specific mutation of the NF1 binding element sequence inhibits complex formation (mutants M1 and M2) (lanes 2 and 3). The binding of recombinant NF1 to mutants M3 and M4 (lanes 4 and 5) shows that reducing the affinity for YY1 (M3) or specifically disabling YY1 binding (M4) did not affect NF1 binding. In M3, the binding affinity for NF1 is increased and the affinity for YY1 binding is reduced (see below). Preincubation with an anti-NF1 antibody (42) reduced the level of shifted complex formed and generated a supershifted complex (compare lanes 6 and 7). However, when a control antibody (anti-YY1) was preincubated with recombinant NF1, the retarded complex was still formed (lane 8). Neither the retarded complex nor the supershifted complex was found when the anti-NF1 antibody was incubated with the WT probe in the absence of recombinant NF1 (lane 9).

Likewise, the specific ability of the element to bind partially purified recombinant his-YY1 (57) was demonstrated (Fig. 2B, right-hand panel). Lane 1 shows the formation of four bands on the WT probe, the upper band being the retarded complex containing the 68-kDa YY1, while two of the lower bands most likely contain proteolytic degradation products, as they are recognized by an anti-YY1 antibody (compare lanes 7 and 8). YY1 has been previously reported to be very susceptible to proteolytic degradation (36). With the M1 mutant no binding of YY1 is observed, as would be predicted for the mutations introduced into the core binding sequences (lane 2). A mutation which affects the NF1 core-binding nucleotides only, M2, did not affect YY1 binding (lane 3), but a mutation in the YY1 binding core (M4) completely impaired YY1 binding (lane 5), while mutation M3 reduced it significantly (lane 4). No YY1 complexes were observed on the WT probe with extract from the his-YY1 expression vector-transformed bacteria that had not been induced with IPTG (isopropyl-β-D-thiogalactopyranoside) (lane 6). In addition, preincubation with an anti-YY1 specific antibody (32) completely impaired formation of the shifted complex (compare lanes 7 and 8), while preincubation with a control antibody (anti-NF1) had no effect on the retarded complexes (compare lanes 7 and 9). No complexes were formed when the WT probe was incubated with the anti-YY1 antibody in the absence of his-YY1 (lane 10).

To examine the tissue differences in binding activity, nuclear extracts were prepared from rat ventral prostate, spleen, liver, and testis and HeLa cells and examined by EMSA. The results with the testis and liver extracts are shown in Fig. 2C and D. EMSA analysis of testis extract revealed a predominance of YY1 binding and little NF1 (similar results were found for the spleen [data not shown]). The binding of YY1 was apparent on probes WT, M2, and M3, as predicted (Fig. 2C, left-hand panel, lanes 2, 4, and 5). Where the YY1 core element had been mutated (M1 and M4), YY1 binding was lost, but NF1 binding, although weak, now became enhanced (multiple NF1 complexes were formed [lanes 5 and 6]). In the absence of added tissue extract no complexes were detected (lane 1). Preincubation with the anti-YY1 antibody selectively inhibited YY1 complex formation (compare lanes 7 and 9). Preincubation with an anti-NF1 antibody had no effect on the retarded YY1 complex (compare lanes 7 and 8). In addition, the YY1-DNA complex migrated to the same distance as the major retarded complex seen with recombinant his-YY1 (data not

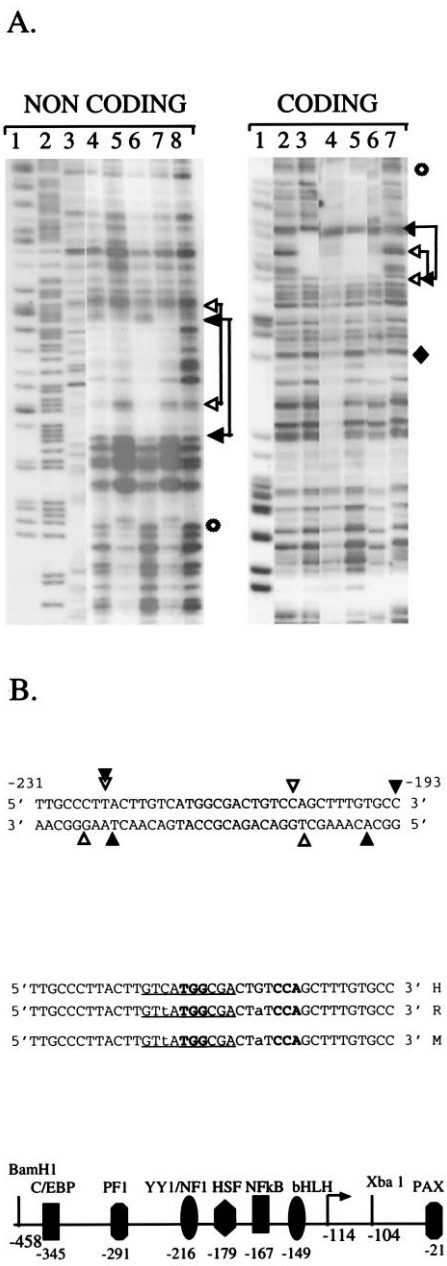

FIG. 1. (A) DNase I footprinting analysis of the human p53 promoter (−458 to −104). Noncoding strand: lane 1, Maxam and Gilbert chemical sequencing G reaction; lane 2, G+A reaction; lanes 3 and 8, DNase I-digested [^{32}P]phosphate-labeled DNA fragment, with bovine serum albumin (BSA) (40 μg); lane 4, rat ventral prostate nuclear extract (40 μg of protein); lane 5, rat spleen extract (40 μg); lane 6, rat liver extract (40 μg); lane 7, rat testis extract (40 μg). Coding strand: lane 1, G reaction; lanes 2 and 7, probe with BSA (40 μg); lane 3, rat ventral prostate nuclear extract (40 μg of protein); lane 4, rat spleen extract (40 μg); lane 5, rat liver extract (40 μg); lane 6, rat testis extract (40 μg). The boundaries of the two footprints over the composite element are marked by arrows. Open circle, beginning of the USF and NF-κB footprints (−153 to −186); diamond, beginning of the C/EBP footprint (−311 to −348). Filled arrows, footprint boundaries for the prostate and liver; open arrows, boundaries of the spleen and testis footprint. (All images in this article were scanned into Adobe Photoshop, version 4.0, with a Microtek Scanmaker IIHR. The images were then imported into Microsoft Powerpoint, version 4.0, for labeling and printing.) (B) Sequence of the footprinted region showing the boundaries of the two types of footprint (upper panel). The boundaries of the putative YY1 and NF1 footprints on the human promoter are indicated by open and filled arrowheads, respectively. The middle panel shows the alignment of this sequence between the human (H), rat (R), and mouse promoters (M). The core sequences of the YY1 and NF1 elements are underlined and boldfaced, respectively. The diagram in the lower panel shows the position of this footprint in relation to the major start site for transcription and other previously reported factor binding sites. The human p53 promoter is numbered by the convention adopted by Tuck and Crawford (62).

shown). Therefore, with the testis extract the binding on this element is dominated by YY1, with very little NF1 binding being seen. This was confirmed by carrying out a series of competition experiments (Fig. 2C, right-hand panel). Preincubation with a 50× excess of a previously characterized YY1-binding element oligonucleotide from the c-*myc* promoter ($YY1_{Myc}$ [50]) greatly reduced binding of the YY1 retarded complex to the WT probe while increasing the intensity of a group of larger retarded complexes (compare lanes 2 and 3). The larger complexes were selectively eliminated by using a previously characterized NF1-binding element oligonucleotide (from the adenovirus replication origin [$NF1_{AV}$] [43]) (lane 4). On the M2 probe, which can bind only to YY1, the YY1 retarded complex was eliminated with the $YY1_{Myc}$ oligonucleotide while the $NF1_{AV}$ had no effect (lanes 5 to 7). Note that, when YY1 binding is inhibited by competition with $YY1_{Myc}$, the larger NF1 complexes appear only on the WT probe and not on M2 (compare lanes 3 and 6). With M4 (which can bind only NF1) as the probe, competition with $YY1_{Myc}$ had no effect on the larger retarded complex, while competition with $NF1_{AV}$ inhibited complex formation (lanes 8 to 10). Therefore, with the testis extract, binding is dominated by YY1, with NF1 binding becoming prominent only when YY1 binding has been inhibited either by mutation or by competition.

Conversely, with the liver extract, both NF1 and YY1 complexes were detected on the WT probe (similar results were obtained with HeLa cell extracts [data not shown]). NF1-containing complexes were detected on the WT, M3, and M4 probes (Fig. 2D, left-hand panel, lanes 2, 5, and 6) but not with probes M1 and M2 (lanes 3 and 4). YY1 complexes were found on the WT and M2 probes (lanes 2 and 4) and not on the M1, M3, and M4 probes (lanes 2, 5, and 6). Preincubation with the anti-NF1 antibody further identified the predominant upper band as an NF1 complex and did not affect YY1 binding (compare lanes 7 and 8). Conversely, preincubation with the anti-YY1 antibody eliminated YY1 binding but did not affect NF1 binding (compare lanes 7 and 9). The identities of the NF1 and YY1 complexes in liver extracts were further confirmed in competition experiments (Fig. 2D, right-hand panel). On the WT probe, preincubation with the competing $NF1_{AV}$ oligonucleotide inhibited formation of the NF1 complexes but had no effect on YY1 complex formation (lane 3). Preincubation with excess $YY1_{Myc}$ oligonucleotide inhibited YY1 complex formation but had no effect on NF1 complex formation (lane 4). With the M2 probe, which can bind only YY1, the retarded complex is eliminated with the $YY1_{Myc}$ oligonucleotide while the $NF1_{AV}$ oligonucleotide had no effect (lanes 5 to 7). With M4 as probe, which can bind only NF1, competition with the $YY1_{Myc}$ oligonucleotide had no effect on the putative NF1 retarded complex, while competition with the $NF1_{AV}$ oligonucleotide inhibited complex formation (lanes 8 to 10). It is noteworthy that, while multiple NF1 complexes are formed on the WT probe (Fig. 2C, right-hand panel, lane 3, and Fig. 2D, right-hand panel, lane 2), only the largest of these complexes are formed on the M4 probe (Fig. 2C and D, right-hand panels, lanes 8). This may be determined by the C-to-A mutation at base 12 in the oligonucleotide, which could affect specificity of NF1 species binding to the neighboring core TGG element (Fig. 2A).

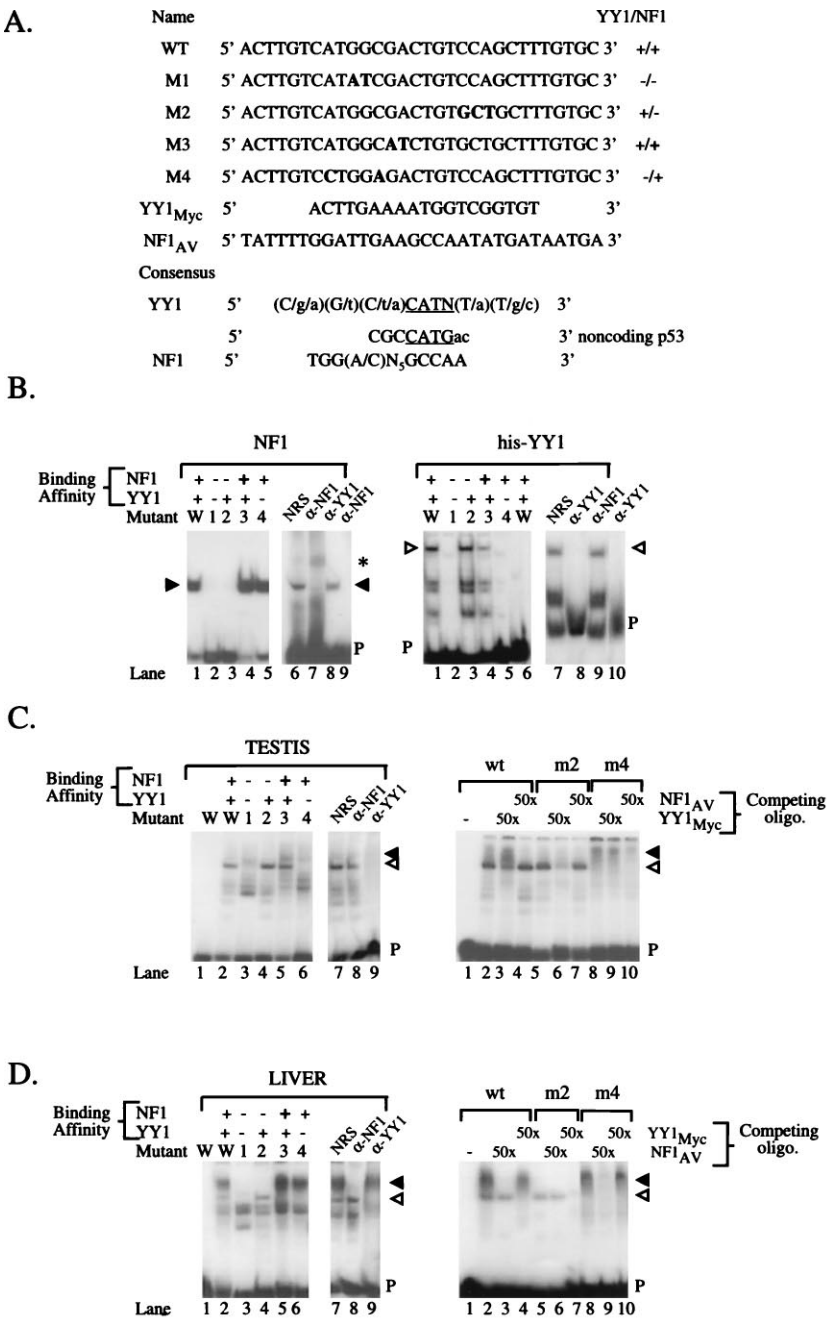

FIG. 2. (A) Composite YY1/NF1 binding element in the human p53 promoter with the wild-type (WT) and the mutation series (M1 to M4) sequences and their predicted specificities. The sequences of the EMSA competitor oligonucleotides, $YY1_{Myc}$ (50) and $NF1_{AV}$ (43), are shown. A consensus YY1 sequence (24) is compared with the p53 sequence. A consensus NF1 sequence is also shown (22). (B) EMSA analysis of recombinant NF1 and recombinant YY1 binding to the composite element. Left-hand panel: lanes 1 to 5, recombinant NF1 (~4 ng of protein) and probes WT, M1, M2, M3, and M4, respectively; lane 6, WT probe, recombinant NF1, and normal rabbit serum (NRS) (1 μl); lane 7, WT probe, recombinant NF1, and anti-NF1 antibody (α-NF1) (1 μl); lane 8, WT probe, recombinant NF1, and anti-YY1 antibody (3 μl); lane 9, WT probe and anti-NF1 antibody (1 μl). Right-hand panel: lanes 1 to 5, his-YY1 extract (6 μl) and probes WT, M1, M2, M3, and M4, respectively; lane 6, WT probe and extract of uninduced his-YY1-expressing bacteria (6 μl); lane 7, WT probe, his-YY1 extract, and cell culture medium (3 μl); lane 8, WT probe, recombinant YY1, and anti-YY1 monoclonal antibody (α-YY1) (3 μl); lane 9, WT probe, recombinant YY1, and anti-NF1 antibody (1 μl); lane 10, WT probe and anti-YY1 antibody (3 μl). The predicted binding affinity of each mutant for YY1 and NF1 is indicated above each lane. Closed arrowhead, YY1 complex; open arrowhead, NF1 complex; asterisk, α-NF1/NF1-DNA supershifted complex. (C) EMSA analysis of testis extract binding to the composite element. Left-hand panel: lane 1, WT probe, no extract; lanes 2 to 6, nuclear extract (10 μg protein) and probes WT, M1, M2, M3, and M4, respectively; lane 7, WT probe, nuclear extract (10 μg), and NRS (1 μl); lane 8, WT probe, extract, and anti-NF1 antibody (1 μl); lane 9, WT probe, extract, and anti-YY1 antibody (3 μl). YY1 and NF1 retarded complexes are indicated by open and filled arrowheads, respectively. Right-hand panel (competition experiments with testis extract): lane 1, WT probe with no extract; lanes 2 to 4, WT probe with extract (10 μg protein); lane 3, preincubation with 50× $YY1_{Myc}$; lane 4, preincubation of 50× $NF1_{AV}$; lanes 5 to 7, M2 probe and extract (10 μg of protein); lane 6, 50× $YY1_{Myc}$; lane 7, 50× $NF1_{AV}$; lanes 8 to 10, M4 probe and extract (10 μg of protein); lane 9, 50× $YY1_{Myc}$; lane 10, 50× $NF1_{AV}$. (D) EMSA analysis of liver extract binding to the composite element. The lanes in the left-hand panel correspond to those in left-hand panel C. Right-hand panel (competition experiments with liver extract): lane 1, WT probe with no extract; lanes 2 to 4, WT probe with extract (10 μg of protein); lane 3, preincubation with 50× $NF1_{AV}$; lane 4, preincubation with 50× $YY1_{Myc}$; lanes 5 to 7, M2 probe and extract; lane 6, 50× $NF1_{AV}$; lane 7, 50× $YY1_{Myc}$; lanes 8 to 10, M4 probe and extract; lane 9, 50× $NF1_{AV}$; lane 10, 50× $YY1_{Myc}$.

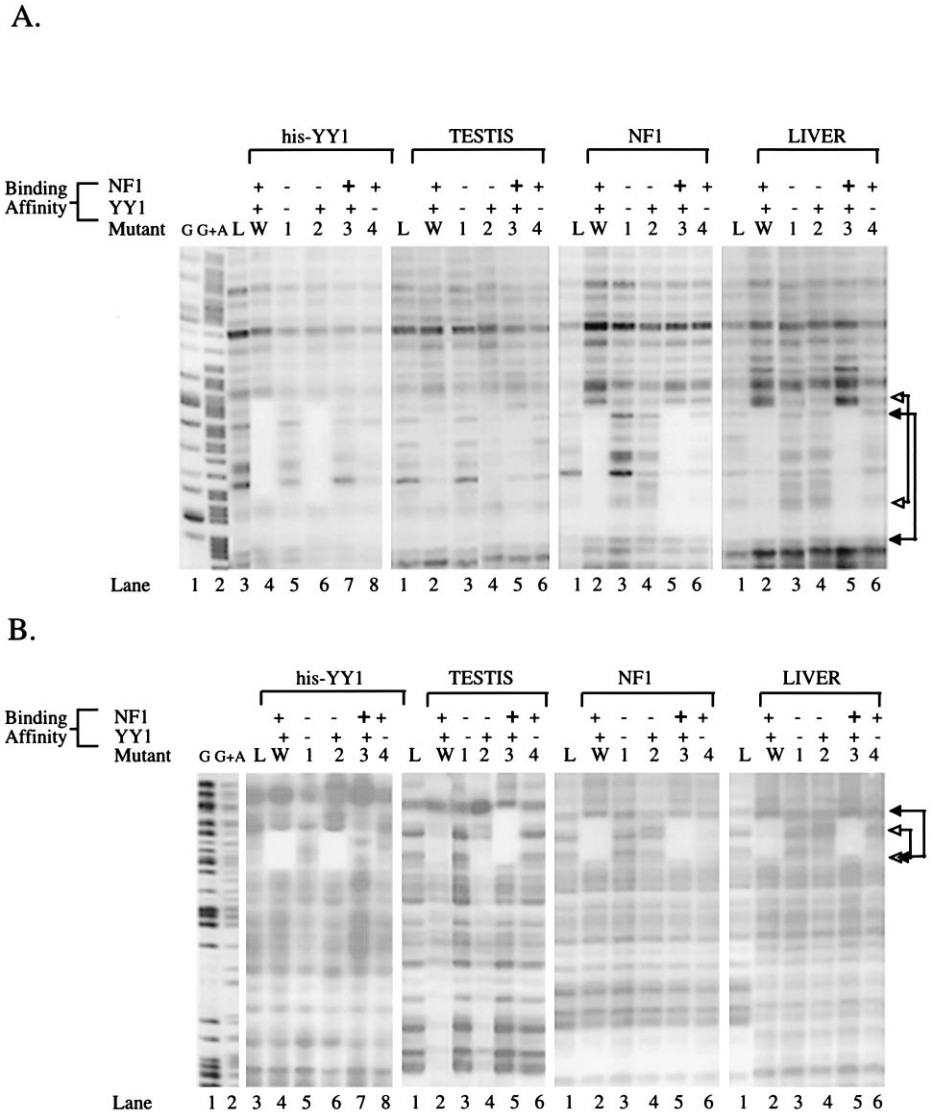

FIG. 3. DNase I footprinting analysis of YY1 and NF1 binding to the composite element (WT) and mutation series (M1 to M4). (A) Noncoding strand. Lanes 1 and 2, G and G+A Maxam and Gilbert sequence; lane 3, [^{32}P]phosphate-labeled fragment (WT) with bovine serum albumin (BSA) (40 μg); lanes 4 to 8, his-YY1 extract (50 μl) and DNA fragments containing elements WT, M1, M2, M3, and M4, respectively. Testis, NF1, and liver extract lanes: lanes 1, WT probe and BSA (40 μg); lanes 2 to 6, either 40 μg of tissue nuclear extract or 50 ng of NF1 and DNA fragments WT, M1, M2, M3, and M4, respectively. (B) Coding strand. The lanes correspond to those in panel A. The DNA fragments (M1 to M4) were generated by site-directed mutagenesis of the wild-type promoter to yield the composite element mutations M1 to M4 (Fig. 2A) and were excised by cutting the internal *Bam*HI and *Xba*I sites to [^{32}P]phosphate end label for footprinting. The boundaries of the YY1 and NF1 footprints on the human promoter are indicated by open and filled arrows, respectively. The binding affinities (as determined by EMSA [Fig. 2]) for YY1 and NF1 are indicated above each lane.

To further test whether the differences shown in Fig. 1 represent alternate occupancy of the site by YY1 and NF1, the series of selective YY1/NF1 binding mutations (M1 to M4) were introduced into the p53 promoter by site-directed mutagenesis (see Materials and Methods) and the resulting promoter fragments were subjected to DNase I footprinting analysis (Fig. 3). Similar footprints on the wild-type element and footprint pattern, over the mutant series, were obtained with the testis extract and recombinant his-YY1, on both the noncoding (Fig. 3A) strand and the coding strand (Fig. 3B): YY1 type footprints are apparent for both recombinant YY1 and testis extract on the WT fragment and fragment M2. No footprint was seen with testis extract or recombinant YY1 on mutant M1 or M4 in which the YY1 core binding element had been mutated (testis lanes 3 and 6). The exception was the footprint on mutant promoter M3 (which has a reduced capacity for binding YY1 and enhanced capacity for binding NF1 (Fig. 2B). With M3 no footprint is seen with recombinant YY1 but an NF1 type footprint is seen with the testis extract (Fig. 3B, his-YY1 lane 7 and testis lane 5).

In contrast, the footprints and footprint pattern over the series of mutants obtained with the liver extract were identical to those obtained for purified recombinant NF1: on WT and M3 (liver lanes 2 and 5) an NF1 type footprint is seen, and no footprints (with recombinant NF1 or with liver extract) were seen on mutants M1 and M2 in which the NF1 core element is mutated (liver lanes 3 and 4). Taken together, these results strongly suggest that the differences seen with this footprint are due to alternate binding of the transcription factors YY1 and NF1 to this site. In addition, we demonstrate that it is possible

to manipulate a switch from the binding of one factor to the other by selective mutation of the element (e.g., Fig. 2C and 3, lower panels, testis; compare lanes 4 and 5). It is also interesting that the liver extract which contains both NF1 and YY1, as shown in EMSA (Fig. 2D), on footprinting exhibits only NF1 binding. This is most likely due to the different requirements of these two binding systems to provide a positive signal and is most probably due to the presence of higher concentrations of NF1 than YY1 in the liver extracts.

The binding of YY1 and NF1 to this composite element is mutually exclusive. Differences in the "points of contact" between YY1 and NF1 on the element were established by methylation interference analysis (Fig. 4). With recombinant NF1, methylation of $GG_{-214/-213}$ and G_{-207} (coding strand) in the sequence 5′-GTCAT**GG**CGACT**G**CCA-3′ proved essential for NF1 binding (lane 5). In contrast, the methylation interference pattern seen with retarded YY1 complexes is selectively dependent on additional contact with G_{-219} and G_{-211} in the sequence 5′-**G**TCAT**GG**C**G**ACT**G**TCCA-3′ (lane 3), which were not necessary for NF1 binding, as well contacts with $GG_{-214/-213}$ and G_{-207}. (The major retarded complex in the testis extract had been identified in EMSA studies to be YY1 [Fig. 2C] and was used for this analysis.)

On the noncoding strand, recombinant NF1 binding was selectively characterized by the partial requirement for interaction with G_{-204} and absolute requirement for interaction at G_{-205} as well G_{-209} (lane 10), which were not necessary for YY1 binding (lane 8). The guanidines G_{-212} and G_{-217} are involved in binding of both YY1 and NF1 (lanes 8 and 10), and the YY1 pattern shows a partial binding interference at G_{-209} (lane 8). On this binding element YY1 contacts a large number of G's compared with other previously reported overlapping sites, for example, the YY1/SRF binding sites in the c-*fos* promoter (19). The YY1 binding element within the p53 promoter fits the consensus sequence proposed by Hyde-DeRuyscher et al. (24) but also contacts additional G's (e.g., G_{-207} [coding strand]) outside this central YY1 binding core, 5′-CG CCATGac-3′ (24). The different patterns of G's required for binding YY1 and NF1 further emphasize the specificity and difference in the interactions of bound NF1 and YY1 with the composite p53 element.

The fact that some of the guanidines are necessary for binding both transcription factors suggests that their binding to this site is mutually exclusive. This was directly tested by carrying out a factor binding competition assay (34): as the NF1 and his-YY1 complexes have different sizes, this can be performed by EMSA under probe limiting conditions, with the concentration of one of the factors kept constant while the other one is titrated up. From Fig. 5, it can be seen that increasing concentrations of his-YY1 decreased NF1 binding in a dose-dependent manner (lanes 3 to 8), and likewise, increasing concentrations of NF1 decreased his-YY1 binding in a dose-dependent fashion (lanes 10 to 13). These results strongly suggest that YY1 and NF1 bind to the composite element in an independent and mutually exclusive fashion.

Differential YY1/NF1 occupancy of the composite element affects p53 expression in HeLa cells. In order to test whether this composite site plays a role in maintaining the expression of the p53 promoter and whether YY1 and/or NF1 activate or repress the p53 promoter by binding to this site, a series of promoter-CAT reporter constructs were generated by using the M1-to-M4 mutant promoter fragment series (see above). HeLa cells were transiently transfected with the wild-type construct (WT-CAT [p5DU 356-CAT] [62]), and significant CAT gene expression was observed (Fig. 6A, lane 1), as would be predicted from previous studies (21). HeLa cells were chosen

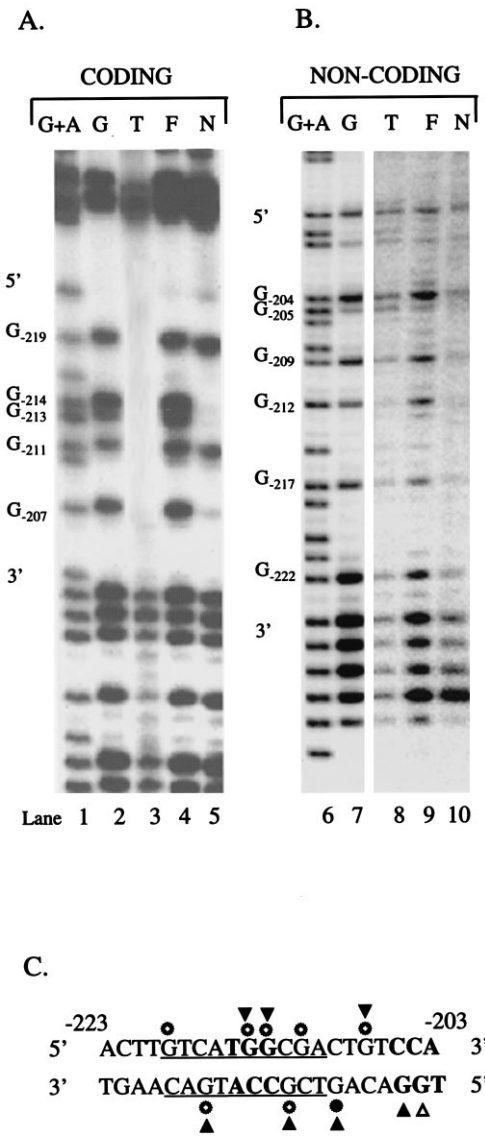

FIG. 4. Methylation interference analysis of YY1 and NF1 binding to the composite element. (A) Coding strand. The wild-type oligonucleotide WT (Fig. 2A) was subcloned into the *Sma*I site of pBS KS 1 in both orientations. After *Not*I digestion, both fragments were strand specifically labeled with Klenow. The labeled probe was partially methylated and incubated with rat testis nuclear extract (50 μg) (T) or recombinant NF1 (10 ng) (N); free and retarded probes were separated by EMSA, and the probes were recovered and cleaved at G. Lane 1, G+A sequence; lane 2, G sequence; lane 3, testis extract, bound probe; lane 4, free probe; lane 5, recombinant NF1, bound probe. (B) Noncoding strand. The lanes correspond to those in panel A. (C) The sequence of the DNA covered in the methylation interference analysis. The core sequences of the YY1 and NF1 elements are underlined and boldfaced, respectively. The G's that are crucial to binding YY1 and NF1 are indicated by open circles and filled arrowheads, respectively. Filled circle, partial interference of YY1 binding; open arrowhead, partial interference of NF1 binding.

for these assays, as we could detect both YY1 and NF1 in HeLa cell nuclear extracts by EMSA (data not shown), which confirmed similar findings from other laboratories (20, 40).

Mutation M1-CAT, in which both YY1 binding and NF1 binding are disabled, reduced activity by 64% (mean) of the wild-type expression, suggesting that either YY1 binding or NF1 binding or both are involved in maintaining the expression of the wild-type promoter. Mutant M2-CAT, which per-

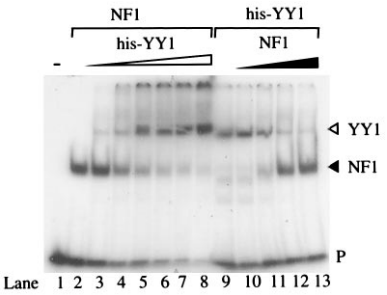

FIG. 5. YY1 and NF1 occupy the composite element in a mutually exclusive manner. A factor binding competition assay-EMSA is shown. Lane 1, WT probe, no extract; lanes 2 to 8, WT probe with recombinant NF1 (5 ng) and his-YY1 extract (0, 2.5, 5, 10, 15, 20, and 25 μl, respectively); lanes 9 to 13, WT probe with his-YY1 extract (10 μl) and recombinant NF1 (0, 2.5, 5, 10, and 20 ng, respectively).

mits only YY1 binding showed a level of CAT gene expression (lane 3) similar to that for M1-CAT. This could suggest that YY1, at the levels available in HeLa cells, cannot drive the p53 promoter strongly. However, as demonstrated below (see Fig. 7B, lanes 1 and 2), while YY1 can bind to mutant M2, it cannot transactivate when bound. Therefore, the overall functional effect of mutation M2 on p53 promoter activity is the same as that of M1. The M3 and M4 mutations, which principally affect YY1 binding, did not cause a significant reduction in CAT expression (lanes 4 and 5). The results of this experiment suggest that both YY1 and NF1 can potentially contribute to the expression of the p53 promoter in HeLa cells by binding on this element. The degree to which YY1 and NF1 contribute may depend on the physiological state of the cells.

To directly test whether YY1 activates or represses the p53 promoter by binding to this element, increasing concentrations of a YY1 expression vector, pSV-YY1 (46), were cotransfected with WT-CAT. This elevated CAT expression from the wild-type promoter in a dose-dependent fashion (Fig. 6B, upper panel, lanes 1 to 4). It did not activate the M1-CAT or M4-CAT construct, neither of which binds YY1 (lanes 8 to 15). Cotransfection of increasing quantities of an expression vector containing YY1 cloned in the opposite orientation, pSV-oYY1, with WT-CAT (46) had a significantly smaller effect than pSV-YY1 (Fig. 6B, upper panel, lanes 5 to 7). This effect of YY1 was not occurring by an interaction solely at the transcription start site, as cotransfection of YY1 with −37 TK-CAT (which contains only a TATAA-dependent minimal promoter [38]) had no activating effect (lanes 16 and 17). Cotransfection of increasing concentrations of an NF1 expression vector, pRSV-CTF1/NF1 (3), also elevated CAT expression from the wild-type promoter in a dose-dependent fashion (Fig. 6B, lower panel, lanes 1 to 4) but not from the M1-CAT and M2-CAT mutant promoters, which do not bind NF1 (lanes 8 to 15). Cotransfecting pCMV-NF1 with −37 TK CAT had no effect (lanes 16 and 17). Thus, both YY1 and NF1 can increase the activity of the p53 promoter by binding to the composite element and potentially contribute to its basal activation. Therefore, in contrast to its effects on a range of other composite binding elements (e.g., YY1/NF-κB [36] and YY1/SRF [19]), YY1 does not have a repressor effect when bound to this site.

Differential effect of YY1/NF1 occupancy of the composite element on E1A induction of p53 promoter activity in HeLa cells. E1A has been reported to increase cellular levels of p53, as determined by an increase in p53 protein and mRNA levels (9). It was of interest to demonstrate the potential of E1A to regulate cellular p53 at the level of gene expression and to investigate the role of the composite element in modulating induced p53 promoter activity by E1A. The effect of transient coexpression of E1A 12S and E1A 13S variants on the activity of the promoter-CAT reporter constructs was studied in HeLa cells (Fig. 7A). E1A expression significantly increased the activity of each of the promoters tested; the 13S E1A caused a 4-fold (mean) induction of WT-CAT expression, while the 12S E1A caused a 14-fold (mean) induction. This finding suggests that the additional transactivating domain, CR3, present in the 13S variant does not contribute to the effect detected. The CR3 domain in 13S E1A is thought to be the major E1A transcriptional activator of a number of cellular and viral genes (32). This places the p53 gene in a small subclass of promoters which include the c-*myc* (23) and PCNA promoters (41), which do not require this domain for maximal E1A induction.

When neither YY1 or NF1 can bind to the composite element (with M1-CAT), the E1A induction is reduced by approximately 55% for both the 12S and the 13S isoforms. This suggests that this site does contribute to enhanced p53 gene expression and that either YY1, NF1, or both may partly mediate the E1A induction. When YY1 binding is disabled but NF1 can bind to the promoter (M4-CAT), the 12S E1A induction is still reduced by over 55%. This suggests that YY1 binding can potentially account for 55% of the E1A induction driven through this composite site. However, our evidence for this claim is indirect. The remaining 45% of the induction may be due either to a general amplification of promoter activity proportional to the basal level, mediated through the basal transcription complex, or to a specific effect mediated through another, as yet unidentified binding element. The effect of E1A on the M3-CAT construct was similar to the effect on M4-CAT (data not shown). The effect on M2-CAT is considered below (Fig. 7B).

We attempted to demonstrate directly that YY1 bound to the composite element can mediate E1A induction of p53 promoter activity. When cotransfection experiments were carried out with M2-CAT (mutant M2 binds YY1 but not NF1 [Fig. 2B], and M2-CAT has low basal activity [Fig. 6A]), we found that the YY1 caused a minimal induction of M2-CAT activity (Fig. 7B, left-hand panel, lanes 1 and 2): it caused a 1.4-fold induction compared with an 8-fold induction of WT-CAT (Fig. 6B, upper panel). Thus, YY1 can bind to the M2 mutant element but will not transactivate when bound. Expression of 12S E1A produced a mean 6-fold induction of M2-CAT over basal level (Fig. 7B, left-hand panel, lane 3), but cotransfection with 12S E1A and the YY1 expression vector gave a mean 18.6-fold induction (lane 4). This strongly suggests a synergy between YY1 and E1A. When M4-CAT, which cannot bind YY1, was cotransfected with YY1, no induction was seen (Fig. 7B, middle panel, lane 2). Expression of 12S E1A produced a mean fivefold induction of M4-CAT over basal level (lane 3), but cotransfection with 12S E1A and the YY1 expression vector gave a mean ninefold induction (lane 4), again suggesting a synergistic interaction between YY1 and E1A but indicating that it is not dependent on YY1 being bound to DNA. With M1-CAT, which is activated by neither YY1 or NF1, a similar result was obtained (right-hand panel). We conclude that, when YY1 is overexpressed, it can synergize with E1A to increase the activity of the p53 promoter without binding to a YY1-binding element.

Lee et al. (32) have recently characterized a specific interaction between YY1 and transcriptional modulator p300 and the possible formation of a three-protein complex composed of YY1, p300, and E1A in mediating transcription. E1A binds p300 through its amino-terminal domain. Lewis et al. (35) and

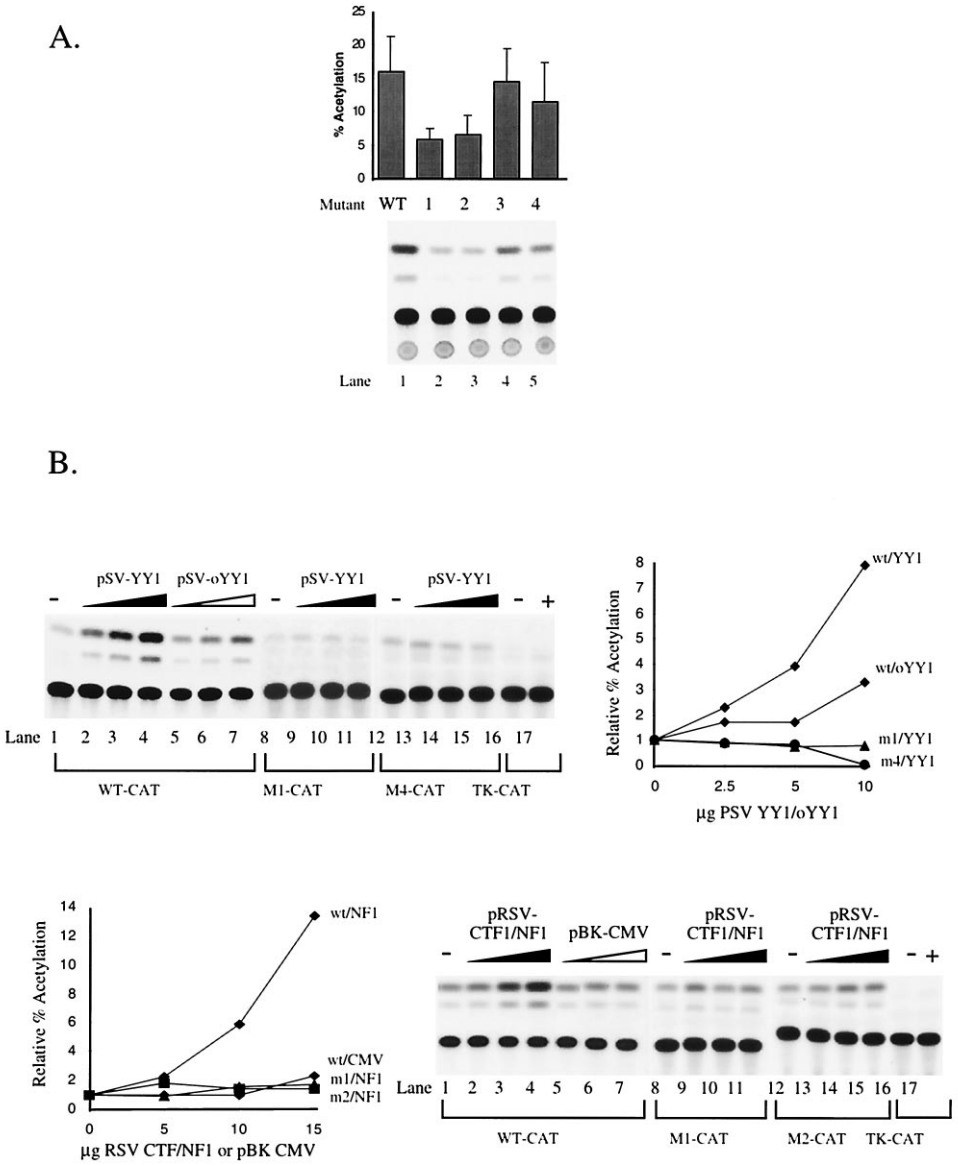

FIG. 6. (A) Differential YY1/NF1 occupancy of the composite element affects promoter activity in HeLa cells. HeLa cells were independently transiently transfected with each of five promoter-CAT constructs (5 μg) containing the human p53 promoter sequences −458 to −104 in pPL2-CAT with the composite element in the wild-type configuration (WT-CAT) or configurations M1 to M4 (M1-M4-CAT). A representative autoradiograph is shown, and the results of four independent experiments, each carried out in triplicate, are presented. Two independent preparations of each plasmid DNA were used. The data are mean percent chloramphenicol acetylation with standard errors of the means. (B) Both YY1 and NF1 can independently activate the p53 promoter by binding to the composite element. Upper panel: HeLa cells were cotransfected with 0, 2.5, 5, and 10 μg of pSV-YY1 (pSV-E1 [46]) and WT-CAT (lanes 1 to 4); 2.5, 5, and 10 μg of pSV-oYY1, an expression vector with YY1 in reverse orientation (pSV-rE1 [46]) and WT-CAT (lanes 5 to 7); 0, 2.5, 5, and 10 μg of pSV-YY1 and M1-CAT (lanes 8 to 11); 0, 2.5, 5, and 10 μg of pSV-YY1 and M4-CAT (lanes 12 to 15); and 0 and 10 μg of pSV-YY1 and −37 TK-CAT (38) (lanes 16 and 17). Lower panel: HeLa cells were cotransfected with 0, 5, 10, and 15 μg of pRSV-CTF/NF1 (3) and WT-CAT (lanes 1 to 4); 5, 10, and 15 μg of pBK CMV (empty vector) and WT-CAT (lanes 5 to 7); 0, 2.5, 5, and 10 μg of pRSV-CTF/NF1 and M1-CAT (lanes 8 to 11); 0, 2.5, 5, and 10 μg of pRSV-CTF/NF1 and M2-CAT (lanes 12 to 15); and 0 and 15 μg of pRSV-CTF/NF1 and −37 TK-CAT (38) (lanes 16 and 17). In both cases representative autoradiographs are shown. The results of three independent experiments were graphed as mean relative CAT activities (compared with activity in the absence of an expression vector).

Lee et al. (33) have also identified two domains within YY1 which can interact directly with two domains in E1A (in the amino terminus and in CR3). As 12S E1A does not contain CR3, it has only one YY1 binding domain, in the amino terminus (amino acids 15 to 35). An expression vector encoding a 12S E1A mutant, dl2-36 (23), which has an amino-terminal deletion impairing its ability to bind to p300 (32) and to directly interact with YY1 (35), was used for this study. Cotransfection of pdl2-36 M12S E1A with pSV-YY1 and M2-CAT gave no induction of CAT expression over basal level (Fig. 7B, left-hand panel, lane 5), suggesting a direct YY1-E1A interaction or an involvement of p300 in the synergistic effect of YY1/E1A on the human p53 promoter. Similarly, no E1A induction was observed when pdl2-36 M12S E1A was cotransfected with M4-CAT in the presence of overexpressed YY1 or with WT-CAT (results not shown). This result suggests that all the E1A effects observed require the N-terminal domain of E1A. This specific requirement for the N-terminal E1A domain has been previously reported for YY1-E1A interactions in two other model systems (32, 35).

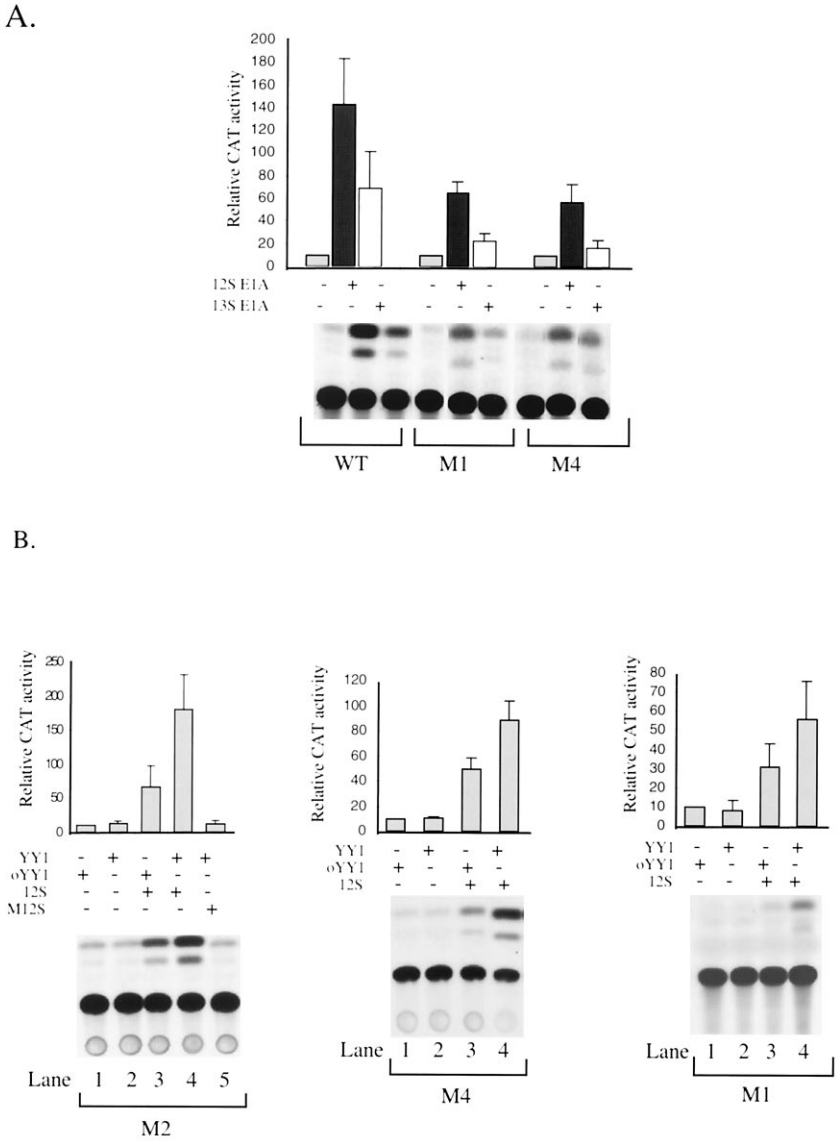

FIG. 7. Effect of E1A 12S and E1A 13S expression on p53 promoter activity: role of the composite element. (A) HeLa cells were independently transiently cotransfected with constructs WT-, M1-, and M4-CAT (5 μg) and carrier plasmid (pBK CMV), pSV-E1A (12S), or pSV-E1A (13S) (5 μg). A representative autoradiograph is shown, and the results of three independent experiments are presented as relative CAT activities (compared with the basal CAT activity in the absence of E1A) (means with standard errors of the means). The presence or absence of either 12S or 13S E1A is indicated. (B) Effect of YY1 overexpression on E1A induction of p53 promoter activity. Left-hand panel: HeLa cells were independently transiently cotransfected with M2-CAT (5 μg) and carrier plasmid (10 μg of pSV-oYY1) (lane 1); M2-CAT (5 μg) and pSV-YY1 (10 μg) (lane 2); M2-CAT (5 μg) and pSV E1A (12S) (3 μg) (lane 3); M2-CAT (5 μg), pSV-YY1 (10 μg), and pSV E1A 12S (3 μg) (lane 4); or M2-CAT (5 μg), pSV-YY1 (10 μg) and pdl2-35 E1A 12S (M12S [23]) (3 μg) (lane 5). Middle panel: as above except that M4-CAT was used. Right-hand panel: as above except that M1-CAT was used. Representative autoradiographs are shown, and the results of three or four independent experiments are presented as relative CAT activities (compared with basal M2-, M4-, or M1-CAT activity) (means with standard errors of the means).

It is noteworthy that we could obtain no evidence for a synergistic action of E1A with NF1 on this promoter (results not shown).

DISCUSSION

On footprinting of the human p53 promoter, a dominant footprint was found with all tissue extracts tested. However, the footprint, which spans from −227 to −194 on the promoter (numbering of Tuck and Crawford [62]) is slightly, but significantly, different for different tissue extracts. The sequence covered by this footprint contains two overlapping transcription factor binding sites, for YY1 and NF1. Purified recombinant NF1 and partially purified his-YY1 were shown to bind to the site by both EMSA and footprinting analysis (Fig. 2B and 3). In addition, the predominant binding of YY1, in testis extracts, and binding of both NF1 and YY1 in liver extracts to this site were confirmed by the ability of specific antibodies to impair their binding (Fig. 2C and D).

The NF1 binding site conforms to a classical NF1 consensus 5′-TGG(A/C)N_5GCCA-3′ (22) and, as such, was previously partially characterized in the mouse p53 promoter (18). YY1 binding elements are much more diverse, and recently a number of consensus sequences have been proposed by three independent groups (24, 59, 68). All three consensus sequences

have a 5′-CATN-3′ core, which is flanked by variable bases in variable numbers. It has been proposed that this high degree of heterogeneity in DNA recognition sites may arise because of the variation in interaction of the four YY1 zinc fingers with DNA (24). This YY1 binding element within the p53 promoter fits the consensus sequence proposed by Hyde-DeRuyscher et al. (24) (Fig. 2A). A comparison of the sequences of the human, rat, and mouse p53 promoters (Fig. 1B, middle panel) (7) shows that both the YY1 and the NF1 elements are conserved. (The only non-conserved base corresponds to the N in the YY1 consensus.) This predicts a significant potential role for both YY1 and NF1 in the regulation of p53 promoter function.

Methylation interference analysis showed that the YY1 and NF1 binding sites are overlapping, and by a factor binding competition experiment (Fig. 5) (34) it was established that the binding of NF1 to the site and the binding of YY1 were mutually exclusive. Composite or shared YY1 binding sites have been described for a number of other promoters, e.g., a YY1/MGF binding site in the β-casein gene promoter (39) and a YY1/NF-κB binding site in the serum amyloid A1 gene promoter (36). In addition, as here, with all reported overlapping sites which bind YY1 the second factor always associates as a dimer, i.e., it contacts the DNA on a symmetrical element, while YY1, being a monomer, binds asymmetrically (19, 36).

By creating a series of mutations within the site (Fig. 2A), it was possible to generate a null element (mutant M1), a YY1-only binding element (M2), an NF1-only binding element (M4), and an element with apparent increased binding affinity for NF1 and reduced affinity for YY1 (M3). The mutants were characterized by their abilities to bind recombinant YY1 and NF1 (Fig. 2B and 3) and were used to define relative YY1 and NF1 binding potential for this site in liver and testis nuclear extracts. In liver extracts NF1 binding predominates the EMSA profile, with some YY1 binding (Fig. 2D), and only an NF1 footprint is seen (Fig. 3). YY1 binding, however, becomes more significant on EMSA when NF1 binding is inhibited (mutant M2; Fig. 2D, liver), but there would seem to be too low a concentration of YY1 to generate a footprint (Fig. 3, liver). In contrast, in the testis extract (and spleen [data not shown]) YY1 binding predominates, and a strong NF1 complex in EMSA is seen only when YY1 binding is compromised (with mutants M4 and M3 [Fig. 2C]). Interestingly, with mutant M3, which increases affinity for NF1 and reduces YY1 binding, an NF1 footprint can be seen with the testis extract (Fig. 3, lower panel, testis, lane 5), contrasting with the YY1 footprint on the wild-type site. Thus, the series of mutants have shown interesting changes in occupancy of the site with extracts that contain both YY1 and NF1 but in differing relative amounts. Some previous studies (1) have reported testicular tissue-specific expression of the p53 gene. This forms an interesting correlation with the predominant occupancy of the element on the p53 promoter by YY1 in testis extracts.

Transient transfection of the WT-CAT construct (p5DU 356-CAT [62]) which contains 344 bp of the human p53 promoter upstream of the major reported transcriptional start site into HeLa cells showed significant reporter gene expression (Fig. 6A). This activity was reduced by 68% when neither YY1 nor NF1 could bind to the composite element (M1-CAT), suggesting that this composite element plays a functional role in the basal regulation of the p53 promoter. Intermediate CAT activities were seen with mutants M3-CAT and M4-CAT, which would confirm the potential for both YY1 and NF1 to contribute positively to basal promoter activity. Interestingly, in the mouse p53 promoter the start site of transcription has been mapped to a position ~100 bp upstream from the start site in the human p53 promoter (7, 62). In addition, neither promoter contains a TATA box. This would place the YY1/NF1 composite element at the mouse p53 start site. YY1 can act as an initiator in some cellular and viral promoters (56). However, it is very unlikely that this YY1/NF1 site is acting as an initiator element in the human p53 promoter, as, when YY1 cannot bind, p53 expression is reduced by only ~30% and, when both factors cannot bind, ~30% of the basal promoter activity remains.

On all previously reported composite sites which bind YY1 and another factor in a mutually exclusive manner, YY1 has acted as a repressor either by decreasing transcription through its transrepressor domain (24), by displacing an activator from the site, or both. When YY1 was transiently coexpressed in HeLa cells with the WT-CAT construct, a dose-dependent increase in CAT activity was seen (Fig. 6B, upper panel). This is, therefore, the first time that YY1 has been reported to activate the p53 promoter and also the first reported case of a composite YY1 binding element on which it acts as an activator. YY1 has been shown to activate a number of other promoters which include the Myc (50) and dihydrofolate reductase (14) promoters and also the immunoglobulin H μ enhancer (13), but the binding elements involved are simple (i.e., nonoverlapping) YY1 binding sites. Shrivastava and Calame (59) attempted to define a consensus sequence for YY1 activator elements and YY1 repressor elements. The p53 YY1 site does not fit either consensus but does contain the core 5′-CCATN-3′ sequence and fits the general consensus proposed by Hyde-DeRuyscher et al. (24) (Fig. 2A). It remains a puzzle how YY1 can bind efficiently to mutant M2 but fail to transactivate (Fig. 2, 4, and 7B). Possibly, it fails to bind on the M2 site in vivo, or else, mutations of bases which we have considered outside the core element are important for its transactivating function.

The contribution of occupancy of the composite element to induced p53 promoter function was studied with E1A as an inducer (Fig. 7). Previous studies have shown E1A to increase cellular p53 levels and p53 mRNA levels (9). Expression of either 12S E1A or 13S E1A significantly increased p53 promoter CAT activity, demonstrating the potential of E1A to regulate cellular p53 at a transcriptional level. Two variants of E1A are produced by alternate splicing: (i) 12S, which contains two activation domains, CR1 and CR2, and (ii) 13S, which contains three activation domains, CR1, CR2, and CR3. The 12S E1A was more than three times as effective an inducer of the p53 promoter as the 13S E1A (Fig. 7A). This is unusual, as with most viral and cellular genes tested in previous studies the 13S variant gives the strongest induction. This would suggest that the p53 gene belongs to a small subset of genes that are induced to a higher level by the 12S variant (23, 41). E1A induction of p53 promoter activity was ~55% reduced by the M1 mutation, which blocks both YY1 and NF1 binding (Fig. 7A). This suggests that occupation of the element contributes significantly to the E1A effect.

It seems likely that YY1 can modulate the E1A effect by binding to the composite element. Mutation M4, which inhibits YY1 binding, reduced the E1A induction to the same degree as mutation M1, which blocks binding of both YY1 and NF1 (Fig. 7A). This indirectly points to YY1 contributing significantly to the effect. With mutant M2, which binds only YY1, overexpression of YY1 did not increase CAT activity levels, but it significantly amplified the E1A effect (Fig. 7B, left-hand panel). However, this effect is probably only partially dependent on YY1 being bound to DNA, because when YY1 was overexpressed in two independent situations in which it could not bind to the p53 promoter, the E1A effect was also amplified (M4- and M1-CAT [Fig. 7B, middle and left-hand pan-

els]). Thus, the data presented here suggest that with the p53 promoter two distinguishable YY1/E1A interactive events are detectable. At lower cellular YY1 concentrations, i.e., with endogenous YY1 only, YY1 which is bound to the composite element interacts with E1A and promoter activation is triggered. With higher cellular YY1 concentrations no association of YY1 with the promoter is required for the synergistic interaction with E1A to occur. It is probable that these YY1/E1A interactions occur by a single mechanism but that in the presence of lower cellular concentrations of YY1 a specific DNA binding event could be required to provide a high local concentration of the factor. Three recent reports (32, 33, 35) describe positive E1A modulation of YY1 transactivation by direct or indirect protein-protein interactions. Lee et al. (32) reported E1A modulation of YY1 transactivation via the transactivator p300. They provided evidence to support the potential formation of a three-protein complex (YY1-p300-E1A) in mediating this effect. Lewis et al. (35) and Lee et al. (33) demonstrate that E1A can interact directly with YY1 to increase its transcriptional activity. Both the direct action of YY1 and the p300-mediated effect target the N-terminal amino acids of 12S E1A which are deleted in the E1A mutant dl2-36 (32, 35). As no E1A induction was seen on the p53 promoter with this mutant E1A, we conclude that all of the E1A effects detected in this study occur either by a direct YY1-E1A interaction, by an interaction with p300, or both. It is interesting to note that Hiebert et al. (23) show that the dl2-36 mutant 12S E1A still modulates E1A effects that involve E2F and Rb; thus, this mutation does not have a general inhibitory effect on E1A's transcriptional activating functions.

With regard to the mechanism of the interactions noted here it is simplest to envisage the following: (i) E1A becoming tethered to the basal transcription complex or an upstream bound factor on the p53 promoter to exert its YY1-independent transcriptional enhancement (Fig. 7A) (this effect requires the N-terminal domain of E1A), (ii) when YY1 can bind to the composite site, it would bind and then interact with E1A, tethered as above, and further enhance transcriptional drive (again, this effect requires the N-terminal domain of E1A), and (iii) when YY1 cannot bind productively to the composite site but is overexpressed, it would interact with E1A, tethered as above, to increase transcriptional drive, but the complex formed might become stabilized by other YY1 protein-protein interactions. As indicated above, other studies support a mechanism involving direct or indirect (via p300) protein-protein associations between YY1 and E1A being responsible for these enhanced transcriptional effects (32, 33, 35). Furthermore, the study of Lewis et al. (35) demonstrates that E1A tethered to DNA, in a simple promoter (as a GAL4/E1A chimera) could, in the absence of a YY1 DNA-binding element, bind a YY1/VP16 chimera (through a direct YY1-E1A interaction) with resultant enhancement of transcription. The YY1-E1A transcriptional enhancement noted here (Fig. 7B) did not require the presence of the additional transactivator function provided by VP16 in the model of Lewis et al. (35). Further studies will be required to explain YY1's additional competence as an activator in the context of the p53 promoter.

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REFERENCES

1. **Almon, E., N. Goldfinger, A. Kapon, D. Schwartz, A. Levine, and V. Rotter.** 1993. Testicular tissue-specific expression of the p53 suppressor gene. Dev. Biol. **156:**107–116.
2. **Andrews, N. C., and D. F. Faller.** 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res. **19:**2499.
3. **Apt, D., Y. Liu, and H.-U. Bernard.** 1994. Cloning and functional analysis of spliced isoforms of human nuclear factor 1-X: interference with transcriptional activation by NF1/CTF in a cell-type specific manner. Nucleic Acids Res. **22:**3825–3833.
4. **Ausubel, F. A., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1987. Current protocols in molecular biology, vol. 1 and 2. Greene Publishing Associates and John Wiley and Sons, Boston.
5. **Barak, Y., T. Juven, R. Haffner, and M. Oren.** 1993. mdm2 expression is induced by wild type p53 activity. EMBO J. **12:**461–468.
6. **Bauknecht, T., F. Jundt, I. Herr, T. Oehler, H. Delius, Y. Shi, P. Angel, and H. zur Hausen.** 1995. A switch region determines the cell type-specific positive or negative action of YY1 on the activity of the human papillomavirus type 18 promoter. J. Virol. **69:**1–12.
7. **Bienz-Tadmor, B., R. Zakut-Houri, S. Libresco, D. Givol, and M. Oren.** 1985. The 5′ region of the p53 gene: evolutionary conservation and evidence for a negative regulatory element. EMBO J. **4:**3209–3213.
8. **Borgmeyer, U., J. Nowock, and A. E. Sippel.** 1984. The TGGCA-binding protein: eukaryotic nuclear protein recognizing a symmetrical sequence on double-stranded linear DNA. Nucleic Acids Res. **12:**4295–4311.
9. **Braithwaite, A., C. Nelson, A. Skulimowski, J. McGovern, D. Pigott, and J. Jenkins.** 1990. Transactivation of the p53 oncogene by E1A gene products. Virology **177:**595–605.
10. **Deffie, A., H. Wu, V. Reinke, and G. Lozano.** 1993. The tumor suppressor p53 regulates its own transcription. Mol. Cell. Biol. **13:**3415–3423.
11. **Dittmer, D., S. Pati, G. Zambetti, S. Chu, A. K. Teresky, M. Moore, C. Finlay, and A. J. Levine.** 1993. Gain of function mutations in p53. Nat. Genet. **4:**42–46.
12. **El-Deiry, W. S., T. Tokino, V. E. Velculescu, D. B. Levy, R. T. Parsons, J. M. Trent, D. Lin, W. E. Mercer, K. W. Kinzler, and B. Vogelstein.** 1993. WAF1, a potential mediator of p53 tumor suppression. Cell **75:**817–825.
13. **Ephrussi, A., G. M. Church, S. Tonegawa, and W. Gilbert.** 1985. B lineage-specific interactions of an immunoglobulin enhancer with cellular factors in vivo. Science **227:**134–140.
14. **Farnham, P. J., and A. L. Means.** 1990. Sequences downstream of the transcription initiation site modulate the activity of the murine dihydrofolate reductase promoter. Mol. Cell. Biol. **10:**1390–1398.
15. **Field, S., and S. K. Jang.** 1990. Presence of a potent transcription activating sequence in the p53 protein. Science **249:**1046–1049.
16. **Frain, M., G. Swart, P. Monaci, A. Nicosia, S. Stämpfli, R. Frank, and R. Cortese.** 1989. The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. Cell **59:**145–157.
17. **Gedrich, R. W., and D. A. Engel.** 1995. Identification of a novel E1A response element in the mouse c-*fos* promoter. J. Virol. **69:**2333–2340.
18. **Ginsberg, D., M. Oren, M. Yaniv, and J. Piette.** 1990. Protein-binding elements in the promoter region of the mouse p53 gene. Oncogene **5:**1285–1290.
19. **Gualberto, A., D. LePage, G. Pons, S. L. Mader, K. Park, M. L. Atchison, and K. Walsh.** 1992. Functional antagonism between YY1 and the serum response factor. Mol. Cell. Biol. **12:**4209–4214.
20. **Hariharan, N., D. E. Kelley, and R. P. Perry.** 1991. δ, a transcription factor that binds to downstream elements in several polymerase II promoters, is a functionally versatile zinc finger protein. Proc. Natl. Acad. Sci. USA **88:**9799–9803.
21. **Haupt, Y., S. Rowan, E. Shaulian, K. H. Vousden, and M. Oren.** 1995. Induction of apoptosis in HeLa cells by trans-activation-deficient p53. Genes Dev. **9:**2170–2183.
22. **Hay, R. T.** 1985. The origin of adenovirus DNA replication: minimal DNA sequence requirement in vivo. EMBO J. **4:**421–426.
23. **Hiebert, S. W., M. Lipp, and J. R. Nevins.** 1989. E1A-dependent transactivation of the human MYC promoter is mediated by the E2F factor. Proc. Natl. Acad. Sci. USA **86:**3594–3598.
24. **Hyde-DeRuyscher, R. P., E. Jennings, and T. Shenk.** 1995. DNA binding sites for the transcriptional activator/repressor YY1. Nucleic Acids Res. **23:**4457–4465.
25. **Jackson, D. A., K. E. Rowader, K. Stevens, C. Jiang, P. Milos, and K. S. Zaret.** 1993. Modulation of liver-specific transcription by interactions between hepatocyte nuclear factor 3 and nuclear factor 1 binding DNA in close apposition. Mol. Cell. Biol. **13:**2401–2410.
26. **Kawamura, H., K. Nagata, Y. Masamune, and Y. Nakanishi.** 1993. Phosphorylation of NF1 in vitro by cdc2 kinase. Biochem. Biophys. Res. Commun. **192:**1424–1431.
27. **Kawasaki, T., Y. Tomita, R. Watanabe, T. Tanikawa, T. Kumanishi, and S. Sato.** 1994. mRNA and protein expression of p53 mutations in human bladder cancer cell lines. Cancer Lett. **82:**113–121.
28. **Kley, N., R. Y. Chung, S. Fay, J. P. Loeffler, and B. R. Seizinger.** 1992.

Repression of the basal c-*fos* promoter by wild-type p53. Nucleic Acids Res. **220:**4083–4087.
29. **Kouzarides, T.** 1995. Functions of pRb and p53: what's the connection? Trends Cell Biol. **5:**448–450.
30. **Kuipers, O. P., H. J. Boot, and W. M. De Vas.** 1991. Improved site-directed mutagenesis method using PCR. Nucleic Acids Res. **19:**4558–4560.
31. **Lane, D. P.** 1992. p53, guardian of the genome. Nature (London) **358:**15–16.
32. **Lee, J.-S., K. M. Galvin, R. H. See, R. Eckner, D. Livingston, E. Moran, and Y. Shi.** 1995. Relief of YY1 transcriptional repression by adenovirus E1A is mediated by E1A-associated protein p300. Genes Dev. **9:**1188–1198.
33. **Lee, J.-S., R. H. See, K. M. Galvin, J. Wang, and Y. Shi.** 1995. Functional interactions between YY1 and adenovirus E1A. Nucleic Acids Res. **23:**925–931.
34. **Lee, T.-C., K.-L. Chow, P. Fang, and R. J. Schwartz.** 1991. Activation of skeletal α-actin gene transcription: the cooperative formation of serum response factor-binding complexes over positive *cis*-acting promoter serum response elements displaces a negative-acting nuclear factor enriched in replicating myoblasts and nonmyogenic cells. Mol. Cell Biol. **11:**5090–5100.
35. **Lewis, B. A., G. Tullis, E. Seto, N. Horikoshi, R. Weinmann, and T. Shenk.** 1995. Adenovirus E1A proteins interact with the cellular YY1 transcription factor. J. Virol. **69:**1628–1636.
36. **Lu, S.-Y., M. Rodriguez, and W. S.-L. Liao.** 1994. YY1 represses rat serum amyloid A1 gene transcription and is antagonized by NF-κB during acute-phase response. Mol. Cell. Biol. **14:**6253–6263.
37. **Marx, J.** 1994. New link found between p53 and DNA repair. Science **266:**1321–1322.
38. **McKnight, S. L., and R. Kingsbury.** 1982. Transcriptional control signals of a eukaryotic protein-coding gene. Science **217:**316–324.
39. **Meier, V. S., and B. Groner.** 1994. The nuclear factor YY1 participates in repression of the β-casein gene promoter in mammary epithelial cells and is counteracted by mammary gland factor during lactogenic hormone induction. Mol. Cell. Biol. **14:**128–137.
40. **Morgan, W. D., G. T. Williams, R. I. Morimoto, J. Greene, R. E. Kingston, and R. Tjian.** 1987. Two transcriptional activators, CCAAT-box-binding transcription factor and heat shock transcription factor, interact with a human hsp70 gene promoter. Mol. Cell. Biol. **7:**1129–1138.
41. **Morris, G. F., and M. B. Mathews.** 1991. The adenovirus E1A transforming protein activates the proliferating cell nuclear antigen promoter via an activating transcription factor site. J. Virol. **65:**6397–6406.
42. **Mul, Y. M., and P. C. Van der Vilet.** 1992. Nuclear factor 1 enhances adenovirus DNA replication by increasing the stability of a preinitiation complex. EMBO J. **11:**751–760.
43. **Nagata, K., R. A. Guggenheimer, and J. Hurwitz.** 1983. Specific binding of a cellular DNA replication protein to the origin of replication of adenovirus DNA. Proc. Natl. Acad. Sci. USA **80:**6177–6181.
44. **Natesan, S., and M. Z. Gilman.** 1993. DNA bending and orientation-dependent function of YY1 in the c-fos promoter. Genes Dev. **7:**2497–2509.
45. **Novak, A., N. Goyal, and R. M. Gronostajski.** 1992. Four conserved cysteine residues are required for the DNA binding activity of nuclear factor 1. J. Biol. Chem. **267:**12986–12990.
46. **Park, K., and M. L. Atchison.** 1991. Isolation of a candidate repressor/activator, NF-E1 (YY-1, δ), that binds to the immunoglobulin κ 3′ enhancer and the immunoglobulin heavy-chain mE1 site. Proc. Natl. Acad. Sci. USA **88:**9804–9808.
47. **Pavletich, N. P., and C. O. Pabo.** 1993. Crystal structure of a five-finger GLI-DNA complex: new perspectivies on zinc fingers. Science **261:**1701–1707.
48. **Rein, T., R. Forster, A. Krause, E. L. Winnacker, and H. Zorbas.** 1995. Organization of the alpha-globin promoter and possible role for nuclear factor 1 in an alpha-globin-inducible and noninducible cell line. J. Biol. Chem. **270:**19643–19650.
49. **Reisman, D., and V. Rotter.** 1993. The helix-loop-helix containing transcription factor USF binds to and transactivates the promoter of the p53 tumor suppressor gene. Nucleic Acids Res. **21:**345–350.
50. **Riggs, K. J., S. Saleque, K. K. Wong, K. T. Merrell, J. S. Lee, Y. Shi, and K. Calame.** 1993. Yin-yang 1 activates the c-myc promoter. Mol. Cell. Biol. **13:**7487–7495.
51. **Robidoux, S., P. Gosselin, M. Harvey, S. Leclerc, and S. L. Guérin.** 1992. Transcription of the mouse secretory protease inhibitor p12 gene is activated by the developmentally regulated positive transcription factor Sp1. Mol. Cell. Biol. **12:**3796–3806.
52. **Rosenthal, N.** 1987. Identification of regulatory elements of cloned genes with functional assays. Methods Enzymol. **152:**704–720.
53. **Rosselli, F., A. Ridet, T. Soussi, E. Duchaud, C. Alapetite, and E. Moustacchi.** 1995. p53-dependent pathway of radio-induced apoptosis is altered in Fancomi anemia. Oncogene **10:**9–17.
54. **Rotter, V., D. Schwartz, E. Almon, N. Goldfinger, A. Kapon, A. Meshorer, L. A. Donehower, and A. J. Levine.** 1993. Mice with reduced levels of p53 protein exhibit the testicular giant-cell degenerative syndrome. Proc. Natl. Acad. Sci. USA **90:**9075–9079.
55. **Roy, B., J. Beamon, E. Balint, and D. Reisman.** 1994. Transactivation of the human p53 tumor suppressor gene by c-Myc/Max contributes to elevated mutant p53 expression in some tumors. Mol. Cell. Biol. **14:**7805–7815.
56. **Seto, E., Y. Shi, and T. Shenk.** 1991. YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. Nature (London) **354:**241–245.
57. **Shi, Y., E. Seto, L.-S. Chang, and T. Shenk.** 1991. Transcriptional repression by YY1, a human GLI-Krüppel-related protein, and relief of repression by adenovirus E1A protein. Cell **67:**377–388.
58. **Shiio, Y., T. Yamamoto, and N. Yamaguchi.** 1992. Negative regulation of Rb expression by the p53 gene product. Proc. Natl. Acad. Sci. USA **89:**5206–5210.
59. **Shrivastava, A., and K. Calame.** 1994. An analysis of genes regulated by the multifunctional transcriptional regulator Yin Yang-1. Nucleic Acids Res. **22:**5151–5155.
60. **Stuart, E. T., R. Haffner, M. Oren, and P. Gruss.** 1995. Loss of p53 function through PAX-mediated transcriptional repression. EMBO J. **14:**5638–5645.
61. **Sun, X., H. Shimizu, and K.-I. Yamamoto.** 1995. Identification of a novel p53 promoter element involved in genotoxic stress-inducible p53 gene expression. Mol. Cell. Biol. **15:**4489–4496.
62. **Tuck, S. P., and L. Crawford.** 1989. Characterization of the human p53 gene promoter. Mol. Cell. Biol. **9:**2163–2172.
63. **Uittenbogaard, M. N., H. A. Giebler, D. Reisman, and J. K. Nyborg.** 1995. Transcriptional repression of p53 by human T-cell leukemia virus type I Tax protein. J. Biol. Chem. **270:**28503–28506.
64. **Waston, C. J., K. E. Gordon, M. Robertson, and J. A. Clark.** 1991. Interaction of DNA-binding proteins with a milk protein gene promoter in vitro: identification of a mammary gland-specific factor. Nucleic Acids Res. **19:**6603–6610.
65. **Wigler, M., R. Sweet, G. K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel.** 1979. Transformation of mammalian cells with genes from prokaryotes and eukaryotes. Cell **16:**777–785.
66. **Wu, H., and G. Lozano.** 1994. NF-κB activation of p53. J. Biol. Chem. **269:**20067–20074.
67. **Wu, X. W., and A. L. Levine.** 1994. p53 and E2F-1 cooperate to mediate apoptosis. Proc. Natl. Acad. Sci. USA **91:**3602–3606.
68. **Yant, S. R., W. Zhu, D. Millinoff, J. L. Slightom, M. Goodman, and D. L. Gumucio.** 1995. High affinity YY1 binding motifs: identification of two core types (ACAT and CCAT) and distribution of potential binding sites within the human β globin cluster. Nucleic Acids Res. **23:**4353–4362.
69. **Yichun, D. A., Y. Liu, and H.-U. Bernard.** 1994. Cloning and functional analysis of spliced isoforms of human nuclear factor 1-X: interference with transcriptional activation by NF1/CTF in a cell-type specific manner. Nucleic Acids Res. **22:**3825–3833.