The Activation Domains, the Proline-rich Domain, and the C-terminal Basic Domain in p53 Are Necessary for Acetylation of Histones on the Proximal p21 Promoter and Interaction with p300/CREB-binding Protein*

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The p53 transcription factor contains two separate tandem activation domains (AD1 and AD2), a proline-rich domain (PRD), and a C-terminal basic domain (BD). Previously, we have shown that these domains are necessary for transcriptional activity. To further characterize the role of these domains in transactivation, we analyzed the regulation of p21, a well characterized p53 target gene, by various p53 mutants deficient in one or more of these domains. We found that the induction of endogenous p21 is compromised by AD1-deficient p53 (p53(AD1/H11546)), AD2-deficient p53 (p53(AD2/H9004)), both AD1- and AD2-deficient p53 (p53(AD1 AD2/H11546)), p53(ΔPRD), which lacks PRD, and p53(ΔBD), which lacks BD. However, p53(AD2/H9004), p53(ΔPRD), and p53(ΔBD) are still capable of activating exogenous p21 promoter to an extent comparable with that of wild-type p53. Thus, we performed chromatin immunoprecipitation assay to measure the DNA binding ability of various p53 mutants in vivo. We found that like wild-type p53, these p53 mutants are capable of binding to the p53 response elements in the p21 promoter. In contrast, we found that the extent of acetylated histones on the p21 promoter, especially the proximal promoter, and the amount of interaction with p300/CREB-binding protein, which contain histone acetyltransferase activity, directly correlate with the activity of p53 to induce endogenous p21. Furthermore, we showed that down-regulation of p300/CBP by short interference RNA markedly decreases the ability of p53 to induce endogenous p21. These data lead us to hypothesize that when p50 binds to the responsive element(s) of a target gene, its ability to interact with histone acetyltransferase-containing proteins and subsequently the acetylation of histones bound to the proximal promoter dictate the induction level of a target gene.

In the eukaryotic nucleus, DNA exists in a highly organized chromatin. The basic structural unit is the nucleosome, consisting of DNA wrapped around an octamer of histones (two each of H2A, H2B, H3, and H4). The packaged nucleosome is the natural barrier to most regulatory proteins due to restriction of access to DNA (1–5). Therefore, activation of transcription requires disruption of the highly dense structure of chromatin to increase the accessibility of DNA to transcription factors. It is believed that disruption of chromatin structure depends on chromatin-modifying complexes, which include two major groups, the ATP-dependent remodeling complexes and the histone modification enzymes. Histone acetyltransferase is one of the well characterized histone modification enzymes that acetylates histone tails and introduces negative charge to histones, thereby reducing the interaction between histones and DNA and promoting accessibility of DNA to the transcriptional machinery (3, 6). Therefore, increased acetylation of histones on promoters often leads to transcriptional activation. In mammalian cells the histone acetyltransferase-containing transcription co-activators p300/CBP and PCAF acylate histones after being recruited by sequence-specific DNA binding transcription factors (7). This is underscored by evidence that p300/CBP can interact with many transcription factors, such as c-Jun, JunB, JunD, MyoD, STATs (signal transducers and activators of transcription), and p53, and increase the transcriptional activity of these proteins (7).

The p53 tumor suppressor gene is the most frequent target for genetic alterations in human cancers, with mutations occurring in almost 50% of all human tumors (8, 9). In response to environmental and intracellular stresses, including DNA damage, ionizing irradiation, and hypoxia, p53 is rapidly stabilized and accumulated (10). The activated p53 induces many target genes, including p21, Bax, PUMA, and MCG10, which mediate p53-dependent cell cycle arrest and/or apoptosis (11–15).

The p53 protein consists of two N-terminal activation domains (AD1 within residues 1–42 and AD2 within residues 43–63), a proline-rich domain (PRD, within residues 64–93), a sequence-specific DNA binding domain (within residues 102–292), and an extreme C-terminal basic domain (BD within residues 364–393) (16). The transcription co-activators p300/CBP have been found to interact with p53 activation domains and enhance the ability of p53 to activate p21 or mdm2 (17–21). A dominant-negative CBP mutant can suppress the p53-dependent induction of p21 (21). Mutant p53 with a double point mutation in AD1 and AD2, each of which is deficient in transactivation, is unable to interact with CBP in vitro (21). In addition, the oncoprotein E1A suppresses p53-dependent induction of p21, probably through disruption of the interaction between CBP and p53 (20). All this evidence suggests that the
transcriptional activity of p53 depends on its interaction with p300/CBP.

The C terminus of p53 has been shown to play a critical role in regulation of p53 functions. For example, the DNA binding ability of p53 is increased after deletion of the C-terminal domain, phosphorylation of serine residues in this domain, or binding of an antibody specific to this domain (10). More recent studies show that p500/CBP and PCAF can acetylate lysine residues within the C-terminal domain in response to p53-acetylation agents (22–28). Acetylated p53 has a higher DNA binding affinity to short oligonucleotides containing a p53 binding site from the p21 promoter in vitro (23, 24) but not to a long DNA fragment (27).

In this study, we used stable cell lines to analyze differential regulation of p21 by wild-type p53 or various mutants. We showed that although all of the p53 mutants used in this assay are compromised in inducing endogenous p21 gene, both wild-type p53 and various p53 mutants have an equivalent sequence-specific DNA binding ability. We found that the ability of p53 mutants to increase acetylation of histones H3 and H4 is impaired. We also found that the compromised transcriptional activity of p53 mutants correlates with the acetylation level of histones H3 and H4 is impaired. We also found that the compromised transcriptional activity of p53 mutants correlates with the acetylation level of histones H3 and H4 on the region proximal to the TATA box. We used the RNA interference technique to confirm and extend previous studies showing that p300/CBP and PCAF can acetylate lysine residues in p53. For example, the DNA binding affinity of p53 is increased after deletion of the C-terminal domain in response to p53-acetylation agents (22–28). Acetylated p53 has a higher DNA binding affinity to short oligonucleotides containing a p53 binding site from the p21 promoter in vitro (23, 24) but not to a long DNA fragment (27).

**Experimental Procedures**

**Cell Lines—**H1299 cell lines, which can be induced to express wild-type p53, p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), or p53(AD3) (29), were described previously (28, 29). Except p53(AD1) and p53(AD2), wild-type p53 and the other four mutants are tagged with an HA at the N terminus.

**Luciferase Assay—**The luciferase reporter under the control of the p21 promoter with two p53-responsive elements was described previously (23, 24). Except p53(AD1), p53(AD2), and p53(AD3), wild-type p53 and the other four mutants were transfected into H1299 cells with 1 ml of cold lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, proteinase inhibitor mixture) and sonicated to generate 200–1000-bp DNA fragments. After clarification by centrifugation, the supernatant was diluted 10-fold with a dilution buffer (1% Triton-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1, proteinase inhibitor mixture) and incubated with anti-p53, anti-acetylated histone H3, and anti-acetylated histone H4, respectively, at 4 °C overnight. The immunocomplexes were then eluted with the elution buffer (1% SDS, 0.1 mM NaHCO3) for 15 min at room temperature. The DNA was purified by phenol/chloroform and precipitated by ethanol and resuspended in 50 μl of TE. 2.5 μl of DNA sample was used as a template for PCR amplification. To amplify the regions containing p53-responsive element 1 (RE1) and RE2 and the region proximal to TATA box in the p21 promoter, PCRs were performed with the following pairs of primers: p53 RE1, forward primer, 5′-CAGGCTTTGCTCTGATTG-3′, and reverse primer, 5′-TTGAC-GATACGGTCAAGGA-3′; p53 RE2, forward primer, 5′-GGTGTGCTACTGTCGCTCC-3′, and reverse primer, 5′-CACTGAAAGAATAAAC-3′; p21 TATA, forward primer, 5′-TTATGTGGGCCGCTTCTCGGA-3′, and reverse primer 5′-CTGTTAGAATGAGCCCCTTCTTT3′.

**RNA Immunoprecipitation—**Total RNA was isolated using Trizol reagent (Invitrogen). Northern blot analysis was performed as described (30). The p21 and glyceraldehyde-3-phosphate dehydrogenase probes were prepared as described previously (28, 29). Except p53(AD1) and p53(AD2), wild-type p53 and the other four mutants are tagged with an HA at the N terminus.

**Protein Extraction and Immunoblotting—**Small portions of cells, which were used for RNA isolation or luciferase assay, were lysed with 2% SDS sample buffer (33). Protein was resolved by 8% SDS-PAGE gel. The expression level of wild-type p53, p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), or p53(AD3) for an internal control, 25 ng of the Renilla luciferase vector, pRL-CMV (Promega, Madison, WI), was cotransfected. Dual luciferase assays were performed in triplicate according to the luciferase vector, pRL-CMV (Promega, Madison, WI), was cotransfected. The expression level of wild-type p53, p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), or p53(AD3) was determined by anti-HA antibody (Santa Cruz). p53(R249S) and p53(R249S) were detected by an equal loading.

**Chromatin Immunoprecipitation Assay (ChIP)—**1 × 108 H1299 cells, which were uninduced (−) and induced (+) to express p53, p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), p53(AD1), p53(AD2), or p53(AD3), were washed 3 times with cold phosphate-buffered saline. Genomic DNA and proteins were cross-linked by the addition of formaldehyde (1% final concentration) directly into culture medium and incubated for 10 min at room temperature. The cross-linked cells were then washed with cold phosphate-buffered saline three times and collected by scraping. The cells were pelleted by centrifugation, resuspended with 1 ml of cold lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, proteinase inhibitor mixture), and sonicated to generate 200–
p21 Is Differentially Regulated by Wild-type p53 and Various p53 Mutants—In the process of delineating p53 functional domains, we generated a number of cell lines that could be induced to express wild-type p53 or various p53 mutants. We found that the level of p21 induced by p53 in these cell lines varied (28, 29, 35). To systematically analyze the induction of p21 in these cell lines, we performed Northern blot analyses and compared the level of p21 induction. We found that p21 was strongly induced in the presence of wild-type p53. In contrast, p53(R249S), which is mutated in the DNA binding domain, was inert in up-regulation of p21 (Fig. 1A), consistent with the previous finding (36). We also found that induction of p21 was diminished by mutation of activation domain I (p53(AD1PRD)) or activation domain II (p53(AD2PRD)) (Fig. 1A), suggesting that both AD1 and AD2 are important to, if not necessary for, the transcriptional activity of p53. This was further supported by the evidence that simultaneous double point mutations in both AD1 and AD2 abrogated the induction of p21 (Fig. 1A). In addition, p53(ΔBD), which lacks the C-terminal basic domain, and p53(ΔPRD), which lacks the proline-rich domain, had a reduced activity in inducing p21 (Fig. 1A), although both mutants are capable of inducing cell cycle arrest (29, 35). To rule out the possibility that differential transcriptional activity of wild-type p53 and various p53 mutants on p21 was due to different expression level of p53 in these stable cell lines, we performed Western blot analysis and found that the level of these p53 mutants was comparable with that of wild-type p53 (Fig. 1B).

p53(ΔPRD) and p53(ΔBD), Both of Which Have an Impaired Activity in Inducing Endogenous p21, Are as Potent as Wild-type p53 in Activating the Transiently Transfected p21 Promoter—The activity of luciferase under the control of a responsive element of a transcription factor has been widely used to measure the transcriptional activity (37). Here, we used a luciferase reporter under the control of the p21 promoter containing two p53-responsive elements to determine the transcriptional activity of wild-type p53 and various p53 mutants. We found that p53(R249S) was inactive, and p53(AD1PRD) and p53(AD1AD2PRD) showed a very weak transcriptional activity on the p21 promoter (Fig. 1C). However, p53(AD2PRD) significantly activated the p21 promoter (Fig. 1C), which is different from the result obtained by Northern blot

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FIG. 2. Wild-type p53 and various mutants with an intact DNA binding domain have similar sequence-specific DNA binding activity. A, schematic representation of the p21 promoter, the location of the p53-responsive elements (p53RE1 and p53RE2), the TATA box, and the location of various primers used for ChIP assay. B and D, wild-type p53 and various mutants with an intact DNA binding domain bound to
analysis (Fig. 1A, p21 panel). Additionally, we found that p53(ΔBD) and p53(ΔPRD) had equivalent, if not more, transcriptional activity on the p21 promoter compared with wild-type p53 (Fig. 1C). That both mutants induced much less endogenous p21 than wild-type p53 (Fig. 1A, p21 panel) suggests the luciferase assay does not faithfully reveal the transcriptional activity of p53 and various mutants in inducing the endogenous p21 gene. This is not surprising since a transiently transfected promoter is more accessible to an activator than the same endogenous promoter. To ensure an equivalent expression level of wild-type p53 and various p53 mutants, we also performed Western blot analysis and found that their expression was comparable (Fig. 1D). Furthermore, we determined the level of endogenous p21 induced by transiently expressed wild-type p53 and various mutants (Fig. 1D, p21 panel). We found that p21 was highly induced by wild-type p53, but little, if any, by p53(R249S), p53(AD1’), p53(AD2’), p53(AD1’AD2’), and p53(ΔPRD). Although p21 was slightly induced by p53(ΔBD), the level of its induction was significantly less by p53(ΔBD) than by wild-type p53. This is consistent with the data observed in the stable cell lines (Fig. 1A).

**Fig. 3.** **Histones are acetylated on the region when bound by p53.** A and C, the histones H3- or H4-DNA complexes bound to the region containing p53 RE1 (A) and p53 RE2 (C) were captured by anti-acetylated histone H3 or anti-acetylated histone H4. B and D, the fold increase of acetylated histones bound to the region containing p53 RE1 (B) and p53 RE2 (D). The fold increase was the product of the amount of PCR products in the presence of p53 divided by that in the absence of p53.
Wild-type p53 and Various p53 Mutants with an Intact DNA Binding Domain Have Similar Sequence-specific DNA Binding Activity—Except the tumor-derived mutant p53(R249S), all of the mutants used in this study have an intact DNA binding domain. Because the DNA binding ability of p53 is necessary for, and correlated with, p53 transcriptional activity (10, 16, 38), it is possible that some p53 mutants have an altered DNA binding ability, which leads to decreased induction of endogenous p21. To test this, we used ChIP-PCR to investigate the ability of wild-type p53 and various mutants to bind the p53-responsive elements in the p21 promoter. Because two p53-responsive elements (RE1 and RE2) exist in the p21 promoter (Fig. 2A), we designed two pairs of primers to amplify them separately. We found that anti-p53 antibodies (pAb1801 and pAb421) were able to pull down both RE1 and RE2 in the p21 promoter when wild-type p53 was expressed. However, neither RE1 nor RE2 was captured by a control antibody (Fig. 2, B and D), suggesting that wild-type p53 specifically binds both RE1 and RE2. We also found that p53(AD1/H11002), p53(AD2/H11002), and p53(AD1−AD2−) had an equivalent ability to bind RE1 and RE2 compared with wild-type p53 (Fig. 2, B–E). These results are not surprising since mutations in either AD1 or AD2 do not affect the specific DNA binding ability of p53. Furthermore, both p53(ABD) and p53(APRD) had an equivalent ability to bind both RE1 and RE2 as wild-type p53 (Fig. 2, B–E), although their ability to induce p21 was significantly diminished. In addition, we also used anti-HA antibody to capture p53(ADBD)-DNA complex in the ChIP assay, and similar results were obtained (data not shown). As expected, p53(R249S), which had a mutation in the p53 DNA binding domain, was incapable of binding RE1 or RE2 (Fig. 2, B–E).

Histones Are Acetylated on the Region When Bound by p53—When transcription factors bind to specific sequences within a promoter, they often recruit a co-activator with histone acetyltransferase activity to the promoter, leading to acetylation of the N-terminal tails of histones H3 and H4. Acetylation can disrupt the interaction of core histones with DNA, thereby increasing the accessibility of the promoter to the basal transcriptional machinery and facilitating the initiation of transcription (3, 4). Because the ability of p53 mutants to bind both RE1 and RE2 in the p21 promoter was not compromised, we decided to determine whether wild-type p53 and various mutants can equivalently increase the acetylation of histones on the regions containing RE1 and RE2. As shown in Fig. 3, A and C, acetylation of histones H3 and H4 on both RE1 and RE2 was increased significantly when wild-type p53 was expressed, suggesting that wild-type p53 can recruit histone acetyltransferases to RE1 and RE2. However, the ability of p53(AD1−), p53(AD2−), or p53(AD1−AD2−) to increase acetylation of histones on the RE1 and RE2 regions was significantly decreased compared with wild-type p53 (Fig. 3, A and C). Therefore, we also used anti-HA antibody to capture p53(ADBD)-DNA complex in the ChIP assay, and similar results were obtained (data not shown). As expected, p53(R249S), which had a mutation in the p53 DNA binding domain, was incapable of binding RE1 or RE2 (Fig. 2, B–E).
tones H3 and H4 on both RE1 and RE2 was severely compromised compared with wild-type p53 (Fig. 3, A–D). This indicates that these mutants probably recruit less histone acetyltransferase activity to RE1 and RE2 than wild-type p53 despite showing equivalent DNA binding ability as wild-type p53. This is further supported by our finding that the ability of p53(AD1/BD) or p53(ΔPRD) to increase acetylation of histones H3 and H4 on both RE1 and RE2 was also impaired, albeit to a lesser extent than that of p53(AD1−), p53(AD2−), or p53(AD1−AD2−) (Fig. 3, A–D). Nevertheless, once p53 binds to its responsive element, histones bound on the region containing the responsive element become accessible for acetylation. Interestingly, we noticed that histone H3 was always acetylated to a higher extent than histone H4 regardless of whether wild-type p53 or various mutants were induced (Fig. 3, B and D, compare histones H3 and H4 panels).

The Level of Histone Acetylation on the Proximal p21 Promoter Correlates with the Degree of Induction of Endogenous p21 by p53—The exposure of the proximal promoter, including the TATA box, to the basal transcriptional machinery is a prerequisite for transcriptional initiation (2, 5). One of the critical processes of chromatin structural disruption is acetylation of histones on the proximal promoter containing the TATA box. Because some p53 mutants, such as p53(AD1−AD2−), were deficient in inducing endogenous p21 but still showed some, although compromised, activity to increase the acetylation of histones on RE1 and RE2 (Fig. 3), we examined whether these p53 mutants are capable of increasing acetylation of histones on the region proximal to the TATA box. As shown in Fig. 4, A–B, acetylation of both histones H3 and H4 on the proximal p21 promoter was significantly increased when wild-type p53 was induced. Acetylation of histone H3 was also increased upon induction of p53(ΔBD) or p53(ΔPRD) (Fig. 4, A–B), although to a lesser extent than that after induction of wild-type p53. However, little, if any, increase in histone H3 or H4 acetylation was found when p53(AD1−), p53(AD2−), or p53(AD1−AD2−) was induced (Fig. 4, A–B). Thus, upon p53 expression, the extent of histone acetylation on the proximal

**Fig. 6. Wild-type p53 and various mutants differentially interact with p300/CBP.** A, cell extracts were prepared from ~2 × 10⁶ cells that were induced to express wild-type p53 or various mutants and immunoprecipitated with anti-HA (lanes 1 and 3–6) or anti-p53 antibodies (pAb1801 and pAb421) (lane 2). 1% of the eluted immunocomplexes was used for Western blot analysis to determine the level of p53 captured by immunoprecipitation, and 90% was used to measure the level of CBP and p300. B, the relative level of wild-type p53 and various mutants captured by immunoprecipitation. The amount of wild-type p53 was set as 100%. The relative level of mutant p53 is the product of the amount of mutant p53 divided by that of wild-type p53. C, the relative level of p300 that interacts with p53. The relative level of p300 that interacts with mutant p53 is the product of the amount of p300 associated with mutant p53 divided by that associated with wild-type p53. D, the relative level of CBP that interacts with p53. The calculation was performed as in C.
p21 promoter correlates with the degree of induction of the endogenous p21 gene.

\textit{p53 Induction of Endogenous p21 Is Impaired by Downexpression of p300/CBP—}p300 and CBP, two histone acetyltransferase-containing co-activators, have been found to physiologically interact with p53 and increase the ability of p53 to induce endogenous p21 or activate promoters of several p53 target genes (17, 19, 20). A recent study also showed that the amount of p300/CBP binding to the p21 promoter correlates with the level of acetylation of histones H3 and H4 (22), indicating that the recruitment of p300/CBP to the p21 promoter by p53 is probably responsible for histone acetylation and subsequent activation of p21. To address what roles p300/CBP play in the p53-dependent induction of p21, we used siRNAs targetting the p300/CBP mRNAs. We found that p300/CBP were specifically knocked down upon transfection of plasmids expressing p300/CBP siRNAs (Fig. 5, lanes 1 and 4) but not a control plasmid (lanes 2 and 5). Interestingly, we found that the ability of wild-type p53 to induce p21 was much less in cells lacking p300/CBP than in control cells (Fig. 5, compare lane 1 with lanes 2–3). Thus, our data are consistent with, and extend, the previous finding that the dominant-negative CBP decreases the induction of endogenous p21 by p53 (21).

The Interaction of p300/CBP with Various p53 Mutants Is Compromised—Our study showed that down-regulation of p300/CBP impairs the induction of endogenous p21 by wild-type p53 (Fig. 5). Additionally, we showed that p53 mutants, which are compromised in inducing p21, are also compromised in increasing the acetylation of histones on the p21 promoter (Figs. 3–4). These findings prompt us to determine whether the compromised ability of various p53 mutants to increase the acetylation of histones on the p21 promoter and to induce p21 is due to their decreased interaction with p300/CBP. To test this, we performed immunoprecipitation with anti-HA or anti-p53 antibodies to capture p53-p300/CBP complexes and Western blot analysis with anti-CBP and anti-p300 antibodies to detect the amount of p300/CBP in the p53 immunocomplexes (Fig. 6). We found that wild-type p53 and p300/CBP interacted \textit{in vivo} (Fig. 6, lane 1), consistent with the previous observation (17, 20, 21, 23). In addition, we found that p53(AD1 and p53(ADPRD) were capable of interacting with p300/CBP (Fig. 6A). However, the amount of p300/CBP interacting with these two mutants was much less than that interacting with wild-type p53 (Fig. 6, A, C, and D), although the amount of wild-type p53 and various p53 mutants in the immunocomplexes was comparable (Fig. 6B). In contrast, p53(AD1), p53(AD2), and p53(AD1 AD2) showed almost no interaction with p300/CBP (Fig. 6, A, C, and D). This suggests that mutations in AD1 and AD2 and deletions of PRD and BD decrease the interaction between p53 and p300/CBP, although such mutations and deletions do not interfere with the sequence-specific DNA binding ability of p53. We would like to note that the amount of p300/CBP, which interacts with wild-type p53 and various mutants, is nearly proportional to the extent of histone acetylation on the proximal p21 promoter (compare Fig. 4B with Fig. 6, C–D).

\textbf{DISCUSSION}

The transcriptional activity of p53 is regulated by various functional domains in p53 (10, 16, 28, 29, 35, 39). Because most tumor-derived p53 mutants have a mutation in the DNA binding domain that inevitably leads to p53 dysfunction (10, 40, 41), many studies have focused on whether various domains in p53 regulate the sequence-specific DNA binding activity of the core DNA binding domain. Indeed, several different experimental approaches have shown that the extreme C-terminal basic domain regulates the activity of the core DNA binding domain in \textit{vitro}. For example, the core DNA binding activity is increased when the basic domain is deleted, phosphorylated, acetylated, or bound by a peptide or an anti-p53 antibody (10, 19). These data lead to a hypothesis that p53 exists in two states, latent and active (42). However, a recent NMR study indicated that both latent and active forms of p53 are identical in conformation (43), suggesting that the basic domain may not have any effect on the DNA binding domain. In this study, we directly tested the DNA binding activity \textit{in vivo} and showed that the core DNA binding activity is not affected by the basic domain (Fig. 2). In addition, we found that p53(ADPRD), p53(AD1), p53(AD2), and p53(AD1 AD2), all of which contain an intact core DNA binding domain, have an unaltered DNA binding ability (Fig. 2). Because these p53 mutants are compromised in the transcriptional activity, especially in inducing endogenous p21, the question is posed of what is responsible for their deficiency?

Disruption of the dense chromatin structure by chromatin remodeling complexes is a prerequisite for transcriptional activation (1, 2, 5). Histone acetyltransferase complexes, which are well defined chromatin remodeling complexes, acetylate the tail of histones and make the promoter accessible to the basal transcriptional machinery (3, 6). Recently, several studies demonstrate that p53 physically interacts with p300/CBP both \textit{in vitro} and \textit{in vivo}, and co-expression of p300/CBP can potentiate p53 to activate the p21 promoter (17, 20, 21, 23). A dominant-negative CBP mutant is able to impair the p53-dependent induction of endogenous p21 (21). In addition, a double-point mutation in the activation domain 1 or the activation domain 2, which abolishes the transcriptional activity of p53, also abolishes the interaction of p53 with CBP \textit{in vitro} (21, 23). Therefore, we have analyzed histone acetylation on the p21 promoter upon expression of p53 \textit{in vivo}. We found that when p53 binds to the responsive elements in the p21 promoter, histone acetylation on the region proximal to the p21 promoter is nearly proportional to the extent of p21 induction by wild-type p53 and various mutants. Furthermore, we found that the extent of histone acetylation on the proximal p21 promoter is also proportional to the amount of p300/CBP that interacts with wild-type p53 and various mutants. Additionally, we found that down-regulation of p300/CBP can lead to impairment of p53-dependent induction of endogenous p21. Thus, our data extend the previous observations and show for the first time that both activation domains in p53 are necessary for interacting with p300/CBP \textit{in vivo}, histone acetylation on the proximal p21 promoter, and subsequently, the promoter accessibility. Furthermore, we found that although the basic domain and the proline-rich domain are not essential for the interaction between p53 and p300/CBP, both domains do influence the extent of this interaction \textit{in vivo} and, subsequently, histone acetylation and the promoter accessibility.

We also mention that histone acetylation on the region surrounding the p53-responsive elements in the p21 promoter is increased by p53(AD1 AD2) (Fig. 3), but the mutant is almost completely deficient in inducing p21 (Fig. 1A) and in interacting with p300/CBP (Fig. 6, A, C, and D). These data suggest that once this mutant binds to the p53-responsive elements, it alters the chromatin structure of these local regions, which concurrently allows for access of some histone acetyltransferase-containing proteins, and subsequently, the acetylation of histones in these regions. However, these histone acetyltransferase-containing proteins are not enough to spread the acetylation to the region proximal to the p21 promoter. Because the proximal promoter is the region to which the basal transcriptional machinery binds, lack of chromatin remodeling on, and subsequently, lack of accessibility of, the proximal p21
promoter are probably responsible for the deficiency of p53/AD1–AD2 to induce endogenous p21.

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The Activation Domains, the Proline-rich Domain, and the C-terminal Basic Domain in p53 Are Necessary for Acetylation of Histones on the Proximal p21 Promoter and Interaction with p300/CREB-binding Protein

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