Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5206–5210, June 1992
Biochemistry

Negative regulation of Rb expression by the p53 gene product

(tumor suppressor genes/transcriptional regulation)

YUZURU SHIIO*, TADASHI YAMAMOTO†, AND NOBUO YAMAGUCHI*

Departments of *Virology and †Oncology, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minatoku, Tokyo 108, Japan

Communicated by Robert A. Weinberg, February 25, 1992

ABSTRACT **Mutation of the p53 gene is one of the most frequent genetic changes found in human cancers. Recent experiments indicated that p53 might contain a transcription-activating domain, which functions when directed to a promoter. This study shows that wild-type p53 suppresses transcription of the retinoblastoma (Rb) gene. From deletion and mutagenesis experiments, a cis-acting element (GGAAGTGA) susceptible to regulation by p53 was mapped within the Rb promoter. This element overlaps the basal transcription unit of the Rb promoter, suggesting that p53 suppresses Rb transcription through inhibition of the basal promoter activity. The N-terminal acidic and C-terminal basic domains of p53 were both required for this suppression. These findings indicate that p53 can act as a transcriptional regulator *in vivo*.**

Inactivation of the p53 gene and the retinoblastoma (Rb) gene is associated with many types of human cancers (reviewed in refs. 1–3). Their gene products have been shown to suppress transformation and can form complexes with the oncoproteins of the DNA tumor viruses (reviewed in refs. 1–3).

In spite of their importance in the mechanism of carcinogenesis, little is known about the normal functions of p53 and Rb. Recent experiments with a p53–GAL4 fusion protein have suggested that p53 might contain a transcription-activating domain (4, 5). However, no information is available on whether p53 really has a role as a transcriptional regulator *in vivo* and, if so, the target of its transcriptional regulation. In an attempt to identify the target of transcriptional regulation by p53, we examined its effect on the promoter of the Rb gene, in expectation of activation of Rb transcription by p53. As reported here, this was not the case; wild-type p53 suppressed transcription of the Rb gene. A cis-acting element susceptible to regulation by p53 was mapped within the Rb promoter. This element overlaps the basal transcription unit of the Rb promoter, suggesting that p53 suppresses Rb transcription through inhibition of the basal promoter activity.

MATERIALS AND METHODS

Plasmids. Human wild-type and mutant p53 cDNAs were excised from pC53-SN3 and pC53-SCX3 (6), respectively, by *Bam*HI digestion and cloned in the sense orientation between the two *Xho* I sites of pME18S vector (7), which is an SRα promoter (8)-based eukaryotic expression vector. The resulting constructs were termed pME18S-SN3 and pME18S-SCX3, respectively. pME18S-p53(82–393), pME18S-p53(160–393), or pME18S-p53(1–326) was derived from pME18S-SN3 by deleting the fragment between the *Nco* I site at amino acid 1 of p53 and the *Cfr*10I site at amino acid 82, between the *Nco* I sites at amino acids 1 and 160, or between the *Ssp* I site at amino acid 327 and the *Pst* I site in the polylinker sequence of pME18S-SN3, respectively. The expression of p53 proteins from all of these constructs was verified by immunoblot and indirect immunofluorescence with the anti-p53 monoclonal antibody PAb122 (ATCC TIB 116), which recognizes an epitope at the C terminus (9), or PAb1801 (Ab-2, Oncogene Science, Uniondale, NY), which recognizes an epitope at the N terminus (10).

pRbCAT2 contains human Rb promoter sequence between −1546 and +186 (relative to the major start site of transcription) linked to the chloramphenicol acetyltransferase (CAT) gene (11). The Rb promoter does not have a TATA box, and previous analysis demonstrated that there are multiple start sites of transcription and the region responsible for the basal level of transcription is located from +72 to +93 downstream of the major start site (11, 12). The 5′ deletion constructs of pRbCAT2 (Δ−567CAT, Δ−125CAT, or Δ+99CAT) were generated by deleting the sequence between −1546 and −567 (*Stu* I site), −1546 and −125 (*Apa*LI site), or −1546 and +99 (*Sac* II site), respectively. The 3′ deletion constructs (+104ΔCAT, +89ΔCAT, +80ΔCAT, and +53ΔCAT) were generated by exonuclease III digestion and retain the *Hin*dIII site at the junction of the Rb sequence and the CAT gene. Δ(+75–+99)CAT was generated by deleting the sequence between the two *Sac* II sites at +75 and +99.

Mutagenesis of +89ΔCAT was performed by replacing the *Sac* II (at +75)–*Hin*dIII (at the junction of the Rb sequence and the CAT gene) fragment by annealed oligonucleotides with the mutations. The sequences of all mutated plasmids were verified by sequencing through the mutated region.

pSV2CAT contains the simian virus 40 (SV40) promoter/enhancer sequence linked to the CAT gene as described (13). Transfer of the p53 control element to pSV2CAT was performed by inserting the annealed oligonucleotide containing two copies of the sequence between +76 to +89 of the Rb promoter into the *Pvu* II site just 5′ to the SV40 enhancer in pSV2CAT. The resulting constructs were termed P2CAT and P4CAT, which contain two and four copies of +76 to +89 sequence, respectively.

Cell Culture. HeLa cells (ATCC CCL 2), Saos-2 cells (ATCC HTB 85), and CV-1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. TIG-3 cells (14) were obtained from the Japanese Cancer Research Resources Bank (Tokyo) and were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum.

DNA Transfections and CAT Assays. Cells (5×10^5 per 6-cm plate) were transfected by the calcium phosphate precipitation technique (15) with 2 μg of each reporter construct and 6 μg of each effector construct. CAT assays were performed using equivalent amount of protein extract as described (13). All CAT assays were repeated more than twice and the reproducibility of the results was confirmed. Only the representative data are shown in each figure.

Immunoblot. HeLa cells were transfected with each p53 construct and cell lysates were prepared 45 hr after trans-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAT, chloramphenicol acetyltransferase; SV40, simian virus 40; Rb, retinoblastoma.

fection. Twenty micrograms of protein from each sample was separated by electrophoresis in 10% SDS/polyacrylamide gels and was electrophoretically transferred to nitrocellulose filters. The blot was incubated with the anti-p53 monoclonal antibody PAb122 or PAb1801 followed by incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dakopatts, Glostrup, Denmark).

RESULTS

Suppression of Rb Transcription by the Wild-Type p53 Protein. In transient-expression assays using HeLa cells (Fig. 1), cotransfection of pRbCAT2 with the wild-type p53 construct, pME18S-SN3, resulted in significantly less CAT activity than that on its cotransfection with the pME18S vector. In contrast, cotransfection of pRbCAT2 with the mutant p53 construct, pME18S-SCX3, resulted in enhanced CAT activity. In four independent experiments, we observed an average of 4-fold reduction and 6-fold increase in CAT activity with the wild-type p53 and mutant p53, respectively. HeLa cells express the E6 protein of human papillomavirus type 18 (16), and this E6 protein has been shown to form a complex with the p53 protein (17). To rule out the possibility that this transcriptional regulation is a special feature of the p53 protein in complex with the E6 protein, we used the human osteosarcoma cell line Saos-2 for transient-expression assays. Saos-2 cells do not contain the human papillomavirus E6 coding region (unpublished data) and have no endogenous p53 (18). Transcription from the Rb promoter was suppressed by wild-type p53 and was enhanced by mutant p53 in Saos-2 cells as in HeLa cells (Fig. 1).

To exclude the possibility that the suppression by wild-type p53 is due to global suppression of transcription, we tested the effect of p53 on the SV40 promoter, using pSV2CAT (13) as a reporter. Although cotransfection of pSV2CAT with the mutant p53 construct, pME18S-SCX3, led to an increase in CAT activity, cotransfection of pSV2CAT with the wild-type p53 construct, pME18S-SN3, had no effect (Fig. 1). From these observations, we conclude that human wild-type p53 does not suppress Rb transcription through a general block of RNA polymerase II-dependent transcription.

Location of the p53 Control Regions in the Rb Promoter. To locate the DNA element(s) in the Rb promoter that mediates the transcriptional regulation by wild-type p53, we tested a series of 5′ and 3′ deletion mutants of the Rb promoter for p53 sensitivity in cotransfection assays (Fig. 2). The transcription of all 5′ deletion constructs down to +99 was suppressed by wild-type p53. With 3′ deletion constructs, the transcription of +89ΔCAT, a deletion construct up to +89, was suppressed by wild-type p53, whereas that of +80ΔCAT, a deletion construct up to +80, was not affected. These data indicate that there are two regions susceptible to regulation by p53 (p53 control regions) in the Rb promoter, one between +80 and +89 and the other between +99 and +186. To confirm the existence of two p53 control regions in the Rb promoter, we tested Δ(+75–+99)CAT, which has a deletion between the two *Sac* II sites (+75 and +99) of the Rb promoter. This construct, which lacks the upstream p53 control region (between +80 and +89), was still suppressed by wild-type p53 (Fig. 2).

Identification of the p53 Control Element in the Rb Promoter. Sequence comparison of the two putative p53 control regions revealed the common sequence G(G/C)AA(G/C)TGA at +80 to +87 and at +121 to +114 (Fig. 3*A*). We next examined the effect of mutations in the upstream candidate element. Four kinds of mutations were introduced into +89ΔCAT, which contains the upstream element (between +80 and +89) but not the downstream element (between +99 and +186); M1, M2, and M4 mutations disrupt the GGAAGTGA sequence, and M3 is a mutation outside this sequence (Fig. 3*B*). As shown in Fig. 3*C*, M1, M2, and M4 abolished the suppression by wild-type p53, whereas M3 had

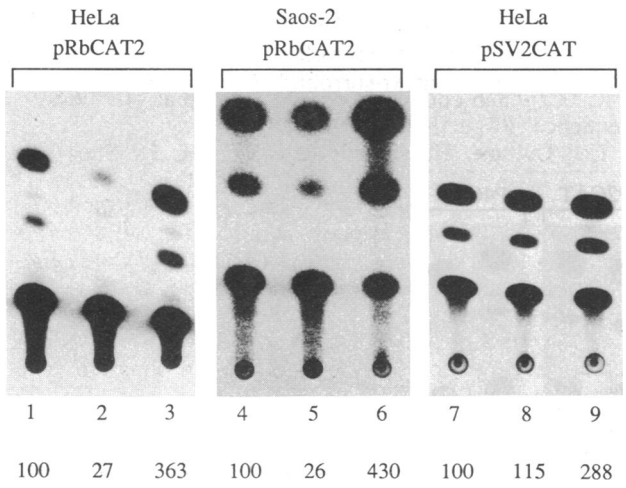

FIG. 1. Effect of human p53 on Rb transcription. pRbCAT2 (lanes 1–6) or pSV2CAT (lanes 7–9) was cotransfected with pME18S vector (lanes 1, 4, and 7), pME18S-SN3 (lanes 2, 5, and 8), or pME18S-SCX3 (lanes 3, 6, and 9) into HeLa cells (lanes 1–3 and 7–9) or Saos-2 cells (lanes 4–6). The relative CAT activities (CAT activity after cotransfection with the control vector is defined as 100%) are shown under each lane.

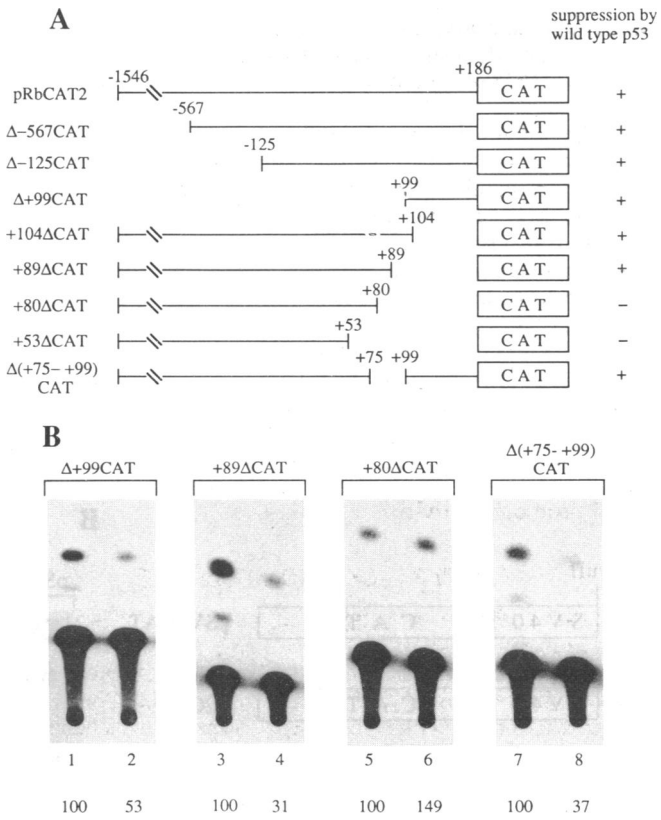

FIG. 2. Deletion analysis of the Rb promoter. (*A*) Summary of mapping of the p53 control regions. Significant suppression by wild-type p53 in cotransfection assay in HeLa cells is indicated by "+". (*B*) Effect of human wild-type p53 on transcription of RbCAT deletion constructs. The effector construct is pME18S vector (lanes 1, 3, 5, and 7) or pME18S-SN3 (lanes 2, 4, 6, and 8). The RbCAT deletion constructs were as follows: Δ+99CAT, lanes 1 and 2; +89ΔCAT, lanes 3 and 4; +80ΔCAT, lanes 5 and 6; and Δ(+75–+99)CAT, lanes 7 and 8.

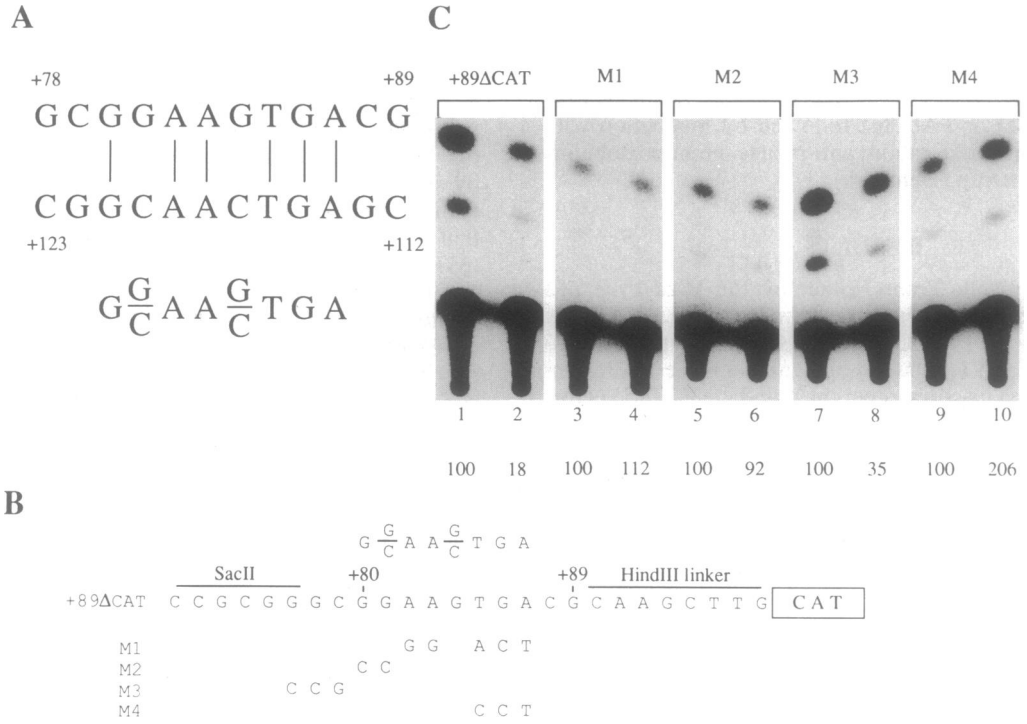

FIG. 3. p53 control element in the Rb promoter. (*A*) Comparison of sequences of the two candidate p53 control elements. The sequence around the downstream element (+112 to +123) is shown as a complementary sequence in reverse orientation. Vertical bars denote matched bases. (*B*) Mutagenesis of +89ΔCAT. The sequence of +89ΔCAT around the upstream p53 control element is shown with the G(G/C)AA(G/C)TGA sequence at the top. *Sac* II denotes the *Sac* II site at +75 of the Rb promoter. Mutations introduced into +89ΔCAT are shown below the sequence of +89ΔCAT. (*C*) Effect of mutations in the p53 control element. +89ΔCAT (lanes 1 and 2) and mutated versions of +89ΔCAT (lanes 3 and 4, M1; lanes 5 and 6, M2; lanes 7 and 8, M3; and lanes 9 and 10, M4) were cotransfected with pME18S vector (lanes 1, 3, 5, 7, and 9) or pME18S-SN3 (lanes 2, 4, 6, 8, and 10) into HeLa cells.

no effect. These data clearly indicate the importance of the GGAAGTGA sequence for the response to wild-type p53. We have termed this element "the p53 control element." In addition, M1, M2, and M4 significantly reduced the basal level of transcription of the Rb promoter, but M3 had no effect, indicating that the element responsible for the basal level of transcription of the Rb promoter also resides in the GGAAGTGA sequence. Deletion or mutation of this sequence resulted in a 10-fold reduction in the level of Rb promoter activity (unpublished data).

To further confirm the authenticity of the p53 control element, annealed oligonucleotide that contains two copies of the +76 to +89 region (including the GGAAGTGA sequence) was inserted directly upstream from the heterologous SV40 enhancer/promoter in pSV2CAT (Fig. 4*A*). Whereas transcription of pSV2CAT was not affected by wild-type p53, the presence of the p53 control element conferred suppression by p53 to pSV2CAT in a copy-number-dependent manner (Fig. 4*B*). The effect of transfer of the p53 control element to pSV2CAT was also observed in Saos-2 cells, CV-1 cells, and human primary embryonic fibroblast TIG-3 cells, all of which are human papillomavirus E6 negative (unpublished data), suggesting that the p53 control element works regardless of the presence of the E6 protein. Although the effect of transfer of the p53 control element was rather weak, this may reflect the difference in the context surrounding the inserted element.

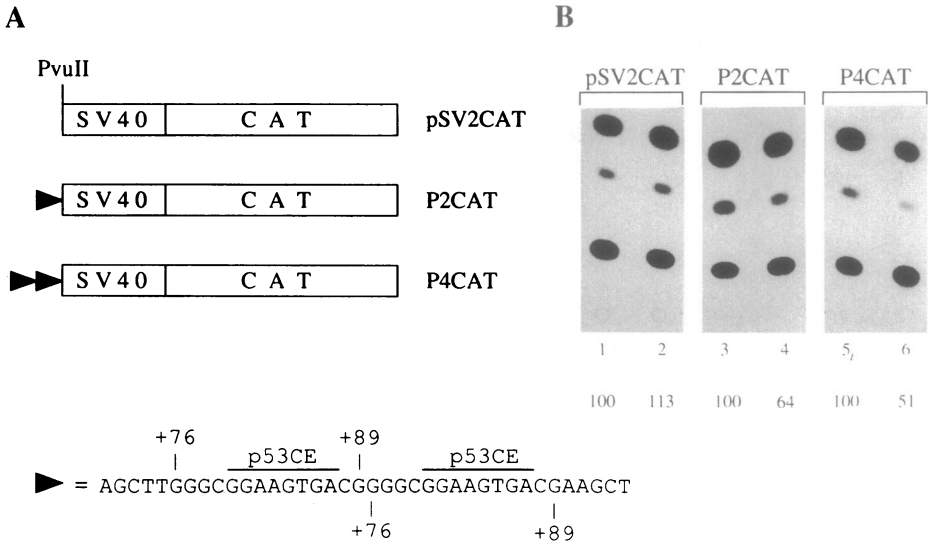

FIG. 4. Transfer of the p53 control element to pSV2CAT. (*A*) Schematic representation of pSV2CAT and its derivatives. (*B*) Effect of transfer of the p53 control element to pSV2CAT. pSV2CAT (lanes 1 and 2), P2CAT (lanes 3 and 4), or P4CAT (lanes 5 and 6) was cotransfected with pME18S vector (lanes 1, 3, and 5) or pME18S-SN3 (lanes 2, 4, and 6) into HeLa cells.

Requirement of N-Terminal Acidic and C-Terminal Basic Domains of p53 for Suppression of Rb Transcription. Analyses of the hydropathic profile, secondary structure potential, and charge distribution of the p53 proteins from various species suggest that this protein may have three domains (1, 19, 20): (*i*) a highly charged acidic N-terminal region, consisting of the first 80 amino acids in human p53, that is predicted to form an α-helical structure; (*ii*) a hydrophobic proline-rich domain between amino acid residues 81 and 150 in human p53; and (*iii*) a highly charged basic C-terminal region that can form helix–turn–helix motifs (residues 319–393 in human p53). To determine which domain of p53 is necessary for suppression of Rb transcription, we constructed three kinds of human p53 deletion mutants (Fig. 5*A*). The expression of p53 proteins from these constructs was verified by immunoblotting (Fig. 5*B*). All of these deletion mutants failed to suppress Rb transcription in HeLa cells (Fig. 5*C*). Similar results were obtained in Saos-2 cells (unpublished data). These findings indicate that the N-terminal acidic and C-terminal basic domains of p53 are both required for suppression of Rb transcription. In addition, p53(1–326) activated Rb transcription more strongly than p53(82–393) and p53(160–393), and the rate of activation was significantly different among three deletion mutants and a point mutant, SCX3. This differential effect was also observed in Saos-2 cells (unpublished data).

DISCUSSION

In this study, we have shown that wild-type p53 suppresses Rb transcription through the cis-acting sequence in the Rb promoter. The GGAAGTGA sequence susceptible to regulation by p53 is present at +80 to +87 of the Rb promoter. Similar sequence is also present at +121 to +114. Although we have not yet proved the authenticity of this downstream element (+121 to +114), this element might be responsible for the suppression of Δ+99CAT and Δ(+75–+99)CAT by p53.

The GGAAGTGA sequence overlaps the element responsible for the basal level of Rb transcription. This suggests that p53 suppresses Rb transcription through inhibition of the basal promoter activity. In the case of transcriptional suppression by Rb, the retinoblastoma control element in the c-*fos* promoter overlaps the element responsible for the basal level of transcription of the c-*fos* promoter, and deletion of this element resulted in a 10-fold reduction in the level of c-*fos* promoter activity (21). Another element called Yi in the murine thymidine kinase promoter was recently suggested to be a target of transcriptional suppression by Rb (35) and this element also overlaps the element responsible for the basal level of transcription of the thymidine kinase promoter (22). Thus the possibility arises that p53 and Rb may suppress transcription of the target gene through inhibition of the basal promoter activity, which is yet to be verified.

The biological significance of suppression of Rb transcription by p53 is unknown. One possibility is that p53 and Rb belong to different growth-regulatory systems and that p53 suppresses Rb transcription through a negative feedback mechanism between the two independent growth-regulatory systems. Alternatively, this phenomenon might explain how p53 regulates the cell cycle. Stimulation of quiescent cells with serum results in an increase in p53 mRNA and protein, with maximum levels found late in G_1 or early in S phase (23, 24), the time point coincident with the phosphorylation of the Rb protein that is assumed to be a regulatory event leading to the inactivation of its growth-suppressing functions (25, 26). If the increased p53 protein suppresses Rb transcription and reduces the load of the Rb kinase(s), this would be another mechanism to down-regulate the activity of the Rb protein, promoting cells to enter S phase.

The mutant p53 we used (p53-SCX3) has a mutation, valine to alanine, at residue 143, in the region of the activating mutations (27). This mutant failed to suppress Rb transcription. Two other transformation-competent p53 mutants, p53-176 (28) and p53-KH215 (29), also showed loss of ability to suppress Rb transcription (unpublished data). There may be a direct correlation between the transcriptional regulator activity of p53 and its ability to suppress transformation. A similar possibility has been suggested from the results of p53–GAL4 fusion experiments (5).

The experiments with the p53 deletion mutants revealed that N-terminal acidic and C-terminal basic domains of p53 are required for suppression of Rb transcription. The N-terminal domain of p53 contains an "acidic blob" structure characteristic of many transactivators (30–32). In the GAL4 fusion system, this acidic domain of p53 strongly activated transcription in mammalian cells (4). Our results confirm the importance of the acidic domain of p53 for transcriptional regulation. The C-terminal basic domain of p53 can form helix–turn–helix motifs (1, 19, 20) and may bind to DNA. Our finding that the C-terminal basic domain of p53 is necessary for suppression of Rb transcription raises the intriguing possibility that wild-type p53 may exert its suppressive effect by binding to a specific DNA sequence through its C-terminal basic domain. In addition, the rate of activation of Rb transcription was significantly different among three deletion mutants and a point mutant, SCX3. One interpretation of this differential effect is that multiple functions of the p53 protein

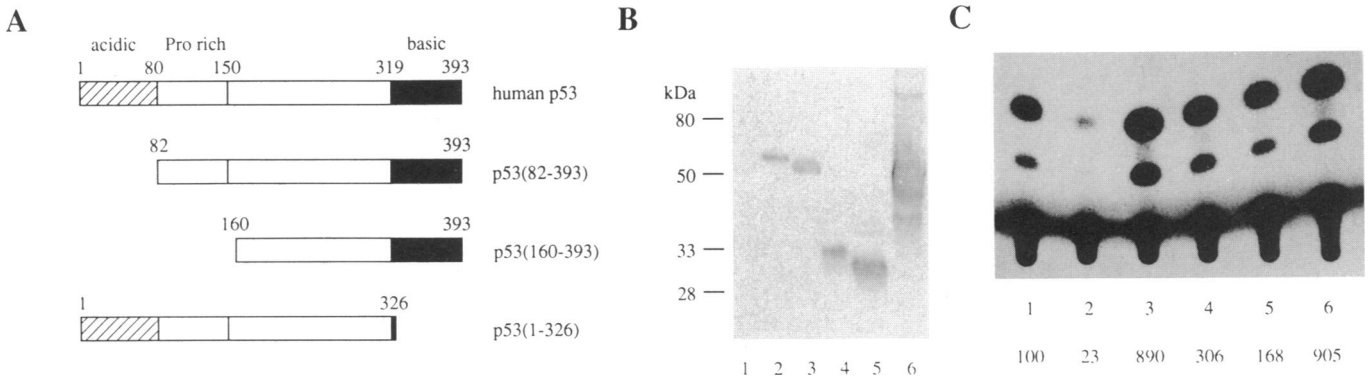

FIG. 5. Effect of p53 deletion mutants on Rb transcription. (*A*) Schematic representation of the domains of the p53 protein and the p53 deletion mutants used. Numbering corresponds to the amino acid sequence. (*B*) Immunoblot for the p53 proteins after transfection of HeLa cells with each p53 expression construct. p53 expression constructs were as follows: pME18S vector, lane 1; pME18S-SN3, lane 2; pME18S-SCX3, lane 3; pME18S-p53(82–393), lane 4; pME18S-p53(160–393), lane 5; pME18S-p53(1–326), lane 6. The anti-p53 antibodies used are PAb122 (lanes 1–5) and PAb1801 (lane 6). (*C*) Levels of CAT activity after cotransfection of pRbCAT2 with pME18S vector (lane 1), pME18S-SN3 (lane 2), pME18S-SCX3 (lane 3), pME18S-p53(82–393) (lane 4), pME18S-p53(160–393) (lane 5), or pME18S-p53(1–326) (lane 6) into HeLa cells.

are required for suppression of Rb transcription and that the effect of loss of one function may be different from that of another function. This differential effect of p53 deletion mutants may serve as a model system to elucidate the role of each p53 domain. The molecular mechanism by which p53 regulates transcription of the target gene is yet to be established, but the present findings should provide clues to the role of p53 in human carcinogenesis.

After having prepared this manuscript, Bargonetti *et al.* (33) reported that wild-type p53 binds to the GC box region of SV40 DNA. Just in the center of the DNase I-protected region, there exists a "GGAACTGG" sequence (+57 to +64 of SV40 DNA) that fits well the G(G/C)AA(G/C)TGA sequence we have proposed (33). Furthermore, two human DNA sequences shown to bind to wild-type p53 (34) also contain the sequences closely resembling the G(G/C)AA(G/C)TGA sequence. These findings further support the authenticity of the proposed p53 control element.

pSV2CAT contains the SV40 promoter/enhancer region, including the above-mentioned p53 binding site (33), but transcription of this construct was not affected by wild-type p53 in HeLa cells. However, in some cell lines transcription of pSV2CAT was suppressed by wild-type p53 (unpublished data). The reason for this difference is unknown, but the difference in transcriptional milieu may affect the action of p53 on the p53 binding sites. In any case, the fact that at least one promoter does not respond to p53 in HeLa cells argues against a general shutoff of the transcriptional machinery.

We thank Dr. Wen-Hwa Lee for pRbCAT2 and Dr. Bert Vogelstein for pC53-SN3 and pC53-SCX3. We are grateful to Drs. Katsuhisa Nakajima and Dong Wan Kim for helpful advice. CV-1 cells and TIG-3 cells were obtained from the Japanese Cancer Research Resources Bank. This work was supported by Grants-in-Aid from the Ministry of Education, Science, and Culture of Japan.

1. Levine, A. J. & Momand, J. (1990) *Biochim. Biophys. Acta* **1032**, 119–136.
2. Marshall, C. J. (1991) *Cell* **64**, 313–326.
3. Weinberg, R. A. (1991) *Science* **254**, 1138–1146.
4. Fields, S. & Jang, S. K. (1990) *Science* **249**, 1046–1049.
5. Raycroft, L., Wu, H. & Lozano, G. (1990) *Science* **249**, 1049–1051.
6. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. V. & Vogelstein, B. (1990) *Science* **249**, 912–915.
7. Maruyama, K. & Yokota, T. (1991) in *Shin-seikagaku-jikken-koza*, eds. The Japanese Biochemical Society (Tokyo-kagaku-dojin, Tokyo), Vol. 7, pp. 123–133 (in Japanese).
8. Takebe, Y., Seiki, M., Fujisawa, J.-I., Hoy, P., Yokota, K., Arai, K.-I., Yoshida, M. & Arai, N. (1988) *Mol. Cell. Biol.* **8**, 466–472.
9. Gurney, E. G., Harrison, R. O. & Fenno, J. (1980) *J. Virol.* **34**, 752–763.
10. Banks, L., Matlashewski, G. & Crawford, L. (1986) *Eur. J. Biochem.* **159**, 529–534.
11. Hong, F. D., Huang, H.-J. S., To, H., Young, L.-J. S., Oro, A., Bookstein, R., Lee, E. Y.-H. P. & Lee, W.-H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5502–5506.
12. Sakai, T., Ohtani, N., McGee, T. L., Robbins, P. D. & Dryja, T. P. (1991) *Nature (London)* **353**, 83–86.
13. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
14. Matsuo, M., Kaji, K., Utakoji, T. & Hosoda, K. (1982) *J. Gerontol.* **37**, 33–37.
15. Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. (1978) *Cell* **14**, 725–731.
16. Banks, L., Spence, P., Androphy, E., Hubbert, N., Matlashewski, G., Murray, A. & Crawford, L. (1987) *J. Gen. Virol.* **68**, 1351–1359.
17. Werness, B. A., Levine, A. J. & Howley, P. M. (1990) *Science* **248**, 76–79.
18. Masuda, H., Miller, C., Koeffler, H. P., Battifora, H. & Cline, M. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7716–7719.
19. Pennica, D., Goeddel, D. V., Hayflick, J. S., Reich, N. C., Anderson, C. W. & Levine, A. J. (1984) *Virology* **134**, 477–482.
20. Soussi, T., Caron de Fromentel, C. & May, P. (1990) *Oncogene* **5**, 945–952.
21. Robbins, P. D., Horowitz, J. M. & Mulligan, R. C. (1990) *Nature (London)* **346**, 668–671.
22. Dou, Q.-P., Fridovich-Keil, J. L. & Pardee, A. B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1157–1161.
23. Reich, N. C. & Levine, A. J. (1984) *Nature (London)* **308**, 199–201.
24. Milner, J. & Milner, S. (1981) *Virology* **112**, 785–788.
25. Ludlow, J. W., DeCaprio, J. A., Huang, C.-M., Lee, W.-H., Paucha, E. & Livingston, D. M. (1989) *Cell* **56**, 57–65.
26. Mittnacht, S. & Weinberg, R. A. (1991) *Cell* **65**, 381–393.
27. Finlay, C. A., Hinds, P. W., Tan, T.-H., Eliyahu, D., Oren, M. & Levine, A. J. (1988) *Mol. Cell. Biol.* **8**, 531–539.
28. Zakut-Houri, R., Oren, M., Bienz, B., Lavie, V., Hazum, S. & Givol, D. (1983) *Nature (London)* **306**, 594–597.
29. Tan, T.-H., Wallis, J. & Levine, A. J. (1986) *J. Virol.* **59**, 574–583.
30. Ma, J. & Ptashne, M. (1987) *Cell* **48**, 847–853.
31. Triesenberg, S. J., Kingsbury, R. C. & McKnight, S. L. (1988) *Genes Dev.* **2**, 718–729.
32. Hollenberg, S. M. & Evans, R. M. (1988) *Cell* **55**, 899–906.
33. Bargonetti, J., Friedman, P. N., Kern, S. E., Vogelstein, B. & Prives, C. (1991) *Cell* **65**, 1083–1091.
34. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C. & Vogelstein, B. (1991) *Science* **252**, 1708–1711.
35. Dou, Q.-P., Markell, P. J. & Pardee, A. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3256–3260.