p53 Basic C Terminus Regulates p53 Functions through DNA Binding Modulation of Subset of Target Genes*  

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Background: The role of the conserved basic C terminus of p53 remains elusive.  
Results: The C terminus of p53 controls target gene selectivity and is needed for cellular responses.  
Conclusion: The C terminus of p53 is required for full p53 function.  
Significance: This provides insight into the basis for target gene selectivity by p53 as well as determination of cell fate outcomes.

The p53 gene encodes a transcription factor that is composed of several functional domains: the N-terminal transactivation domain, the central sequence-specific DNA binding domain, the tetramerization domain, and the highly basic C-terminal regulatory domain (CTD). The p53 CTD is a nonspecific DNA binding domain that is subject to extensive post-translational modifications. However, the functional significance of the p53 CTD remains unclear. The role of this domain in the regulation of p53 functions is explored by comparing the activity of ectopically expressed wild-type (WT) p53 protein to that of a truncated mutant lacking the 24 terminal amino acids (Δ24). Using quantitative real time PCR and chromatin Immunoprecipitation experiments, a p53 CTD deletion is shown to alter the p53-dependent induction of a subset of its target genes due to impaired specific DNA binding. Moreover, p53-induced growth arrest and apoptosis both require an intact p53 CTD. These data indicate that the p53 CTD is a positive regulator of p53 tumor suppressor functions.

p53 is a well characterized tumor suppressor protein whose activity is regulated tightly (1). This protein consists of several domains, each specialized in different functions. The N terminus contains its transactivation domain; the central region of the protein corresponds to its sequence-specific DNA binding domain, which is followed by a tetramerization domain; a relatively short but highly basic domain constitutes the C terminus. The p53 carboxyl-terminal domain (CTD) is conserved highly conservatively short but highly basic domain constitutes the C terminus. The p53 carboxyl-terminal domain (CTD) is conserved highly in vertebrates but shows significant divergence in lower organisms, suggesting a functional significance that appeared at a late stage during evolution (2).

Initial investigations into p53 CTD function suggested that the CTD was at best not required for p53 tumor suppressor function (3) or perhaps a negative regulator of p53 activity (3, 4). Later, others showed that this basic region (containing six lysines in humans, seven in mice) was a target of MDM2-mediated ubiquitination as part of an autoregulatory negative feedback loop leading to the degradation of p53 and consequent down-regulation of its activities (5). Furthermore, studies using the CTD in EMSA experiments showed that p53 binds DNA with higher affinity without its C-terminal moiety (4, 6).

More recently, however, several laboratories used either in vitro transcription assays to show that a ΔCTD mutant does not activate p21 transcription (7) or cellular chromatin immunoprecipitation experiments to demonstrate that this mutant does not bind to its target genes in the context of chromatin (8). Together, these data argue that the p53 CTD is, in fact, required for promoter binding in a cellular context. This conclusion was supported by the finding that the p53 CTD binds to DNA in a nonspecific manner and stimulates p53 linear “fast sliding” on DNA, thereby facilitating the search by the core domain for specific binding sites (8–11).

In addition to being ubiquitinated, the six lysines in the human p53 CTD are all modified by one of several additional post-translational marks, including acetylation, methylation, or sumoylation (reviewed in Ref. 12). It was first thought that the acetylation of these lysines counteracts their ubiquitination, thus stabilizing the protein and enabling p53 activation (13–16). More recently, it has been proposed that p53 CTD acetylation may play a key role in what has been called antirepression, a mechanism by which p53 is released from repressive interactions with factors such as MDM2 or MDMX (17, 18). Nevertheless, mouse models in which either six or seven of these lysines were mutated to arginines revealed no obvious phenotype (19, 20). The p53 CTD is also phosphorylated on several residues, but the relevance of these phosphorylations also is unclear (12, 21). An S389A mutation in the mouse (Ser-392 in humans) has indeed only minor defects in p53 functions (22).

The expression of alternatively spliced forms of the p53 CTD has been shown in the mouse (23, 24) and later in humans (25, 26), but the several alternative protein isoforms encoded by the human p53 gene have been described fully only recently (27). There are at least three different CTDs encoded by the WT p53 gene: p53α CTD (full-length protein), p53β CTD (which has a 52-amino acid C-terminal deletion), and p53γ CTD (which has a 47-amino acid C-terminal deletion) (28). Although their different roles remain to be determined fully (29, 30), they have been reported to be differentially expressed across a subset of
human cancers (31, 32), raising the intriguing possibility that they play a role in tumor development.

Although an important body of data has been generated over the years from studying the p53 CTD, its functional significance remains elusive. Here, we compare the activity of ectopically expressed wild-type p53 to that of a truncated mutant lacking the terminal 24 amino acids (Δ24) and show that deletion of the p53 CTD affects the ability of p53 to bind to the promoters of a subset of its target genes. Furthermore, p53Δ24 is unable to induce critical p53 functions, such as apoptosis or cell cycle arrest. These data support an obligatory and positive role for the p53 CTD in p53 tumor suppressor functions.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—p53 WT or mutant constructs were all derived from a pCMV-FLAG-p53 plasmid, a gift from Dr. Wei Gu. The Δ24 deletion was created in the p53 moiety by introducing a stop codon at the 370 position with oligonucleotide-directed mutagenesis using the Pfu-DNA polymerase and the following pair of primers: 5′-ACTCAGCACTGTTAGTCCTAAAAAGGTC-3′ and 5′-GACCTTTTTGGACTACGGTGCTGAGT-3′. The p53prom-p53 plasmid was derived from the pcDNAs5/FRT expression vector (Invitrogen). Briefly, a BamHI-p53-cDNA-BamHI fragment from the pC53-SN3 plasmid (a gift from Dr. Bert Vogelstein) was cloned in the BamHI site of the multiple cloning site of pcDNAs5/FRT. The CMV promoter was removed by cutting the resulting vector with MluI and Nhel. An MluI/p53-promoter-Nhel fragment was then inserted in these sites after being generated by PCR from the hp1p-luc plasmid (a gift from Dr. David Reisman) using the following primers 5′-GAATTCACGCGTGAGTAGGCCAAGGGAAGCATGCAGG-3′ and 5′-GAATTCGCTAGCCAATCCAGGGAAGCGAGTTTTCCTTTGGACTACAGGTGGC-3′. All constructions were verified by DNA sequencing.

**Cell Lines and Drug Treatments**—H1299, SAOS2, U2OS, and HCT116 cell lines were purchased from ATCC. Cells were grown on coverslips, were fixed with formaldehyde (4% in PBS) and permeabilized with 0.1% Triton X-100 in PBS. The anti-p53 monoclonal (DO1) was used at 1/1000 dilution in PBS containing 0.1% Triton X-100. After incubation at room temperature for 2 h, the coverslips were washed three times in PBS 0.1% Triton X-100 and then incubated 1 h at room temperature with Alexa Fluor 594-conjugated donkey anti-mouse IgG at concentration recommended by the supplier (Molecular Probes, Invitrogen Detection Technologies) (1:500). After incubation, the coverslips were washed one time in PBS, 0.1% Triton X-100, and three times in PBS. Nuclei were counterstained with DAPI (Sigma). After staining, the coverslips were mounted in Fluoromount-G (Electron Microscopy Sciences) and analyzed with a Nikon E-700 epifluorescence photomicroscope (Tokyo, Japan) using a Diagnostic Instruments, Inc. RT-SE SPOT digital camera. Image enhancement software (Adobe Photoshop CS5) was used to balance signal strength and to separate signal from noise.

**TUNEL Assay**—TUNEL assay was performed using the *in situ* cell death detection kit, TMR red from Roche Applied Science, and following the manufacturer’s instructions.

**Bromodeoxyuridine (BrdU) Incorporation Assay**—Cells were pulsed with BrdU, which was added to the culture medium at a 10 μM final concentration for 6 h before harvesting. Cells were fixed for 30 min with 4% paraformaldehyde (Sigma) and washed three times with PBS. Cells were then incubated 30 min with 2 M HCl and neutralized for 2 min with 0.1 M sodium borate, pH 8.5, at room temperature. Cells were permeabilized with 0.1% Triton X-100, 2% BSA in PBS for 30 min at room temperature, and incubated with mouse anti-BrdU antibody (BD Biosciences). After three washes with PBS/BSA, cells were incubated with the secondary antibody Alexa Fluor 647 goat anti-mouse IgG (Molecular Probes, Invitrogen Detection Technologies). DNA was counterstained with DAPI (Sigma) before mounting the slides.

**Colony Formation Assay**—Each of the plasmids expressing p53prom-p53 WT, p53prom-p53Δ24 or an empty vector was...
co-transfected with a plasmid conferring puromycin resistance into exponentially growing HCT116 cells using Lipofectamine™ reagent and Plus™ reagent (Invitrogen) following the manufacturer’s instructions. Forty-eight hours post-transfection, the cells were subjected to puromycin (2 μg/ml) selection for 12–14 days. The colonies were fixed and stained with Giemsa (Sigma-Aldrich).

RESULTS

To clarify the role of the p53 CTD, the activity of exogenously overexpressed p53 WT was compared with the activity of a deletion mutant that lacks the terminal 24 amino acids (Δ24) in a p53-null cell line (H1299, colon carcinoma) (Fig. 1A).

Under conditions in which the protein levels of p53 WT and Δ24 were similar, the induction of several p53 target genes was assessed by immunoblotting. As shown in Fig. 1B, in the absence of stimuli such as DNA damage or hypoxia, overexpression of p53 WT is sufficient to induce expression of p53 target genes, including p21, MDM2, or BAX. Deletion of the last 24 amino acids of p53, however, has a distinct effect on p53 target gene expression levels. In contrast to MDM2 and BAX, p21 is poorly induced by p53Δ24. To assess whether or not this effect was due to a change in p53 localization, we performed immunofluorescence experiments (Fig. 1C). After transfection in H1299 cells, p53 WT and p53Δ24 are indeed both localized in the nucleus. Together, these results argue that the p53 CTD may, in fact, influence p53 target gene selectivity.

The mRNA levels of several p53 target genes were then assessed by quantitative RT-PCR and normalized to that of GAPDH (Fig. 2). A representative matching immunoblot is presented in Fig. 2D. The mRNA expression levels of five of eight target genes were different and suggested a requirement for the p53 CTD in their transcriptional regulation (Fig. 2, A and B). The remaining three genes (Fig. 2C) did not show any statistically significant difference between the WT and Δ24 induction. BAX, a proapoptotic gene (34), and MDM2 were not affected by the deletion of p53 CTD, in agreement with what was seen at the protein level. GADD45A, a prosurvival target gene that contributes to the maintenance of genomic stability (35), also seemed unaffected by p53 CTD deletion. As expected, p21 mRNA and protein levels showed a clear correlation. Other target genes that showed a decreased induction by p53Δ24 were the proapoptotic genes PUMA (36, 37) and PIG3 (38) and the prosurvival genes TIGAR (39) and SESN2 (40). These data suggest that deletion of the p53 CTD influences its transactivation functions but does not discriminate between proapoptotic or prosurvival genes. To further demonstrate the importance of the p53 CTD in p53 transactivation functions, the mRNA levels of several p53 target genes were also assessed in a second p53-null cell line (SAOS2, osteosarcoma) (supplemental Fig. 2). As observed in H1299, the p53Δ24 mutant had a decreased (supplemental Fig. 2A) or a similar (supplemental Fig. 2C) activity on several target genes. A representative immunoblot shown in supplemental Fig. 2D confirms these observations. However, in the case of PUMA and SESN2, no statistically significant difference between the WT and the Δ24 induction was observed (supplemental Fig. 2B). Taken together, these data suggest that
the role that the p53 CTD plays in p53 transactivation functions is cell type specific for a subset of its target genes. The p53 CTD is a nonspecific DNA binding domain that has been shown to facilitate sequence-specific p53 binding to its target genes. It has also been shown to recruit transcriptional cofactors that exert selective influences on p53 target genes. To further understand how the p53 CTD may be involved in target gene selectivity, we performed a series of ChIP experiments.

The p21 gene is under the control of a promoter that contains two p53 responsive elements, namely the 5′ and 3′ sites. There are two additional p53-responsive elements in the first intron of the gene, sites B and C (Fig. 3A). First, the binding of p53 to the 5′ and 3′ site was assessed using either a monoclonal antibody to p53 (DO1) or no antibody as a control. In both cases, under conditions where p53 WT and p53Δ24 proteins were expressed at comparable levels (Fig. 3C), deletion of the last 24 amino acids of the protein impaired its binding to these responsive elements (Fig. 3A, left panels). Next, using a polyclonal antibody to p53 (FL393), the binding of p53 to the four p53 responsive elements of the p21 gene was assessed. As seen in Fig. 3A (5′ site and 3′ site, middle panels; sites B and C, right panels), deletion of the p53 CTD impairs p53 occupancy on all four sites. This is

FIGURE 2. p53Δ24 fails to transactivate a subset of target genes. H1299 p53-null cells were transfected with 1 μg of a p53 (WT or mutant) expressing vector. 24 h after transfection, cells were collected and p53 target genes mRNA levels were quantitated by quantitative RT-PCR. p53 target genes were subdivided in three categories according to whether their expression was affected by the deletion of p53 C terminus (A) or not (B and C) and to whether their expression was affected comparably with that of SAOS2 cells (A and C) or not (B) (see supplemental Fig. 1). *, p < 0.05; N.S., non-significant. A representative matching immunoblot is presented in D.

in agreement with a decrease in p21 mRNA expression, as shown in Fig. 2A. To confirm that this impaired DNA binding ability was not restricted to p21 responsive elements alone, the binding of p53 to PUMA was also assessed (supplemental Fig. 1A). Similar to what was observed for p21, p53Δ24 was seen to bind with less affinity to the p53-responsive elements of this target gene compared with the full-length protein.

Next, the binding of p53 to the responsive elements of the target genes whose expression were not affected by the deletion of the last 24 amino acids was assessed. For MDM2, p53 has been shown to bind to two responsive elements located in the first intron, in proximity to the second transcription start site and exon 2 (Fig. 3B). These two responsive elements are too close to each other to be discriminated by PCR after ChIP. Thus, the combination of primers used for this experiment encompasses both sites. Using two different antibodies (monoclonal DO1 or polyclonal FL393), p53Δ24 was observed to have lost part of its ability to bind to the responsive elements, compared with the WT protein (~2-fold less). These data do not correlate with the results seen with MDM2 mRNA induction (Fig. 2B). This apparent discrepancy could be explain by the fact that the impaired ability of p53Δ24 to bind to the MDM2 promoter might still be sufficient to induce the transcription of this gene. Indeed, p53Δ24 was also shown to have impaired binding to p53-responsive element in the first intron of the BAX gene (supplemental Fig. 1B), although it remained able to induce BAX both at the mRNA and the protein levels (Figs. 1B and 2B).

FIGURE 3. p53 CTD is needed to bind to p21 promoter but not to MDM2 promoter. A, the different p53-responsive elements of the p21 promoter region and the primers used for real-time PCR are depicted. H1299 p53-null cells were transfected with 1 μg of a p53 (WT or Δ24) expressing vector. 24 h after transfection, a ChIP assay was carried out using a p53 antibody (DO1 or FL393 as indicated) or no antibody as a control. The recovered DNA was subjected to a real-time PCR using primers that amplify a portion of the promoter containing the indicated responsive elements. Black bars represent p53 antibody values (DO1 or FL393), and gray bars represent no antibody values. ∗, same as A at the MDM2 promoter region. A representative matching immunoblot is presented in C. *, p < 0.05; N.S., non-significant; Ab, antibody.
p53 Basic C Terminus Regulates Target Gene Selectivity

Taken together, these data argue that the CTD is essential for the binding of p53 to all the target genes tested here, although to a extent that varies from one gene to another. MDM2 appears to be the target gene for which p53Δ24 retains the highest binding capability.

To further explore the biological significance of these results, two sets of experiments were performed to assess two major cellular outcomes in response to p53, apoptosis, and cell cycle arrest. First, p53-induced apoptosis was assessed by measuring DNA fragmentation via labeling the terminal end of nucleic acids (TUNEL assay). This assay was performed after cotransfecting H1299 with expression vectors for GFP and either p53 WT or Δ24 (Fig. 4, A and B). As shown in Fig. 4A, in cells in which GFP is expressed (and presumably p53), only the WT protein is able to induce apoptosis. After quantification (Fig. 4B), it appears that p53 WT was able to induce apoptosis in ~15% of the GFP-positive cells, whereas the Δ24 mutant did not appear to be significantly different from the control experiment (<5%).

Second, the ability of p53 to induce a cell cycle arrest was assessed by monitoring DNA synthesis by means of a BrdU incorporation assay (Fig. 4, C and D). Again, among the GFP-positive cells, fewer BrdU-positive cells were observed after transfection with a plasmid expressing 53WT than with one for p53Δ24 (Fig. 4C). Quantification of these findings (Fig. 4D) showed 44% of the cells growing after transfection with p53 WT compared with 62% with p53Δ24. Although the difference in the observed effect between the WT and the mutant was less striking than that which we had observed in the TUNEL assay, it was still statistically significant (p < 0.05). These results suggest that the CTD is critical for both activities of p53.

There was a concern that the levels of p53 being achieved in these experiments may be non-physiological. To address this, the expression level achieved with the pCMV-p53 construct was compared with that of a p53prom-p53 plasmid, where the p53 cDNA is under the control of the native p53 promoter. This resulted in low levels of p53 expression in transfected H1299 cells, as shown in Fig. 5A. Protein expression levels in H1299 transfected with either pCMV-p53 or p53prom-p53 were compared with those of two cancer cell lines (HCT116 colon cancer and U2OS osteosarcoma cells) after induction of p53 by the DNA damaging agent doxorubicin. To take into account the transfection efficiency of H1299 cells (~20%), five times more extract from H1299 cells than from HCT116 or U2OS cell lines (50 μg versus 10 μg) were loaded on the gel. The levels of p53 achieved after transfection with pCMV-p53 were clearly higher than those achieved by treating the cells with doxorubicin (compare lane 6 with lanes 2 and 4). Using the same amount of transfected DNA (2 μg), the levels achieved after transfection with p53prom-p53 were more comparable with those achieved by stimulating endogenous expression of p53 with doxorubicin. An induction of both MDM2 and p21 at the protein level was also observed. p21 expression levels in cells transfected with the p53prom-p53 WT or p53Δ24 plasmids were also assessed by immunoblotting (Fig. 5B). Under conditions in which p53 WT and p53Δ24 levels were comparable, the ability of the p53Δ24 mutant to induce p21 was impaired, in agreement with what was seen with the pCMV-p53Δ24 plasmid (Figs. 1B and 2B).

Next, the levels of p21, PUMA, MDM2, and BAX mRNA were assessed by quantitative RT-PCR (Fig. 5C). At physiologically relevant levels of p53 expression, p53Δ24 was observed to be defective in the induction of several of its target genes compared with p53 WT (p21, PUMA). Moreover, and contrary to what has been seen with the pCMV constructs, p53Δ24 was shown to be unable to induce BAX, demonstrating that the spectrum of target genes that require the p53 CTD for activation was broader under conditions in which p53 was expressed at physiological levels.

To put these data in a biological context, a colony formation assay was then performed using the same p53 promoter-based plasmids. As shown in Fig. 5D, about three times the number of colonies were observed after transfection with p53Δ24 compared with p53 WT. This number of colonies was comparable with the control, again underscoring an essential role for the CTD in p53 function.

DISCUSSION

Deciphering p53 CTD functions has long been a challenge (41). In this study, it is shown that the CTD is needed for all p53 activities that were tested and that this effect is likely due to impaired DNA binding to a subset of its target genes. In the past, contradictory studies have fueled controversy over the positive or negative regulatory function of the p53 CTD. The results presented here confirm a positive role for the p53 CTD in p53 functions. The Δ24 deletion mutant does not bind to its target elements within the p21 promoter or the first intron of PUMA and fails to activate their transcription in transfection experiments (CMV plasmids, Figs. 2 and 3; p53 promoter plasmids, Fig. 5). These results therefore demonstrate that the CTD plays an important role in p53 DNA binding and transcriptional activity. The impaired DNA binding properties observed on deletion of the p53 CTD results to compromised p53-dependent cellular responses (Fig. 4).

The findings presented here are in agreement with numerous previous studies. For example, it has been shown that the p53 CTD is needed for p53-mediated apoptosis (42–44), a result in accordance with the data presented here in Fig. 4, A and B. The data in Fig. 3 and supplemental Fig. 1 argue that the p53 CTD is needed for p53 to bind to the promoter of several of its target genes, in agreement with other reports (7, 8, 45).

In previous studies, a slightly different mutant, the p53Δ30, has been utilized. The p53Δ30 and p53Δ24 constructs express a truncated protein that includes the amino acids 1–363 and the amino acids 1–369, respectively. None of the supplemental six amino acids present in p53Δ24 are basic and the so-called C-terminal basic domain can thus be defined by the peptide spanning the amino acids 370–393. However, at least one of the amino acids present in p53Δ24 and not in p53Δ30 has been shown to be post-translationally modified. Serine 366 is in fact one of several residues phosphorylated by CHK2 after DNA damage, a modification that seemed only required for the activation of a subset of p53 target genes (46). Nevertheless, in all of the reports cited above, p53Δ30 seems to behave like p53Δ24, and in both cases, the p53 CTD serves as a positive regulator for p53 functions.
Conversely, at least two reports in which the p53Δ30 and not the p53Δ24 have been utilized showed a negative role for the p53 CTD in p53 functions (47, 48). This discrepancy could be explained by the different experimental settings used in these reports. In their study, Harms et al. (47) demonstrate that the p53 CTD acts as a negative regulator of the p53 target gene IGFBP3 activation. However, most of the data were generated by overexpressing several p53 deletion mutants (including the p53Δ30) in the breast cancer cell line MCF7. These cells are not mutated for p53 and the expression of the endogenous wild-type p53 might modulate the effects of the transfected mutant p53. This also is reminiscent of the differences observed in the present study between two different p53-null cell lines. Indeed, the data showed Fig. 2 and supplemental Fig. 2 argue that the

**FIGURE 4.** p53 functions are impaired by its C terminus deletion. H1299 p53-null cells were cotransfected with 1 μg of a p53 (WT or Δ24 as indicated) expressing vector or an empty vector and 2 μg of a GFP-expressing vector. 24 h after transfection, a TUNEL (A) or a BrdU incorporation (B) assay was carried out and cells positive for apoptosis or BrdU incorporation, respectively, were visualized by fluorescence microscopy. For each point, two representative fields are shown. Quantification was realized by counting TUNEL (B) or BrdU (D)-positive cells within the GFP-positive pool of cells and plotted on a graph. *, p < 0.05. Representative immunoblots for p53 and GFP are shown for both assays (B and D).
role the p53 CTD plays in p53 functions is cell type-specific. On the other hand, Sauer et al. (48) employed the same cell lines as the ones that have been used in the present study. However, in their studies, p53 WT or mutants were overexpressed by means of adenoviral infection, which is known to generate high levels of expression in mammalian cells. They show that the p53 CTD has a negative impact on several p53 functions (48). The differences in expression levels might account for the different outcomes observed here in terms of p53 functions. Together with the data presented here, these studies allude to the limitations of cell culture based approaches and further stress the need for a more physiological model, such as a transgenic mouse, to study the role of the p53 CTD in vivo. In fact, whereas the C-terminal lysines post-translational modifications originally were shown to be dispensable during embryogenesis and did not significantly affect p53 activity in mouse embryonic fibroblasts (19, 20), a recent report shows that these modifications contribute to hematopoietic stem cells homeostasis and confirms the tissue and condition-specific effects of the post-translationally modified C-terminal lysines in vivo (49).

During the past 10 years, p53 has been shown to be part of a family of proteins, illustrated by the discovery of several isoforms (27) and two paralogs, p63 and p73 (50). p53 is thought to be the last member of the family to have emerged during evolution. Drosophila p53 and the Caenorhabditis elegans p53 (CEP-1) are functionally closer to p63/p73 than to p53, highlighting the relevance of p53 in higher organisms (51–53). Despite the high conservation of their DNA binding domain, these proteins have distinct biological functions and the sequence of their CTDs is conserved poorly. This indicates that each CTDs may play an important and distinct role in specifying the biological functions of each family member (2). This specificity is exemplified by the observations reported here that suggest that the basic p53 CTD is indeed of great importance.

![FIGURE 5. Physiological levels of p53Δ24 fail to induce p53 target genes and repress cell growth.](image-url)

A. p53, p21, and MDM2 protein levels of transfected H1299 p53-null cells were compared with those of doxorubicin-treated HCT116 colon carcinoma cells and U2OS osteosarcoma cells. H1299 p53-null cells were transfected with 2 μg of a p53-expressing vector (pCMV-FLAG or p53 promoter (p53prom)-based plasmid). 24 h after transfection, protein levels of indicated proteins were assayed by immunoblotting. HCT116 and U2OS were treated with 0.2 and 0.1 μg/ml of doxorubicin (DOX), respectively, and cells were harvested 24 h post-treatment. To take into account the transfection efficiency (~20%), gels were loaded with five times more H1299 extract than HCT116 or U2OS extracts (50 μg versus 10 μg, respectively). B. H1299 p53-null cells were transfected with 1 μg of a p53prom-p53 WT, p53Δ24, or empty expressing vector. 24 h after transfection, protein levels of indicated proteins were assayed by immunoblotting. C. H1299 p53-null cells were transfected with 1 μg of a p53prom-p53 (WT or Δ24)-expressing vector. 24 h after transfection, cells were collected, and p21, PUMA, MDM2, and BAX mRNA levels were quantitated by quantitative RT-PCR. *, p < 0.05; N.S., non-significant. D. H1299 cells cotransfected with p53prom-p53 WT, Δ24 or empty vectors along with a puromycin resistance conferring plasmid were subjected to antibiotic selection for 2 weeks, and the remaining colonies were stained subsequently with GIEMSA (upper panel) and counted (lower panel). Representative plates are shown. *, p < 0.05. N.S., non-significant.
for p53 biological functions. This is in contrast to another member of the p53 family that also possesses a basic CTD, the p73γ isoform, which has been shown to be less efficient than other p73 isoforms that lack the basic CTD in activating p21 and inhibiting colony formation (54). Another study showed that p53β, a p53 isoform that lacks the basic CTD, is unable to transactivate p21 and poorly induces p53-dependent apoptosis (27). However, the same study also showed that p53β could potentiate p53 full-length protein activities, suggesting that the interplay between different p53 isoforms is more complex than expected and needs further investigation (27).

An additional intriguing question is how p53 is able to discriminate between such separate cell fates as cell cycle arrest, DNA repair, or apoptosis. In the past, this question has received special attention, since the recapitulation of an apoptotic rather than cell cycle arrest or senescent effect would be of great therapeutic interest. A significant body of literature has shown that p53 activates different subsets of target genes depending not only on the nature and/or duration of the stimuli, but also the cell type (reviewed in Refs. 55 and 56). According to a selective binding model, the ability of p53 to recognize its genomic-binding sites should be modulated in a gene-specific manner. An alternative model, a selective context model, proposes that p53 binds all accessible binding sites and that specificity is achieved at subsequent regulatory steps. At least four aspects of the interactions between p53 and its target genes have been suggested to take part in either of these models of p53-dependent cell fate determination: 1) the strength of individual p53 responsive elements, 2) post-translational modifications affecting the p53 protein, and more specifically its CTD, 3) p53 binding partners, and 4) the epigenetic landscape of p53 target genes (55, 56). According to the results presented here characterizing a mutant p53 with deletion of the CTD, none of these models are adequate. The p53 CTD is needed for both proapoptotic and prosurvival activities (Figs. 4 and 5D), and this correlates with a failure of p53Δ24 to induce either proapoptotic genes (PUMA, PIG3) or prosurvival genes (p21, TIGAR, SESN2) (Fig. 2).

Surprisingly, however, p53Δ24 was as efficient as p53 WT in inducing other proapoptotic (BAX) or prosurvival (GADD45A) target genes. This apparent discrepancy could be explained by the different expression levels of several target genes after p53 induction. For example, a 4- to 5-fold induction is seen with p21 as compared with a modest 2-fold induction with GADD45A. The same effect is observed with PUMA and BAX. These observations might signify that certain p53 target genes are more potent effectors than others for specific p53-dependent cellular responses. Intriguingly, the p53 CTD does not appear to be needed for MDM2 transactivation but may be required for DNA binding to its promoter (Figs. 2B and 3B). The quantitative RT-PCR results are in agreement with a previous study from the Gu laboratory (18) showing that a non-acetyllatable mutant of p53 is defective for the induction of all tested target genes except MDM2, alluding to the specificity of the p53-MDM2 regulatory loop. The apparent discrepancy between the transactivation and the DNA binding properties of p53 for MDM2 could be explained by the fact that a p53 mutant lacking the last 24 amino acids is still able to bind the MDM2-responsive elements, although to a lesser extent than p53 WT (Fig. 3B). In fact, although statistically significant, the difference between p53 WT and p53Δ24 in the binding to the MDM2 promoter is mild (only 1.75-fold difference) when the p53 monoclonal antibody DO1 is used to immunoprecipitate p53. This difference is actually not significant when another p53 antibody is used (the polyclonal antibody FL393). Nevertheless, this suggests that there is a threshold above which the binding of p53 to its responsive elements is sufficient to activate the transcription of its target genes. In fact, a similar phenomenon is observed with BAX (Fig. 2C and supplemental Fig. 1B), although the difference in binding is more significant than that of observed with MDM2. Attempting to rigorously address the role of DNA binding is complicated by the fact that each gene has varying numbers of multiple response elements. Taken together, the data are consistent with impaired DNA binding contributing to the defective transcriptional output of the truncated p53, at least in part. This does not rule out a role for the C terminus beyond gene occupancy.

Why the p53 CTD is selectively required to bind to different target genes and activate their transcription remains an unanswered question. This study argues that the p53 CTD is dispensable for the activation of three target genes: BAX, MDM2, and GADD45A. It has already been shown that BAX levels in thymocytes from a mouse expressing a mutated p53 (p53S18A) and treated with ionizing radiation are comparable with the levels of wild-type cells (57). This mutation, however, was shown to reduce the apoptotic response and induce late-onset lymphomas (58). Similarly, p53 CTD deletion is shown here not to affect BAX expression but to be critical for the induction of p53-dependent apoptosis. As for MDM2, the p53 CTD seems to be required for DNA binding to the responsive element in the first intron of the BAX gene. However, this does not correlate with what is seen at the mRNA level. There is at least one other p53 binding site in this gene, namely in the BAX promoter (34, 59). It would be of interest to test whether p53Δ24 is still able to bind to that responsive element, which could account for the similar induction of BAX that is seen with p53 WT and p53Δ24.

In the case of MDM2, the p53-responsive element has been suggested to have a unique architecture and to be nucleosome-free (60). It has been shown to be preprimed for transcriptional activation by p53. Therefore, the CTD and its DNA tracking function (8, 10) might be dispensable for the efficient binding of the p53 core domain to the responsive element and subsequently the transactivation of the MDM2 gene. It also would be interesting to study the p53-responsive elements of BAX and GADD45A in this regard.

In conclusion, we have shown that the p53 CTD is an essential part of the protein and is pivotal in both proapoptotic and prosurvival p53 activities. Further studies will be needed to understand fully how the p53 CTD selectively regulates p53-dependent transcription on different subsets of its targets.

Acknowledgments—We thank Dr. Wei Gu (Columbia University, New York, NY), Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD), and Dr. David Reisman (University of South Carolina, Columbia, SC) for vectors. Lois Resnick-Silverman, Luis Carvajal, Luciana Giono, Wendy Liu, Melissa Mattia, Emir Senturk, Crystal Tonnessen, and Caleb Lee are thanked for help and support.
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