The Transactivation Domains of the p53 Protein

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The p53 tumor suppressor is a transcriptional activator, with discrete domains that participate in sequence-specific DNA binding, tetramerization, and transcriptional activation. Mutagenesis and reporter studies have delineated two distinct activation domains (TADs) and specific hydrophobic residues within these TADs that are critical for their function. Knockin mice expressing p53 mutants with alterations in either or both of the two TADs have revealed that TAD1 is critical for responses to acute DNA damage, whereas both TAD1 and TAD2 participate in tumor suppression. Biochemical and structural studies have identified factors that bind either or both TADs, including general transcription factors (GTFs), chromatin modifiers, and negative regulators, helping to elaborate a model through which p53 activates transcription. Posttranslational modifications (PTMs) of the p53 TADs through phosphorylation also regulate TAD activity. Together, these studies on p53 TADs provide great insight into how p53 serves as a tumor suppressor.

One of the best-characterized properties of the p53 tumor suppressor is its ability to activate gene transcription. Over the years, ever-increasing numbers of direct p53-bound and activated genes have been identified, facilitated recently through genomic approaches. These genes have been shown to be involved in various p53 functions, including cell-cycle arrest/senescence, apoptosis, regulating metabolism, DNA repair, and inhibiting cell migration and invasion (Vousden and Prives 2009; Bieganski et al. 2014). Although we comprehend now that p53 transcriptional activation potential is fundamental for its function as a tumor suppressor, this understanding developed over a period of many years. In this review, we describe the studies that illuminated the role for p53 as a

transcriptional activator, the importance of p53 transcriptional activation function in vivo, and the mechanisms through which p53 transcriptional activation domains act. Collectively, these studies have helped define the molecular underpinnings of p53 tumor suppressor function.

p53 IS A TRANSCRIPTIONAL ACTIVATOR

By 1989, it was clear that p53 plays an important role in tumor suppression (Levine et al. 2004). However, the molecular mechanisms underlying p53's transformation suppression activity remained elusive. p53 was known to be nuclear and to have the capability to bind DNA through its highly conserved sequence-specific DNA-binding domain (DBD) (Dippold et al. 1981;

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Bargonetti et al. 1991, 1993; Kern et al. 1991; Pavletich et al. 1993), but a significant clue that p53 might be a transcription factor came from analysis of the composition of the p53 amino acid sequence. In particular, the amino terminus of p53 is highly acidic (~20%), a feature reminiscent of the acidic activation domains of other transcription factors such as Fos, GAL4, and the glucocorticoid receptor (Fig. 1) (Pennica et al. 1984; Ma and Ptashne 1987; Hollenberg and Evans 1988; Lech et al. 1988). In addition, p53 carries a proline-rich domain (PRD), another characteristic of transcriptional activation domains (TADs) such as in CTF/NF-1 (Mermod et al. 1989). Of note, although the overall sequence of the p53 amino terminus is not highly conserved between species (Fig. 1A), the acidic character is conserved (Soussi and May 1996).

Direct evidence of p53 transactivation potential came first from experiments aimed at determining whether fusing either full-length or truncated p53 to the heterologous DBD of yeast GAL4 was sufficient to promote activation of a reporter carrying GAL4 binding sites. Indeed, fusion of either human or mouse p53 sequences to the GAL4 DBD, which itself has no transactivation potential, conferred transactivation activity in both yeast and mammalian cells (Fields and Jang 1990; O'Rourke et al. 1990; Raycroft et al. 1990, 1991; Hulboy and Lozano 1994). Initially, such experiments showed that the p53 amino-terminal 73 residues are sufficient to confer activation on the GAL4 DBD, to an extent similar to the potent herpes simplex virus VP16 TAD (Fields and Jang 1990). These experiments established that the p53 protein contains transactivating sequences, supporting the idea that it is a transcriptional activator. Experiments to further define the p53 TAD revealed that a fusion of GAL4 to p53 residues 1–42 displayed activity comparable to that of GAL4 full-length p53, suggesting that p53 TAD activity is localized within amino acids 1–42 (Unger et al. 1992). Next, to correlate transcriptional activity with tumor suppression, GAL4 fusions to various p53 tumor-derived mutants were generated. Interestingly, certain tumor-derived p53 mutants—known

as conformation mutants because they disrupt p53 structure (mouse p53^{A135V}, human p53^{V143A}, p53^{R175H})—were unable to support reporter activation (Raycroft et al. 1990, 1991). In contrast, tumor-derived p53 mutants known as contact mutants (human p53^{R273H}, mouse p53^{R245W}), which simply alter key residues required for p53 to interact with DNA but without affecting p53 structure, could still confer activation potential on the GAL4 DBD (Fields and Jang 1990; Raycroft et al. 1991; Unger et al. 1992). Collectively, these studies pinpointed regions of p53 with transactivation potential and suggested that the molecular mechanism accounting for p53-mediated tumor suppression is p53's ability to activate transcription (Fields and Jang 1990; O'Rourke et al. 1990; Raycroft et al. 1990, 1991; Unger et al. 1992; Hulboy and Lozano 1994). This work was complemented by subsequent studies showing that wild-type human and mouse p53 can bind defined DNA elements and activate transcription from specific promoters in *in vitro* transcription or transfection assays, whereas tumor-derived p53 mutants fail to do so (Farmer et al. 1992; Zambetti et al. 1992).

The generation of chimeras in which the p53 TAD was replaced with the heterologous VP16 TAD bolstered the notion that p53 is a transcriptional activator. Although deleting the p53 amino-terminal ~79 amino acids disrupts p53 function, reconstitution with an alternate TAD permits p53 function. *In vitro* assays showed that a p53–VP16 chimera can induce cell-cycle arrest or apoptosis in p53-deficient fibroblasts and suppress transformation (Reed et al. 1993; Pietenpol et al. 1994; Attardi et al. 1996). In addition, studies of cells from knockin mice expressing a p53–VP16 fusion showed that this chimera is capable of activating many classical p53 targets as well as cell-cycle arrest and senescence responses (Johnson et al. 2008). The fact that a different, well-defined TAD restored biological function to a p53 mutant lacking its own amino terminus strongly supported the idea that transactivation potential, rather than some other activity of the amino terminus, is critical for p53 biological function.

Figure 1. Protein sequence alignment of the p53 transactivation (TAD) domains in various species using the Clustal Omega program. (A) The domain structure of full-length p53 is shown at the top for reference. The full 85 amino acids originally used to characterize the two TADs are shown, although the carboxy-terminal portion of this region is now considered the proline-rich domain (PRD). Blue shading indicates the degree of identity of residues between proteins of different species, generated by the percentage identity feature in Jalview 2.8 software, with darker blue signifying identity in more species. The positions of residues 22, 23, 53, and 54 are indicated. (B) Human p53 TAD residues 1–85 with acidic (yellow) and hydrophobic amino acids (pink) highlighted. The four key hydrophobic residues at positions 22, 23, 53, and 54 that are critical for TAD transcriptional activity are shown with a black bar above them. (C) Human p53 TAD residues 1–85 indicating serines and threonines that are targets of phosphorylation and affect p53 TAD interactions with binding partners. DBD, DNA-binding domain; TET, tetramerization domain; Basic, basic amino acid-rich region.

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SPECIFIC HYDROPHOBIC RESIDUES ARE CRITICAL FOR p53 TRANSACTIVATION DOMAIN FUNCTION

Once the amino-terminal 42 amino acids were defined as sufficient for transcriptional activation, subsequent studies aimed to identify residues within this region critical for transactivation. Like the VP16 TAD, the p53 TAD has numerous acidic amino acids interspersed with bulky hydrophobic residues (Fig. 1B). Thus, a vast array of p53 TAD single, double, and multiple point mutants altering acidic and/or hydrophobic residues within the first 42 amino acids of p53 were analyzed for their transcriptional activity using reporter assays (Lin et al. 1994). Interestingly, these studies revealed that the acidic residues in the p53 amino terminus are not critical for p53 transactivation, although they do contribute to efficacy of transactivation. Instead, there are specific hydrophobic residues—primarily L22 and W23 of human p53—that are required for transactivation (but not for DNA binding). Notably, although each of the single-point mutations at codon 22 or 23 had a modest effect on transactivation, combined L22Q and W23S mutations resulted in loss of reporter activation. Similarly, mutagenesis of GAL4 DBD-p53(1-73) fusions showed that, although the acidic and proline residues contribute to efficient transactivation, the hydrophobic amino acids F19, L22, W23, L25, and L26 are the most crucial for activation (Chang et al. 1995). Interestingly, hydrophobic residues had been shown to be important for function of other transcription factors such as VP16, NTF-1, Sp1, and Gcn4 (Cress and Triezenberg 1991; Attardi and Tjian 1993; Gill et al. 1994; Drysdale et al. 1995). Collectively, these studies also advanced an explanation for the observation that p53 mutations in cancer tend to occur in the DBD rather than the TAD. In contrast to the DBD, which can be inactivated with one amino acid change, mutations in multiple TAD residues are required to significantly affect transactivation potential. There is, therefore, a much greater probability of sustaining one mutation in the DBD during cancer development than multiple alterations in the TAD (Lin et al. 1994).

Given that the human p53^{L22Q,W23S} mutant was found to be severely compromised for transactivation, it provided a useful tool to assess the role of p53 transcriptional activation for p53 biological function. In a variety of studies, the human p53^{L22Q,W23S} mutant and the analogous p53^{L25Q,W26S} mouse mutant were found to be totally defective in different p53 cellular functions, including DNA damage or oncogene-induced apoptosis or suppression of colony formation, leading to the conclusion that transcriptional activation potential is crucial for p53 to induce cellular responses (Sabbatini et al. 1995; Attardi et al. 1996; Chao et al. 2000). Interestingly, this mutant sometimes displayed activity in apoptosis or in transformation suppression in vitro, leading to the idea that transcriptional activation may not always be required for p53 function (Haupt et al. 1995; Sabbatini et al. 1995; Chen et al. 1996; Walker and Levine 1996; Zhu et al. 1998, 2000; Venot et al. 1999; Kokontis et al. 2001; Baptiste et al. 2002; Baptiste-Okoh et al. 2008). Alternatively, this context-dependent activity may relate instead to the fact that the p53^{L22Q,W23S} mutant possesses residual transcriptional activity on certain genes (Zhu et al. 1998, 2000; Venot et al. 1999; Brady et al. 2011). Together, these findings suggested the importance of transcriptional activation for p53 function in at least some contexts and foreshadowed a complexity to TAD function that was later clarified in mouse experiments in vivo (discussed below).

p53 HAS TWO DISCRETE TRANSCRIPTIONAL ACTIVATION DOMAINS

Shortly after these studies functionally defined the p53 TAD as between residues 1–42, it was shown that p53 actually has two TADs within the amino terminus. This idea was originally proposed on the realization that there is a striking similarity between p53 residues 1–83 and the activation domain of VP16 (residues 413–490), which contains two independent TADs (Cress and Triezenberg 1991; Regier et al. 1993; Chang et al. 1995; Candau et al. 1997). The ability of p53 residues 40–83 to serve as an independent TAD was therefore investigated.

p53 amino acids 1–40 and either 43–73 or 40–83 were fused to the GAL4 DBD, and these chimeras were assayed for their ability to activate reporters in yeast and mammalian cells. Indeed, GAL4–p53 (1–40), GAL4–p53 (43–73), and GAL4–p53 (40–83) have transactivation potential in reporter assays (Chang et al. 1995; Candau et al. 1997). Examination of the roles of the two TADs in the context of full-length p53, using p53^{Δ1–39} and p53^{Δ34–78} mutants, showed that these two mutants have similar activities, albeit to a significantly reduced extent relative to wild-type p53 (Candau et al. 1997). These studies indicated that the two subdomains (henceforth referred to as TAD1 and TAD2) are both functional in the context of full-length protein, and, moreover, that the two domains act synergistically rather than additively. As with the L22 and W23 residues critical for TAD1 function, two consecutive hydrophobic residues in TAD2 (W53, F54), which are surrounded by acidic amino acids, were found to be critical for TAD2 activity. Together, these findings showed that there is a discrete second TAD in p53 that contributes significantly to p53 transcriptional activity and defined W53 and F54 as key residues for TAD2 function.

These studies were expanded by subsequent investigations of the biological activities of these mutants in vitro (Zhu et al. 1998, 2000; Venot et al. 1999). In one study, p53^{ΔN1–42} and p53^{L22Q,W23S}, but not p53^{ΔN1–63}, induced apoptosis, suggesting further the existence of a second TAD between residues 43 and 63 (Zhu et al. 1998, 2000). In another study, p53^{L22Q,W23S} retained the ability to suppress colony growth and trigger apoptosis, consistent with residual transactivation function on some genes (Venot et al. 1999). To determine whether p53 TAD2 accounted for biological activities in these studies, a quadruple mutant—p53^{L22Q,W23S,W53Q,F54S}—as well as a p53^{W53Q,F54S} mutant were generated and evaluated functionally in several p53-based assays (Venot et al. 1999). p53^{W53Q,F54S} activated reporters to an extent intermediate between wild-type p53 and the p53^{L22Q,W23S} mutant, while p53^{L22Q,W23S,W53Q,F54S} failed to transactivate reporters. p53^{L22Q,W23S} and p53^{W53Q,F54S} each induced apoptosis and colony suppres-

sion almost equivalently but not as well as wild-type p53. p53^{ΔN1–42+W53Q,F54S} and p53^{L22Q,W23S,W53Q,F54S} were both unable to induce cell-cycle arrest, apoptosis, colony suppression, or target gene expression, bolstering the functional significance of residues 53 and 54 and the second TAD (Zhu et al. 1998; Venot et al. 1999). Notably, the demonstration that abolishing transcriptional activity through mutation of both TADs incapacitates p53 biological activity underscores the importance of transcriptional activation activity for p53 function.

FUNCTIONAL ANALYSIS OF p53 TRANSACTIVATION DOMAINS IN VIVO

To definitively understand the role of the two p53 TADs for p53 function in vivo, a panel of p53 TAD mutant knockin mouse strains was generated (Johnson et al. 2005; Brady et al. 2011). Mutations previously characterized in in vitro assays (Candau et al. 1997; Zhu et al. 1998, 2000; Venot et al. 1999) were introduced into the first (L25Q, W26S), second (F53Q, F54S), or both TADs (L25Q, W26S, F53Q, F54S). A major strength of this approach is that mutants are expressed under the control of the p53 promoter, hence with the proper spatial and temporal expression profile. In addition, a lox-stop-lox element was introduced upstream of the p53 coding sequence to allow conditional activation of the mutant alleles by Cre recombinase expression. Analysis of the p53^{L25Q,W26S} mutant, both in cells derived from the mice and in mice, provided an additional perspective on why this mutant showed variable activity in vitro. Specifically, this mutant displayed selective transactivation activity both by genome-wide expression profiling and by analysis of expression of individual genes. For example, p53^{L25Q,W26S} is severely compromised in activating *p21*, *Noxa*, and *Puma* but not *Bax* or a number of novel p53-regulated genes (Fig. 2) (Johnson et al. 2005; Brady et al. 2011). Moreover, p53^{L25Q,W26S} is unable to mount responses to acute DNA damage, either cell-cycle arrest in DNA-damaging agent-treated mouse fibroblasts or apoptosis in radiosensitive tissues in vivo on irradiation, suggesting that full tran-

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Figure 2. Molecular models for p53 transactivation at different target genes based on p53 TAD mutant phenotypes. (A) Table summarizing properties of the indicated mouse p53 TAD mutants relative to wild-type p53. + indicates wild-type activity and – indicates lack of activity. +/– indicates selective transactivation, with severely compromised activation of most p53 target genes and intact transactivation of a small subset of p53 target genes. Acute DNA-damage responses encompass cell-cycle arrest and apoptosis in response to DNA damage. Embryonic lethality is in the context of one mutant allele and one p53 null allele. (B) Models for p53 action in responses to acute DNA damage versus tumor suppression. p53 TAD1 activity is required for robust transactivation of canonical p53 target genes, such as p21, Puma, and Noxa, that mediate p53 DNA-damage responses, including apoptosis and cell-cycle arrest (i). In contrast, TAD1 is dispensable for activation of some p53 target genes, such as Abhd4, Bax, and Sidt2, and for tumor suppression, as either TAD1 or TAD2 suffices for these responses (ii). In model i, p53 uses TAD1 to interact with cofactor X to drive expression of TAD1-dependent genes. In model ii, p53 uses both TADs to interact with cofactor Y to activate genes associated with tumor suppression, as based on the observation that mutation of either TAD alone does not compromise expression of these genes.

scriptional activity is critical for the ability to drive responses to acute DNA damage. In contrast, the $p53^{L25Q,W26S}$ mutant can induce apoptosis in response to nongenotoxic stresses such as hypoxia and serum starvation (Johnson et al. 2005). Additionally, this mutant is completely competent to respond to oncogenic signals, inducing senescence in HRasV12-expressing MEFs and suppressing cancers of different types in vivo, including non-small-cell lung cancer, T- and B-cell lymphoma, and medulloblastoma (Brady et al. 2011; Jiang et al. 2011). These findings suggest that selective transcriptional activation suffices for p53-mediated tumor suppression. This notion is consistent with the lack of tumor suppressor activity of a $p53^{L25Q,W26S}$ mutant also carrying the DBD mutation A135V, which perturbs targeting to p53 consensus sites (Jimenez et al. 2000; Nister et al. 2005). However, based on these original studies, it remained possible that transcriptional activation-independent activities of p53 account for p53 function in these contexts and, therefore, it was necessary to investigate the biological activity of the transactivation-dead $p53^{L25Q,W26S,F53Q,F54S}$ mutant. Although the $p53^{F53Q,F54S}$ mutant showed no apparent compromise in transactivation or biological activities, the $p53^{L25Q,W26S,F53Q,F54S}$ mutant failed to show activity in activating target genes, inducing responses to DNA damage, or suppressing B- and T-cell lymphomas and lung cancer (Brady et al. 2011; Jiang et al. 2011). These observations thus show that transcriptional activity is crucial for p53 biological function and highlight the importance of TAD2 for tumor suppression, at least in the context of TAD1 mutation.

The study of these mutant mouse strains has also shed light on p53 function in embryonic development. Unrestrained p53 function during development, as occurs with ablation of either of the two p53 negative regulators, Mdm2 or MdmX, results in embryonic lethality, at E5.5 and E7.5–11.5, respectively, because of inappropriate p53-driven apoptosis or cell-cycle arrest in embryos (Jones et al. 1995; Montes de Oca Luna et al. 1995; de Rozieres et al. 2000; Parant et al. 2001; Finch et al. 2002; Migliorini et al. 2002). To assess whether the deleterious

effects of unbridled p53 activity during development relies on p53 transcriptional activation function, the TAD mutants were expressed during development. Importantly, mutation at the residues 25;26 disrupt p53 interaction with Mdm2 (Lin et al. 1994); therefore, the proteins carrying these amino acid alterations are stabilized and can be studied as if in a *Mdm2* nullizygous background. Each $p53^{LSL-m/+}$ mouse strain—where *m* denotes any mutant—was crossed to mice carrying a CMV–Cre transgene, to allow ubiquitous expression of the particular mutant throughout the developing organism, along with wild-type p53. Interestingly, although the expression of $p53^{F53Q,F54S}$ failed to affect development, expression of $p53^{L25Q,W26S}$ induced embryonic lethality at ~E10.5 associated with neural tube closure defects (Johnson et al. 2005; Van Nostrand et al. 2014). Moreover, expression of $p53^{L25Q,W26S,F53Q,F54S}$ provoked lethality at ~E13.5, associated with a host of phenotypes, including coloboma (fissure in the retina), heart outflow tract defects, and aberrant outer ear and semicircular canal formation (Van Nostrand et al. 2014). Notably, these phenotypes were not observed in $p53^{LSL-25,26,53,54/-}$; CMV–Cre embryos, which develop into viable adults (although they ultimately succumb prematurely to cancer), suggest that there is a genetic interaction between $p53^{L25Q,W26S,F53Q,F54S}$ and wild-type p53 that leads to the specific constellation of phenotypes observed. Furthermore, the viability of $p53^{LSL-25,26,53,54/-}$ mice indicates that transcriptional activation is critical for p53 to induce developmental defects. Intriguingly, the specific spectrum of phenotypes resembles those seen in a human syndrome known as CHARGE (coloboma heart defects atresia of the choanae retarded growth genitourinary tract defects and ear defects), which is associated with mutations in the Chd7 chromatin remodeler (Jongmans et al. 2006). Although the transactivation-dead $p53^{L25Q,W26S,F53Q,F54S}$ mutant does not itself compromise development, the $p53^{L25Q,W26S}$ mutant retains adequate function through TAD2 to cause lethality even in the context of one p53 null allele, again underscoring the functionality of the second TAD. Collectively, these

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studies revealed a complexity to TAD function in vivo, with a role for TAD1 in responses to acute DNA damage and for both TADs in tumor suppression and developmental phenotypes (Fig. 2). A mechanistic understanding of the basis for these intriguing observations necessitates a detailed biochemical analysis of interacting partners, which we will discuss next.

p53 TRANSACTIVATION-DOMAIN-INTERACTING PROTEINS

Transcriptional activators stimulate gene transcription through a precisely orchestrated series of steps. After binding to specific DNA sites via their sequence-specific DBDs, transcriptional activators must then open the adjacent chromatin and recruit the transcriptional machinery to promote RNA synthesis. Typically, through their TADs, transcriptional activators attract a variety of proteins engaged in different steps of transcription, including histone modification, chromatin remodeling, and transcriptional initiation and elongation (Lemon and Tjian 2000). Accordingly, the amino-terminal p53 TADs interact with proteins involved in different steps of transcription, as outlined below (Table 1). Moreover, the p53 TADs are important for interactions with the p53 inhibitors Mdm2, MdmX, and E1B, providing another level of transcriptional regulation.

p53 TADs Enhance Recruitment and Activity of General Transcription Factors

Transcription of eukaryotic genes requires the assembly of the general transcription factors (GTFs) and Pol II on gene promoters to form the so-called preinitiation complex (PIC) (Murakami et al. 2013). The first GTF to nucleate PIC formation is transcription factor IID (TFIID, comprising the TATA-box-binding protein [TBP] and multiple TBP-associated factors [TAFs]), which binds to the TATA box, a promoter element important for directing proper transcription initiation (Conaway and Conaway 1993). p53 can interact directly with different components of the TFIID complex to promote transcriptional initiation (Seto et al. 1992; Chen

et al. 1993b; Liu et al. 1993; Truant et al. 1993; Lin et al. 1994; Chang et al. 1995; Horikoshi et al. 1995; Lu and Levine 1995; Thut et al. 1995). The wild-type human p53 TADs (residues 1–73) interact with TBP via conserved hydrophobic residues, as F19R, L22R;W23S, or L25R;L26S mutations significantly decrease TBP binding (Chang et al. 1995). Interestingly, L22Q;W23S mutations in the context of full-length p53 do not impair interaction with TBP (Lin et al. 1994), suggesting that TBP might bind to other p53 domains beyond TAD1 such as the carboxyl terminus (Horikoshi et al. 1995). p53 TAD1 also interacts with two additional subunits of the TFIID complex, TAF6 and TAF9 (formerly known as TAF70 and TAF32, respectively), and these interactions are abolished by the L22A;W23A, L22Q;W23S, or L14Q;F19S mutations in TAD1 (Lu and Levine 1995; Thut et al. 1995). The correlation between the ability of the p53 TADs to interact with TFIID components and to activate transcription suggests that these interactions are important for transcriptional activity. Beyond TFIID, the p53 TADs also interact with the GTF transcription factor IIH (TFIIH) (Xiao et al. 1994). Human p53 TAD residues 20–73 are sufficient for binding to the pleckstrin homology (PH) domains of the p62 and Tfb1 subunits of the human and yeast TFIIH complexes, respectively (Di Lello et al. 2006). In contrast, TFIIH does not bind to p53 TAD1 (residues 1–40), suggesting that it interacts specifically with TAD2, a notion borne out by NMR structural studies of the Tfb1/p53 complex. Together, these studies suggest that TAD1 and TAD2 cooperate to recruit both TFIID and TFIIH to promoters to stimulate transcriptional initiation.

In addition to contacting the general transcription machinery directly, p53 communicates with the GTFs via the Mediator (also known as TRAP [thyroid hormone receptor-associated protein] or SMCC [SRB- and MED-containing coactivator complex]), an evolutionarily conserved, multisubunit complex that acts as a central scaffold in the PIC, transmitting signals from transcriptional activators directly to Pol II to enhance transcriptional initiation (Conaway and Conaway 2013). Medi-

Table 1. Summary of p53 TAD-interacting proteins

Interaction partner	Category	Interaction site on p53	References
<i>Transcriptional machinery components</i>			
TBP	GTF	TAD1	Seto et al. 1992; Chen et al. 1993b; Liu et al. 1993; Truant et al. 1993; Lin et al. 1994; Chang et al. 1995; Horikoshi et al. 1995
TAF6	GTF	TAD1	Thut et al. 1995
TAF9	GTF	TAD1	Lu and Levine 1995; Thut et al. 1995
TFIIH (p62 and Tfb1)	GTF	TAD2	Xiao et al. 1994; Di Lello et al. 2006; Okuda and Nishimura 2014
Mediator (Med17)	Coactivator	TAD1	Ito et al. 1999; Meyer et al. 2010
<i>Chromatin modifiers</i>			
p300/CBP	HAT	TAD1 and TAD2	Avantaggiati et al. 1997; Gu et al. 1997; Lill et al. 1997; Scolnick et al. 1997; Teufel et al. 2007; Feng et al. 2009; Lee et al. 2009, 2010a,b; Miller Jenkins et al. 2015
GCN5	HAT	TAD2	Gamper and Roeder 2008
PRMT1	Arginine methyltransferase	TAD1	An et al. 2004
<i>DNA metabolism^a proteins</i>			
PC4	DNA metabolism and transcription	TAD2	Rajagopalan et al. 2009
RPA (RPA70N)	DNA metabolism and transcription	TAD2	Dutta et al. 1993; He et al. 1993; Li and Botchan 1993; Bochkareva et al. 2005
HMGBl	DNA metabolism and transcription	TAD2	Rowell et al. 2012
<i>p53 Inhibitors</i>			
MDM2	E3 ubiquitin ligase	TAD1 and TAD2	Momand et al. 1992; Oliner et al. 1992; Chen et al. 1993a; Lin et al. 1994; Kussie et al. 1996; Blommers et al. 1997
MDMX	RING domain protein	TAD1 and TAD2	Shvarts et al. 1996; Hu et al. 2006; Popowicz et al. 2008
E1B	Adenoviral oncoprotein	TAD1	Lin et al. 1994

^aDNA metabolism comprises the cellular processes of DNA replications, recombination, and repair.

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ator subunits Med1 and Med 17 (originally known as TRAP220 and TRAP80, respectively) interact directly with wild-type p53, although only Med17 binds the isolated p53 TAD1 while Med1 binds to the p53 carboxy-terminal domain (Ito et al. 1999). Accordingly, the p53^{L22Q;W23S} mutant can interact with Med1 but not with Med17, suggesting that p53 may recruit Mediator components via multiple points of interaction. Electron microscopy analysis showed that p53 TAD binding to Med17 triggers a dramatic shift in Med17 architecture, such that a large “pocket” opens up in Med17 at a site where Pol II is known to bind, leading to the model that this p53-induced structural shift in Med17 promotes Pol II transitioning to its productive elongation state (Meyer et al. 2010). These studies collectively thus helped establish a mechanism for p53-mediated transactivation in which the p53 TADs both directly and indirectly—through Mediator—recruit and activate core transcription machinery.

p53 TADs Interact with Chromatin Modifiers

Before p53 can recruit and activate GTFs, p53 interacts with chromatin-modifying proteins to open chromatin. The best-studied p53-interactors in this category are CREB-binding protein (CBP) and p300, histone acetyltransferase (HAT) paralogs important for p53-mediated transactivation (Avantaggiati et al. 1997; Gu et al. 1997; Lill et al. 1997; Scolnick et al. 1997). Initial studies showed that p300 and CBP stably complex with p53 and enhance p53 transactivation, suggesting that these proteins function as coactivators of p53. Beyond promoting chromatin opening by histone acetylation, p300/CBP binding to p53 TADs, such as in response to DNA damage, also stabilizes p53 by inducing acetylation of p53 at multiple sites in the carboxyl terminus, thereby preventing p53 ubiquitylation at these sites and consequent degradation (Rodriguez et al. 2000; Li et al. 2002; Brooks and Gu 2003).

p53 interacts with multiple conserved domains in p300 and CBP, including the KIX (CREB and Myb interaction domain), TAZ1/CH1 and TAZ2/CH3 (transcriptional adaptor

zinc-binding domain; cysteine-histidine rich), and IBiD (interferon response-binding domain; also known as nuclear receptor coactivator-binding domain or NCBBD) domains (Teufel et al. 2007). p53 TAD1 and TAD2 (amino acids 1–57) can synergistically interact with each domain of p300/CBP. Because p53 binds DNA as a tetramer with four available TAD1/TAD2 domains, the p53 TADs may make multivalent interactions with the four domains of p300/CBP. Deletion of either TAD or mutation of L22Q;W23S or W53Q;F54S in the context of full-length p53 diminishes binding to CBP (Gu et al. 1997; Scolnick et al. 1997). Moreover, analysis of p53 residues 1–57 revealed that the L22Q;W23S;W53Q;F54S mutations—which render p53 transactivation-dead—abrogates interactions with each of the four p300 domains (Teufel et al. 2007). Thus, loss of p300/CBP binding may explain the inactivity of the p53^{L25Q;W26S;F53Q;F54S} mutant in transactivation and biological function.

p53 TADs are also known to interact with various other chromatin-modifying enzymes. For example, two components of the human STAGA HAT complex—GCN5 and TAF9—directly interact with p53 TAD residues 1–73 (Gamper and Roeder 2008). This interaction is required for loading of STAGA onto promoters to allow chromatin opening and p53-mediated transactivation. Mutation of either p53 TAD1 (L22Q;W23S) or TAD2 (W53Q;F54S) results in weakened STAGA binding to p53, whereas the L22Q;W23S;W53Q;F54S mutations affect the interaction more severely, supporting the idea that STAGA binding may be key for transactivation. These findings are consistent with earlier observations showing that p53 TADs require GCN5 HAT activity for transcriptional activation in yeast (Candau et al. 1997). p53 also regulates chromatin state by binding the PRMT1 arginine methyltransferase, via residues 1–43, thereby promoting p300/CBP acetyltransferase mediated to enhance p53 target gene expression (An et al. 2004). Together, these studies show that p53 TADs directly recruit various specialized regulators to alter the chromatin landscapes in the vicinity of p53 target promoters, preparing for PIC nucleation.

p53 Transactivation Domain Interactions with Negative Regulators

Beyond interacting with transcriptional machinery components, the p53 TADs bind several negative regulators of p53 that suppress its ability to activate transcription. For example, the p53 amino-terminal 52 amino acids interact with the Mdm2 (Murine double minute) protein, an E3 ubiquitin ligase that inhibits p53-mediated transactivation both by binding and concealing the p53 TAD and by targeting p53 for ubiquitin-mediated proteolysis (Momand et al. 1992; Oliner et al. 1992; Chen et al. 1993a; Haupt et al. 1997; Honda et al. 1997; Kubbutat et al. 1997). Mdm2 directs p53 degradation via the proteasome by ubiquitylating multiple lysines in the carboxyl terminus of p53 (Rodriguez et al. 2000). Mutagenesis studies defined F19, W23, and L26 as key human p53 residues for interacting with Mdm2 and its human ortholog, HDM2, a notion supported later by structural studies (Lin et al. 1994; Kussie et al. 1996; Blommers et al. 1997) (see below). Interestingly, the phenomenon of a TAD overlapping a degron has been observed in a host of transcriptional activators, including Myc, β -catenin, c-Jun, and E2F (Salghetti et al. 2000, 2001; Kim et al. 2003; Lipford and Deshaies 2003; Muratani and Tansey 2003). Ubiquitin-mediated proteolysis can actually enhance the activity of transactivators, as exemplified by the observation that p53 and components of the proteasome interact and are recruited to the *p21* promoter to promote transcription (Zhu et al. 2007). Although the rationale for coupling activator destruction and transcriptional activation is not completely clear, it has been proposed that proteolytic destruction of the activator may allow replacement of “spent” activators with new ones (Geng et al. 2012).

MdmX (also known as Mdm4) and its human counterpart HDMX are also negative regulators of p53 (Shvarts et al. 1996). Unlike Mdm2, MdmX inhibits p53-mediated transactivation but does not directly affect p53 protein stability, as it lacks E3 ubiquitin ligase activity. Similarly to Mdm2, MdmX directly binds p53 TAD residues 1–52, and mutations of

L22Q;W23S abolish this interaction (Hu et al. 2006). By binding to p53, Mdm2 and MdmX can inhibit p53 acetylation by coactivators such as p300/CBP, thereby dampening p53 transcriptional activity (Kobet et al. 2000; Sabbatini and McCormick 2002). Another well-established negative regulator of p53 is adenoviral E1B 55-kDa protein, which binds the amino-terminal 123 amino acids of p53, thereby masking the TADs and promoting oncogenic transformation (Lin et al. 1994). Interestingly, hydrophobic residues W23, K24, and P27 in p53 are critical for the interaction with E1B, suggesting that E1B may also inhibit p53 TADs by blocking sites for critical coactivators.

STRUCTURAL ANALYSES OF THE p53 TRANSCRIPTIONAL ACTIVATION DOMAINS

To gain a deeper understanding of the function of p53 TADs, a variety of structural biology approaches were used to examine the TADs in isolation and complexed with different interacting proteins. As with other transcription factors, it was expected that the p53 TADs in isolation would be unstructured, adopting a clear structure only when complexed with specific interacting proteins (Wright and Dyson 1999). Indeed, in the absence of a binding partner, the p53 TAD regions are easily digested by proteases, suggesting a loosely folded conformation (Pavletich et al. 1993). The unstructured nature of TADs has been proposed to facilitate interactions with multiple different protein partners and to allow TADs to be targeted for posttranslational modification (PTM) by various enzymes (Oldfield et al. 2008). PTMs can promote or hinder p53 interactions with specific protein partners, thereby regulating p53 function (Meek and Anderson 2009; Teufel et al. 2009).

The structure of the p53 TADs was first solved in the context of an Mdm2–p53 complex by X-ray crystallography. Minimal regions of both Mdm2 and p53 TAD1 (amino acids 15–29) sufficient for interacting were cocrystallized (Kussie et al. 1996). Mdm2 was found to form a deep hydrophobic cleft into which the p53 peptide inserts as an amphipathic α -helix.

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The Mdm2–p53 interaction is mediated by three highly conserved hydrophobic residues in human p53: F19, W23, and L26. Nuclear magnetic resonance (NMR) analyses of a p53 peptide comprising amino acids 17–24 also highlighted the critical role of residues F19 and W23 as well as L22 in the p53–MDM2 interaction (Blommers et al. 1997). As these p53 residues are important both for interactions with transcriptional machinery components and activating transcription, this study suggested further that p53 may bind transcriptional cofactors through an amphipathic α -helical structure as well, which was subsequently borne out (see below). Collectively, these experiments provided the first information on p53 TAD structure.

The observation that p53 TADs are natively unfolded was confirmed by several studies of the structure of the p53 TADs in isolation. Circular dichroism and NMR analysis on full-length p53 as well as purified TADs in isolation (residues 1–93) revealed that p53 TADs are largely devoid of secondary and tertiary structures under physiological conditions (Bell et al. 2002; Dawson et al. 2003; Wells et al. 2008). However, some NMR analyses of p53 residues 1–73 suggested that although the p53 TADs lack tertiary structure, they do contain some secondary structure, specifically an amphipathic α -helix between T18 and L26 and two nascent amphipathic turns, between M40 and M44 (turn 1) and D48 and W53 (turn 2) (Botuyan et al. 1997; Lee et al. 2000). The significance of these elements is suggested by the known importance of these residues for TAD function. Moreover, the unbound TAD1 α -helix resembles the Mdm2-bound TAD1, suggesting that binding of p53 to targets simply tightens the α -helix into a more stable form. Interestingly, subsequent NMR experiments showed that Mdm2 not only binds the α -helix in TAD1 but also the turn regions in TAD2, which, on Mdm2 binding, form a fully stable TAD2 helix that inserts into the Mdm2 cleft, although with lower affinity than TAD1 (Chi et al. 2005; Shan et al. 2012).

Various subsequent studies examined p53 TAD1 or TAD2 complexed with other proteins, including MdmX, TFIIH, p300, and CBP

(Di Lello et al. 2006; Teufel et al. 2007; Popowicz et al. 2008; Feng et al. 2009; Lee et al. 2009, 2010a,b; Miller Jenkins et al. 2015). The crystal structure of the p53-binding domain of MdmX bound to p53 TAD1 residues 15–29 revealed that the p53 TAD1 hydrophobic residues F19, W23, and L26 form the primary contact surfaces for this interaction, as with the p53–Mdm2 interaction (Popowicz et al. 2008). The structure of p53 TAD2 complexed to the PH domain of yeast TFIIH (Tfb1) was solved by NMR (Di Lello et al. 2006), and although unstructured when unbound, p53 TAD2 residues 47–55 form an amphipathic α -helix on binding to Tfb1. Three hydrophobic residues (I50, W53, and F54) in this amphipathic α -helix make crucial contacts with Tfb1, providing an explanation for any compromise in TAD2 transcriptional activity arising from mutations in these residues. Interestingly, NMR studies suggested that p53 TAD2 binds the human TFIIH subunit p62 through a different structure from Tfb1—an extended string-like conformation rather than an amphipathic α -helix (Okuda and Nishimura 2014). Considerable attention has also been focused on solving the structure of the p53 TADs in complex with the KIX, TAZ1/CH1, TAZ2/CH3, or NCBD domains of p300 and CBP. In one study, the NMR structure of the p300 TAZ2 domain and p53 TAD1 showed that p53 forms an α -helix that interacts with TAZ2 via residues F19, L22, and L25 (Miller Jenkins et al. 2015). The NMR structure of the p300 TAZ2 domain complexed with p53 TAD2 residues 35–59 showed that TAD2 also forms an α -helix that interacts with TAZ2 via I50, W53, and F54 (Feng et al. 2009). Similarly, the NMR structures of both p53 TADs with either the KIX or NCBD domain of CBP revealed that the two p53 TADs fold into a pair of helices (F19–L25 and P47–W53) on interacting with these CBP domains (Lee et al. 2009, 2010b). These studies collectively also highlighted how PTMs on p53 can enhance p53 interactions with p300/CBP, as discussed below.

An additional interesting structural aspect of p53 TADs came to light on characterizing their interaction with the single-stranded DNA (ssDNA)-binding proteins RPA (replica-

tion protein A), PC4 (positive cofactor 4), and high mobility group B1 (HMGB1), proteins involved in transcription, DNA replication, DNA recombination, and DNA repair (Wold 1997; Bochkareva et al. 2005; Rowell et al. 2012; Mortusewicz et al. 2015). Initial biochemical studies showed that p53 TAD residues 1–73 are sufficient for interaction with RPA (Dutta et al. 1993; He et al. 1993; Li and Botchan 1993). NMR analysis of the p53 TAD–RPA complex revealed that p53 TAD residues 47–57 adopt an amphipathic α -helical structure on interaction and that this interaction resembles that of RPA with ssDNA (Bochkareva et al. 2005). Specifically, in this complex, p53 residues I50 and F54 are reminiscent of the DNA bases while the side chain carbonyl groups mimic the phosphate backbone of DNA. Similarly, p53 TAD2 (residues 35–57) adopts an α -helical conformation on interaction with PC4 in a manner reminiscent of the PC4–ssDNA interaction (Rajagopalan et al. 2009). These findings have advanced the model that p53 TADs mimic ssDNA structure, which may help recruit DNA metabolism factors that may facilitate PIC formation or liberate p53 from RPA at sites of DNA damage, where ssDNA accumulates. The NMR structure of the high mobility group B1 (HMGB1) protein A-box and p53 residues 1–93 also revealed that p53 TAD2 adopts an amphipathic α -helical structure on binding to the A-box, again mimicking ssDNA, but in this case HMGB1 is proposed to facilitate p53 binding to DNA (Rowell et al. 2012).

THE IMPACT OF p53 AMINO-TERMINAL PHOSPHORYLATION ON TRANSACTIVATION DOMAIN FUNCTION

The studies of p53 TAD-interacting partners have provided great support for the idea that phosphorylation of p53 TADs plays a large part in regulating these interactions. Diverse kinases can phosphorylate the p53 TADs, including ATM, ATR, Chk2, MAPK, HIPK, and various CDKs (Jenkins et al. 2012). Phosphorylation of sites within the amino-terminal TADs (Fig. 1C) in response to DNA-damage signals

not only relieves p53 from negative regulation by Mdm2 and MdmX but also facilitates transcriptional activity by enhancing binding to certain interacting partners, including TFIIH and CBP/p300 (Lambert et al. 1998; Sakaguchi et al. 2000; Schon et al. 2002; Di Lello et al. 2006; Polley et al. 2008; Ferreón et al. 2009; Jenkins et al. 2009, 2012). Numerous studies showed that phosphorylation of single or multiple p53 TAD residues including S15, T18, S20, S33, S37, S46, or T55 increases the binding affinity to individual domains of p300/CBP (Lambert et al. 1998; Polley et al. 2008; Feng et al. 2009; Ferreón et al. 2009; Jenkins et al. 2009, 2012; Teufel et al. 2009; Lee et al. 2010a). Interestingly, heptaphosphorylation of both TADs at S15, T18, S20, S33, S37, S46, and T55 induces 40-fold and 80-fold more binding of p53 to the TAZ1/CH1 and TAZ2/CH3 domains of p300, respectively (without effects on KIX or I β Id), as well as a 24-fold reduction of binding to Mdm2 (Teufel et al. 2009). These studies thus provide a mechanism for phosphorylation-mediated replacement of Mdm2 with p300 or CBP, thereby stabilizing p53 and enhancing p53 transactivation. Phosphorylation of either S46 or T55 in TAD2 also enhances p53 binding to p62 and Tfb1 subunits of TFIIH, suggesting another mechanism by which p53 phosphorylation enhances transcription (Di Lello et al. 2006). The significance of these interaction studies is borne out in numerous biological studies. For example, in microarray studies of cells expressing p53 with mutations in all serines of the TADs (S6, S9, S15, S20, S33, S37, S46), a significant decrease in the number of p53-induced genes is observed relative to cells expressing wild-type p53 (Ohki et al. 2007). Moreover, some studies in cultured cells and in knockin mice with mutations in specific phosphorylation sites support the importance of these phosphorylation sites for transactivation of p53 target genes and p53 biological function (Mayr et al. 1995; Lohrum and Scheidtmann 1996; Siliciano et al. 1997; Chao et al. 2003; Sluss et al. 2004). For example, cells from p53^{S18A/S18A} knockin mice show similar DNA-damage-induced p53 stabilization and DNA binding as cells expressing wild-type p53, but target gene activation and

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apoptosis are compromised (Chao et al. 2003; Sluss et al. 2004). Thus, PTM of the TADs provides an important mechanism for modulating p53 transcriptional activity.

SUMMARY AND PERSPECTIVES

Since the original description of p53 function as a transcriptional activator, many studies have delved into the intricacies of this function. Although many molecular details remain to be fully elucidated, the studies summarized here have illuminated how p53 transcriptional activation function contributes to its biological roles as well as the mechanisms through which p53 activates transcription. Analyses of p53 TAD mutant knockin mice have revealed the importance of p53 transactivation activity in various p53 biological functions, including DNA-damage responses, promoting developmental phenotypes, and tumor suppression. Interestingly, different TADs are required in different contexts, suggesting that different transcriptional networks are involved in diverse biological responses. Genomic studies will continually add to this picture by elaborating target genes involved in different p53 responses. As a transcription factor, p53 acts by stimulating chromatin remodeling and the recruitment of the transcription machinery to enhance transcriptional initiation and elongation of a host of genes. Biochemical and structural biological studies have revealed critical TAD-interacting partners through which p53 acts to enhance transcription. Future studies will not only continue to decipher the targets most central to different p53 responses, but also elucidate how the two TADs act mechanistically at different classes of genes and whether modulating TADs can be of any therapeutic benefit. Intriguingly, the role of TAD1 is most important for acute DNA-damage responses, but dispensable for tumor suppression, suggesting that its inhibition could help spare cancer patients some of the side effects of genotoxic cancer therapies. Further unraveling of the molecular underpinnings of p53 transcriptional activation will ultimately lead to improved cancer therapeutics.

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