The p53 protein contains several functional domains necessary for inducing cell cycle arrest and apoptosis. The C-terminal basic domain within residues 364–393 and the proline-rich domain within residues 64–91 are required for apoptotic activity. In addition, activation domain 2 within residues 43–63 is necessary for apoptotic activity when the N-terminal activation domain 1 within residues 1–42 is deleted (ΔAD1) or mutated (AD1Δ). Here we have discovered that an activation domain 2 mutation at residues 53–54 (AD2Δ) abolges the apoptotic activity but has no significant effect on cell cycle arrest. We have also found that p53-(ΔAD2), which lacks activation domain 2, is inert in inducing apoptosis. p53-(AD2ΔBD), which is defective in activation domain 2 and lacks the C-terminal basic domain, p53-(ΔAD2ΔBD), which lacks both activation domain 2 and the C-terminal basic domain, and p53-(ΔPRDΔBD), which lacks both the proline-rich domain and the C-terminal basic domain, are also inert in inducing apoptosis. All four mutants are still capable of inducing cell cycle arrest, albeit to a lesser extent than wild-type p53. Interestingly, we have found that deletion of the N-terminal activation domain 1 alleviates the requirement of the C-terminal basic domain for apoptotic activity. Thus, we have generated a small but potent p53-(ΔAD1ΔBD) molecule. Furthermore, we have determined that at least two of the three domains (activation domain 1, activation domain 2, and the proline-rich domain), are required for inducing cell cycle arrest. Taken together, our results suggest that activation domain 2 and the proline-rich domain form an activation domain for inducing pro-apoptotic genes or inhibiting anti-apoptotic genes. The C-terminal basic domain is required for maintaining this activation domain competent for transactivation or transrepression.

Activation of p53 leads to at least two well defined cellular responses: cell cycle arrest and apoptosis (1–4). Based on these activities and other characteristics (1, 5), the p53 protein can be divided into several functional domains. These are activation domain 1 within residues 1–42 (6–8), activation domain 2 within residues 43–63 (9–11), the proline-rich domain within residues 64–91 (12), the sequence-specific DNA-binding domain within residues 100–300 (1), the nuclear localization signal within residues 316–325 (13), the tetramerization domain within residues 334–356 (14), which also contains a nuclear export signal (15), and the C-terminal basic domain within residues 364–393 (1, 5).

p53 is frequently mutated in cancers. Mutations in the p53 DNA binding domain or certain mutations in the nuclear localization signal and tetramerization domain that indirectly affect DNA binding abrogate or diminish p53 activity in cell cycle arrest and apoptosis (1, 5). The proline-rich domain has been shown to be required for efficient growth suppression (12). Recent experiments indicate that the proline-rich domain is necessary for apoptosis but not cell cycle arrest (16–18). In addition, the proline-rich domain plays an important role in the induction of several endogenous target genes, but is not required for activation of the exogenously introduced promoters of these target genes (17). These results suggest that the proline-rich domain may participate in the induction of cellular target gene(s) responsible for mediating apoptosis. However, the role of other p53 functional domains (especially the N-terminal activation domain 1 and the C-terminal basic domain) in apoptosis is still not certain. Earlier reports have shown that in some experimental protocols (19–21) including our own (22), p53 transactivation activity is dispensable for apoptosis. It should be noted that this conclusion is based at least in part on the observation that an activation domain 1-deficient mutant (a double point mutation at residues 22–23, AD1Δ) is capable of inducing apoptosis (21, 22). Recently, we and others have shown that p53-(AD1Δ) contains an intact activation domain 2 (9–11), and therefore, p53-(AD1Δ) is still competent in transactivation (10). Furthermore, when both activation domain 1 and activation domain 2 are mutated (a quadruple point mutation at residues 22–23 and 53–54, AD1ΔAD2Δ), the resulting protein is inert in transactivation and in inducing cell cycle arrest and apoptosis (9–11).

The C-terminal basic domain has been subjected to extensive analysis, and all evidence suggests that the basic domain is a regulatory domain. This basic domain can regulate the DNA binding activity when it is phosphorylated (1, 5), acetylated (23–25), deleted (26), or associated with anti-p53 antibody (26, 27) or peptides derived from the C terminus of p53 (28, 29). Interestingly, the mechanism by which these latter peptides enhance p53 DNA binding activity is the ability of the peptides to interact with three separate domains in p53, that is, the proline-rich domain (30), the DNA binding domain (31), and the C-terminal basic domain (30, 31). The C-terminal basic domain also interacts with several cellular proteins, such as TFIIH subunits XBP and XPD (32, 33), and Werner syndrome protein (WRN) (34, 35), which all lead to efficient induction of p53-mediated apoptosis. These results support a hypothesis that the C-terminal basic domain is a negative regulatory do-

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main whose effect on the DNA binding activity can be alleviated by interacting with other cellular proteins, peptides derived from the p53 C terminus, or other modifications. However, several groups have shown that p53-(ABD), which lacks the C-terminal basic domain, has a reduced ability to induce several cellular target genes and becomes incapable of inducing apoptosis (22, 32, 36). These results suggest that the C-terminal basic domain can regulate p53 activity both positively and negatively.

In this study, we show that activation domain 2 and the proline-rich domain form an activation domain for inducing pro-apoptotic genes or inhibiting anti-apoptotic genes. The C-terminal basic domain is required for maintaining this activation domain competent for transactivation or transrepression. We also found that an activation domain capable of inducing at least partial cell cycle arrest can be formed by activation domain 1 plus activation domain 2, activation domain 1 plus the proline-rich domain, or activation domain 2 plus the proline-rich domain. The ability of these activation domains to induce cell cycle arrest can be enhanced by the presence of the C-terminal basic domain.

EXPERIMENTAL PROCEDURES

Plasmids and Mutagenesis—Mutant p53 cDNA constructs were generated by polymerase chain reaction, and mutations were confirmed by DNA sequencing. All p53 proteins were tagged at their N termini with the influenza hemagglutinin (HA) peptide recognizable by anti-HA antibody 12CA5 (Roche Molecular Biochemicals), an antibody (10, 17, 22), with anti-p53 monoclonal antibody Pab240, which was amplified independently and ligated through an internal StuI site (37), and 3'-end primer C59 (TTCACTGGACCTGCTTCTTGCCTATATC). To generate p53-(AD2'), cDNA fragments encoding amino acids 1–59 and 60–393 was amplified independently and ligated through an internal Avel site. The 3'-end primer encoding amino acids 1–59 was amplified by 5'-end primer 5HA (GATCGAATTCACATGGTCTAGTGGTTCAATATC), and the 3'-end primer C393 (GATCGAATTCATGGTCTAGTGGTTCAATATC). This cDNA fragment encoding amino acids 60–393 was amplified by 5'-end primer N60 (ACTGAAGCTTCTTCAGTGCTCTGTTGTTCAATATC). The cDNA fragment encoding amino acids 41–393 but lacks residues 43–63 was amplified by 5'-end primer AD2' (TGGCAATGGATGATCCCCTGTCGTCTTCTGT) and 3'-end primer C393. To generate p53-(AD2), a cDNA fragment that encodes residues 41–393 but lacks residues 43–63 was amplified by 5'-end primer AD2 (TGGCAATGGATGATCCCCTGTCGTCTTCTGT) and 3'-end primer C393. To generate p53-(AD1A), p53-(AD2), a cDNA fragment that encodes residues 41–393 at a SphI site was amplified by 5'-end primer AP5 (TTGCAATGGATGATCCCCTGTCGTCTTCTGT) and 3'-end primer N60 (ACTGAAGCTTCTTCAGTGCTCTGTTGTTCAATATC). This fragment was then used to replace the HA-tagged wild-type p53 from residues 41–393 at a BsrD1 site. To generate p53-(ΔAD2ΔPRD), a cDNA fragment that encodes residues 41–393 but lacks residues 43–63 was amplified by 5'-end primer AP5 (TTGCAATGGATGATCCCCTGTCGTCTTCTGT) and 3'-end primer C393. This fragment was then used to replace the HA-tagged wild-type p53 from residues 41–393 at a BsrD1 site. p53-(ΔAD1), p53-(ΔAD1ΔPRD), p53-(ΔAD1ΔPRDΔBD), and p53-(ΔAD1Δ1ΔAD2ΔBD) were generated as described previously (10, 17, 22). To generate p53-(ΔA1D1PRD), p53-(ΔAD1PRD) cDNA was amplified by 5'-end primer N43 (GATCGAATTCACCATGGGTCACCATAGTGTCATCGACCCTTGATCTCCTGCCG) and 3'-end primer C393. To generate p53-(ΔAD2) (BD), p53-(ΔAD2ΔBD), p53-(ΔAD1ΔPRDΔBD), p53-(ΔAD1ΔPRDΔBDΔBD), and p53-(ΔAD2ΔPRD) were replaced with the corresponding cDNA fragment in p53-(ΔAD2ΔPRDΔBD), and p53-(ΔAD2ΔPRDΔBDΔBD) cDNA fragments starting from the StuI site in p53-(AD2'), p53-(AD2), p53-(ΔPRD), p53-(ΔAD1ΔPRD), p53-(ΔAD1ΔPRDΔBD), p53-(ΔAD1Δ1ΔAD2ΔBD), and p53-(ΔAD2ΔPRD) were replaced with the corresponding DNA fragment in p53-(ΔAD2ΔPRDΔBD) cDNA fragments.

The above mutant p53 cDNAs were cloned separately into a tetracycline-responsive plasmid expression vector pUHD10-3, at its EcoRI site (37), and the resulting plasmids were used to generate cell lines that inducibly express p53.

Cell Lines—H1299 and MCF7 cell lines that express inducible proteins of interest were generated as described previously (10, 17, 22). The H1299 cell lines p53-3, p53-(R248S)-4, p53-(AD1)-2, p53-(AD2)-3, p53-(ΔAD1Δ1ΔAD2)-4, and p53-(ΔAD2)-5, and p53-(ΔAD1)-2 were used as described previously (10, 17, 22).

Western Blot Analysis—Western blot analysis was performed as described (10, 17, 22), with anti-p53 monoclonal antibody Pab240, anti-HA monoclonal antibody 12CA5 (Roche Molecular Biochemicals), anti-actin polyclonal antibody (Sigma), and anti-p21 monoclonal antibody (Ab-1) (Oncogene Research Products, Cambridge, MA).

Growth Rate Analysis, Trypan Blue Dye Exclusion Assay, DNA Histogram Analysis, and Annexin V Staining—Growth rate analysis, trypan blue dye exclusion assay, and DNA histogram analysis were performed as described previously (10, 17, 22). Propidium iodide and RNase A were purchased from Sigma. Fluorescein isothiocyanate-labeled annexin V was purchased from Roche Molecular Biochemicals, and staining was performed as described by the manufacturer.

RESULTS

The Activity of Activation Domain 2 Is Necessary for Inducing Apoptosis—Previously, we have shown that the activity of activation domain 2 is required for inducing apoptosis when a double point mutation at residues 22–23 or deletion of the N-terminal 42 amino acid residues renders activation domain 1 dysfunctional (10). To further determine the function of activation domain 2 in apoptosis, we generated an activation domain 2-deficient mutant, p53-(AD2'), which contains a double point mutation at residues 53–54. We then established several cell lines that inducibly express this mutant in p53-null H1299 lung carcinoma cells. Western blots from two representative cell lines, p53-(AD2')-6 and -8, are shown in Fig. 1A. After normalization to the levels of actin protein expressed, we found that the levels of p53 protein in p53-(AD2')-6 and -8 cells were comparable with that in p53-3 and HA-p53-15 cells, which express wild-type p53 and HA-tagged wild-type p53, respectively (Fig. 1A, upper two panels, compare lanes 5–8 with lanes 1–4). To determine the transcriptional activity of p53-(AD2'), we measured the level of p21 protein induced by p53-(AD2'). Surprisingly, we found that the ability of p53-(AD2') to induce p21 was severely diminished (Fig. 1A, p21 panel, lanes 5–8). These results are similar to that observed for the activation domain 1-deficient mutant (6, 10, 22). In contrast, p21 was strongly induced by wild-type p53 and HA-tagged wild-type p53 (Fig. 1A, p21 panel, lanes 1–4).

One of the hallmarks for p53 when overexpressed in cells is growth suppression (1–3). The HA-tagged wild-type p53 protein in HA-p53-15 cells, like the untagged wild-type p53 in p53-3 cells (10, 22), inhibits cell proliferation (data not shown). To determine the activity of p53-(AD2') in H1299 cells, the growth rate of p53-(AD2')-6 cells was determined over a 5-day period. When induced to express p53-(AD2') cells failed to multiply (Fig. 1B), but visible microscopic cell death was not significantly increased (data not shown).

Previously, several studies have shown that the C-terminal basic domain is necessary for inducing apoptosis but not cell cycle arrest (22, 32). To determine whether this domain has any effect on the ability of p53-(AD2') to induce growth suppression, we generated p53-(AD2ΔBD), which is deficient in activation domain 2 and has a deletion of the C-terminal basic domain. We then established several cell lines that inducibly express p53-(AD2ΔBD). Western blots from three representative cell lines, p53-(AD2ΔBD)-2, -8, and -9, are shown in Fig. 1A. We found that the levels of p53 in these cells were comparable with that in HA-p53-15 and p53-(AD2ΔBD)-1 cells (Fig. 1A, upper two panels, compare lanes 5–8 and 9–16). p53-(AD2ΔBD)-1 cells are derived from H1299 cells that inducibly express p53-(ΔBD), which lacks the C-terminal basic domain (22). Similarly, the transcriptional activity of p53-(AD2ΔBD) was determined by measuring the level of p21 induced. We found that, like p53-(AD2'), the ability of p53-(AD2ΔBD) to induce p21 was significantly diminished (Fig. 1A, p21 panel, compare lanes 11–16 with lanes 1–4). In contrast, p21 was strongly induced by p53-(ΔBD) (Fig. 1A, p21 panel, lanes 9–10), consistent with previous reports (22, 32). Nevertheless, growth rate analysis showed that p53-(AD2ΔBD) was still capable of inhibiting cell

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growth (Fig. 1C), albeit to a lesser extent than p53-(AD2)
(Fig. 1B).

To determine whether the growth suppression by p53-
(AD2) is because of cell cycle arrest, apoptosis, and/or both,
we performed a DNA histogram analysis and an annexin V
staining assay. When induced to express the mutant
p53-(AD2) for three days, we found that the percentage of cells in
S phase decreased from 35 to 8% whereas cells in G1 increased
from 49 to 75%, suggesting that p53-(AD2) arrested cells
primarily in G1 (Fig. 1, D–E). However, no apparent apoptosis
was detected by either DNA histogram analysis (Fig. 1,
D–E) or annexin V staining (Fig. 1, F–G). Thus, the activity in activa-
tion domain 2 is necessary for inducing apoptosis. As a positive
control, we analyzed p53-3 and HA
z p53-15 cells. When induced
to express wild-type or HA-tagged p53 for three days, we found
that both p53-producing cells were arrested primarily in G1
and underwent apoptosis, consistent with previous reports (10,
22). We also analyzed p53-(AD2ΔBD)-9 cells. We found that
no significant apoptosis was observed, and cells primarily ar-
rested in G1 when induced to express p53-(AD2ΔBD) (data not
shown).

To determine the activity of the entire activation domain 2
(residues 43–62), we generated p53-(AD), which lacks the
entire activation domain 2 and p53-(ΔAD2ΔBD), which in turn
lacks activation domain 2 and the C-terminal basic domain. We
then established several cell lines that inducibly express p53-
(ΔAD2) and p53-(ΔAD2ΔBD), respectively (Fig. 2, A and C). We
found that p53-(ΔAD2) and p53-(ΔAD2ΔBD) suppressed cell
proliferation (Fig. 2, B and D), albeit to a lesser extent than
p53-(AD2) and p53-(AD2ΔBD) (Fig. 1, B and C). Further-
more, we found that cells were arrested primarily in G1 but did
not undergo apoptosis when induced to express these p53 mu-
tants (data not shown, Table I). However, we found that
p21 was not significantly induced (Fig. 2,
A and C), suggesting that
p53-dependent cell cycle arrest in G1 can be mediated by a
gene(s) other than p21.

The Proline-rich Domain Contributes to the Ability of p53 to
Induce Cell Cycle Arrest—Previously, we and others have
shown that the proline-rich domain (16–18) and the C-terminal
basic domain (22, 32) are necessary for inducing apoptosis but
not cell cycle arrest. To determine whether both domains are
dispensable for inducing cell cycle arrest, we generated p53-
(ΔPRDΔBD), which lacks both the proline-rich domain and the
C-terminal basic domain. We then established several cell lines
that inducibly express this mutant. Western blots from three
representative cell lines, p53-(ΔPRD)-2, -6, and -7, are
shown in Fig. 2E. We found that the level of p53 expressed in
p53-(ΔPRDΔBD)-2 cells was comparable with that in p53-3,
HA-p53-15, and p53-(ΔBD)-1 but slightly lower than that in
p53-(ΔPRD)-5, which inducibly expresses a p53 mutant lacking

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**Fig. 1.** The activity of activation domain 2 is necessary for inducing apoptosis. A, levels of p53, p21, and actin were assayed by Western blot analysis in cell lines as shown above the blots. Cell extracts were prepared from non-induced cells (−) and cells induced to express p53 for 24 h (+). HA-tagged p53 was detected with 12CA5 antibody. p53 was detected with anti-p53 monoclonal antibody Pab240. p21 was detected with anti-p21 monoclonal antibody (Ab-1). Actin was detected with anti-actin polyclonal antibody. B and C, growth rates of p53-(AD2)-6 and p53-(AD2ΔBD)-9 cells in the absence (○) or presence (□) of p53 over a 5-day period. D and E, DNA content was quantified by propidium iodide staining of fixed cells that were non-induced (− p53) or induced (+ p53) to express p53-(AD2) for 3 days. F and G, apoptotic cells were quantified by propidium iodide-annexin V staining of cells that were non-induced (− p53) or induced (+ p53) to express p53-(AD2) for 3 days.
the proline-rich domain (Fig. 2E, p53 panel). To determine whether p21 can be induced, we found that p53-(ΔPRΔΔBD) was much less potent in inducing p21 than wild-type p53, HA-tagged p53, p53-(ΔBD), or p53-(ΔPRΔD) (Fig. 2E, p21 panel). However, when the DNA binding activity was determined in vitro, we found that p53-(ΔPRΔΔBD) was as potent as wild-type p53 in binding to the ribosomal gene cluster p53 response element (data not shown). This suggests that deletion of both the proline-rich domain and the C-terminal basic domain does not affect the activity of the p53 DNA binding domain. Growth rate analysis showed that p53-(ΔPRΔDΔ) had a much reduced ability to suppress cell proliferation (Fig. 2F). In addition, DNA histogram analysis and annexin V staining assay showed that a partial arrest in G1, but no apoptosis, was detected in p53-(ΔPRΔΔBD)-2 cells (data not shown).

p53-(ΔAD1ΔBD) Is Small but Potent in Inducing Cell Cycle Arrest and Apoptosis—We and others have shown that p53-(ΔBD), which lacks the C-terminal basic domain, is inactive in inducing apoptosis (22, 32, 36) whereas p53-(ΔAD1), which lacks activation domain 1 (residues 1–42), is very active (10). To determine whether the C-terminal basic domain is necessary for p53-(ΔAD1) to induce apoptosis, we generated p53-(ΔAD1ΔBD), which lacks activation domain 1 and the C-terminal basic domain. We then established several cell lines that inducibly express p53-(ΔAD1ΔBD). Western blots from three representative cell lines, p53-(ΔAD1ΔBD)-3, -6, and -7, are shown in Fig. 3A. We found that the level of p53 expressed in these cells was comparable with that in p53-3, HA-p53-15, and p53-(ΔBD)-1 cells, but lower than that in p53-(ΔAD1)-2 cells (Fig. 3A, p53 panel). p53-(ΔAD1)-2 cells are derived from H1299 cells that inducibly express p53-(ΔAD1), which lacks activation domain 1 (10). We found that p53-(ΔAD1ΔBD) retained the ability to induce p21. Induction of p21 by p53-(ΔAD1ΔBD) was greater than induction by p53-(ΔAD1) but less than induction by wild-type p53 and p53-(ΔBD) (Fig. 3A, p21 panel). Growth rate analysis showed that cells failed to multiply when induced to express p53-(ΔAD1ΔBD) (Fig. 3, B and C). Microscopic examination showed that the p53-expressing cells detached from plates and shrank to form apoptotic bodies (data not shown). DNA histogram analysis showed that the percentage of cells in S phase decreased from 35 to 11% but the percentage of cells in G1 increased from 55 to 75%, suggesting that these cells arrested primarily in G1 (Fig. 3, D–E). We also found that the number of cells with a sub-G1 DNA content was much less potent in inhibiting p53-(ΔAD1)-1 than in p53-(ΔAD1)-2 cells. However, when stained for annexin V, we found that the percentage of stained cells increased from 5 to 7%, suggesting that these cells also underwent apoptosis (Fig. 3, F–G).

To further confirm the ability of p53-(ΔAD1ΔBD) to induce apoptosis, we generated several MCF7 breast carcinoma cell lines that inducibly express wild-type p53 and p53-(ΔAD1ΔBD). Western blots from one representative cell line that inducibly expresses wild-type p53 (MCF7-p53-24) and two that inducibly express p53-(ΔAD1ΔBD) (MCF7-p53-
Activated p53 Mutants—To further define the role of activation domain 1, activation domain 2, and the proline-rich domain in inducing cell cycle arrest and apoptosis, we generated six p53 mutants that are dysfunctional in two or three of the four functional domains (AD1ΔBD)-7 and -15) are shown in Fig. 4A. We found that the level of p53 induced in MCF7-p53-(AD1ΔBD)-7 and -15 cells was slightly lower than in MCF7-p53-24 cells (Fig. 4A, p53 panel). When the level of p21 was measured to determine the transcriptional activity of p53-(AD1ΔBD), we found that p53-(AD1ΔBD) was potent in transactivation (Fig. 4A, p21 panel). This result is similar to that obtained in H1299 cells (Fig. 3A). Growth rate analysis showed that cells failed to multiply when induced to express wild-type p53 or p53-(AD1ΔBD) (Fig. 4, B–C). Microscopic examination showed that the p53-expressing cells detached from plates and shrank to form apoptotic bodies (data not shown). DNA histogram analysis showed that the percentage of cells that had a sub-G1 DNA content was increased from 3 to 37% by wild-type p53 (Fig. 4, D–E) and from 4 to 49% by p53-(AD1ΔBD) (Fig. 4, F–I). In addition, annexin V staining assay showed that the percentage of the annexin V-stained cells was increased from 7 to 28% by wild-type p53 and from 9 to 29% by p53-(AD1ΔBD). These data indicate that p53-(AD1ΔBD) is a potent apoptotic inducer.

At Least Two of the Three Domains, i.e. Activation Domain 1, Activation Domain 2, and the Proline-rich Domain Are Re-quired For Inducing Cell Cycle Arrest—To further define the role of activation domain 1, activation domain 2, and the proline-rich domain, and the C-terminal basic domain in inducing cell cycle arrest and apoptosis, we generated six p53 mutants that are dysfunctional in two or three of the four functional domains.
7–8). p53-(AD1) (lanes 5–6), p53-(AD1AD) (lanes 9–10), p53-(AD2) (lanes 13–14), and p53-(AD2–AD) (lanes 15–16) were extremely weak in inducing p21 and BAX (2-fold or less). It should be mentioned that p53-(AD2) is extremely potent in inducing G1 arrest (see Fig. 1, D–E), suggesting that a gene(s) other than p21 is responsible for this. Furthermore, when activation domain 1 and the basic domain were deleted, the ability of p53-(AD1AD) to induce p21 and BAX was partially restored (lanes 11–12), consistent with the result detected by Western blot analysis (Fig. 3A).

DISCUSSION

p53 induces apoptosis but the underlying mechanism remains unclear. To determine this mechanism, two major questions need to be addressed. What domains in p53 are required and is p53 transcriptional activity necessary for inducing apoptosis? Previous attempts to answer these questions have been inconclusive, because different experimental systems have been used (1, 2). These include various types of cell lines and methods to express p53 (transient versus stable, ectopic versus inducible) and different types of p53 mutants (temperature-sensitive mutant versus wild-type p53; point mutations versus deletion mutations). To avoid these problems, we have applied the tetracycline inducible expression system to stably express various p53 mutants in p53-null H1299 cells. On the basis of the results obtained in this study (Table I) and several previous studies (11, 12, 16, 32, 38–40), including our own (10, 17, 22), we propose the following model for p53 functional domains in apoptosis (Fig. 7). First, p53 DNA binding activity is necessary for apoptosis because mutants that are defective in the DNA binding and tetramerization domains are inert. Second, activation domain 2 and the proline-rich domain can form an activation domain for transactivating pro-apoptotic genes or transrepressing anti-apoptotic genes, because mutation or deletion in either one of the domains abrogates the apoptotic activity. Third, activation domain 1 is not required because deletion of or mutation in activation domain 1 (p53-(AD1), p53-(AD1AD)) has little effect on apoptosis. Fourth, the C-terminal basic domain is necessary for maintaining p53 competent in inducing apoptosis, probably by relieving the inhibitory activity of activation domain 1, because p53-(AD1AD) is capable of inducing apoptosis.

Several p53 inducible genes, such as BAX (41), KILLER/DR5 (42), and several PIGs (43), may participate in the apoptotic process. These genes can be induced by either p53-(ADPRD) (17) or p53-(AD2) (data not shown), both of which are active in inducing cell cycle arrest but not apoptosis, suggesting that these genes are not required or insufficient for inducing apoptosis. Recent evidence has shown that p53 can repress specific genes, such as MAP4 (44). It is possible that transrepression of anti-apoptotic genes plays an important role in p53-mediated
apoptosis. Therefore, the cell lines that inducibly express the p53 mutants described in this study, especially p53-(AD1AD2BD), can be used to identify and determine whether a cellular gene is necessary for mediating p53-dependent apoptosis.

p53 transcriptional activity has been shown to be necessary for inducing cell cycle arrest (1, 2, 4, 45). In this study, we extend this observation. We found that an activation domain capable of inducing at least partial cell cycle arrest can be formed by activation domain 1 plus activation domain 2, activation domain 1 plus the proline-rich domain, or activation domain 2 plus the proline-rich domain (Table I). When two of the three domains, i.e. activation domain 1, activation domain 2, and the proline-rich domain, become dysfunctional, the ability to induce cell cycle arrest is abrogated (Table I). It should be mentioned that p53-(AD1BD) is defective in inducing cell cycle arrest although two functional domains, i.e. activation domain 2 and the proline-rich domain are still intact (22). However, when part or all of the residues for activation domain 1 are deleted, as in p53-(AD1–23) and p53-(AD1D1), the ability to induce cell cycle arrest is retained. This suggests that the presence of the mutated activation domain 1 may mask the activity of, or inhibit the interaction of, a potential co-activator (or an adaptor) with the activation domain formed by activation domain 2 and the proline-rich domain necessary for transactivation or transrepression.

The search for mediators of p53-dependent cell cycle arrest has identified many cellular p53 target genes (1, 4, 46). p21cip1/waf1, a well characterized cyclin-dependent kinase inhibitor, can mediate cell cycle arrest in G1 when overexpressed (22, 47–51). Previous studies have shown that p53-(AD1), which is deficient in inducing p21, is incapable of inducing arrest in G1, consistent with the hypothesis that p21 plays an important role in mediating p53-dependent arrest in G1 (22, 40). In this study, we found that p53-(AD2) is extremely active in inducing arrest in G1, suggesting that activation domain 1, but not activation domain 2, plays an important role in inducing cell cycle arrest. However, p21 is only slightly induced by p53-(AD2) (Fig. 1A). Because p53-(AD1AD2), which is deficient in both activation domain 1 and activation domain 2, is inert in inducing cell cycle arrest (9–11), this suggests that a gene(s) responsible for arrest by p53-(AD2) must be induced. This is not surprising because DNA damage-induced G1 arrest is delayed but not abolished in p21-null fibroblasts from p21-deficient mice (52, 53). Therefore, the cell line that inducibly expresses p53-(AD2) can be used to identify other novel gene(s) responsible for G1 arrest.

Previously, several studies have shown that the p53 protein can be cleaved by cellular proteases in cells treated with DNA damaging agents, which leads to formation of several smaller polypeptides with molecular masses ranging from 35–50 kDa (54–58). In addition, the cleavage of p53 is concomitant with the
onset of apoptosis in cells treated with DNA damaging agents, suggesting that the cleaved p53 polypeptides are potent in p53 activity and may participate in the apoptotic process (58). Interestingly, one of the cleaved p53 polypeptides, p50, is p53-(ΔN23), which lacks the N-terminal 23 residues (58). We have shown previously that p53-(ΔN23) is active in inducing cell cycle arrest and apoptosis (10). Thus, the cellular machinery can generate an active but smaller p53 polypeptide that would not be subject to negative regulation by MDM2 (59–63). It is not clear whether p53-(ΔAD1BD) is an in vivo cleavage product of p53. However, because p53-(ΔAD1BD) lacks the MDM2 binding site, it would not be subjected to the negative regulation by MDM2. Thus, p53-(ΔAD1BD) represents a small but potent, apoptosis-inducing form of p53. Recent clinical trials have shown that adenoviruses expressing p53 are effective in treating some advanced forms of human cancers (64, 65). We suggest that p53-(ΔAD1BD) is a good candidate to replace the larger, unwieldy wild-type p53 in cancer gene therapy.

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REFERENCES

1. Ko, L. J., and Prives, C. (1996) Genes Dev. 10, 1054–1072
2. Levine, A. J. (1997) Cell 89, 323–331
3. Agarwal, M. L., Taylor, W. R., Chernov, M. V., Chernov, O. B., and Stark, G. R. (1998) J. Biol. Chem. 273, 1–4
4. Chen, X. (1999) Mol. Med. Today 5, 387–392
5. Giaccia, A. J., and Kastan, M. B. (1998) Nature Genet. 19, 305–308
6. Lin, J., Chen, J., Elenbaas, B., and Levine, A. J. (1994) Genes Dev. 8, 1235–1246
7. Unger, T., Mietz, J. A., Scheffner, M., Yee, C. L., and Howley, P. M. (1993) Mol. Cell. Biol. 13, 5186–5194
8. Chang, J., Kim, D. H., Lee, S. W., Choi, K. Y., and Sung, Y. C. (1995) J. Biol. Chem. 270, 25014–25019
9. Cauda, R., Scholnick, D. M., Darpino, P., Ying, C. Y., Halazonetis, T. D., and Berger, S. L. (1997) Oncogene 15, 807–816
10. Zhu, J., Zhou, W., Jiang, J., and Chen, X. (1998) J. Biol. Chem. 273, 13303–13306
11. Venot, C., Maratrat, M., Sierra, V., Conseiller, E., and Debussche, L. (1999) Oncogene 18, 2405–2410
12. Walker, K. K., and Levine, A. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15335–15340
13. Shaulsky, G., Goldfinger, N., Tosky, M. S., Levine, A. J., and Rotter, V. (1991) Oncogene 6, 2055–2060
14. Sturzbecher, H. W., Brain, R., Addison, C., Rudge, K., Remm, M., Grimaldi, M., Keenan, E., and Jenkins, J. B. (1992) Oncogene 7, 1513–1523
15. Stommel, J. M., Marbelen, N. D., Jimenez, G. S., Moll, U. M., Hope, T. J., and Wahl, G. M. (1999) EMBO J. 18, 1660–1672
16. Sakamuro, D., Sabbatini, P., White, E., and Prendergast, G. C. (1997) Oncogene 15, 887–898
17. Zhan, J., Zhang, J., Zhou, W., Zhu, K., and Chen, X. (1999) Oncogene 18, 2149–2155
18. Venot, C., Maratrat, M., Dureuil, C., Conseiller, E., Bracco, L., and Debussche, L. (1998) EMBO J. 17, 4668–4679
19. Caelies, C., Helberg, A., and Karin, M. (1994) Nature 370, 220–223
20. Wagner, A. J., Kokontis, J. M., and Hay, N. (1994) Genes Dev. 8, 2817–2830
21. Haupt, Y., Rowan, S., Shaulian, E., Vousden, K. H., and Oren, M. (1995) Genes Dev. 9, 2170–2183
22. Chen, X., Ko, L. J., Jayaraman, L., and Prives, C. (1996) Genes Dev. 10, 2438–2451
23. Lee, L., Scholnick, D. M., Trievel, R. C., Zhang, H. B., Marmarstein, R., Halazonetis, T. D., and Berger, S. L. (1999) Mol. Cell. Biol. 19, 1202–1209
24. Gu, W., and Roeder, R. G. (1997) Cell 90, 556–606
25. Sakaguchi, K., Herrera, E., Saito, S., Miki, T., Bustin, M., Vassilev, A., Anderson, C. W., and Appella, E. (1998) Genes Dev. 12, 2831–2841
26. Hupp, T. R., Meek, D. W., Midley, C. A., and Lane, D. P. (1992) Cell 71, 875–886
27. Halazonetis, T. D., Davis, L. J., and Kandel, A. N. (1993) EMBO J. 12, 1021–1028
