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Transcriptional activation of the human proliferating-cell nuclear antigen promoter by p53

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ABSTRACT **Proliferating-cell nuclear antigen (PCNA) is a DNA damage-inducible protein that performs an essential function in DNA replication and repair as an auxiliary factor for DNA polymerases δ and ε. Examination of the human PCNA promoter DNA sequence revealed a site with homology to the consensus DNA sequence bound by p53. PCNA promoter fragments with this site intact bound p53 *in vitro* and were transcriptionally activated by wild-type p53 in transient expression assays in SAOS-2 cells. The resident p53-binding site could be functionally substituted by a previously described p53-binding site from the ribosomal gene cluster. A plasmid expressing a mutated version of p53 derived from a patient with Li–Fraumeni syndrome failed to activate the PCNA promoter in the cotransfection assay. In different cell types, activation of the PCNA promoter by the p53-binding sequence correlated with the status of p53. Activation of the PCNA promoter by wild-type p53 depends upon the level of p53 expression. This concentration dependence and cell type specificity reconciles the observations presented here with prior results indicating that wild-type p53 represses the PCNA promoter. These findings provide a mechanism whereby p53 modulates activation of PCNA expression as a cellular response to DNA damage.**

In human cancers, frequent mutations in the gene encoding the p53 tumor suppressor protein highlight the importance of p53 in the control of cell growth and suppression of neoplastic transformation (1–4). Wild-type p53 is a nuclear phosphoprotein that can activate or repress transcription (2, 3, 5). In accord with its role in the regulation of cell proliferation, wild-type p53 induces growth arrest and suppresses transformation by a variety of cellular and viral oncogenes (3, 4). In contrast, mutant alleles of p53 cooperate with activated *ras* in cotransfection assays to transform primary cells (3, 4). Agents that damage cellular DNA cause an increase in cellular p53 levels, and elevated p53 levels correlate with cell cycle arrest or with apoptosis (5–9). Cells lacking wild-type p53 lost the ability to arrest after DNA damage (8). Mice lacking the p53 gene develop normally but are prone to an early onset of cancer (10). Moreover, thymocytes from mice with an ablated p53 gene are resistant to induction of apoptosis by irradiation (11). These observations and others (for review, see refs. 1–4, 12, and 13) suggest that p53 regulates DNA replication, DNA repair, and programmed cell death; however, the mechanisms by which p53 elicits these important cellular functions remain unknown.

The preponderance of data suggests that the regulatory activities of p53 are related to its function as a transcription factor (1–4). Activation of transcription by wild-type p53 correlates with its binding to a specific DNA sequence (2, 3). The consensus DNA sequence specific for p53 binding consists of two copies of the inverted repeat sequence [RRRC(A/T)(A/T)GYYY] separated by 0–13 nucleotides (2, 3). A number of genes that contain a p53-binding site are transcriptionally activated *in vivo* and *in vitro* by p53 (2, 3, 5). The DNA binding and transcriptional activation properties of p53 may be regulated by its posttranslational modifications or by interactions of p53 with other cellular or viral proteins (3). Transcriptional repression by wild-type p53 *in vivo* and *in vitro* appears to be mediated [with some exceptions (14, 15)] through a more general mechanism that involves DNA elements of the basal promoter (3, 16). Wild-type p53 represses transcription from minimal promoters containing a TATA motif, whereas initiator-directed transcription from minimal promoters lacking a TATA box resists repression by p53 (16). The TATA-mediated mechanism for p53 repression of transcription is consistent with the observation that p53 can bind directly to the TATA-binding protein of the transcription factor TFIID complex (17–19). Although TFIID is also required for transcription initiation of TATA-less promoters (20, 21), the transcription initiation pathways of promoters with and without a TATA motif may differ sufficiently to provide the means for distinct responses to p53 (22).

Relevant target genes for the transcriptional regulatory functions of p53 must be identified to fully understand how p53 controls cell growth and suppresses neoplastic transformation. The proliferating-cell nuclear antigen (PCNA), or DNA polymerase δ auxiliary factor, is an essential DNA replication protein (23) that is increased in abundance in proliferating cells (24–26). Consistent with the role of PCNA in DNA replication, its promoter is activated in HeLa cells by coexpression of the adenovirus E1A protein (27). Sequences 50 nucleotides upstream of the transcription initiation site with homology to the consensus site bound by the activating transcription factor mediate activation of the PCNA promoter by E1A (27, 28). Mutant E1A proteins that fail to induce DNA synthesis in quiescent cells also fail to activate the PCNA promoter in HeLa cells (29).

PCNA is also required for DNA repair (30–33) and in this capacity it is induced concomitantly with increasing p53 levels in human skin cells exposed to ultraviolet radiation (34). The DNA repair function of PCNA and its coexpression pattern with p53 suggest that the PCNA promoter is a target for transcriptional regulation by p53. Moreover, PCNA can be found in a quaternary complex with cyclins, cyclin-dependent kinases, and p21, a recently identified inhibitor of cyclin-dependent kinases that is transcriptionally regulated by p53 (35, 36). p21 inhibits PCNA DNA replication activity by direct binding (37) and permits PCNA DNA repair functions (32).

We have identified a sequence in the PCNA promoter that is homologous to the DNA consensus sequence bound by p53. This sequence bound p53 *in vitro* and mediated activation of the PCNA promoter by wild-type p53 upon coexpression in p53-deficient SOAS-2 cells. In contrast, a PCNA promoter

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Abbreviations: PCNA, proliferating-cell nuclear antigen; CAT, chloramphenicol acetyltransferase; RGC, ribosomal gene cluster.

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construct with the p53-binding site deleted was repressed by coexpression of wild-type p53. Replacement of the p53-binding site with a heterologous p53 binding site restored activation by wild-type p53. Expression of an oncogenic mutant form of p53 failed to activate expression from any of the PCNA promoter–chloramphenicol acetyltransferase (CAT) constructs in the coexpression assay. The p53-binding sequence activated the PCNA promoter in cells containing, but not in cells lacking, wild-type p53. Activation of the PCNA promoter by wild-type p53 depended upon the level of p53 expression. These observations suggest a cellular response to DNA damage (9, 34, 38) whereby p53 activates expression of PCNA.

MATERIALS AND METHODS

Plasmids. The PCNA CAT construct containing nucleotides −249 to +62 was described (39) and was referred to as +60. The PCNA–CAT construct containing nucleotides −213 to +62 was prepared by digesting with *Eag* I and cloning the desired restriction fragment between the *Xho* I and *Hin*dIII sites of pBACAT. The sequence of the upstream border of −213 PCNA–CAT from the common *Xho* I site is ctcgaggGGCCGGG (with PCNA promoter sequences in uppercase type). As described for the −249 to +62 PCNA–CAT construct, the downstream border was formed at the *Nru* I site (+62, previously +60; ref. 39) in the PCNA promoter with the addition of a *Hin*dIII linker. The RGC213 PCNA–CAT construct was prepared by inserting wild-type p53-binding sequences from the ribosomal gene cluster (RGC) into a *Xho* I site upstream of the −213 PCNA–CAT construct as shown below (with the RGC sequence underlined).

ctcgagTTTTGCCTGGACTTAGCCTGGCCTTG

213

CCTTTTCtcgaggGGCCGGG

Cells. SAOS-2 cells were obtained from American Type Culture Collection (ATCC). Monolayer cultures were grown in McCoy's medium with 15% (vol/vol) fetal bovine serum, penicillin (100 μg/ml), and streptomycin (100 μg/ml). HeLa cells were obtained from American Type Culture Collection. Monolayer cultures were grown in Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum, and penicillin and streptomycin as above. CREF cells were obtained from P. Fisher (Columbia University). Monolayer cultures were grown in DMEM, 5% fetal bovine serum, and penicillin and streptomycin as above. Baby rat kidney (BRK) cells were prepared and grown as described (40).

Transfection Assays. Transfection assays of all cell types were as described (39) with the exception of SAOS-2 cells. For SAOS-2, cells at 50–60% confluence in 6-cm dishes were transfected by the calcium phosphate method (39). Each transfection mixture contained 10 μg of the reporter plasmid and 0.5 μg of the p53 expression plasmid—human wild-type or mutant p53 [pC53-SN3 (41) and pCMVp53E258K, respectively]—driven by the human cytomegalovirus virus immediate-early promoter. In the absence of the p53-expressing plasmid in the transfection, 0.5 μg of pCMV12S.FS, a plasmid containing the human cytomegalovirus promoter directing expression of a nonfunctional E1A mRNA (27), was included as control. After 3.5 h, the DNA precipitate was removed and the cells were exposed to 15% (vol/vol) glycerol for 1 min followed by the addition of fresh medium. Cells were harvested 48 h after transfection. A 100-μl freeze–thaw extract was prepared from each plate of transfected cells and 50 μl of each extract was assayed for CAT activity as described (27). One CAT unit is defined as 1% conversion of chloramphenicol to its acetylated form per h per 100-μl reaction mixture (50-μl cell extract) at 37°C.

Gel Mobility Shift Assays. Epitope-tagged wild-type human p53 (42, 43) was purified from baculovirus-infected insect cells by immunoaffinity methods as described (44). The binding reaction was as follows: 1 μg of purified p53 prepared was incubated with bovine serum albumin (22 μg/ml)/0.2 mM dithiothreitol/20 mM Tris, pH 8.0/50 mM NaCl/5% glycerol/0.02 Nonidet P-40/poly(dI·dC) (6.67 μg/ml)/10^4 cpm of ^{32}P-labeled DNA in a final volume of 15 μl. In some experiments, 1 μl of PAb421 ascites fluid (Oncogene Science) was added. After 30 min at 22°C, the binding reaction mixtures were resolved on a 5% polyacrylamide gel containing 0.5× TBE. The gel was electrophoresed for 1 h at 200 V and dried for autoradiography. Double-stranded oligonucleotide probes for the gel shift assays were end-labeled with [^{32}P]dCTP and the Klenow fragment of DNA polymerase I (45).

RESULTS

Examination of the PCNA promoter between nucleotides −217 and −236 revealed that 18 out of 20 nucleotides match the p53-binding consensus.

p53 consensus sequence RRRCWWGYYYRRRCWWGYYY

PCNA promoter sequence GAACAAGTCCGGGCATATGT

−217 −236

R is a purine, Y is a pyrimidine, and W is A or T. To determine whether p53 can bind to this site *in vitro*, gel mobility shift

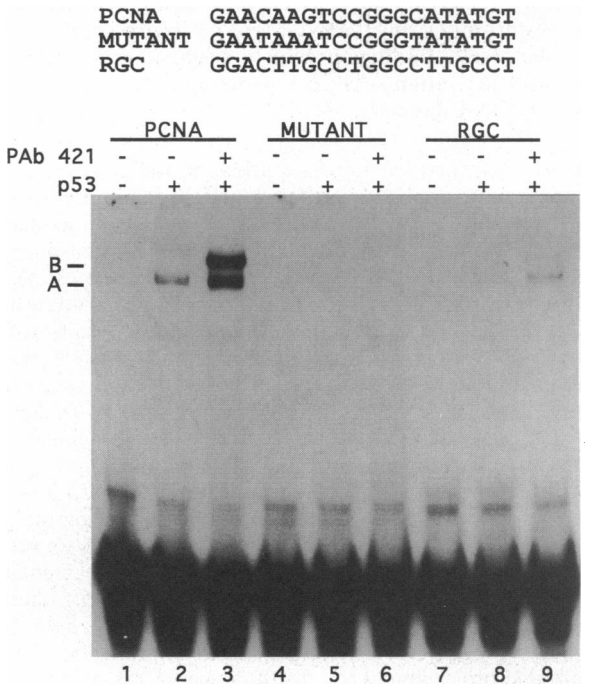

FIG. 1. Gel mobility shift assays with oligonucleotides corresponding to the PCNA promoter. Double-stranded oligonucleotides corresponding to the wild-type PCNA promoter sequence (PCNA), a mutated version designed to disrupt p53 binding (MUTANT), or the p53 binding sequence from the RGC were employed in gel mobility shift assays with purified wild-type human p53. The wild-type PCNA oligonucleotide was incubated without protein (lane 1), with purified human p53 (lane 2), or with purified p53 and monoclonal antibody PAb421 (lane 3). Similar analyses were performed with the mutated PCNA sequence (lanes 4–6) and the RGC motif (lanes 6–9). Band A is a specific complex formed with the wild-type human p53 and the PCNA promoter sequence in lanes 2 and 3. Band B is a slower migrating complex formed upon addition of monoclonal antibody specific for p53.

assays were performed with a double-stranded oligonucleotide corresponding to the PCNA promoter sequence (Fig. 1). Wild-type human p53 purified from insect cells infected with a recombinant baculovirus produced a specific complex (band A, lane 2). A slower-migrating complex (band B) appeared upon addition of a monoclonal antibody, PAb421, specific for p53 to the gel shift assay (Fig. 1, lane 3). Similar complexes were formed when the PCNA promoter oligonucleotide was replaced by an oligonucleotide corresponding to the p53-binding site from the RGC (46). No such complexes were formed with a mutant PCNA sequence harboring changes in the conserved nucleotides C4 and G7 that were expected (47) to disrupt p53 binding (Fig. 1, lanes 5 and 6). These data indicate that sequences in the PCNA promoter bind wild-type human p53 specifically; consideration of the relative band intensities (compare lanes 2 and 3 with lanes 8 and 9) suggests that the affinity of p53 for the PCNA sequence is greater than that for the RGC sequence.

Several studies have explored the regulation of human PCNA expression by wild-type p53 with various results (16, 48–51). Since DNA sequences that bind p53 mediate transcriptional activation by the protein (2), one might expect that binding of p53 to the PCNA p53-binding site would activate transcription from the PCNA promoter. To test this prediction, we first determined whether wild-type p53 affects transcription from the PCNA promoter in SAOS-2 cells. This human osteosarcoma cell line is devoid of p53 as a result of a gene deletion (41). SAOS-2 cells were employed in two previous reports indicating that the PCNA promoter is repressed by coexpression of wild-type p53 (48, 49). A cotransfected plasmid encoding wild-type human p53 stimulated PCNA–CAT expression approximately 4.5-fold (Fig. 2*B*) from a reporter plasmid containing PCNA promoter sequences from nucleotides −1265 to +62 directing CAT gene expression (−1265 PCNA–CAT, Fig. 2*A*). The stimulation was greatest at low amounts (0.25 and 0.5 μg) and essentially disappeared with higher amounts of the p53 expression vector. A similar response was obtained with a shorter form of the promoter (−249 PCNA–CAT; Fig. 2*A*), which retains the p53-binding site (Fig. 2*C*). Further truncation of the promoter, giving the −213 PCNA–CAT construct (Fig. 2*A*), removes this site and abrogated the stimulation entirely (Fig. 2*C*). Comparable results were also obtained in HeLa cells that are also p53-deficient (data not shown).

The results shown in Fig. 2 suggested that p53 stimulates the PCNA promoter in a concentration-dependent fashion and implicated the p53-binding site in the response. The data of Table 1 support these inferences. To confirm that the p53-binding site is required, a previously defined heterologous p53-binding site from the RGC (46) was inserted upstream of position −213 in place of the natural p53-binding site, to produce the RGC213 reporter construct (Fig. 2*A*). This substitution restored transcriptional activation by wild-type p53 to the −213 promoter (Table 1) even though the homology between the p53-binding site in RGC and the p53-binding site in the PCNA promoter is only 8 of 20 nucleotides. To confirm that p53 mediated the stimulation, we assayed a transdominant mutant of p53 with a Glu → Lys change at amino acid 258 (52, 53). Cotransfection with a plasmid expressing this mutant p53 had little effect on any of the PCNA–CAT promoter constructs (Table 1). We conclude that sequences between positions −249 and −213 within the PCNA promoter that bind p53 *in vitro* mediate activation by wild-type p53 *in vivo*.

To explore the generality of this finding, we assayed the two PCNA–CAT constructs containing (−249) and lacking (−213) the p53-binding site (Fig. 2*A*) in three different cell lines that display variable activity of wild-type p53 (Table 2). As before (Table 1) in SAOS-2 cells, which lack p53, removal of the p53-binding site activated PCNA–CAT expression by about 75% (Table 2). In HeLa cells, p53 is bound to the human papillomavirus E6 protein, which targets p53 for degradation (54).

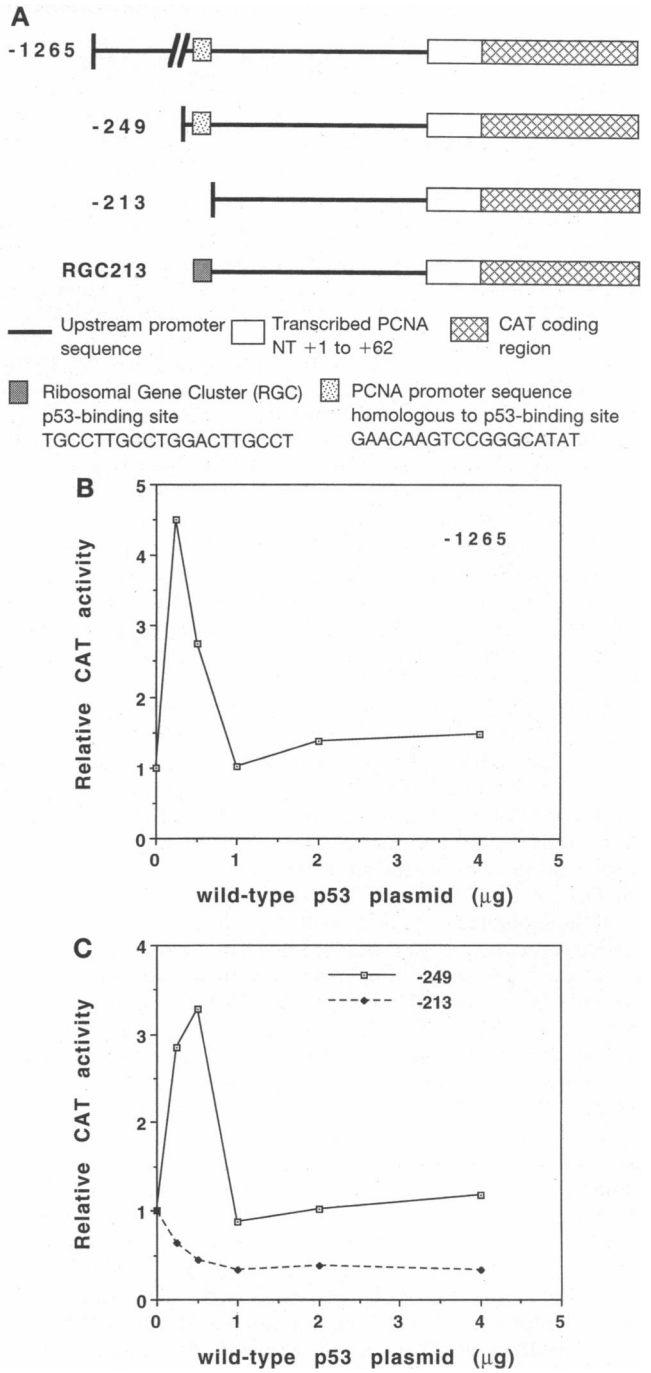

FIG. 2. Low levels of wild-type p53 expression activate the PCNA promoter via the PCNA p53-binding site. (*A*) Diagram of PCNA–CAT constructs. Cloned human PCNA promoter fragments were fused to the CAT reporter gene in pBACAT. (*B*) The −1265 to +62 PCNA–CAT construct (10 μg) was cotransfected with increasing amounts of the wild-type p53-expressing plasmid (pC53-SN3) into SAOS-2 cells. The CAT activity obtained in the absence of the p53-expressing plasmid was normalized to one. The results shown are from a single experiment performed in duplicate. (*C*) PCNA–CAT constructs (10 μg) with (−249 to +62 PCNA–CAT) and without (−213 to +62 PCNA–CAT) the p53-binding site were cotransfected with increasing amounts of the wild-type p53-expressing plasmid into SAOS-2 cells. The CAT activity obtained with each PCNA–CAT construct in the absence of the p53-expressing plasmid was normalized to one. The results shown are from a single transfection performed in duplicate. Similar results were obtained upon repetition of the experiment.

CAT expression from the −213 construct was reduced by one-third relative to that from the −249 construct in HeLa

Table 1. Wild-type p53 activates PCNA expression in SAOS-2 cells

PCNA–CAT construct	CAT expression			
	Control	+ wt p53	+ mutant p53	*n*
−249	12.7 ± 5.6	31.5 ± 11.2	11.2 ± 4.1	5
−213	24.1 ± 7.6	6.2 ± 1.4	19.3 ± 5.7	4
RGC213	24.6 ± 1.9	94.1 ± 22.9	18.6 ± 4.5	3

Values for CAT expression are the average values for CAT activity in relative units with standard deviation for the indicated number of experiments (*n*). Each experiment was performed in duplicate.

cells (Table 2), indicating that removal of the p53-binding site in the PCNA promoter exerted little effect on PCNA–CAT activity in these cells. In contrast to these relatively small effects, in primary baby rat kidney (BRK) cell cultures removal of the p53-binding site from the PCNA promoter reduced PCNA–CAT expression by about 4-fold (Table 2). This observation supports the view that the p53 binding site in the PCNA promoter mediates transcriptional activation in cells expressing wild-type p53. Removal of the p53 binding site also reduced PCNA–CAT expression by about 3-fold in CREF cells (Table 2). The status of p53 in CREF cells appears to be wild type by two criteria: an authentic p53 binding site can activate transcription in CREF cells (see below) and CREF cells are a clonal derivative of rat embryo fibroblasts (55) that undergo wild-type p53-mediated apoptosis upon expression of the adenovirus E1A 243R protein (56, 57). Thus, the PCNA promoter sequences that bind p53 *in vitro* mediate activation of the promoter in cells with wild-type p53 expression (CREF and BRK cells) and these sequences do not activate the promoter in cells lacking wild-type p53 expression (HeLa and SAOS-2 cells).

If the sequence from nucleotides −236 to −217 in the PCNA promoter constitutes a p53-binding site that mediates cell-type-specific transcriptional activation by wild-type p53, introduction of a heterologous p53-binding site should restore activity to the −213 PCNA–CAT construct in the same cell-type-specific manner. To test this prediction the RGC213 construct (Fig. 2*A*) was also assayed in the same four cell types (Table 2). In BRK and CREF cells, the heterologous p53 binding site restored promoter activity in the RGC213 construct to levels comparable to those obtained with the −249 construct in these two cell types (Table 2). In SAOS-2 and HeLa cells, however, insertion of the heterologous p53 binding site had no effect on, or slightly stimulated, the promoter activity of the −213 construct (Table 2). We conclude from these data that the PCNA promoter sequence can be functionally replaced by a previously defined wild-type p53-binding sequence from the RGC in cells with wild-type p53 expression (BRK and CREF cells). Moreover, these observations suggest that the principal function of these sequences in the PCNA promoter correlates with p53 binding. In the absence of functional p53 (HeLa and SAOS-2 cells), deletion of the PCNA p53-binding sequence or its replacement with the RGC sequence has minimal effect. On the other hand, this site is responsible for stimulation of PCNA promoter expression in cells that contain functional p53.

Table 2. Activation of PCNA–CAT expression by PCNA sequences between −249 and −213 depends on the status of p53 in a given cell type

Cell type	p53 status	CAT expression			
		−249	−213	RGC213	*n*
HeLa	Bound by HPV E6	1.0	0.66 ± 0.17	1.54 ± 0.5	3
SAOS-2	Absent	1.0	1.72 ± 0.96	2.35 ± 0.86	3
BRK	wt	1.0	0.24 ± 0.08	1.32 ± 0.26	3
CREF	wt?	1.0	0.34 ± 0.07	1.18 ± 0.39	6

CAT values are normalized to those obtained with the −249 construct with CMV12S.FS in the different cell types. The average relative CAT activity with standard deviation of the indicated PCNA–CAT construct is shown with the indicated number of experiments (*n*). Each experiment was assayed in duplicate. HPV, human papillomavirus.

DISCUSSION

Our data indicate that wild-type p53 binds to a site between nucleotides −217 and −236 in the human PCNA promoter and activates expression. These observations are compatible with the functions of p53, as an inducer of DNA repair (6, 8, 33), and PCNA, as a DNA repair protein (30–32). Thus, activation of the PCNA promoter by p53 would constitute a cellular response to DNA damage and is consistent with previous observations indicating that DNA damaging agents activate expression of both p53 and PCNA (G.F.M., unpublished results and refs. 6, 9, 34, and 38).

p53 has multiple and far-reaching effects on cells and its effects on the human PCNA promoter can vary from cell type to cell type and with p53 concentration. The results shown here provide a basis for understanding the various effects reported (16, 48–51). In a study of the human glioblastoma cell line GM47.23, which is transformed with a gene expressing wild-type p53 from a steroid inducible promoter, induction of p53 expression reduced PCNA mRNA levels (51). However, it was not clear whether this reduction was a direct effect of wild-type p53 or an indirect consequence of p53-induced growth arrest, and the step in PCNA mRNA biosynthesis that was inhibited was not identified. In the human cervical epithelial cell line C33A, coexpression of wild-type p53 had no effect on human PCNA–CAT expression (16). Additional studies showed that wild-type p53 repressed expression from a human PCNA–CAT reporter in HeLa and Vero cells (50). These results were expanded in subsequent experiments demonstrating a 2- to 4-fold reduction in PCNA–CAT expression by wild-type p53 in SAOS-2 cells (48, 49), the p53-minus cell line employed in the experiments described herein. Mutant p53 transactivated PCNA–CAT expression in HeLa and SAOS-2 cells through a sequence with homology to an activating transcription factor binding site in the PCNA promoter (48, 49). The mouse PCNA promoter is also repressed by coexpression of wild-type p53 (58), but no sequence that mediates transcriptional repression of the PCNA promoter by p53 has been identified (16, 50, 58).

On the other hand, we show here that transcriptional activation of the PCNA promoter by wild-type p53 in SAOS-2 cells is mediated by sequences that bind p53 *in vitro*. Both the −249 and the longer −1265 PCNA–CAT constructs were activated in a dose-dependent fashion (Fig. 2). These cells were chosen as an experimental model because the absence of p53 expression in SAOS-2 cells precludes complicated interactions of the transiently expressed wild-type protein with endogenous wild-type or mutant p53. The data shown in Fig. 2 indicate a possible explanation for the discordant reports of p53 regulation of PCNA expression; i.e., the level of p53 protein affects the response of the PCNA promoter. Furthermore, this concentration-dependent response of the PCNA promoter to p53 raises the possibility of differential cellular responses to DNA damage in which p53 can either activate or repress PCNA expression. Such transcriptional regulation of a DNA repair protein by p53 would be consistent with its functions as an activator of DNA repair (e.g., in the activation of the PCNA promoter) and an inducer of apoptosis (e.g., in the repression of the PCNA promoter). However, it is not likely that p53 acts alone to regulate the PCNA promoter, independent of other cellular factors. For example, p53 also activates expression of MDM-2, which can, in turn, antagonize the transcriptional effects of p53 (59). The binding affinity of p53 to DNA can also

be modulated (60, 61). So the concentration of p53 is likely to be only one of many variables in the regulation of PCNA expression.

The demonstration that PCNA, a DNA replication and repair protein, is regulated by p53 provides an ideal model system for understanding the functions of p53 as a regulator of DNA synthesis and repair processes. In addition, the recent finding that PCNA is associated with cell cycle regulatory proteins (62–64) suggests a mechanism whereby cellular PCNA levels are linked to cell cycle progression. Since cyclin-dependent kinases can also alter p53 binding affinities for some DNA sequences (60), it will be interesting to determine whether p53 binding to PCNA promoter sequences is altered by PCNA-associated kinases.

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1. Oren, M. (1992) *FASEB J.* **6,** 3169–3176.
2. Vogelstein, B. & Kinzler, K. W. (1992) *Cell* **70,** 523–526.
3. Zambetti, G. P. & Levine, A. J. (1993) *FASEB J.* **7,** 855–865.
4. Donehower, L. A. & Bradley, A. (1993) *Biochim. Biophys. Acta* **1155,** 181–205.
5. Farmer, G., Bargonetti, J., Zhu, H., Friedman, P., Prywes, R. & Prives, C. (1992) *Nature (London)* **358,** 83–86.
6. Zhan, Q., Carrier, F. & Fornace, A. J. (1993) *Mol. Cell. Biol.* **13,** 4242–4250.
7. Lu, X. & Lane, D. P. (1993) *Cell* **75,** 765–778.
8. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. & Fornace, A. J. (1992) *Cell* **71,** 587–597.
9. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. W. (1991) *Cancer Res.* **51,** 6304–6311.
10. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Butel, J. S. & Bradley, A. (1992) *Nature (London)* **356,** 215–221.
11. Lowe, S. W., Schmitt, E. M., Smith, N. W., Osborne, B. A. & Jacks, T. (1993) *Nature (London)* **362,** 847–852.
12. Prives, C. & Manfredi, J. J. (1993) *Genes Dev.* **7,** 529–534.
13. Harris, C. G. & Hollstein, M. (1993) *N. Engl. J. Med.* **329,** 1318–1327.
14. Agoff, S. N., Hou, J., Linzer, D. I. & Wu, B. (1993) *Science* **259,** 84–87.
15. Shiio, Y., Yamamoto, T. & Yamaguchi, N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5206–5210.
16. Mack, D. H., Vartikar, J., Pipas, J. M. & Laimins, L. A. (1993) *Nature (London)* **363,** 281–283.
17. Liu, X., Miller, C. W., Koeffler, P. H. & Berk, A. J. (1993) *Mol. Cell. Biol.* **13,** 3292–3300.
18. Seto, E., Usheva, A., Zambetti, G. P., Momand, J., Horikoshi, N., Weinmann, R., Levine, A. J. & Shenk, T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12028–12032.
19. Martin, D. W., Munoz, R. M., Subler, M. A. & Deb, S. (1993) *J. Biol. Chem.* **268,** 13062–13067.
20. Smale, S. T., Schmidt, M. C., Berk, A. J. & Baltimore, D. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4509–4513.
21. Pugh, B. F. & Tjian, R. (1991) *Genes Dev.* **5,** 1935–1945.
22. Ragimov, N., Kruskopf, A., Navot, N., Rotter, V., Oren, M. & Aloni, Y. (1993) *Oncogene* **8,** 1183–1193.
23. Prelich, G., Kostura, M., Marshak, D. R., Mathews, M. B. & Stillman, B. (1987) *Nature (London)* **326,** 471–475.
24. Matsumoto, K., Moriuchi, T., Koji, T. & Nakane, P. (1987) *EMBO J.* **6,** 637–642.
25. Jaskulski, D., Gatti, C., Travali, S., Calabretta, B. & Baserga, R. (1988) *J. Biol. Chem.* **263,** 10175–10179.
26. Almendral, J. M., Huebsch, D., Blundell, P. A., Macdonald-Bravo, H. & Bravo, R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1575–1579.
27. Morris, G. F. & Mathews, M. B. (1991) *J. Virol.* **65,** 6397–6406.
28. Labrie, C., Morris, G. F. & Mathews, M. B. (1993) *Mol. Cell. Biol.* **13,** 1697–1707.
29. Kannabiran, C., Morris, G. F., Labrie, C. & Mathews, M. B. (1993) *J. Virol.* **65,** 507–515.
30. Nichols, A. F. & Sancar, A. (1992) *Nucleic Acids Res.* **20,** 2441–2446.
31. Shivji, M. K. K., Kenny, M. K. & Wood, R. D. (1992) *Cell* **69,** 367–374.
32. Li, R., Waga, S., Hannon, G., Beach, D. & Stillman, B. (1994) *Nature (London)* **371,** 534–537.
33. Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C. Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M. & Fornace, A. J. (1994) *Science* **266,** 1376–1380.
34. Hall, P. A., McKee, P. H., Menage, H. D., Dover, R. & Lane, D. P. (1993) *Oncogene* **8,** 203–207.
35. El-Deiry, W., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W. & Vogelstein, B. (1993) *Cell* **75,** 817–825.
36. Harper, J. W., Adami, G. R., Wei, N., Keyomars, K. & Elledge, S. J. (1993) *Cell* **75,** 805–816.
37. Waga, S., Hannon, G., Beach, D. & Stillman, B. (1994) *Nature (London)* **369,** 574–578.
38. Celis, J. E. & Madsen, P. (1986) *FEBS Lett.* **209,** 277–283.
39. Morris, G. F. & Mathews, M. B. (1990) *J. Biol. Chem.* **265,** 16116–16125.
40. Moran, E., Grodzicker, T., Robert, R. J., Mathews, M. B. & Zerker, B. (1986) *J. Virol.* **57,** 765–775.
41. Diller, L., Kassel, J., Nelson, C. E., Gryka, M. A., Litwak, G., Gebhardt, M., Bressac, B., Ozturk, M., Baker, S., Vogelstein, B. & Friend, S. H. (1990) *Mol. Cell. Biol.* **10,** 5772–5781.
42. Bischoff, J. R., Casso, D. & Beach, D. (1992) *Mol. Cell. Biol.* **12,** 1405–1411.
43. Grussenmyer, T., Scheidtmann, K. H., Hutchinson, M. A., Eckhart, W. & Walter, G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7952–7954.
44. Spaargaren, M. & Bischoff, J. R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12609–12613.
45. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
46. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman,P., Prives, C. & Vogelstein, B. (1991) *Science* **252,** 1708–1711.
47. El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. & Vogelstein, B. (1992) *Nat. Genet.* **1,** 44–49.
48. Deb, S., Jackson, C. T., Subler, M. A. & Martin, D. W. (1992) *Virology* **66,** 6164–6170.
49. Jackson, P., Ridgway, P., Rayner, J., Noble, J. & Braithwaite, A. (1994) *Biochem. Biophys. Res. Commun.* **203,** 133–140.
50. Subler, M. A., Martin, D. W. & Deb, S. (1992) *J. Virol.* **66,** 4757–4762.
51. Mercer, W. E., Shields, M. T., Lin, D., Appella, E. & Ullrich, S. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1958–1962.
52. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A. & Friend, S. H. (1990) *Science* **250,** 1233–1238.
53. Srivastava, S., Wang, S., Tong, Y. A., Pirollo, K. & Chang, E. H. (1993) *Oncogene* **8,** 2449–2456.
54. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J. & Howley, P. M. (1991) *Cell* **63,** 1129–1136.
55. Fisher, P. B., Babiss, L. E., Weinstein, B. & Ginsberg, H. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3527–3531.
56. Debbas, M. & White, E. (1993) *Genes Dev.* **7,** 546–554.
57. Lowe, S. W. & Ruley, H. E. (1993) *Genes Dev.* **7,** 535–545.
58. Yamaguchi, M., Hayashi, Y., Matsuoka, S., Takahashi, T. & Matsukage, A. (1994) *Eur. J. Biochem.* **221,** 227–237.
59. Momand, J., Zambetti, G. P., Olson, D. C., George, D. & Levine, A. J. (1992) *Cell* **69,** 1237–1245.
60. Wang. Y. & Prives, C. (1995) *Nature (London)* **376,** 88–91.
61. Jayaraman, L. & Prives, C. (1995) *Cell* **81,** 1021–1029.
62. Zhang, H., Xiong, Y. & Beach, D. (1993) *Mol. Biol. Cell* **4,** 897–906.
63. Xiong, Y., Zhang, H. & Beach, D. (1993) *Genes Dev.* **7,** 1572–1583.
64. Xiong, Y., Zhang, H. & Beach, D. (1992) *Cell* **71,** 505–514.