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Sequence-specific transcriptional activation is essential for growth suppression by p53

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ABSTRACT **Although several biochemical features of p53 have been described, their relationship to tumor suppression remains uncertain. We have compared the ability of p53-derived proteins to act as sequence-specific transcriptional (SST) activators with their ability to suppress tumor cell growth, using an improved growth-suppression assay. Both naturally occurring and *in vitro* derived mutations that abrogated the SST activity of p53 lost the ability to suppress tumor cell growth. Additionally, the N- and C-terminal ends of p53 were shown to be functionally replaceable with foreign transactivation and dimerization domains, respectively, with concordant preservation of both SST and tumor-suppressive properties. Only the central region of p53, conferring specific DNA binding, was required to suppress growth by such hybrid proteins. The SST activity of p53 thus appeared to be essential for the protein to function as a tumor suppressor.**

Studies of human and murine p53 have provided evidence that the wild-type (wt) p53 protein can suppress cell proliferation, whereas mutant versions of p53 lose this ability (1). The existence of mature p53 "knockout" mice suggests that p53 is not essential for progression through the cell cycle, but the increased incidence of tumors in these animals confirms that expression of p53 can block uncontrolled cell division (2).

Biochemical studies have suggested several potential mechanisms underlying such p53-mediated growth suppression. First, p53 may function as a transcription factor. p53 has sequence-specific DNA-binding properties (3), and the N terminus of p53 is a potent transcriptional activator (TA) when fused to the DNA-binding domain of yeast protein GAL4 (4, 5). As a result of these two related activities (DNA binding and TA), wt p53 can stimulate the expression of reporter genes regulated by promoters containing p53 binding sites in both mammalian and yeast cells. Importantly, at least some mutants of p53 appear to have lost this activity (reviewed in ref. 6).

Conversely, p53 may mediate its growth inhibitory effect by acting as a transcriptional repressor. The wt p53 can repress a wide variety of cellular and viral promoters (reviewed in ref. 7), in particular those containing a TATA-box motif (8). Transcriptional repression mediated by wt p53 is unlikely to involve the direct binding of p53 protein to promoter sequences, because none of these promoters contain p53 binding sites. Instead, overexpression of wt p53 may modify the nuclear environment, perhaps by sequestering factors necessary for transcription (reviewed in ref. 9).

The relationship between the biochemical properties of p53 and its tumor-suppressive abilities has remained speculative. To determine the relationship between p53 sequence-specific transcriptional (SST) activity and p53-mediated tumor suppression, we have analyzed these activities by using an extensive set of *in vivo* and *in vitro* derived p53 mutants.

MATERIALS AND METHODS

Yeast and Mammalian Expression Vectors. Information on the construction of the expression vectors, including the sequences of primers used in PCR amplification, is available from the authors upon request.

Yeast and Mammalian Reporter Vectors. S201, 9H, and 3H are 326-, 467-, and 126-bp human genomic fragments, respectively, each containing a single p53 binding site (10). S201-lacZ, 9H-lacZ, and 3H-lacZ were constructed by inserting these genomic p53-binding DNA fragments into the *Sal* I site of the pCZ plasmid containing a minimal promoter and the *lacZ* reporter gene (11). WG½-lacZ, WG1-lacZ, and WG2-lacZ were derived by inserting either a half, one, or two copies of the oligonucleotide 5′-AGGCATGCCTAGGCATGCCT-3′ (10) into the *Sal* I and *Xho* I sites of pCZ. p53CON1 and p53CON2 were produced by ligating either one or two copies of 5′-GGACATGCCCGGGCATGTCC-3′ (12) into the *Sal* I site of pCZ. PG16-lacZ and PG16-CAT contained 16 copies of the human genomic p53 binding site 5′-CCTGCCTGGACTTGCCTGG-3′ (3).

Yeast Strains and β-Galactosidase Assays. The reporter and p53 expression vectors were introduced into *Saccharomyces cerevisiae* strain YPH681 (a generous gift of P. Hieter, Johns Hopkins University School of Medicine), and at least two clones were assayed for each transfection. β-Galactosidase assays were performed as described (13).

Cell Culture, Transfection, and Chloramphenicol Acetyltransferase (CAT) Analysis. For transient transfection, cultures of H1299 lung adenocarcinoma cells (graciously provided by A. Fornace, National Institutes of Health) or SW480 colorectal carcinoma cells were transfected with 2.5 μg of pCEP4-based expression vectors and 0.5 μg of CAT reporter vectors plus 25 μg of Lipofectin (Bethesda Research Laboratories). Cells were harvested at 24 hr for SST activity measurement and at 48 hr for the transcriptional repression experiments. CAT activity of the lysates was measured as described (14). For growth-suppression assays, cell cultures at 50% confluence were transfected with 5 μg of the indicated plasmids, and hygromycin B (450 μg/ml) was added 24 hr later. Colonies of 20 cells were counted after 10 days of selection.

Western Analysis. All the expression vectors directly compared in this study produced similar levels of p53-derived proteins, as assessed by Western analysis of yeast or transiently transfected H1299 cell lysates. Western blot analysis was performed with either PAb240 (for VP16–p53 hybrid protein detection) or PAb1801 (for all other proteins) antibodies (Oncogene Sciences).

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Abbreviations: SST, sequence-specific transcriptional; TA, transcriptional activator; CAT, chloramphenicol acetyltransferase; CC, coiled-coil; wt, wild type.

RESULTS

Tumor-Derived p53 Mutants Have Diminished SST Activity. Since the initial observations of wt p53 SST activity, several studies have suggested that some tumor-derived mutant forms of p53 retain such activity (15–17). If SST activity were essential for wt p53 growth suppression, it is difficult to envision how mutants which retain this activity could endow cells with a selective growth advantage *in vivo*. To better understand these discordant results, the effect of mutation on the ability of p53 to activate gene expression from a variety of p53 binding sites was examined.

A yeast-based transcription assay was used to test the ability of wt and mutant p53 to activate transcription from three different genomic DNA fragments containing p53 binding sites. *S. cerevisiae* cells were stably cotransformed with (*i*) a plasmid containing a *lacZ* reporter gene downstream of genomic fragments and (*ii*) a galactose-inducible expression vector that produced wt or one of four mutant forms of p53 (each representing one of the four "hotspots" in the central domain). Induction of wt p53 resulted in >100-fold elevation of β-galactosidase activity from reporter plasmids containing any of the three genomic DNA fragments (Table 1). The ability of p53 mutants to activate transcription from the human genomic fragments was about 100-fold lower than wt in every case (Table 1). Expression levels of the p53 mutants were similar to that of wt p53 (data not shown).

The ability of mutant forms of p53 to activate transcription from previously studied artificial binding sites was also tested. The reporter plasmids: WG½-lacZ, WG1-lacZ, and WG2-lacZ contained a half, one, or two copies, respectively, of the 20-bp consensus binding site defined by El-Deiry *et al.* (10), while p53CON1-lacZ and p53CON2-lacZ contained one or two copies of the palindromic consensus sequence defined by Funk *et al.* (12); PG16-lacZ contained 16 copies of the C_{BE} genomic binding site described by Kern *et al.* (13). Each of these reporters was stably transformed into yeast and SST activity was monitored by β-galactosidase production. After induction of wt p53, there was a marked increase in β-galactosidase activity from all the engineered binding sites containing a full consensus site (Table 1). Expression of the p53 mutants encoded by pRS-143 and pRS-273 resulted in increased β-galactosidase from the WG2-lacZ, p53CON1-lacZ, and p53CON2-lacZ reporters. Mutant forms of p53 were unable to induce appreciable levels of β-galactosidase activity from the human genomic fragment C_{BE} construct, even though it contained 16 copies of the binding site.

Tumor Cell Growth Suppression and SST Activity. Having confirmed that tumor-derived mutant forms of p53 have a dramatic reduction in SST activity when assayed with a subset of p53 binding sites, we tested the relationship of this activity to p53-mediated growth suppression using the four *in vivo* derived p53 mutants described above. Two cell lines were used for growth-suppression assays. H1299 cells are null for both alleles of p53, circumventing any interfering effects of endogenous p53 and facilitating the detection of protein produced from transfected expression vectors. The SW480 cell line, containing the p53 $Arg^{273} \rightarrow$ His mutant, was also used. The growth of this cell line is inhibited by expression of wt p53 (18). p53 genes were cloned into an expression vector (pCEP4) which can be maintained extrachromosomally because it contains an Epstein–Barr virus origin of replication and encodes the viral replication protein EBNA-1 (19). We hypothesized that the loss of exogenous gene expression would be minimized by using such episomal constructs and that the background levels of colonies in growth-suppression assays would be low. The results described below validated this prediction.

Expression vectors that encoded wt and various mutant forms of p53 were transfected into H1299 and SW480 cells, and hygromycin B-resistant colonies were counted 10 days later. Similar levels of protein were produced by each of the expression vectors in the H1299 cells (data not shown). Cells transfected with pCEP-wt formed at least 30-fold fewer visible colonies than those transfected with the *in vivo* derived mutants (Table 2). Expression of *in vitro* generated mutants containing a $Ser^{392} \rightarrow$ Ala or Asp substitution gave the same result as wt in either assay (data not shown).

The SST activity of the mutants was also analyzed in the H1299 and SW480 cells. One of the p53 binding sites used in the yeast assays (PG16) was placed upstream of a minimal promoter and CAT reporter gene (13). The binding site chosen was one that did not bind to any mutant forms of p53. This reporter was cotransfected with p53 expression vectors and subsequent SST activity was monitored by CAT assay. Corroborating the results in yeast, only expression of wt p53 resulted in measurable CAT activity in mammalian cells (Table 2). We conclude that there is a dramatic difference between mutant and wt p53 SST activity when tested on binding sites from the human genome. These differences primarily reflect sequence-specific DNA binding (data not shown) and correlate with growth-suppressor activity (Table 2).

The Transcriptional Activation Domain of p53 Can Be Replaced by VP16. The domain of p53 required for transcriptional activation has been previously mapped (aa 20–42) by testing hybrid proteins containing the N terminus of p53 fused to the DNA-binding domain of GAL4 (20). To define the N-terminal sequences required for p53-specific DNA binding, we performed the reverse experiment; the transcriptional activation domain from herpes simplex virus VP16 (aa 410–489; ref. 21) was fused to successive N-terminal deletion mutants of p53. Deletion of the first 80 aa of p53 resulted in complete loss of SST activity in both yeast (Fig. 1, construct

Table 1. Effect of *in vivo* mutation on SST activity

Reporter	β-Galactosidase activity, nmol/(min·mg)					
	pRS	pRS-wt	pRS-143*	pRS-175*	pRS-248*	pRS-273*
S201-lacZ	0, 0	732, 859	2, 2	2, 1	1, 4	0, 0
9H-lacZ	1, 0	292, 246	0, 0	0, 3	1, 2	1, 0
3H-lacZ	5, 1	460, 445	7, 4	0, 1	0, 4	0, 0
WG½-lacZ	3, 2	5, 2	4, 3	0, 3	2, 2	2, 2
WG1-lacZ	4, 3	1096, 810	5, 8	2, 3	4, 4	1, 5
WG2-lacZ	1, 1	922, 1014	407, 460	4, 4	4, 3	120, 107
p53CON1-lacZ	10, 12	350, 328	190, 129	5, 13	9, 8	32, 17
p53CON2-lacZ	26, 32	523, 635	211, 231	18, 21	21, 19	197, 237
PG16-lacZ	1, 1	1552, 741	15, 10	2, 0	1, 0	1, 1

S201, 9H, and 3H reporters were constructed with human DNA fragments containing p53 binding sites. The other reporters were constructed with synthetic oligonucleotides representing consensus binding sites (see text). For each case, β-galactosidase activity was assayed in two independent transformants grown at 30°C.
*Containing mutations found in human tumors at the indicated amino acid (see Table 2).

Table 2. Effect of tumor-derived mutations on SST and growth-suppressor activity

Expressed protein	SST activity: β-Galactosidase in yeast,* nmol/(min·mg)	SST activity: CAT in mammalian cells,† % of wt SW480	SST activity: CAT in mammalian cells,† % of wt H1299	Growth suppression, no. of colonies SW480	Growth suppression, no. of colonies H1299
wt	1552, 741	96, 105	100, 100	0, 0	3, 3
Val^{143} → Ala	15, 10	0, 0	0, 0	954, 906	276, 249
Arg^{175} → His	2, 0	0, 0	0, 0	1922, 2018	271, 258
Arg^{248} → Trp	1, 0	0, 0	0, 0	428, 471	81, 93
Arg^{273} → His	1, 1	0, 0	0, 0	1518, 1696	117, 137

*Assayed with PG16-lacZ reporter in two independent clones.
†Assayed with PG16-CAT reporter.

2) and mammalian cells (data not shown). Replacement of the first 80 aa of p53 with the VP16 activation domain resulted in substantial recovery of SST activity of the hybrid protein (Fig. 1, construct 3). Further deletions of the p53 N terminus showed that SST activity was abruptly lost when amino acids distal to residue 100 were deleted (Fig. 1). Levels of SST activity in mammalian cells were in accord with the data from yeast using the analogous constructs (data not shown).

The growth-suppressor activity of these p53 derivatives was also tested. The VP16–p53 hybrids were cloned into pCEP4 expression vectors and transfected into H1299 cells. Proteins retaining substantial SST activity (Fig. 1, constructs 1, 3, and 4) resulted in significant growth suppression, while proteins without SST activity (Fig. 1) had at least 50 times as many colonies. Similar results were obtained with SW480 transfectants (data not shown).

As a further control for specificity in this assay, we tested VP16 hybrids containing aa 80–393 derived from a p53 Arg^{175} → His mutant. This construct had no SST activity and did not suppress growth (compare constructs 3 and 7 in Fig. 1).

The Dimerization Domain of p53 Is Necessary for Transcriptional Activity and Growth Suppression. Residues 363–393 can be removed from p53 without detriment to its ability to specifically bind DNA (22). Accordingly, we found that up to 40 aa from the C terminus of p53 could be deleted with no appreciable effect on SST activity in yeast (Fig. 2*A*). Further deletion to residue 333, however, completely abrogated SST activity, and deletion of residues between 343 and 351 resulted in partial loss of SST function (Fig. 2*A*). Similar results were observed in H1299 cells using analogous constructs (Fig. 2*A*). More importantly, there was a striking inverse correlation between the ability of these p53-derived constructs to inhibit growth and stimulate SST activity. The deletion mutant terminating at aa 333 had >100 times less SST activity and >100 times less growth-suppressive activity than the truncated protein ending at aa 353 (Fig. 2*B*). Proteins encoded by constructs ending between codons 333 and 353 had intermediate activity. There seemed to be a threshold effect for growth suppression, as only p53 mutants conferring >30–40% of the SST activity of wt p53 could suppress growth >10-fold (Fig. 2*B*). Similar results were obtained with SW480 cells, except that the threshold was higher, with strong growth suppression occurring only from mutants conferring

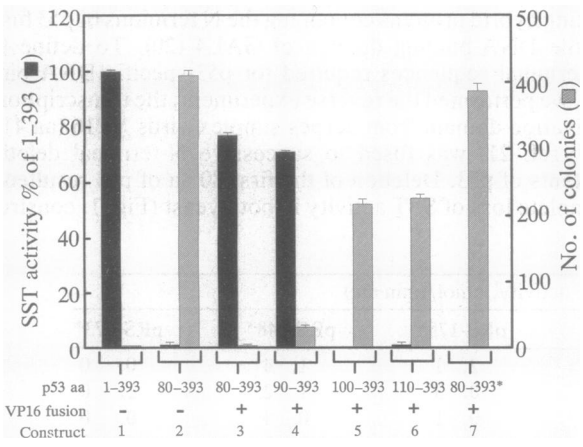

FIG. 1. SST and growth-suppressor activities of VP16–p53 hybrid proteins. For SST activity, lysates were prepared from yeast grown at 30°C expressing wt p53 or the indicated VP16–p53 hybrid proteins and PG16-lacZ reporter activities were assayed. The average and 1 SD from two independent clones are indicated. Growth suppression was assessed by the number of colonies that formed after transfection of H1299 cells with the indicated p53 expression vectors following hygromycin B selection for 10 days. The average and 1 SD derived from two separate transfections are shown. *, VP16 fused N-terminal to codon 80 of p53 Arg^{175} → His mutant.

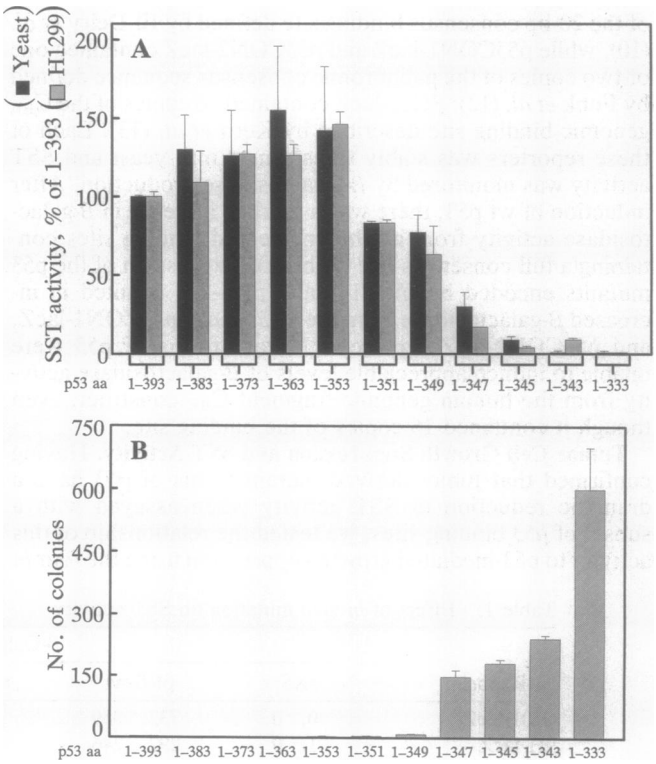

FIG. 2. Activities of p53 C-terminal deletion mutants. (*A*) SST activity in yeast and mammalian cells. For yeast, protein was isolated from cells grown at 30°C containing wt p53 or the indicated C-terminal deletion mutants, and PG16-lacZ reporter gene activities were assayed. For H1299 cells, protein was harvested from cells expressing the identical proteins and PG16-CAT reporter activities were assayed. (*B*) Growth-suppressor activity in H1299 cells. The number of H1299 colonies formed 10 days after transfection with wt or the indicated p53 C-terminal deletion mutant expression vectors is shown. The average and 1 SD calculated from two separate transfections (mammalian cells) or clones (yeast) are shown.

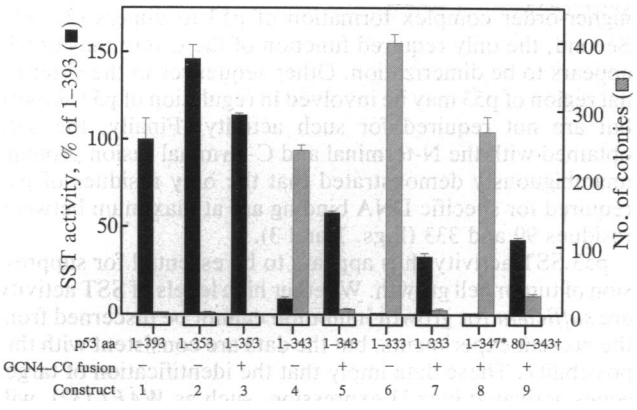

FIG. 3. SST and growth-suppressor activities of p53–CC hybrids. SST activity was measured in H1299 cells following transfection with the indicated p53–CC hybrid expression vectors and PG16-CAT. Growth suppression was determined from the number of colonies formed 10 days after transfection of H1299 with p53–CC hybrid vectors. The average and 1 SD calculated for two separate transfections are depicted. Construct 8, 1–347* represents the p53 Arg^{175} → His mutant fused to GCN4 CC at codon 347. †, Construct 9, the three-component chimeric protein containing the VP16 activation domain, aa 80–343 of p53, and the GCN4 CC domain.

>70–80% of wt SST activity (data not shown). The various C-terminally truncated proteins were expressed at comparable levels (*Materials and Methods*).

The Oligomerization Domain of p53 Can Be Replaced by the Coiled-Coil (CC) Domain of GCN4. The C-terminal deletion analysis described above suggested that SST activity required p53 residues 333–353. Previous studies have suggested that an amphipathic α-helix may exist between positions 334 and 356, providing homooligomerization ability (23). We reasoned that if a required function of the C terminus was to facilitate oligomerization, it might be possible to replace it with a dimerization domain from another protein. To test this possibility, expression plasmids were engineered that encoded C-terminal deletions of p53 fused to the CC dimerization domain of the yeast transcription factor GCN4 (aa 249–281; refs. 24 and 25). The p53–CC hybrid proteins were expressed in H1299 cells and yeast and tested for SST and growth-suppressor activities. Fusing the CC domain to p53 C-terminally truncated proteins at aa 343 or 333 restored SST activity both in mammalian cells (Fig. 3, compare construct 4 with construct 5 and 6 with 7) and yeast (data not shown). The restored SST activity was sufficient to suppress tumor cell growth (Fig. 3).

Because the CC domain of GCN4 could potentially interact with a variety of other cellular proteins containing CC domains, it was important to rule out nonspecific effects of the CC hybrids on cell growth. For this purpose, the C terminus of a p53 Arg^{175} → His mutant was replaced with the CC domain of GCN4. Although its level of expression was similar to that of the analogous wt p53 hybrids (*Materials and Methods*), this hybrid protein did not have any growth-suppressor activity (Fig. 3, construct 8).

Finally, we attempted to replace both the N-terminal and C-terminal domains of p53 with functionally analogous domains from other proteins. The resultant three-component hybrid contained the activation domain of VP16, the central portion of p53 (aa 80–343), and the CC domain of GCN4. This chimera produced about 40% of wt p53 SST activity when measured in mammalian and yeast cells (Fig. 3, construct 9), and this level of SST activity was sufficient to significantly inhibit the growth of H1299 cells (Fig. 3, construct 9).

Transcriptional Repression and Growth Suppression. wt p53 has been shown to repress transcription from several cellular promoters. However, these promoters do not contain p53 binding sites, and it is thought that p53 may reduce transcription by sequestering transcription factors. This suggests that p53 may mediate its growth inhibitory effect through nonspecific transcriptional repression (see Introduction). To address this question, we examined the effect of the engineered p53 mutants on cytomegalovirus promoter activity, previously demonstrated to be repressed by overexpression of p53 in mammalian cells (26, 27).

H1299 cells were cotransfected with p53 expression vectors (Table 3) and a pCMVCAT reporter plasmid (26), and transcriptional repression was monitored by assaying CAT activity 48 hr later. There was some correlation between the SST activity of the p53 derivatives and their ability to repress transcription; constructs with high SST activity had generally higher transcriptional repression activity (Table 3 and data not shown). However, there was a smaller difference between the relative repression activities of the various mutants and their relative SST and growth-suppressor activities. For example, the C-terminal deletion mutants had 100-fold differences in their SST and growth-suppressive activities, but only a 4-fold difference in their transcriptional repression ability (Table 3, constructs 2 and 3). Similarly, there was only a 3-fold difference in transcriptional repression between the p53 derivative truncated at aa 343 and its CC hybrid, despite an 80-fold difference in relative growth suppressive and SST activities (Table 3, constructs 3 and 4). Similar results were observed with the N-terminal deletion mutants. For example, there was little difference between the transcriptional repression exerted by wt p53 and VP16–p53 hybrids, while the SST and growth-suppressive activities differed dramatically (Table 3, compare constructs 1, 5, and 6). The transcriptional

Table 3. Activities of selected p53-derived proteins

Expressed protein		SST activity,*	Growth suppression,†	Repression,‡
p53 aa	Fusion	% of wt	no. of colonies	% of wt
1–393 (wt)	None	100, 100	3, 3	95, 106
1–353	None	101, 182	2, 3	127, 124
1–343	None	1, 1	233, 249	44, 22
1–343	GCN4 CC§	81, 79	1, 0	109, 80
80–393	VP16¶	72, 72	5, 2	130, 124
100–393	VP16‖	2, 1	225, 209	103, 105

Two independent determinations for SST activity, growth suppression, and transcriptional repression are shown.
*Assayed with PG16-lacZ reporter.
†Assayed in H1299 cells.
‡Assayed with pCMV-CAT in H1299 cells.
§Fused C-terminal to codon 343 of p53.
¶Fused N-terminal to codon 80 of p53.
‖Fused N-terminal to codon 100 of p53.

repression mediated by these constructs was therefore not a reliable indicator of their growth-suppressive abilities.

DISCUSSION

Recent studies have suggested that some mutant forms of p53 maintain sequence-specific DNA-binding activity (15–17). In agreement with these studies, we were able to demonstrate that both the 143 and 273 mutants have partial SST activity on artificial binding sites (up to half of that induced by wt p53). However, the present studies show that *all* mutants tested failed to demonstrate SST activity from binding sites found within human genomic DNA fragments (Table 1). We also found that artificial binding sites are not equivalent, with the precise sequence and number of copies apparently determining the affinity for both wt and mutant p53, in accord with the observations of Zhang *et al.* (16). These results suggest that the critical targets of p53 activation in the human genome will be more similar to the previously identified human genomic sequences containing derivative forms of the "consensus" than to artificial sequences which match the consensus perfectly.

The inability of *in vivo* derived mutants to activate transcription from the human genomic sites was associated in all cases with the inability of these mutants to inhibit the growth of tumor cell lines. Furthermore, *in vitro* engineered mutants that had low SST activity, like the tumor-derived mutants, were unable to suppress tumor cell growth. These results are consistent with the idea that a certain level of p53 SST activity is necessary for growth suppression and leads to the prediction that any mutants found *in vivo* will have SST activity below that required for growth suppression.

The necessity of the N-terminal region of p53 for SST activity and growth suppression has been demonstrated in this study and in previous reports (28–30). Consistent with the findings of Reed *et al.* (29), we were able to substitute the N-terminal domain of p53 with the transactivating domain of VP16. Our results additionally demonstrate that the 50 aa following the activation domain are dispensable for function, but that SST activity (and growth suppression) is rapidly lost when additional amino acids are removed.

wt p53 can repress a wide variety of cellular and viral promoters (see Introduction). However, there was much less concordance between the transcriptional repression and growth-suppressor activities of the various mutants examined in the current study than between their respective SST and growth-suppressor activities (Table 3). We have also examined the ability of wt p53 to suppress transcription from the herpes simplex virus thymidine kinase promoter, which is known to be down-regulated by p53 (26). We found that the level of p53-mediated repression from this viral promoter was very sensitive to cell type, the method of transfection, the levels of p53 induced, and the length of time of p53 expression. In parallel studies, few variations in p53 SST activity were observed when the same parameters were changed (data not shown). Overall, our studies suggest that the transcriptional repression mediated by p53 derivatives is not a reliable indicator of their growth-suppressive abilities. However, it is impossible to rule out the possibility that such repression plays some role in tumor suppression in certain instances.

Several studies have suggested that subtle mutations in p53 can affect the conformation of the entire protein. Hence, a remarkable finding of the current study is the extent to which p53 can be modified through the addition of foreign domains and yet still maintain high levels of biological activities. The ability of the p53–CC hybrids to function as a SST activator and growth suppressor has several implications. First, p53 is not required to be a tetramer to perform SST or growth-suppressor activity; the CC domain of GCN4 should limit the higher-order complex formation of p53 to dimers (25, 31). Second, the only required function of the C terminus of p53 appears to be dimerization. Other sequences in the C-terminal region of p53 may be involved in regulation of p53 activity but are not required for such activity. Finally, the data obtained with the N-terminal and C-terminal fusion proteins unambiguously demonstrated that the only residues of p53 required for specific DNA binding are at maximum between residues 90 and 333 (Figs. 1 and 3).

p53 SST activity thus appears to be essential for suppression of tumor cell growth. Whether high levels of SST activity are *sufficient* for growth inhibition cannot be discerned from the present experiments, but the data are consistent with this possibility. These data imply that the identification of target genes activated by p53 expression, such as *WAF1* (32), will prove critical for unraveling the p53 pathway.

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1. Zambetti, G. P. & Levine, A. J. (1993) *FASEB J.* **7**, 855–865.
2. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S. & Bradley, A. (1992) *Nature (London)* **356**, 215–221.
3. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C. & Vogelstein, B. (1991) *Science* **252**, 1707–1711.
4. Fields, S. & Jang, S. K. (1990) *Science* **249**, 1046–1049.
5. Raycroft, L., Wu, H. & Lozano, G. (1990) *Science* **249**, 1049–1051.
6. Vogelstein, B. & Kinzler, K. W. (1992) *Cell* **70**, 523–526.
7. Mercer, W. E. (1992) *Crit. Rev. Eukaryotic Gene Exp.* **2**, 251–263.
8. Mack, D. H., Vartikar, J., Pipas, J. M. & Laimins, L. A. (1993) *Nature (London)* **363**, 281–283.
9. Pietenpol, J. A. & Vogelstein, B. (1993) *Nature (London)* **365**, 17–18.
10. El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. & Vogelstein, B. (1992) *Nat. Genet.* **1**, 45–49.
11. Buchman, A. R., Lue, N. F. & Kornberg, R. D. (1988) *Mol. Cell. Biol.* **50**, 5086–5099.
12. Funk, W. D., Pak, D. T., Karas, R. H., Wright, W. E. & Shay, J. W. (1992) *Mol. Cell. Biol.* **12**, 2866–2871.
13. Kern, S. E., Pietenpol, J. A., Thiagalingam, S., Seymour, A., Kinzler, K. W. & Vogelstein, B. (1992) *Science* **256**, 827–830.
14. Gorman, C. M., Moffa, L. F. & Howard, B. H. (1992) *Mol. Cell. Biol.* **2**, 1044–1051.
15. Chen, J.-Y., Funk, W. D., Wright, W. E., Shay, J. W. & Minna, J. D. (1993) *Oncogene* **8**, 2159–2166.
16. Zhang, W., Funk, W. D., Wright, W. E., Shay, J. W. & Deisseroth, A. B. (1993) *Oncogene* **8**, 2555–2559.
17. Prives, C. & Manfredi, J. J. (1993) *Gene Dev.* **7**, 529–534.
18. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. V. & Vogelstein, B. (1990) *Science* **249**, 912–915.
19. Groger, R. K., Morrow, D. M. & Tykocinski, M. L. (1989) *Gene* **81**, 285–294.
20. Unger, T., Nau, M. M., Segal, S. & Minna, J. D. (1992) *EMBO J.* **11**, 1383–1390.
21. Trizenberg, S. J., Kingsbury, R. C. & McKnight, S. L. (1988) *Genes Dev.* **2**, 718–729.
22. Hupp, T. R., Meek, D. W., Midgley, C. A. & Lane, D. P. (1992) *Cell* **71**, 875–886.
23. Sturzbecher, H.-W., Brain, R., Addison, C., Rudge, K., Remm, M., Grimaldi, M., Keenan, E. & Jenkins, J. R. (1992) *Oncogene* **7**, 1513–1523.
24. Hu, J. C., O'Shea, K., Kim, P. & Sauer, R. T. (1990) *Science* **250**, 1400–1403.
25. Ellenberger, T. E., Brandl, C. J., Struhl, K. & Harrison, S. C. (1992) *Cell* **71**, 1223–1237.
26. Subler, M. A., Martin, D. W. & Deb, S. (1992) *J. Virol.* **66**, 4757–4762.
27. Jackson, P., Bos, E. & Braithwaite, A. W. (1993) *Oncogene* **8**, 589–597.
28. Aoyama, N., Nagese, T., Sawazaki, T., Mizuguhi, G., Nakagoshi, H., Fujisawa, J.-I., Yoshida, M. & Ishii, S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5403–5407.
29. Reed, M., Wang, Y., Mayr, G., Anderson, M. E., Schwedes, J. F. & Tegtmeyer, P. (1993) *Gene Expression* **3**, 95–107.
30. Unger, T., Mietz, J. A., Scheffner, M., Yee, C. L. & Howley, P. M. (1993) *Mol. Cell. Biol.* **13**, 5186–5194.
31. Talanian, R. V., McKnight, C. J. & Kim, P. S. (1990) *Science* **249**, 769–771.
32. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J., Lin, D., Mercer, W. E., Kinzler, K. W. & Vogelstein, B. (1993) *Cell* **75**, 817–825.