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p53 Negatively Regulates *cdc2* Transcription via the CCAAT-binding NF-Y Transcription Factor*

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The p53 tumor suppressor protein regulates the transcription of regulatory genes involved in cell cycle arrest and apoptosis. We have reported previously that inducible expression of the p53 gene leads to the cell cycle arrest both at G_1 and G_2/M in association with induction of p21 and reduction of mitotic cyclins (cyclin A and B) and *cdc2* mRNA. In this study, we investigated the mechanism by which p53 regulates transcription of the *cdc2* gene. Transient transfection analysis showed that wild type p53 represses whereas various dominant negative mutants of p53 increase *cdc2* transcription. The *cdc2* promoter activity is not repressed in cells transfected with a transactivation mutant, $p53^{22/23}$. An adenovirus oncoprotein, E1B-55K inhibits the p53-mediated repression of the *cdc2* promoter, while E1B-19K does not. Since the *cdc2* promoter does not contain a TATA sequence, we performed deletion and point mutation analyses and identified the inverted CCAAT sequence located at −76 as a cis-acting element for the p53-mediated regulation. We found that a specific DNA-protein complex is formed at the CCAAT sequence and that this complex contains the NF-Y transcription factor. Consistently, a dominant negative mutant of the NF-YA subunit, NF-YAm29, decreases the *cdc2* promoter, and p53 does not further decrease the promoter activity in the presence of NF-YAm29. These results suggest that p53 negatively regulates *cdc2* transcription and that the NF-Y transcription factor is required for the p53-mediated regulation.

Inactivation of p53 tumor suppressor gene occurs in over half of all human tumors, implying that loss of this gene represents a fundamentally important step in genomic instability and susceptibility to malignant transformation (1, 2). The underlying mechanism of tumor suppressor activity of p53 resides in part in its ability to bind DNA in a sequence-specific manner to activate gene transcription (2). It has been reported that a substantial number of genes containing the p53-binding site(s) are activated by p53. These include *mdm2* (3, 4), p21/WAF-1 (5), Gadd45 (6), cyclin G (7), *bax* (8), and an insulin-like growth factor-binding protein (IGF-BP3) (9). p21 and Gadd45 were implicated in the p53-mediated cell cycle regulation (10, 11), while *bax* and IGF-BP3 were involved in the induction of apoptosis (8, 9).

In addition to playing a role as a DNA-binding dependent transcription activator, p53 has also been reported to negatively regulate the transcription of a number of genes. These genes include presenilin 1 (12), topoisomerase IIα (13, 14), *map4* (15), O^6-methylguanine-DNA methyltransferase (16), insulin receptor (17), *mdr*-1 (18), hsp70[1] (19), interleukin-6 (20), *bcl2* (21), c-*fos* (22), and other viral and cellular promoters (23). In contrast to the transcription activation by p53, no consensus sequence has been found in the promoters that are repressed by p53. It was initially reported that only the promoters containing a TATA box, but not those containing an initiator element, are repressed by p53 (24). This finding, coupled with the known interaction of p53 with a TATA-binding protein and TATA-binding protein-associated factors, suggested that p53 represses these promoters by squelching TATA-binding protein or TATA-binding protein-associated factors, thus inhibiting efficient initiation of transcription (25–27). However, in at least one case, it has been proposed that p53 represses transcription through interaction with a transcription activator rather than the basic transcription machinery. Repression of hsp70 transcription by p53 is mediated by an interaction between p53 and CCAAT-binding protein (CBF) a transcription activator of the hsp70 promoter (19).

p53 was first shown to mediate cell cycle arrest primarily at the G_1 phase. The G_1 arrest is, in part, mediated by the p53-dependent activation of negative cell cycle regulators such as p21/WAF1 and Gadd45 (5, 6, 10, 11). Recently, however, p53 was implicated in the cell cycle arrest at G_2 as well as at G_1 phase (28, 29). We reported previously that induction of p53 expression in EJ-p53 cells lacking endogenous p53 leads to a cell cycle arrest both at G_1 and G_2/M in association with induction of p21 and reduction of mitotic cyclins (cyclin A and B) and *cdc2* mRNA (30). The *cdc2* gene encodes the $p34^{cdc2}$ protein kinase associated with cyclin B (31–33). The p34/cyclin B complex is required for G_2-M progression in the cell cycle (31). *cdc2* transcription is regulated in a cell cycle-dependent manner, reaching the maximum level at the G_2 phase of the cell cycle and down-regulated in senescent cells (34–37). Various viral and cellular genes, including c-*myb* (38), c-*myc* (39), E2F (34), Fas (40), SV40T (41), and protein phosphatase 2A (42), activate *cdc2* transcription, suggesting that transcriptional regulation of the *cdc2* gene is closely related to cell proliferation, senescence, and apoptosis.

In this study, we describe our findings that p53 negatively

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[1] The abbreviations used are: hsp70, heat shock protein 70; CBF, CCAAT-binding factor; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; C/EBP, CCAAT/enhancer-binding protein.

regulates *cdc2* transcription and that the NF-Y transcription factor bound to the CCAAT sequence of the promoter is required for the p53-mediated regulation.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfection, and Chloramphenicol Acetyltransferase (CAT) Assay—The EJ-p53 cell line was established previously, in which p53 expression is regulated in a tetracycline-dependent manner (30). HepG2 cells were grown in 10% fetal bovine serum/Dulbecco's modified Eagle's medium as described (43). DNA transfection was performed using the $CaPO_4$ coprecipitation procedure (44). After 48 h of transfection, cells were harvested, and proteins were extracted by three cycles of freeze-thawing. The protein concentration of each cell lysate was determined with the Bio-Rad protein assay kit (Bio-Rad). In all transfection experiments, β-galactosidase activity derived from either pCMV-β-gal or pMT-β-gal was used to monitor and to normalize the transfection efficiency. CAT and β-galactosidase assays were carried out according to the protocol described by Gorman *et al*. (45). The promoter activities of reporter constructs were assayed by measuring the radioactivity of acetylated forms using a phosphoimage analyzer (Fuji).

Plasmid Construction—PCR techniques were employed to generate various derivatives of the *cdc2* promoter-CAT fusion construct, starting from pcdc2-*Pst*I provided by B. Calabretta (38). Briefly, pcdc2-*Pst*I, renamed as plasmid pcdc2–937 in this paper, had −937 to +64 of *cdc2* promoter DNA fused to the CAT gene on pUCCAT plasmid (Promega, Madison, WI). Two other plasmids with 5′ sequential deletions in the *cdc2* promoter, pcdc2–764 and pcdc2–568, were identical to pcdc2-*Pvu*II and pcdc2-*Ssp*I, respectively, as described by Ku *et al*. (38). The plasmids with further deletions in the 5′-flanking region were generated by PCR with the following oligomers as forward primers: 5′-TGAACTGTGCCAATGCTGGGA-3′ (bp −306 to −286) for pcdc2−306; 5′-TTTTCTCTAGCCGCC-3′ (bp −155 to −141) for pcdc2−155; and 5′-CTAGCCACCCGGGAA-3′ (bp −119 to −105) for pcdc2−119. PCR was performed using a common reverse primer containing a *Sal*I site (underlined), 5′-TCTAGAGTCGACCTGCCAGGC-3′ (bp +20 to +34) was used. The PCR fragments were ligated into the *Hin*dIII (blunt ended) and the *Sal*I sites of pCAT-basic vector (Promega, Madison, WI). To construct plasmids carrying internal deletions in the *cdc2* promoter region, we carried out PCRs using the following primers: 5′-TACCCGATTGGTGAATCCGGGGCC-3′ (bp −52 to −29) for pcdc2−109/−52; 5′-TGAAACTGCTCGCAC-3′ (bp −11 to +4) for pcdc2−109/−11; 5′-TACCCAGCGTAGCTGGGCTCTGAT-3′ (bp −100 to −77) for pcdc2−109/−100, coupled with the common reverse primer described above. The DNA fragments obtained from PCR replaced the promoter region from *Sma*I (bp −109) to *Sal*I (bp +34) in pcdc2–937. Mutagenesis in the putative protein binding sites was carried out by the overlapping extension PCR method. The PCRs were carried out with two common primers, a forward primer, 5′-GCCAAGCTTAGTGCAGAATC-3′ (bp −932 to −921), carrying a *Hin*dIII (underlined) site, and the common reverse primer carrying a *Sal*I site along with two overlapping oligomers containing mutated core motifs (underlined): 5′-GGCTCTGCCAGCTGCTTTGAAA-3′ (forward) and 3′-ATCGACCCGAGACGGTCGACGAA-5′ (reverse) for pcdc2-dCATmt; 5′-GGCTACCCGGGCAGTGAATCCGG-3′ (forward) and 3′-GATGCCCGATGGGCCCGTCACTTA-5′ (reverse) for pcdc2-pCATmt; 5′-CCCTTTAATATTGTGAGTTTGAAA-3′ (forward) and 3′-GGGAAATTATAACACTCAAACTTT-3′ (reverse) for pcdc2-E2F4mt.

Electrophoretic Mobility Shift Assay—Nuclear lysates were prepared according to the method described by Dignam *et al*. (46). A double-stranded, *in vitro* synthesized DNA fragment containing the distal CCAAT motif (−88 to −64) was labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Nuclear lysates (5 μg) were preincubated for 30 min at 0 °C with 1 μg of poly(dI-dC) (Amersham Pharmacia Biotech) and unlabeled competitor DNA in 25 mM HEPES (pH 7.9), 20 mM KCl, 30 mM NaCl, 0.5 mM EDTA, 0.25 mM dithiothreitol, and 10% glycerol. The end-labeled probe (about 15,000 cpm) was added and incubated for an additional 20 min at room temperature. The DNA-protein complexes were separated on a 6% polyacrylamide gel in 0.25× TBE at 15 mA for 2 h. For antibody supershift assay, 1 μg of each antibody specific for the A or B subunit of NF-Y (KB070 and KB090; Accurate), C/EBP (α, β, δ) (Δ198, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and C/EBPβ (C-19, Santa Cruz Biotechnology) was included in the preincubation mixture. The DNA sequences of *in vitro* synthesized oligomers used in competition assay were as follows: 5′-CTGGGCTCTGATTGGCTGCTTTGAA-3′ for distal CCAAT; 5′-CTGGGCTCTAGCCAGCTGCTTTGAA-3′ for distal CCAATmt; 5′-TACCCGATTGGTGAATCCGGGGCC-3′ for proximal CCAAT; 5′-CCCTTTAGCGCGGTGAGTTTGAAA-3′ for E2F4.

RESULTS

Negative Regulation of cdc2 Transcription by p53—To examine the effect of p53 on *cdc2* transcription, we transfected the *cdc2* promoter-CAT reporter construct (pcdc2-CAT) into HepG2 cells with the plasmid carrying either the wild type p53 or its dominant negative mutant form, p53^{273}. In this experiment, we used two control promoters, pSV-CAT and pG5-CAT. pSV-CAT carries the SV40 early promoter and is repressed by p53 (23). On the other hand, pG5-CAT carries the G5 promoter containing five consecutive p53 binding sequences and is thereby activated by p53 (23, 47). Fig. 1*A* shows the relative CAT activity of the pSV-CAT, pG5-CAT, and pcdc2-CAT in the presence of wild type or mutant p53. The p53 expression decreased the SV40 promoter activity approximately 3.7-fold, while increasing the G5 promoter activity approximately 12-fold. However, the *cdc2* promoter activity was decreased approximately 6.7-fold by the presence of p53. The expression of the mutant p53 (p53^{273}) had virtually no effect on the SV40 promoter but decreased the G5 promoter activity more than 10-fold. The *cdc2* promoter activity was increased approximately 14-fold by the presence of p53^{273}. We examined the effects of other dominant negative mutants on the *cdc2* promoter. Four mutant forms of p53 tested in this study decreased the G5 promoter activity while increasing the *cdc2* promoter activity as with p53^{273} (Fig. 1*B*).

The dominant negative mutants of p53 have been shown to activate transcription of a novel set of genes that are not regulated by wild type p53 (48). This "gain of function" phenotype should be inhibited by double mutations in the transactivation domain of p53 (p53$^{22/23}$) (49). Fig. 1*C* shows the effects of a triple mutant, p53$^{22/23/281}$, on the *cdc2* promoter. p53$^{22/23/281}$ increased the *cdc2* promoter activity similarly as with p53^{281}, indicating that the effect of dominant negative mutations, such as p53^{281}, on the *cdc2* promoter was not affected by the transactivation mutation (p53$^{22/23}$) (Fig. 1*C*). The double mutant, p53$^{22/23}$, increased the *cdc2* promoter activity about 3-fold (Fig. 1*C*).

To eliminate possible artifacts caused by overexpression of p53 in the cotransfection assay, we examined the *cdc2* promoter activity in EJ-p53 cells, in which expression of the wild type p53 gene is regulated by the tetracycline-regulated gene expression system (*tet-off* system) (50). The p53 expression was kept repressed in the presence of tetracycline but was induced upon removal of tetracycline from the culture medium (30). In EJ-p53 cells, the SV40 promoter activity was not significantly repressed by p53 expression (Fig. 2). The G5 promoter was activated (data not shown), while the *cdc2* promoter was repressed by p53 expression in agreement with the results of the cotransfection experiment. These results suggest that p53 specifically repressed *cdc2* transcription.

It has been reported that an adenovirus oncoprotein affects the p53-mediated transcription regulation (51–53). The E1B-55K protein binds to the amino-terminal transactivation domain of p53 and inhibits its transactivation function (53, 54). E1B-19K has been shown to inhibit the p53-mediated transcription repression of the basic promoters containing a TATA or an initiator element but not the transcription activation of promoters containing the p53-binding sites (51, 52). We examined the effects of E1B-19K or -55K on *cdc2* transcription in presence or absence of tetracycline (Fig. 2). In the presence of tetracycline, neither E1B-55K nor -19K affected the *cdc2* promoter activity (Fig. 2). However, repression of the *cdc2* promoter in the absence of tetracycline was inhibited in cells transfected with E1B-55K, suggesting that E1B-55K is capable of inhibiting the p53-mediated transcription repression of the *cdc2* promoter. Unlike the basic promoters, E1B-19K did not

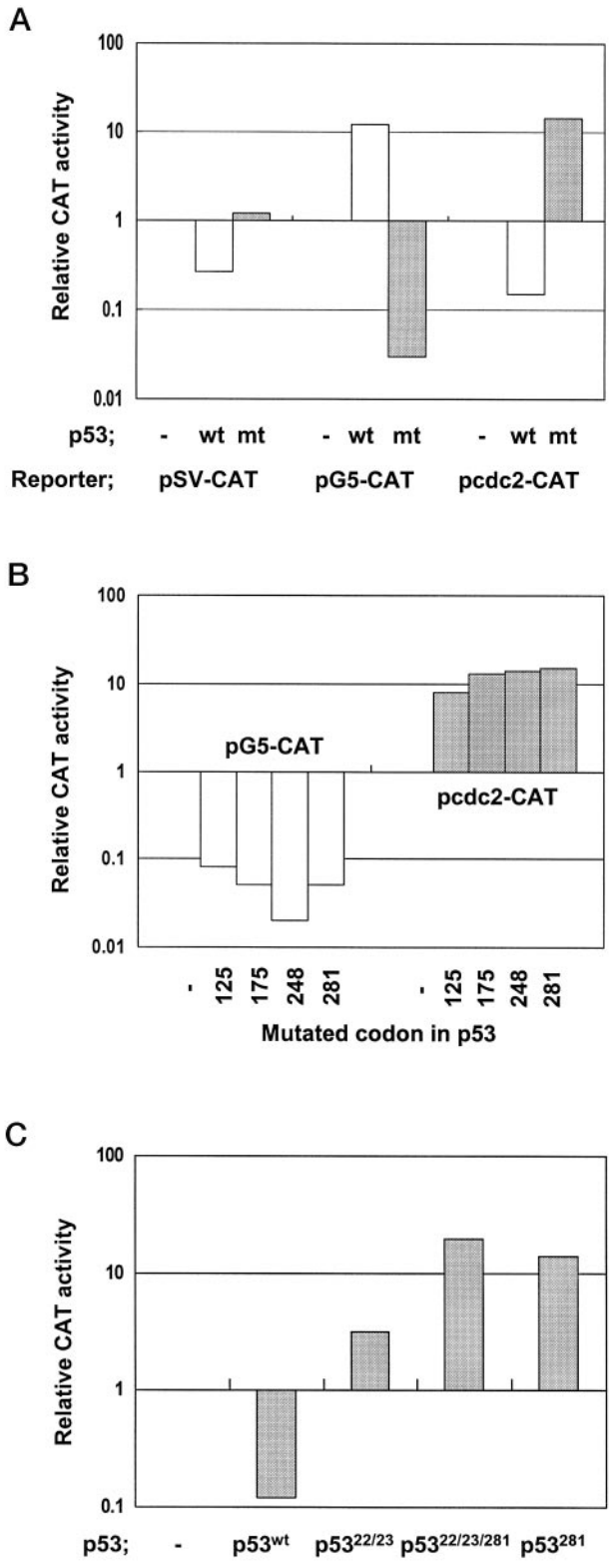

FIG. 1. **Effects of p53 on *cdc2* transcription.** HepG2 cells were cotransfected with 1 μg of the pSV-CAT, pG5-CAT, or pcdc2-CAT reporter plasmid and 2 μg of the wild-type or mutant p53 expression plasmid or the pCMV control vector plasmid. The CAT activity expressed in the cotransfected cells with each reporter plasmid and pCMV vector was defined as 1. Relative CAT activity was calculated as described under "Experimental Procedures." The data represent the averaged results from three independent transfections. *wt*, wild type; *mt*, mutant.

affect repression of the *cdc2* promoter by p53, suggesting that p53 represses the *cdc2* promoter in a manner different from the basic promoters.

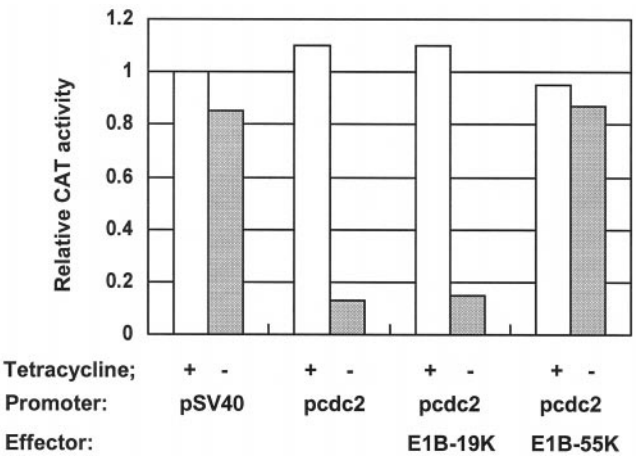

FIG. 2. **Effects of p53 and E1B on *cdc2* transcription.** EJ-p53 cells were transfected with 1 μg of either the pSV-CAT or pcdc2-CAT reporter plasmid and incubated with or without tetracycline (1 μg/ml) for 48 h. To examine the effects of E1B, we cotransfected the pcdc2-CAT plasmid into EJ-p53 cells with either pCMV-E1B-19K or -55K. The CAT activity expressed in cells transfected with the pSV-CAT and grown with tetracycline was defined as 1. Relative CAT activity was calculated as described under "Experimental Procedures." The data represent the averaged results from three independent transfections.

Functional Analysis of Human cdc2 5′-Flanking Sequence—To identify the sequence element(s) needed for the p53-mediated regulation, we analyzed effects of various deletions of the *cdc2* promoters in HepG2 cells. The *cdc2* promoter activity with deletions up to −109 was increased to a level similar to that with the wild type promoter (pcdc2–937) by the p53 mutant, $p53^{273}$ (data not shown, see "Experimental Procedures" for construction of the plasmids). However, the promoters with deletion from either −109 to −52 (pcdc2−109/−52) or −109 to −11 (pcdc2−109/−11) showed a significant reduction in the mutant 53-mediated increase (Fig. 3). Two well characterized protein binding sequences are found between −109 and −52: ets2 centered at −104.5 and an inverted CCAAT sequence (the distal CCAAT) centered at −76. Deletion of ets2 (pcdc2−109/−101) had little effect on the mutant p53-mediated increase. In contrast, the site-directed mutation of the distal CCAAT sequence (dCCAATmt) caused a significant reduction (Fig. 3). Mutations in the proximal CCAAT sequence (at −44) and the E2F-like sequence (at −20) did not affect the mutant p53-mediated increase. We examined the effects of the mutant promoters in EJ-p53 cells. In the absence of tetracycline in the medium, the expression of dCCAATmt-CAT was decreased only about 1.3- fold, while pCCAATmt-CAT was decreased by 7.7-fold, which is a level similar to that of the wild type *cdc2* promoter (Fig. 3). Taken together, these results suggest that the distal CCAAT sequence located at −76 is necessary for regulation of the *cdc2* promoter by p53.

Identification of the Distal CCAAT-binding Protein(s)—We performed a gel shift assay to identify the protein(s) interacting at the distal CCAAT sequence. A 24-bp DNA probe containing the distal CCAAT sequence was incubated with a nuclear lysate of HepG2 cells and was analyzed by a nondenaturing polyacrylamide gel (Fig. 4). In the presence of a random competitor DNA, poly(dI-dC), several high molecular weight bands were detected. The intensity of the uppermost band (band *a*) was reduced markedly in the presence of unlabeled DNA containing the distal CCAAT sequence (dCAT). However, the band *a* was not changed significantly in the presence of unlabeled DNA containing any of the proximal CCAAT sequence (pCAT), E2F4 sequence (E2F4), or mutated distal CCAAT sequence (dCATmt). This result suggests that band *a* contains a protein(s) interacting specifically at the distal CCAAT sequence.

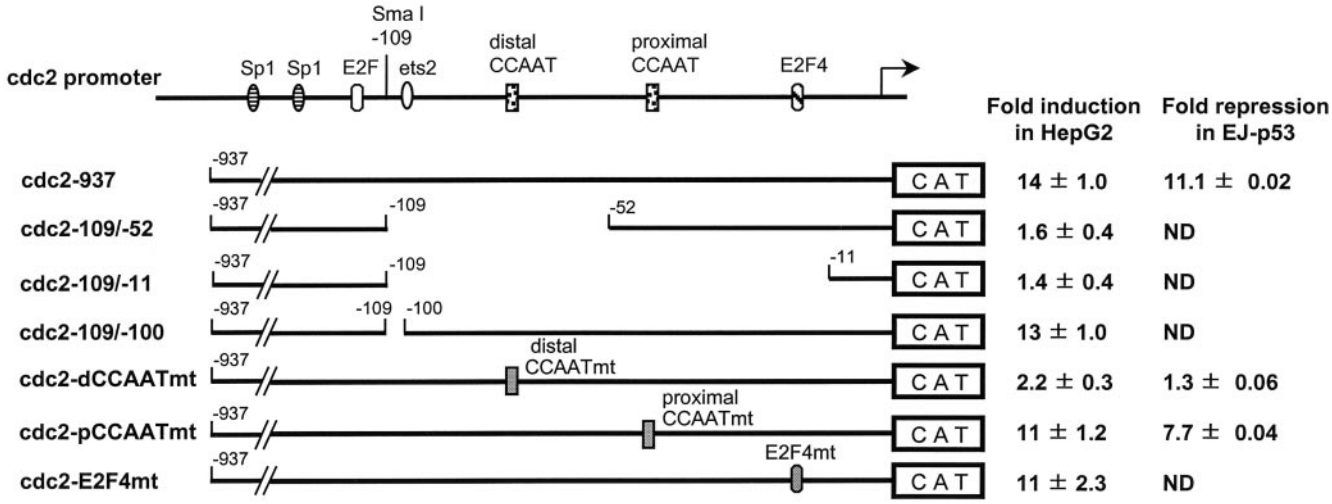

FIG. 3. **Identification of a cis-acting element responsible for the p53-dependent *cdc2* regulation.** The *cdc2* promoter-CAT chimeric constructs used in this series of experiments are presented schematically. The *numbers* indicate the 5′- or 3′-end position of deletion in the promoter (see "Experimental Procedures"). HepG2 cells were cotransfected with 1 μg of the mutant *cdc2* promoter-CAT plasmid and 2 μg of either the pCMV-p53^{273} or the pCMV vector plasmid. The -fold effects were calculated by dividing the CAT activity in the presence of pCMV-p53^{273} by that in the presence of pCMV vector in HepG2 cells. EJ-p53 cells were transfected with the mutant cdc2 promoter-CAT plasmid and incubated with or without tetracycline (1 μg/ml) for 48 h. The -fold effects were calculated by dividing the CAT activity in the absence of tetracycline by that in the presence of tetracycline.

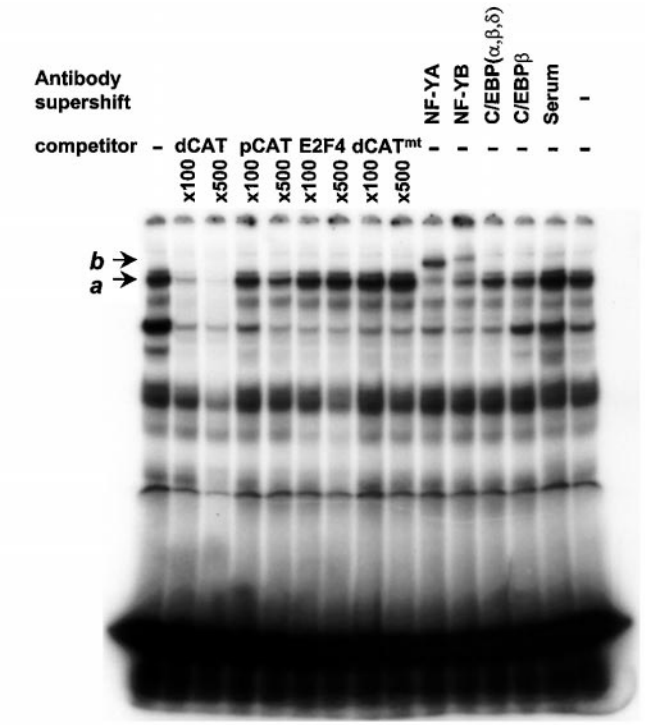

FIG. 4. **Identification of the distal CCAAT binding proteins.** Electrophoretic mobility shift assays of complexes formed by the DNA probe containing the distal CCAAT element of the *cdc2* promoter (the promoter sequences between −88 and −64). An end-labeled DNA probe was incubated with nuclear lysates of HepG2 cells. For the competition assay, a 100- or 500-fold molar excess of competitors containing each protein binding site (dCAT, pCAT, and E2F4) or mutations in the distal CCAAT element (dCATmt) indicated *above* each *lane* was incubated together with the labeled DNA probe. Mobility shift assay was performed in the presence of antibodies raised against various CCAAT-binding proteins. An antibody (1 μg) specific for each protein as indicated *above* each *lane* was preincubated in the nuclear lysate. The *arrows* indicate the complex formed at the distal CCAAT site (*a*) and a supershifted band formed in the presence of the antibody (*b*).

A number of different proteins that bind the CCAAT sequence have been reported: C/EBP isoforms (55), NF-Y (CBF or CP-1) (56), CBF/hsp70 (19), and NF-1 (also known as CTP) (57). To identify the protein binding at the distal CCAAT sequence, we performed a supershift assay using antibodies that recognize NF-Y or C/EBP isoforms (α, β, and δ) in the nuclear extract of HepG2 cells (Fig. 4). The antibodies against A or B subunit of NF-Y led to the formation of a supershifted band (band *b*) at the expense of band *a*, while the antibodies against the C/EBP isoforms did not give rise to such supershifted bands. These results indicate that the complex formed at the distal CCAAT sequence, corresponding to band *a*, contains the heterotrimeric transcription factor, NF-Y.

Effects of a Dominant Negative Mutant of NF-YA—We examined if NF-Y is required for the p53-mediated repression of the *cdc2* promoter by employing a dominant negative mutant form of NF-YA (NF-YAm29) in which three amino acids in the DNA binding domain have been mutated (60). It has been demonstrated that the complex with the mutant NF-YA is functionally inactive both *in vitro* and *in vivo* (58, 59). The dominant negative mutant of NF-YA was introduced together with either the pcdc2-CAT or pSV-CAT into EJ-p53 cells, and the promoter activities in the presence or absence of p53 (under *tet-off* control) were determined (Fig. 5). The NF-YA mutant decreased *cdc2* promoter activity in the presence of tetracycline, while it did not affect SV40 promoter activity (Fig. 5). In the absence of tetracycline, however, *cdc2* promoter activity was not further repressed in the presence of the NF-YA mutant. Contrarily, serum depletion further decreased *cdc2* transcription in the presence of the NF-YA mutant, suggesting that the serum depletion represses the cdc2 promoter independently of CCAAT-binding NF-Y. Taken together, these results suggest that the distal CCAAT sequence and its binding protein, NF-Y, are required for the p53-mediated regulation of *cdc2* transcription.

DISCUSSION

Recent studies implicated the potency of p53 as a transcription repressor in the tumor suppressor function as well as in apoptosis (51, 52, 60). It therefore is of considerable interest to identify endogenous specific target genes that are negatively regulated by p53.

In this study, we investigated a mechanism by which p53

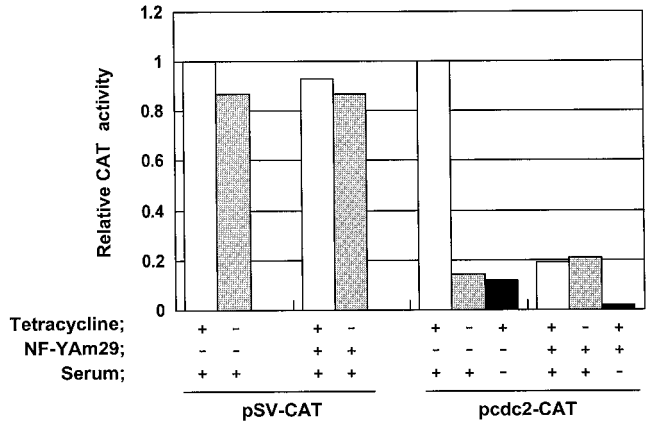

FIG. 5. **Effects of a dominant negative NF-YA mutant on the p53-mediated *cdc2* repression.** A dominant negative mutant form of NF-YA (NF-YAm29) on expression plasmid was cotransfected into EJ-p53 cells with either the pSV-CAT or the pcdc2-CAT reporter plasmid. The cells were incubated with or without tetracycline (1 μg/ml) for 48 h after the transfection. For serum starvation, the cells were incubated with 0.1% of serum for 48 h after transfection. The CAT activity expressed in the cells grown with tetracycline was defined as 1. Relative CAT activity was calculated as described under "Experimental Procedures." The data represent the averaged results from two independent transfections.

negatively regulates transcription of the *cdc2* gene, which encodes a protein kinase associated with cyclin B (31, 33). A transient transfection assay showed that *cdc2* transcription was repressed by wild type p53, while it was increased by various mutants of p53 in HepG2 cells (Fig. 1*A*). We designed two types of experimental setups to verify negative regulation of cdc2 transcription by p53. First, we examined whether the dominant negative mutants of p53 increase *cdc2* promoter activity by the "gain of function" phenotype or by interfering with wild type p53 present in HepG2 cells. Mutations in the transactivation domain ($p53^{22/23}$) was reported to inhibit the "gain of function" phenotype of the dominant negative mutation ($p53^{281}$) (49). However, we found that both the $p53^{281}$ and $p53^{22/23/281}$ mutants increased *cdc2* promoter activity (Fig. 1*C*). Therefore, we suggest that the dominant negative mutants of p53 increase the *cdc2* promoter activity by interfering with the repressive effect of wild type p53 on the *cdc2* promoter rather than exerting through the gain of function phenotype. Second, we employed EJ-p53 cells to confirm the results obtained from the cotransfection assay in HepG2 cells. The p53 expression in EJ-p53 cells is endogenously regulated by the *tet-off* system (30). Cdc2 transcription was repressed in cells growing without tetracycline, while SV40 promoter activity was not (Fig. 2). These results indicate that p53 specifically represses *cdc2* transcription.

In this study, we examined the possibility that the transactivation domain of p53 is required for repression of *cdc2* transcription. The $p53^{22/23}$ mutant did not repress the cdc2 promoter. Interestingly, $p53^{22/23}$ increased *cdc2* promoter activity approximately 3-fold (Fig. 1*C*), suggesting that a heteromeric complex containing wild type p53 and $p53^{22/23}$ cannot repress the *cdc2* promoter. E1B-55K protein has been reported to bind the transactivation domain of p53, thereby blocking its transactivation function (53, 54). Our results showed that E1B-55K inhibits repression of *cdc2* transcription as well as transcription activation of the p53-responsive promoters by p53 (Fig. 2). Although E1B-19K did not affect the p53-mediated repression of the *cdc2* promoter (Fig. 2), it was shown to inhibit the p53-mediated repression of the basic promoters carrying a TATA box or an initiator element (51). These results suggest that p53 represses *cdc2* promoter activity in a manner different from the basic promoters, in which p53 represses the promoters by regulating the basic transcription machinery. Subsequently, we identified the distal CCAAT sequence as a cis-acting element necessary for the negative regulation of *cdc2* promoter by p53 (Fig. 3). It therefore is likely that p53 regulates the *cdc2* promoter by modulating a CCAAT binding protein rather than the basic transcription machinery.

The p53-dependent *cdc2* regulation is somewhat analogous to the regulation of the human hsp70 gene by p53 (19). Transcription of the hsp70 gene is repressed by p53 but derepressed by a mutant form of p53 or E1A, an adenovirus oncoprotein. It was suggested that CBF/hsp70, a transcription activator binding to a CCAAT sequence of the promoter, mediates the p53-dependent repression. CBF/hsp70 interacts with the p53 protein or E1A. The p53 protein is able to compete with E1A for binding to CBF/hsp70. It was suggested that CBF/hsp70 complexed with E1A forms an activator, whereas the same protein complexed with p53 forms a repressor. Recently, it was reported that E1A activates the *cdc2* promoter and that the CCAAT sequence is necessary for this activation (61). Consistently, we found that E1A activated the *cdc2* promoter in the presence of the distal CCAAT sequence when EJ-p53 cells were grown with tetracycline (data not shown). However, this activation was abolished when p53 expression was induced in EJ-p53 cells by removal of tetracycline in the medium (data not shown), suggesting that the *cdc2* promoter is regulated in a manner similar to the hsp70 promoter.

We identified the NF-Y transcription factor as a protein binding to the distal CCAAT sequence on the *cdc2* promoter by a gel shift assay (Fig. 4). Based on our observation that the p53-dependent cdc2 repression is abolished by expression of the dominant negative mutant form of NF-YA, it is postulated that a functional form of NF-Y is necessary for the p53-mediated *cdc2* repression (Fig. 5). Recently, a 110-kDa protein (CBF/cdc2) was identified to interact with the CCAAT sequence of the *cdc2* promoter, and the 110-kDa protein is similar or related to CBF/hsp70 (61). The protein complexes interacting with the CCAAT sequences of the *cdc2* or hsp70 promoter exhibit the same mobility on the polyacrylamide gel (61). The CCAAT sequences could compete with each other for binding of the CBF in a cross-competition experiment (61). Furthermore, NF-Y also bound to the CCAAT sequence on the hsp70 promoter.[2] These results suggest that both CBF/hsp70 and NF-Y bind to the CCAAT sequences in both the *cdc2* and hsp70 promoters. Since p53 interacts with CBF/hsp70, this interaction may affect the transactivation ability of NF-Y. Further studies are required to elucidate a detailed mechanism by which p53 regulates the CCAAT-binding protein complex.

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