Multiple Response Elements and Differential p53 Binding Control *Perp* Expression During Apoptosis

Elizabeth E. Reczek,[1] Elsa R. Flores,[1] Alice S. Tsay,[1] Laura D. Attardi,[2] and Tyler Jacks[1,3]

[1]Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA; [2]Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA; and [3]Howard Hughes Medical Institute, Chevy Chase, MD

Abstract

The p53 tumor suppressor gene responds to cellular stress by activating either cell cycle arrest or apoptosis. A growing number of target genes involved in each of these pathways have been identified. However, the mechanism by which the apoptosis *versus* arrest decision is made remains to be elucidated. *Perp* is a proapoptotic target gene of p53 expressed to high levels in apoptotic cells compared with those undergoing cell cycle arrest. This pattern of expression is unusual among p53 target genes, many of which are induced to similar levels during arrest and apoptosis. Here, we describe the regulation of the *Perp* gene by p53 through at least three response elements in the *Perp* promoter and first intron. These sites are occupied *in vivo* in E1A-expressing mouse embryo fibroblasts undergoing apoptosis but not cell cycle arrest, in contrast to the *p21* 5′ response element, which is occupied during both. The apoptosis-deficient p53 point mutant, p53V143A, displays a selective deficit in binding to the *Perp* elements, demonstrating that p53 can distinguish between *Perp* and *p21* at the level of DNA binding. These results provide mechanistic insight into the selective expression of *Perp* during apoptosis and may provide a useful model for studying the p53-dependent cell cycle arrest *versus* apoptosis decision.

Introduction

The p53 tumor suppressor gene is a critical component of the anticancer defense mechanism (1). At the cellular level, p53 is a central regulator of stress response pathways (2), becoming active in response to a variety of conditions, including abnormal proliferation, hypoxia, DNA damage, nucleotide depletion, and disruption of the mitotic spindle (3–9). Increased levels of active p53 can have many outcomes, including cell cycle arrest, apoptosis, senescence, and differentiation, depending on numerous cell intrinsic and extrinsic factors (10). Following some forms of cellular stress, p53 can induce reversible cell cycle arrest and appears to participate in damage repair mechanisms (11, 12). The ability of p53 to direct damaged or dangerous cells to undergo apoptosis is also critical to its tumor suppressive function (13). However, the mechanisms that determine whether cells will undergo p53-dependent cell cycle arrest or apoptosis following a given stress stimulus remain to be elucidated.

The p53 protein is a transcriptional activator with a transactivation domain at its NH_2 terminus (14), a regulatory region and tetramerization domain at its COOH terminus (15, 16), and a central sequence-specific DNA binding domain (17, 18). The majority of p53 mutations found in human cancers reside within the DNA binding domain, suggesting that sequence-specific binding and transactivation of specific target genes is crucial for the tumor suppressive properties of p53 (17). p53 is capable of inducing expression of several genes containing one or more copies of a p53 binding element, located either within the promoter region (19–24) or in the introns of the responsive gene (5, 25–29). el-Deiry *et al.* (30) defined a consensus sequence for p53 binding consisting of two copies of a 10-bp motif, 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′, separated by 0–13 bp, and most p53-responsive genes identified to date contain a sequence roughly corresponding to this consensus. Transactivation by p53 is required for both arrest and apoptotic activities of p53 (31–34). However, a subset of p53 point mutants shows a selective defect in the transactivation of apoptosis-related target genes and induction of an apoptotic response, suggesting that the apoptosis- and arrest-inducing functions of p53 can be separated (35–41). Additionally, the p53 family members, p63 and p73, have been shown to be required for p53-dependent apoptosis but not cell cycle arrest, and loss of these proteins specifically affects transactivation of apoptosis-specific target genes (42). Other proteins such as ASPP1/2 and JMY have been found to selectively induce transactivation of apoptosis-related p53 target genes and to stimulate p53-dependent apoptosis but not cell cycle arrest (43, 44). Both apoptotic and arrest functions of p53 are likely to be tightly regulated through these and other factors to prevent their inappropriate activation.

Mouse embryo fibroblasts (MEFs) undergo p53-dependent G_1 arrest in response to treatment with DNA damaging agents (5). Expression of the adenoviral E1A oncoprotein induces hyperproliferation and sensitizes wild-type MEFs to undergo p53-dependent apoptosis in response to DNA damage (45, 46), as does expression of c-*myc* or E2F-1 (47–51). The mechanism by which E1A and other oncoproteins can modulate the response of the cell to DNA damage is not fully understood. However, the

Received 8/21/03; revised 11/12/03; accepted 11/14/03.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Grant support: Anna Fuller Fund (E. E. R.), Leukemia and Lymphoma Society of America (E. R. F.), Damon Runyon Cancer Research Foundation (L. D. A.), and NIH and Howard Hughes Medical Institute (T. J.).
Requests for reprints: Tyler Jacks, Center for Cancer Research, Massachusetts Institute of Technology, Room E17-110, 77 Massachusetts Avenue, Cambridge, MA 02139. Phone: (617) 253-0262; Fax: (617) 253-9863.
E-mail: tjacks@mit.edu

transactivation function of p53 is crucial for the apoptotic response in E1A-MEFs, suggesting that transcriptional targets of p53 are likely to be involved (32). We previously reported the cloning of the p53 target gene *Perp*, which is expressed to higher levels in apoptotic E1A-MEFs than in MEFs undergoing G_1 arrest (52). This pattern of expression is unusual for p53 target genes, many of which are induced to similar levels during both cell cycle arrest and apoptosis in this system. Thus, understanding *Perp* regulation could provide insight into the mechanisms through which p53 controls these cellular fates. We described previously the identification of two matches to the consensus p53 binding site in the murine *Perp* promoter (52). Here, we describe the identification of two additional p53 response elements in the *Perp* gene, one in the promoter region and one in intron 1. We demonstrate that at least three of these elements are functional p53 binding sites and can direct a p53 response in reporter assays, supporting the conclusion that *Perp* is a direct transcriptional target of p53. Further, we show that these sites can be distinguished from p53 response elements derived from cell cycle arrest related target genes in several ways, including their affinity for wild-type p53, their ability to bind to and mediate transactivation by apoptosis-deficient point mutants of p53, and their occupation by p53 *in vivo* under conditions of arrest and apoptosis.

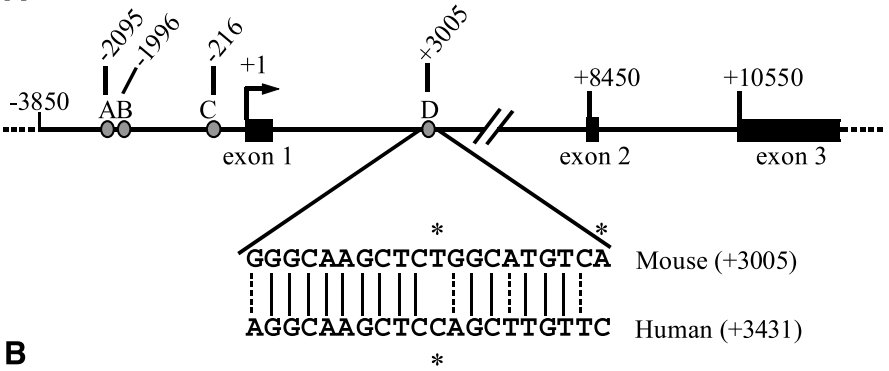
Results

Four Putative p53 Binding Sites Are Present Within the Promoter and First Intron of the Murine Perp Gene

We previously identified two sites (A and C) with significant similarity to the p53 binding site consensus within the *Perp* promoter region (Fig. 1, A and B) and demonstrated that the 4-kb upstream region of the murine *Perp* gene could direct a p53-dependent response (52). Further analysis of the murine *Perp* genomic sequence from −3850 to +11975 revealed the presence of two additional putative sites, one within the promoter region (site B) and one within the first intron (site D; Fig. 1, A and B). Sites A and C each contain a short spacer region between the two half-sites, while sites B and D consist of two half-sites immediately adjacent to one another. Site B contains a third half-site 4 bases upstream. Of the four sites identified, only the intronic site (site D) was conserved in the human *Perp* sequence obtained from the Human Genome Project public database (Fig. 1A). The mouse and human sequences at site D were identical at 14 of 20 residues, and four of six changes were conservative such that the mouse and human sites match the consensus at 18 of 20 and 19 of 20 bases, respectively. Several potential sites were found by sequence analysis of the human promoter region, but these sites have not been tested for function (data not shown).

Radiolabeled oligonucleotide probes representing these four sites were tested for the ability to bind p53 in electrophoretic mobility shift assays (EMSA). As shown in Fig. 2, only oligonucleotide probes corresponding to sites B and D were capable of binding to p53 in extracts from doxorubicin-treated E1A-MEFs. Binding could be competed by a 50-fold excess of an unlabeled consensus oligonucleotide (lanes 7 and 15; Fig. 2) but not by unlabeled mutant oligonucleotide (lanes 8 and 16; Fig. 2), demonstrating the specificity of the interaction. Binding was not observed in the absence of the activating antibody, monoclonal antibody 421 (lanes 5 and 13; Fig. 2), consistent with previous reports (53–56).

A



B

potential site	sequence	position
p53 consensus binding site	RRR**C(A/T)(A/T)G**YYY (0-13 bp) RRR**C(A/T)(A/T)G**YYY	R=A,G Y=C,T
p21 5' site	GAA**CATG**TCC CAA**CATG**TTG	-2281
PERP site A	GCG**CTAG**TCC ACAC AGA**CTAG**ATT	-2095
PERP site B	TTG**CTTG**GTC TCAC ATT**CTAG**CCC AGG**CTTG**CCC	-1996
PERP site C	GCT**CAAG**TGT AGCCTT AGC**CATG**CTC	-216
PERP site D	GGG**CAAG**CTC TGG**CATG**TCA	+3005

FIGURE 1. Cloning and sequencing of the murine *Perp* gene reveals four putative p53 binding sites. **A.** The murine *Perp* genomic locus. *Black boxes,* exons. *Filled circles,* matches to the p53 binding site consensus. Positions are labeled with respect to the transcriptional start site. The alignment between human and mouse *site D* is shown. *Solid lines,* perfect matches. *Dotted lines,* mismatches where both residues are consistent with the consensus. *Asterisks,* mismatches to the consensus sequence. **B.** The sequences of the four putative p53 binding sites from the *Perp* locus. The p53 binding site consensus and the 5′ site from the murine *p21* locus are shown for comparison. *Bold letters,* four core nucleotides of each half-site. *Underlined sequences,* mismatches to the consensus sequence. Half-site sequences separated from one another and from spacer nucleotides are indicated by a *space*.

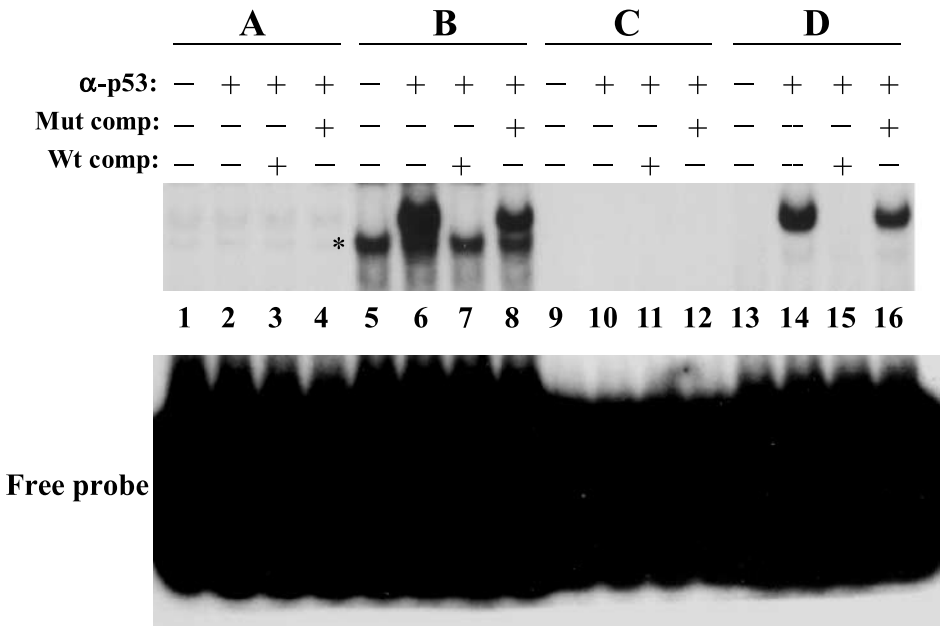


FIGURE 2. *Perp* sites B and D are capable of sequence-specific binding to p53. EMSAs were performed using oligonucleotide probes corresponding to the four *Perp* sites. Lysates from wild-type E1A-MEFs treated with 0.2 μg/ml doxorubicin for 12 h were incubated with the labeled probes in the presence or absence of p53 antibody 421. A 50-fold molar excess of unlabeled wild-type probe (*Wt comp*; *lanes 3*, *7*, *11*, and *15*) or mutant probe (*Mut comp*; *lanes 4*, *8*, *12*, and *16*) was added to demonstrate specificity of binding. *Asterisk,* position of a nonspecific band in *lanes 5*, *6*, *7*, and *8*. *Lower panel,* free probe.

Sites B and D Are Required for Full p53-Dependent Transactivation

Because sites B and D were capable of binding to p53 *in vitro*, we next tested the ability of sites A–D to direct p53-dependent expression of *Perp* in a luciferase reporter assay. The E1A-MEF system, in which *Perp* was originally identified, was not suitable for these assays because the process of transfection resulted in high levels of apoptosis. Therefore, we chose the HIp53 cell line (57), a derivative of the p53-deficient H1299 human lung carcinoma cell line (58) expressing His-tagged, wild-type, human p53 that can be induced by addition of the ecdysone analog, Ponasterone A (PonA). It should be noted that induction of p53 in HIp53 cells results in G_1 arrest, and we were unable to sensitize these cells to apoptosis with irradiation or additional chemical treatments. Therefore, while we do observe endogenous *Perp* expression on p53 induction in these cells (data not shown) and reporters tested were strongly and reproducibly induced, this system is not ideal for distinguishing between arrest- and apoptosis-specific regulation of *Perp*. However, it has been very useful for assessing the contribution of different putative p53 binding sites to p53-dependent transactivation more generally. To address apoptosis-specific regulation, we have relied on chromatin immunoprecipitation (ChIP) experiments in wild-type and E1A-expressing MEFs.

We described previously the construction of the pPERPluc1 vector by insertion of ~4-kb upstream genomic fragment of the *Perp* gene into the pGL3Basic vector (Promega, Madison, WI; 52). While this reporter was strongly induced in p53−/− MEFs when cotransfected with a p53 expression vector (52), we observed minimal induction of pPERPluc1 expression in the HIp53 system (Fig. 3A). This difference is likely due to the modest level of p53 induced by PonA treatment in this system compared with levels achieved through overexpression of p53 by transient transfection (data not shown). In contrast, the pPERPlucFL reporter, containing an 8-kb *Perp* genomic fragment extending from −3850 to +4150, showed 9-fold induction with PonA treatment comparable with the induction of the murine *p21* (59) and rat *Cyclin G* (60) reporters (Fig. 3A).

Because site D was capable of binding p53 in gel shift assays (Fig. 2), we inserted a 1.5-kb fragment of intron 1 (from +2375 to ~+3875) containing site D downstream of the luciferase cassette in the pPERPluc1 vector to generate pPERPlucPS. pPERPlucPS showed a 5-fold induction in HIp53 cells after PonA treatment (Fig. 3A), suggesting that a p53-responsive element was present within this fragment. However, the response of pPERPlucPS was weaker than seen with pPERPlucFL. Thus, an additional regulatory site(s) may be present in the upstream portion of intron 1, or the endogenous spacing of the elements within the *Perp* locus may be important for full functionality. Point mutation of the central cytosine and guanine residues of each half-site of site D to thymidine residues reduced the response of the pPERPlucFL vector by approximately two-thirds (pPERPlucFL-mutD; Fig. 3A) and completely abrogated the response of the pPERPlucPS vector (pPERPlucPS-mutD; Fig. 3A) to PonA treatment. Insertion of an oligonucleotide corresponding to site D into the unresponsive pPERPluc1 vector at a cloning site downstream of the luciferase cassette (pPERPluc1+D) was sufficient to restore the level of induction to ~70% of that seen with the pPERPlucPS vector; a mutant oligonucleotide (pPERPluc1+mutD) had no effect (Fig. 3A). Together, these data strongly implicate site D in the response of *Perp* to p53 but suggest that additional sites or endogenous spacing might be required for full induction of *Perp* in response to p53.

We next examined the ability of sites within the promoter region of *Perp* to enhance the p53 response mediated by site D. To this end, a series of deletions was made in the upstream region of the pPERPlucPS reporter (Fig. 3B). Deleting the region from −2180 to −650, which includes sites A and B, decreased the response by ~30% (pPERPlucPS-650). A

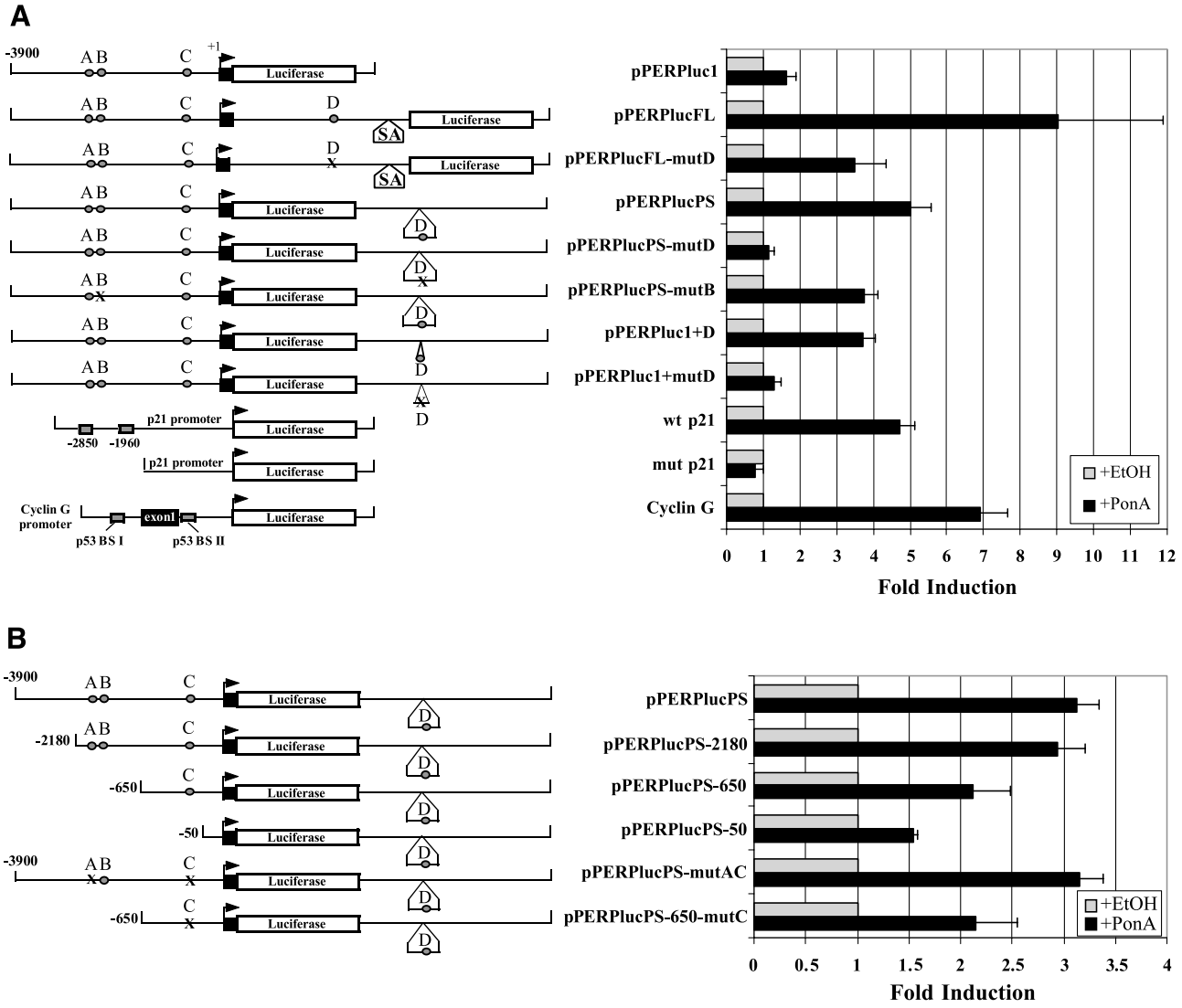


FIGURE 3. *Perp* sites B and D are required for full transactivation by p53. *Left,* Schematics of the relevant luciferase reporter constructs (**A** and **B**). *Black boxes,* exons. *Filled circles,* putative p53 binding sites. *Gray boxes,* binding sites from *p21* and *Cyclin G*. *X,* point-mutated sites. *SA,* insertion of a splice acceptor sequence from *Perp* exon 2. Positions are designated in reference to the transcriptional start site. Reporters were cotransfected with the pRL-null *Renilla* expression vector into HIp53 cells, treated with PonA or ethanol (*EtOH*) control 12 h post-transfection, and assayed for firefly and *Renilla* luciferase activities 12 h later. Firefly activity was normalized to *Renilla* activity. Fold induction is graphed relative to ethanol control. *Columns,* averages of at least three separate experiments each performed with six replicates; *bars,* 1 SD. Differences in levels of induction of pPERPlucPS between **A** and **B** may reflect differences in batches of PonA or HIp53 responsiveness to PonA treatment in experiments performed several months apart.

deletion from −650 to −50 bp, including sites A–C, further decreased expression following p53 induction (pPERPlucPS-50). These data suggest that a p53 response element is present in the region from −2180 to −650 and an additional site may lie in the region from −650 to −50. To determine whether site A, B, or C might mediate the observed response, we point mutated these sites individually and in combination. Point mutation of sites A and C together (pPERPlucPS-mutAC) or site C alone (pPERPlucPS-650-mutC) had no effect on the ability of the reporter to respond to p53 in this system (Fig. 3B). However, point mutation of site B in the pPERPlucPS reporter (pPERPlucPS-mutB) decreased the response to PonA treatment by ~30% (Fig. 3A). These data suggest that site D is responsible for the majority of the response of *Perp* to p53 in this system but that site B might augment or modulate that response. Because the HIp53 cell line fails to undergo apoptosis, however, we cannot rule out the possibility that sites A and C might function under apoptotic conditions.

p53 Is Present at the Perp Locus in Apoptotic but not Cell Cycle Arrested MEFs

Having established the ability of sites B and D to mediate transactivation by p53, we performed ChIP for p53 on wild-type MEFs, wild-type E1A-MEFs, and p53−/− E1A-MEFs in the presence and absence of DNA damage to examine the

functionality of the four putative response elements *in vivo* under conditions of apoptosis and cell cycle arrest (Fig. 4). Twelve hours after doxorubicin treatment, 80% of wild-type MEFs are in G_1 arrest, while only 60% of E1A-MEFs are viable at this time point. In these cells, we examined the 5′ p53 response element from promoter of the *p21* gene, which is induced under conditions favoring both cell cycle arrest and apoptosis, in addition to the four putative sites from the *Perp* locus. Due to the proximity of *Perp* sites A and B, these sites could not be distinguished in this assay.

Association of p53 with the *p21* promoter was strongly stimulated in both wild-type MEFs and wild-type E1A-MEFs following doxorubicin treatment (Fig. 4). In contrast, association of p53 with the *Perp* sites was greatly induced by doxorubicin treatment in wild-type E1A-MEFs but not in wild-type MEFs without E1A. These data correlate well with the observed expression patterns of *p21* and *Perp* in cell cycle arrested and apoptotic MEFs (52) and provide the first mechanistic insight into the differential regulation of *Perp* under these conditions. Interestingly, while site C failed to bind p53 in gel shift experiments (Fig. 2) and point mutation of site C had no effect on the ability of p53 to transactivate *Perp* luciferase reporters in the HIp53 system (Fig. 3B), we observed a strong association of p53 with *Perp* site C *in vivo*. This discrepancy may suggest the presence of a nonconsensus response element within the amplicon used to detect binding to site C or may indicate that additional sequences surrounding the minimal consensus element used for EMSA analysis are required. Alternatively, p53 binding to site C *in vivo* may require association of other factors that are not present in the *in vitro* gel shift conditions or in the context of the reporter assay.

A p53 Point Mutant Deficient in Induction of Apoptosis Fails to Transactivate Perp

We also examined the ability of an apoptosis-deficient, temperature-sensitive p53 mutant (p53V143A) to transactivate the pPERPlucFL reporter. The V143A mutation lies within the DNA binding domain of p53 and shows a temperature-sensitive phenotype with respect to transactivation of some target genes, including *GADD45*, *Cyclin G*, *p21*, and *mdm2* (35). However, p53V143A fails to transactivate target genes involved in apoptosis, including *Bax* and *IGF-BP3*, and is specifically defective in inducing apoptosis at the permissive temperature (35). Consistent with previous reports, in our experiments, the V143A mutant was unable to transactivate any of the target gene reporter constructs tested in transient cotransfection assays in H1299 cells at 37°C. At 32°C, p53V143A transactivated the *Cyclin G* and *mdm2* reporters but still failed to induce expression of the pPERPlucFL and *IGF-BP3* box B reporters (Fig. 5A). These data place *Perp* in the apoptotic category of p53 target genes.

To determine whether the observed differences in transactivation between these reporters were mediated by the p53 response elements themselves or required additional sequences, we examined the ability of wild-type p53 and p53V143A to bind sites B and D from the *Perp* gene and the 5′ *p21* site using gel shift analysis. Consistent with previous reports, p53V143A expressed at 37°C bound much more weakly to all sites examined (Fig. 5B) despite being strongly expressed at levels comparable with wild-type p53 at this temperature (Fig. 5C). At 32°C, p53V143A was capable of binding to all sites. However, p53V143A binding to *Perp* sites B and D was sharply lower than the binding of wild-type p53 to those sites at the same temperature (Fig. 5B; 18- and 11-fold, respectively), consistent with the failure of the point mutant to transactivate pPERPlucFL at 32°C (Fig. 5A). This difference was not due to differences in the expression of wild-type and mutant p53, as levels of both proteins were comparable at 32°C (Fig. 5C). In contrast, binding of p53V143A to the 5′ *p21* probe was robust and was only 2.3-fold lower than wild-type at this temperature (Fig. 5B), correlating with the ability of p53V143A to transactivate the *p21* reporter at 32°C (Ref. 35, and data not shown). Thus, the deficiency in binding of the p53V143A mutant at 32°C compared with the wild-type protein was more pronounced with respect to *Perp* sites B and D than with respect to the 5′ *p21* site, demonstrating that the minimal response element contains sufficient information to allow p53V143A to distinguish between *Perp* and *p21*.

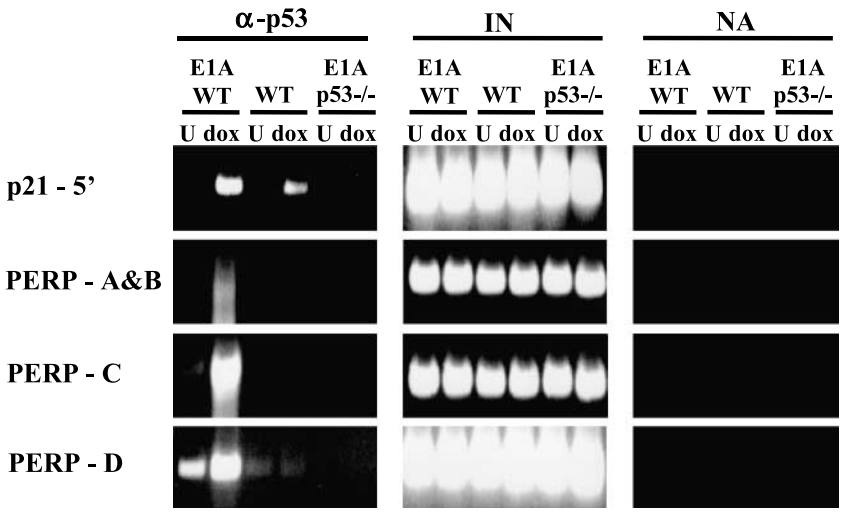


FIGURE 4. Differential binding of p53 at the *Perp* and *p21* sites *in vivo* in apoptotic *versus* cell cycle arrested cells. ChIP analysis for the presence of p53 at the 5′ *p21* response element and *Perp* sites A/B (*A&B*), C, and D either untreated (*U*) or following treatment of wild-type MEFs (*WT*), wild-type E1A-MEFs (*E1A WT*), or p53−/− E1A-MEFs (*E1A p53-/-*) with doxorubicin (*dox*) for 12 h. Twenty percent of total input chromatin (*IN*) and no antibody (*NA*) controls are shown. *Perp* sites *A&B* could not be separated in this assay due to their proximity to one another in the *Perp* promoter. PCR reactions were run on a 2% gel and stained with ethidium bromide to visualize amplified fragments.

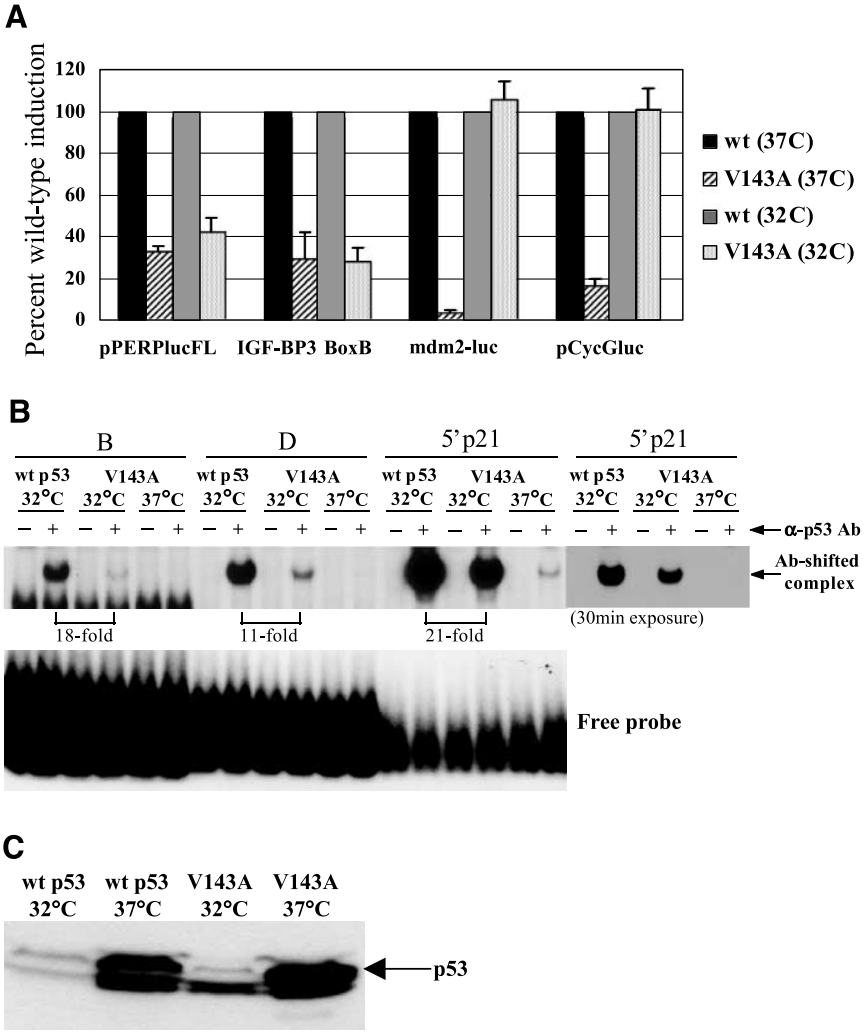


FIGURE 5. The p53V143A point mutant can discriminate between *Perp* and arrest-related target genes. **A.** Relative transactivation of target genes by wild-type p53 (*wt*) and p53V143A (*V143A*) at 37°C and 32°C in transiently transfected H1299 cells. Cells were cotransfected with reporter, the relevant expression vector, and the pRL-null *Renilla* luciferase vector and transferred 3 h after transfection to 32°C or left at 37°C. Data are normalized to activity in the empty vector condition and are plotted as a percent induction by wild-type p53 at each temperature at 24 h. *Columns,* averages of three separate experiments performed with four replicates per condition; *bars,* 1 SD. **B.** EMSA showing a selective defect in the ability of p53V143A to bind probes corresponding to *Perp* sites B and D compared with the 5′ *p21* probe. H1299 cells were transfected with the appropriate expression vector and were transferred 3 h after transfection to 32°C or left at 37°C. Cells were lysed 24 h after temperature shift. Forty micrograms of whole cell lysate were used per well. Probes were counted in a scintillation counter and equal counts of all probes were loaded per lane. *Lower panel,* free probe. **C.** Western blot for p53 demonstrating approximately equal expression of wild-type and mutant p53 at each temperature. Both wild-type and mutant p53 showed significantly higher expression at 37°C compared with 32°C.

Discussion

Given the well-established role of p53 in tumor suppression and the apoptotic response of tumor cells to chemotherapeutic agents, a clear understanding of its many functions will be crucial for rational design of improved treatments. One key outstanding question is the mechanism by which p53 distinguishes between classes of target genes to induce cell cycle arrest or apoptosis when appropriate. In this report, we have dissected the transcriptional control elements of the *Perp* gene to gain insight into this process. As described above, the regulation of the *Perp* promoter by p53 appears to involve a complex interplay between multiple response elements. The data presented here demonstrate the existence of two novel p53 response elements within the murine *Perp* gene, sites B and D, in addition to sites A and C, which we described previously (52). In addition, we show that *Perp* can be distinguished from *p21* at the level of DNA binding *in vivo* and *in vitro*.

Several models have been proposed to explain the different cellular outcomes downstream of p53 activation. In one model, p53 responds to cellular insults in a consistent manner, and the ultimate fate of the cell is determined by the presence of other p53-independent survival or death signals (61). While other factors are undoubtedly involved in the decision, our data argue against such a limited role for p53. We observed differential association of p53 with the *Perp* promoter in ChIP analysis of apoptotic cells compared with cell cycle arrested cells (Fig. 4), suggesting that the activity of p53 itself toward apoptotic targets is stimulated under conditions favoring an apoptotic response. In addition, the temperature-sensitive p53V143A mutant showed a selective defect in binding to *Perp* gel shift probes compared with the 5′ *p21* probe at the permissive temperature, which was reflected in the inability of p53V143A to transactivate *Perp* and other apoptotic target genes but not cell cycle targets at this temperature (Fig. 5). These data agree with previous observations that the cell cycle arrest and apoptotic functions of p53 can be uncoupled through specific point mutations and that loss of apoptotic function is correlated with the inability to transactivate a specific subset of target genes implicated in apoptosis (35, 38, 41). While there is no question that p53-independent factors can influence the eventual cellular outcome, p53 itself can clearly play a direct role as well. Therefore, a second model, in which p53 plays an active role in determining the outcome following a given insult (61), appears to be more consistent with our observations.

Although the mechanisms behind this role of p53 remain largely unclear, the ability of p53 to distinguish between arrest and apoptosis targets requires, at least, that intrinsic differences exist in the regulatory regions of these genes, either within the response element itself or in surrounding sequences. The ability of p53 to distinguish between short oligonucleotide probes corresponding to *Perp* and *p21* sites (Fig. 5B) suggests that the minimal response element, in the absence of surrounding sequences, contains such information. Our understanding of specific sequence requirements for functional p53 response elements within the accepted consensus sequence put forth by el-Deiry *et al.* in 1992 is still rudimentary, but several groups have begun making progress on this front. Recently, Qian *et al.* (62) compared binding sites from 57 known and potential target genes of p53 and noted the presence of spacer regions between half-sites and three or more mismatches relative to the consensus in apoptotic targets, while response elements from cell cycle related target genes generally lacked spacers and had two or fewer mismatches. Inga *et al.* (63) showed that p53 response elements from various target genes could vary by as much as 1000-fold in their ability to direct p53-dependent transactivation in yeast and that the presence of a CATG at the core of one or both half-sites correlated strongly with a high transactivation capacity.

Based on these predictions, the four p53 binding sites we identified at the *Perp* locus would be expected to display a range of affinities for p53. Sites B and D each contain two mismatches (excluding the third half-site in site B) and lack spacers, suggesting that these would be relatively strong sites. In addition, site D contains a CATG core at the 3′ half-site, consistent with it being a particularly strong site. In fact, we observed robust binding to both sites B and D in gel shifts (Figs. 2 and 5B), and a strong requirement for site D for full induction by p53 in reporter assays (Fig. 3). In addition, there was clear occupancy of site D by p53 in ChIP analysis in E1A-MEFs even in the absence of DNA damage (Fig. 4). The requirement for site B in reporter assays was somewhat weaker.

In contrast, sites A and C contain two and four mismatches, respectively, and each has a spacer region, suggesting that these would be relatively weak sites. As predicted, these sites failed to bind p53 in gel shift experiments (Fig. 2) and were dispensable for induction of *Perp* reporters by p53 (Fig. 3). However, site C carries a CATG motif at the core of its 3′ half-site, and p53 was found to associate strongly with site C in ChIP analysis in E1A-MEFs following DNA damage (Fig. 4). Thus, perhaps *in vivo*, the presence of a CATG motif can compensate to some degree for the presence of mismatches and a spacer. Finally, the 5′ *p21* site, to which the *Perp* sites were compared, contains no spacer, has two mismatches, and has a CATG motif at the core of each half-site (Fig. 1B). This site bound very strongly to p53 in gel shift assays (Fig. 5B). It was required for transactivation of *p21* reporters by p53 (Fig. 2A; Ref. 20) and is occupied by p53 during both apoptosis and arrest conditions in ChIP analysis (Fig. 4). These data are consistent with the hypothesis that intrinsic differences in response elements may be partially responsible for determining their affinity for p53. However, the presence of multiple sites with varying characteristics within the *Perp* promoter suggests that a simple model in which cell cycle arrest related targets contain high-affinity sites and proapoptotic targets contain low-affinity sites is insufficient to explain the differential regulation of proarrest and proapoptotic target genes. Instead, the regulation of *Perp* expression, and perhaps that of other target genes, may be achieved through a complex interaction of p53 with multiple sites of varying affinity as well as with other cofactors.

Several groups have recently identified cofactors such as ASPP1/2 and JMY that bind p53 and can selectively stimulate transactivation of apoptosis-related genes without affecting expression of arrest-specific genes (43, 44). Additionally, we have shown previously that the p53 family members, p63 and p73, are required for binding of p53 to response elements in apoptotic targets and induction of apoptosis but not for cell cycle arrest (42). In that report, p63 was shown to be associated with the *Perp* promoter in E1A-MEFs following DNA damage and loss of both p63 and p73 prevented association of p53 with the *Perp* promoter following DNA damage. In contrast, p63 and p73 were not required for binding of p53 to the *p21* and *mdm2* promoters. These data show that p53 family members play an important role in the transactivation of apoptotic targets of p53 and suggest the intriguing possibility that p63, and perhaps p73, might bind to the *Perp* promoter following DNA damage and recruit p53 to the otherwise weak response elements at the *Perp* locus, thereby activating *Perp* to stimulate an apoptotic response.

The regulation of the apoptosis *versus* cell cycle arrest decision by p53 is clearly complex and likely involves an intricate interplay between numerous factors to produce the appropriate cellular response under each set of conditions. Moreover, the large number of apoptosis-related targets of p53 identified to date in various systems may imply that the specific mechanisms differ depending on cell type and specific death stimulus. The results presented here confirm the role of *Perp* as a direct transcriptional target of p53 involved in the apoptosis response. They demonstrate that interaction of p53 with the *Perp* locus occurs through at least three p53 response elements of varying affinities and is regulated at the level of p53 DNA binding. Understanding how these sites function and the mechanisms by which *Perp* expression is enhanced under apoptotic conditions will likely provide valuable insight into the regulation of the critical cell death decision.

Materials and Methods

Cloning and Sequence Analysis of Perp Genomic DNA

A 200-bp *Rsa*I fragment of the *Perp* cDNA was used to probe a murine 129/SvJae genomic λFIX II phage library. Isolated clones were sequenced and intron-exon boundaries were determined from the cDNA sequence. All putative boundaries contain consensus splice donors and acceptors. *Perp* genomic DNA sequence was scanned for sequences matching the p53 consensus sequence (30). The human *Perp* sequence was obtained from the Sanger Center Web site (http://www.sanger.ac.uk/HGP/Chr6/) and was used for comparative sequence analysis with the murine *Perp* sequence. Regions of

high conservation were identified using several Internet-based alignment programs (Bayesian Phylogenetic Footprint Homepage, http://bayesweb.wadsworth.org/cgi-bin/bayes_align12.pl, and PipMaker, http://bio.cse.psu.edu/pipmaker) and the VectorNTI alignment program (Informax, Frederick, MD).

Cell Culture

p53-inducible HIp53 cells (57) were selected in 600 μg/ml G418 and 400 μg/ml Zeocin. Wild-type and p53−/− MEFs were isolated as described. To generate E1A-expressing MEFs, wild-type and p53−/− MEFs were infected with retroviral E1A as described previously (64).

EMSA

EMSAs were performed using the p53 NuShift Plus kit from Active Motif, Carlsbad, CA. Antibody 421 (Ab-1, Oncogene Science, Cambridge, MA) at 1 μg/μl was used in place of the supplied polyclonal anti-p53 antibody. Cells were plated at 2×10^6 cells per 15-cm dish 24 h before treatment. After 12–24 h of treatment, cells were rinsed once in PBS, scraped from the plate, pelleted, and lysed in 100 μl cell lysis buffer [100 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1% NP40] plus protease inhibitors (Complete Mini, Roche, Basel, Switzerland) for 30 min at 4°C. Total protein concentrations were determined using BCA Protein Assay (Pierce Chemical Co., Rockford, IL). Total protein used per binding reaction varied from 40 to 100 ng depending on the experiment but was constant for all lanes in a given experiment. Probes were end labeled with T4 PNK and purified in G-25 Microspin columns (Amersham Pharmacia Biotech, Piscataway, NJ). All probes were in excess and equal counts were used per reaction. Binding reactions were run on a 4% polyacrylamide gel. Oligonucleotide probes were *Perp* site A: 5′-AGAGGCGCTAGTCCACACAGACTAGATTCCTC-3′, *Perp* site B: 5′-TATTTGCTTGGTCTCACATTCTAGCCCAGGCTTGCCCAGA-3′, *Perp* site C: 5′-TGGAGCTCAAGTGTAGCCTTAGCCATGCTCCTTA-3′, *Perp* site D: 5′-CTTCAGATCCTGGGCAAGCTCTGGCATGTCAGGGTGGAGGG-3′, *p21* 5′ site: 5′-GAACATGTCTTGACATGTTC-3′, wild-type competitor: 5′-AGCTGGACATGCCCGGGCATGTCC-3′, and mutant competitor: 5′-AGCTGGATCGCCCCGGGCATGTCC-3′.

Plasmids

Wild-type p53 and p53V143A expression vectors were described previously (65, 66). All *Perp* luciferase reporters were constructed in the pGL3Basic vector. The pPERPluc1 reporter was described previously (52). The pPERPlucFL vector was constructed by insertion of a splice acceptor upstream of the luciferase cassette in pGL3Basic and insertion of a *Bam*HI *Perp* genomic fragment (from −3850 to +4150) into the polylinker region. pPERPlucPS was derived from pPERPluc1 by blunt insertion of a *Pme*I-*Sph*I fragment of intron 1 (from +2377 to +3870) into the downstream cloning site in the pGL3Basic backbone. pPERPlucPS-mutD and pPERPlucPS-mutB vectors were derived from pPERPlucPS through site-directed mutagenesis using the QuikChange kit (Stratagene, La Jolla, CA). Oligonucleotides used in site-directed mutagenesis were *Perp* site A: 5′-CCTTAAGAGGCGTTATTCCACACAGATTATATTCCTCCATATG-3′, *Perp* site B: 5′-GGTCTCACATTTTATCCCAGGTTTTCCCAGAATTAAC-3′, *Perp* site C: 5′-GTGCTTGGAGCTTAATTGTAGCCTTAGCTATTCTCCTTACACTG-3′, and *Perp* site D: 5′-CTTCAGATCCTGGGTAATCTCTGGTATTTCAGGGTGGAGGG-3′. The pPERPlucFL-mutD vector was derived from pPERPlucFL by substitution of the point-mutated *Pme*I-*Sph*I intron 1 fragment for the wild-type fragment. pPERPluc1+D and pPERPluc1+-mutD were constructed using the QuikChange kit to insert an oligonucleotide containing wild-type or mutant site D downstream of the luciferase coding region. The wild-type and mutant *p21* luciferase reporters were derived from *p21* chloramphenicol acetyltransferase reporters (59). The *Bax*, *Cyclin G*, *mdm2*, and *IGF-BP3* reporters were described previously (60). Deletion constructs were derived from pPERPlucPS vector using standard cloning methods. The pRL-null *Renilla* luciferase expression vector (Promega) was used in all luciferase assays.

DNA Transfection and Transactivation Assays

Transient transfections were performed using Fugene6 (Roche) at a 1:1.5 or 1:3 DNA/Fugene6 ratio according to manufacturer's instructions. Luciferase assays were performed using the Dual Luciferase System (Promega). Cells were plated 18–24 h before transfection at 5000 cells/well of a 96-well plate. Fifty nanograms of reporter, 50 ng of pRL-null, and 100 ng of wild-type or mutant p53 were transfected per well. HIp53 cells were treated with 10 μM PonA or an equal volume of ethanol 6–12 h post-transfection. Cells were lysed 12 h post-treatment for HIp53 or 24 h post-transfection for cotransfection experiments with p53V143A in 20 μl/well 1× Passive Lysis Buffer (Promega) by shaking at room temperature for 15 min. The 96-well plates were read on a LB96V plate-reading luminometer (Berthold, OakRidge, TN) with 45 μl injection volumes for each reagent and a 2-s delay followed by a 10-s integration time. Firefly luciferase readings were divided by *Renilla* luciferase readings as a control for variations in cell number and transfection efficiency.

ChIP Assay

After treatment with doxorubicin for 12 h, wild-type MEFs with or without E1A and p53−/− E1A-MEFs were cross-linked with 1% formaldehyde. ChIP was carried out as described previously (42). Primers for the 5′ site of the *p21* promoter and *Perp* sites A/B and C were as described previously (42). Primers for *Perp* site D were 5′-TGAATGTTTGGCTTATATTTGTGGAG-3′ and 5′-CCTTCTTTCAGTGCATACCTCATCCC-3′. Annealing temperature was 60°C. PCR products were electrophoresed on a 1.2% agarose gel and were detected by ethidium bromide staining.

Acknowledgments

We thank M. Oren for *Cyclin G*, *mdm2*, and *IGF-BP3* reporters, J. Pietenpol for the HIp53 cell line, K. Macleod for *p21* chloramphenicol acetyltransferase reporters, and B. Vogelstein for pCMV-wild-type p53 and pCMV-p53V143A expression vectors.

References

1. Levine, A. J. p53, the cellular gatekeeper for growth and division. Cell, *88:* 323–331, 1997.

2. Giaccia, A. J. and Kastan, M. B. The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev., *12:* 2973–2983, 1998.

3. Graeber, T. G., Osmanian, C., Jacks, T., Housman, D. E., Koch, C. J., Lowe, S. W., and Giaccia, A. J. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. Nature, *379:* 88–91, 1996.

4. Guidos, C. J., Williams, C. J., Grandal, I., Knowles, G., Huang, M. T., and Danska, J. S. V(D)J recombination activates a p53-dependent DNA damage checkpoint in SCID lymphocyte precursors. Genes Dev., *10:* 2038–2054, 1996.

5. Kastan, M. B., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, *71:* 587–597, 1992.

6. Linke, S. P., Clarkin, K. C., Di Leonardo, A., Tsou, A., and Wahl, G. M. A reversible, p53-dependent G_0/G_1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev., *10:* 934–947, 1996.

7. Cross, S. M., Sanchez, C. A., Morgan, C. A., Schimke, M. K., Ramel, S., Idzerda, R. L., Raskind, W. H., and Reid, B. J. A p53-dependent mouse spindle checkpoint. Science, *267:* 1353–1356, 1995.

8. Di Leonardo, A., Khan, S. H., Linke, S. P., Greco, V., Seidita, G., and Wahl, G. M. DNA re-replication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. Cancer Res., *57:* 1013–1019, 1997.

9. Minn, A. J., Boise, L. H., and Thompson, C. B. Expression of Bcl-xL and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. Genes Dev., *10:* 2621–2631, 1996.

10. Vousden, K. H. and Lu, X. Live or let die: the cell's response to p53. Nat. Rev. Cancer, *2:* 594–604, 2002.

11. Khanna, K. K. and Jackson, S. P. DNA double-strand breaks: signaling, repair and the cancer connection. Nat. Genet., *27:* 247–254, 2001.

12. Smith, M. L. and Seo, Y. R. p53 regulation of DNA excision repair pathways. Mutagenesis, *17:* 149–156, 2002.

13. Symonds, H., Krall, L., Remington, L., Saenz-Robles, M., Lowe, S., Jacks, T., and Van Dyke, T. p53-dependent apoptosis suppresses tumor growth and progression *in vivo*. Cell, *78:* 703–711, 1994.

14. Lin, J., Wu, X., Chen, J., Chang, A., and Levine, A. J. Functions of the p53 protein in growth regulation and tumor suppression. Cold Spring Harbor Symp. Quant. Biol., *LIX:* 215–233, 1995.

15. Hupp, T. R. and Lane, D. P. Allosteric activation of latent p53 tetramers. Curr. Biol., *4:* 865–875, 1994.

16. Jayaraman, J. and Prives, C. Activation of p53 sequence-specific DNA binding by short single strands of DNA requires the p53 C-terminus. Cell, *81:* 1021–1029, 1995.

17. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science, *265:* 346–355, 1994.

18. Jeffrey, P. D., Gorina, S., and Pavletich, N. P. Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. Science, *267:* 1498–1502, 1995.

19. Oda, E., Ohki, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita, T., Tokino, T., Taniguchi, T., and Tanaka, N. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science, *288:* 1053–1058, 2000.

20. el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. Cell, *75:* 817–825, 1993.

21. Okamoto, K. and Beach, D. Cyclin G is a transcriptional target of the p53 tumor suppressor protein. EMBO J., *13:* 4816–4822, 1994.

22. Zambetti, G. P., Bargonetti, J., Walker, K., Prives, C., and Levine, A. J. Wild-type p53 mediates positive regulation of gene expression through a specific DNA sequence element. Genes Dev., *6:* 1143–1152, 1992.

23. Miyashita, T. and Reed, J. C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell, *80:* 293–299, 1995.

24. Comer, K. A., Dennis, P. A., Armstrong, L., Catino, J. J., Kastan, M. B., and Kumar, C. C. Human smooth muscle α-actin gene is a transcriptional target of the p53 tumor suppressor protein. Oncogene, *16:* 1299–1308, 1998.

25. Thornborrow, E. C., Patel, S., Mastropietro, A. E., Schwartzfarb, E. M., and Manfredi, J. J. A conserved intronic response element mediates direct p53-dependent transcriptional activation of both the human and murine bax genes. Oncogene, *21:* 990–999, 2002.

26. Ren, C., Li, L., Goltsov, A. A., Timme, T. L., Tahir, S. A., Wang, J., Garza, L., Chinault, A. C., and Thompson, T. C. mRTVP-1, a novel p53 target gene with proapoptotic activities. Mol. Cell. Biol., *22:* 3345–3357, 2002.

27. Nakano, K. and Vousden, K. H. PUMA, a novel proapoptotic gene, is induced by p53. Mol. Cell, *7:* 683–694, 2001.

28. Zauberman, A., Flusberg, D., Haupt, Y., Barak, Y., and Oren, M. A functional p53-responsive intronic promoter is contained within the human mdm2 gene. Nucleic Acids Res., *23:* 2584–2592, 1995.

29. Wu, X., Bayle, J. H., Olson, D., and Levine, A. J. The p53-mdm-2 autoregulatory feedback loop. Genes Dev., *7:* 1126–1132, 1993.

30. el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B. Definition of a consensus binding site for p53. Nat. Genet., *1:* 45–49, 1992.

31. Ko, L. J. and Prives, C. p53: puzzle and paradigm. Genes Dev., *10:* 1054–1072, 1996.

32. Attardi, L. D., Lowe, S. W., Brugarolas, J., and Jacks, T. Transcriptional activation by p53, but not induction of the p21 gene, is essential for oncogene-mediated apoptosis. EMBO J., *15:* 3693–3701, 1996.

33. Chao, C., Saito, S., Kang, J., Anderson, C. W., Appella, E., and Xu, Y. p53 transcriptional activity is essential for p53-dependent apoptosis following DNA damage. EMBO J., *19:* 4967–4975, 2000.

34. Jimenez, G. S., Nister, M., Stommel, J. M., Beeche, M., Barcarse, E. A., Zhang, X. Q., O'Gorman, S., and Wahl, G. M. A transactivation-deficient mouse model provides insights into Trp53 regulation and function. Nat. Genet., *26:* 37–43, 2000.

35. Friedlander, P., Haupt, Y., Prives, C., and Oren, M. A mutant p53 that discriminates between p53-responsive genes cannot induce apoptosis. Mol. Cell. Biol., *16:* 4961–4971, 1996.

36. Ishioka, C., Englert, C., Winge, P., Yan, Y. X., Engelstein, M., and Friend, S. H. Mutational analysis of the carboxy-terminal portion of p53 using both yeast and mammalian cell assays *in vivo*. Oncogene, *10:* 1485–1492, 1995.

37. Kobayashi, T., Consoli, U., Andreeff, M., Shiku, H., Deisseroth, A. B., and Zhang, W. Activation of p21WAF1/Cip1 expression by a temperature-sensitive mutant of human p53 does not lead to apoptosis. Oncogene, *11:* 2311–2316, 1995.

38. Rowan, S., Ludwig, R. L., Haupt, Y., Bates, S., Lu, X., Oren, M., and Vousden, K. H. Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. EMBO J., *15:* 827–838, 1996.

39. Sabbatini, P., Chiou, S. K., Rao, L., and White, E. Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. Mol. Cell. Biol., *15:* 1060–1070, 1995.

40. Ryan, K. M. and Vousden, K. H. Characterization of structural p53 mutants which show selective defects in apoptosis but not cell cycle arrest. Mol. Cell. Biol., *18:* 3692–3698, 1998.

41. Ludwig, R. L., Bates, S., and Vousden, K. H. Differential activation of target cellular promoters by p53 mutants with impaired apoptotic function. Mol. Cell. Biol., *16:* 4952–4960, 1996.

42. Flores, E. R., Tsai, K. Y., Crowley, D., Sengupta, S., Yang, A., McKeon, F., and Jacks, T. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature, *416:* 560–564, 2002.

43. Shikama, N., Lee, C. W., France, S., Delavaine, L., Lyon, J., Krstic-Demonacos, M., and La Thangue, N. B. A novel cofactor for p300 that regulates the p53 response. Mol. Cell, *4:* 365–376, 1999.

44. Samuels-Lev, Y., O'Connor, D. J., Bergamaschi, D., Trigiante, G., Hsieh, J. K., Zhong, S., Campargue, I., Naumovski, L., Crook, T., and Lu, X. ASPP proteins specifically stimulate the apoptotic function of p53. Mol. Cell, *8:* 781–794, 2001.

45. Debbas, M. and White, E. Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. Genes Dev., *7:* 546–554, 1993.

46. Lowe, S. W. and Ruley, H. E. Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. Genes Dev., *7:* 535–545, 1993.

47. Hermeking, H. and Eick, D. Mediation of c-Myc-induced apoptosis by p53. Science, *265:* 2091–2093, 1994.

48. Qin, X. Q., Livingston, D. M., Kaelin, W. G., Jr., and Adams, P. D. Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. Proc. Natl. Acad. Sci. USA, *91:* 10918–10922, 1994.

49. Wagner, A. J., Kokontis, J. M., and Hay, N. Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21waf1/cip1. Genes Dev., *8:* 2817–2830, 1994.

50. Wu, X. and Levine, A. J. p53 and E2F-1 cooperate to mediate apoptosis. Proc. Natl. Acad. Sci. USA, *91:* 3602–3606, 1994.

51. Soengas, M. S., Alarcon, R. M., Yoshida, H., Giaccia, A. J., Hakem, R., Mak, T. W., and Lowe, S. W. Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. Science, *284:* 156–159, 1999.

52. Attardi, L. D., Reczek, E. E., Cosmas, C., Demicco, E. G., McCurrach, M. E.,

Lowe, S. W., and Jacks, T. PERP, an apoptosis-associated target of p53, is a novel member of the PMP-22/gas3 family. Genes Dev., *14:* 704–718, 2000.

53. Resnick-Silverman, L., St Clair, S., Maurer, M., Zhao, K., and Manfredi, J. J. Identification of a novel class of genomic DNA-binding sites suggests a mechanism for selectivity in target gene activation by the tumor suppressor protein p53. Genes Dev., *12:* 2102–2107, 1998.

54. Mundt, M., Hupp, T., Fritsche, M., Merkle, C., Hansen, S., Lane, D., and Groner, B. Protein interactions at the carboxyl terminus of p53 result in the induction of its *in vitro* transactivation potential. Oncogene, *15:* 237–244, 1997.

55. Hupp, T. R., Meek, D. W., Midgley, C. A., and Lane, D. P. Regulation of the specific DNA binding function of p53. Cell, *71:* 875–886, 1992.

56. Funk, W. D., Pak, D. T., Karas, R. H., Wright, W. E., and Shay, J. W. A transcriptionally active DNA-binding site for human p53 protein complexes. Mol. Cell. Biol., *12:* 2866–2871, 1992.

57. Flatt, P. M., Tang, L. J., Scatena, C. D., Szak, S. T., and Pietenpol, J. A. p53 regulation of G(2) checkpoint is retinoblastoma protein dependent. Mol. Cell. Biol., *20:* 4210–4223, 2000.

58. Takahashi, T., Carbone, D., Nau, M. M., Hida, T., Linnoila, I., Ueda, R., and Minna, J. D. Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. Cancer Res., *52:* 2340–2343, 1992.

59. Macleod, K. F., Sherry, N., Hannon, G., Beach, D., Tokino, T., Kinzler, K., Vogelstein, B., and Jacks, T. p53-dependent and independent expression of p21 during cell growth, differentiation, and DNA damage. Genes Dev., *9:* 935–944, 1995.

60. Zauberman, A., Lupo, A., and Oren, M. Identification of p53 target genes through immune selection of genomic DNA: the cyclin G gene contains two distinct p53 binding sites. Oncogene, *10:* 2361–2366, 1995.

61. Vousden, K. H. p53: death star. Cell, *103:* 691–694, 2000.

62. Qian, H., Wang, T., Naumovski, L., Lopez, C. D., and Brachmann, R. K. Groups of p53 target genes involved in specific p53 downstream effects cluster into different classes of DNA binding sites. Oncogene, *21:* 7901–7911, 2002.

63. Inga, A., Storici, F., Darden, T. A., and Resnick, M. A. Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. Mol. Cell. Biol., *22:* 8612–8625, 2002.

64. McCurrach, M. E., Connor, T. M., Knudson, C. M., Korsmeyer, S. J., and Lowe, S. W. Bax-deficiency promotes drug resistance and oncogenic transformation by attenuating p53-dependent apoptosis. Proc. Natl. Acad. Sci. USA, *94:* 2345–2349, 1997.

65. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K., and Vogelstein, B. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science, *249:* 912–915, 1990.

66. Kern, S. E., Pietenpol, J. A., Thiagalingam, S., Seymour, A., Kinzler, K. W., and Vogelstein, B. Oncogenic forms of p53 inhibit p53-regulated gene expression. Science, *256:* 827–830, 1992.

Molecular Cancer Research

Multiple Response Elements and Differential p53 Binding Control *Perp* Expression During Apoptosis[1] 1 Anna Fuller Fund (E. E. R.), Leukemia and Lymphoma Society of America (E. R. F.), Damon Runyon Cancer Research Foundation (L. D. A.), and NIH and Howard Hughes Medical Institute (T. J.).

Elizabeth E. Reczek, Elsa R. Flores, Alice S. Tsay, et al.

Mol Cancer Res 2003;1:1048-1057.