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Covalent and noncovalent modifiers of the p53 protein

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Abstract. Despite the massive attention it has received, there is still much to learn about the p53 tumour suppressor protein. Given that it plays complex and multiple roles in cells, it is not surprising that p53 is subjected to an intricate array of regulatory processes. p53 receives signals from cells in multiple ways, leading to its stabilization and activation. The functions of the protein are altered by phosphorylation and other covalent modifications. However, a number of proteins can regulate p53 function dramatically by noncovalent means. p53 is thus subjected to numerous signalling and regulatory pathways which we have only begun to decipher.

Key words. Phosphorylation; DNA bending; redox; stabilization; signalling; C-terminus; N-terminus; modification; DNA binding.

Introduction

Since p53 was shown to be a tumour suppressor in the late 1980s, it has been subjected to a wide and extensive range of experimental approaches extending from X-ray crystallography to clinical studies. It is now clear that this protein plays a pivotal role in the stress response programmes of normal cells, and that loss or mutation of p53 function is highly correlated with tumorigenesis. There is good evidence that p53 is a multifunctional protein which can transmit signals from many forms of genotoxic assault to genes and factors that control aspects of the cell cycle and cell death. Given the role of p53 as a cellular master switch, it is reasonable to assume that it is subject to extensive and complex forms of regulation. The purpose of this article is to review recent advances in our understanding of the regulation of p53. Such progress is built upon a solid foundation of previous reports, and a number of useful reviews have been written that deal with the structure and function of the p53 protein [1–3].

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p53 is a sequence-specific transcriptional activator, and its interactions with DNA and several cellular proteins have been extensively studied. Following stress signals levels of p53 are stabilized through post-transcriptional mechanisms. Additionally, there is good evidence that the intrinsic activity of the p53 protein is altered such that it becomes a more effective transactivator. Like most proteins, p53 is organized into several functional domains (fig. 1) [1–3]. Within its N-terminus (residues 1–~100) are located an activation region (residues 1–~70) as well as a proline-rich region containing five copies (in human p53) of the sequence PXXP (residues ~60–~97). The central conserved region of the protein (residues ~100–~300) contains its sequence-specific DNA-binding domain, and the C-terminal portion contains a flexible linker (residues ~300–~325), a tetramerization domain (residues ~325–~356) and a highly basic stretch at the extreme C-terminus (residues 363–393). Phosphorylation sites, as well as other sites of modification, have been mapped within the N- and C-termini of the protein. Below we discuss the control of p53 function, both by interactions between p53 domains, as well as through interaction of these domains with cellular factors.

Regulation of p53 through its N-terminus

The N-terminus of p53 can be divided into discrete regions. The transcriptional activation region consists of two subdomains, one extending between residues 1 and 43 [4] and the second between residues 40 and 83 [5, 6], the latter being weaker than the former in mammalian cells but the stronger of the two in yeast. The second activation domain overlaps with the PXXP domain, which is located between residues 63 and 97. Deletion of the PXXP domain renders p53 somewhat weaker in transcriptional activation than the wild-type form, and reduces the ability of the protein to suppress growth and induce apoptosis [7–9]. Several p53 phosphorylation sites have been located within the N-terminus. Sites for casein kinase I (CK I): Ser 4, Ser 6 and Ser 9; and DNA-activated protein kinase (DNA-PK): Ser 15 and Ser 37 have been mapped in murine and/or human p53 (reviewed in ref. 10). Ser 33 is also phosphorylated in vivo and at least one protein kinase, the TFIIH-associated trimeric cyclin-activating kinase complex (CAK), consisting of cyclin H, CDK7 and p36/MAT1, has been shown to phosphorylate p53 at that site in vitro [11]. Murine p53 can be phosphorylated by c-jun kinase (JNK) at a corresponding site, Ser 34 [12]. JNK can also phosphorylate human p53 at its N-terminus, although the exact site has not yet been reported [13]. Additionally, murine p53 can be phosphorylated in vitro by mitogen-activated protein kinase (MAPK) [14].

It should be noted that there are a number of other potential threonine and serine phosphate acceptors within p53. Although two-dimensional phosphotryptic mapping has provided strong support for the identification of the known sites, and perhaps can be tentatively used to rule out others, it remains possible that in some cells or conditions novel sites may be utilized. Such sites may indeed turn out to be very interesting. Additionally, whilst the kinases that can phosphorylate existing defined sites in vitro have largely been identified, it remains possible that multiple kinases can phosphorylate a given site. One example is phosphorylation of Ser 15, a site inducibly phosphorylated after DNA damage [15, 16]. It is predicted that multiple protein kinases will prove capable of phosphorylation of this site. The major questions are, then, which protein kinase receives the first signal from DNA damage, and which kinases are actually responsible for phosphorylating p53 after DNA damage? Are there conditions (or cell types) where a given kinase predominates?

p53 N-terminal-interacting proteins

Of the p53 N-terminal-interacting proteins, the most well characterized is the MDM2 protein. The regions of these two proteins that interact have been mapped [17–20], and the crystal structure was solved of a complex between a p53 peptide extending between residues 17 and 27 bound to residues 17–125 of MDM2 [20]. There are a number of reasons why the p53:MDM2 interaction is physiologically relevant. MDM2 and p53 are coimmunoprecipitated from a number of different cells, and a subset of tumours expressing wild-type p53 (soft tissue sarcomas in particular) overexpress MDM2 from amplified genes (reviewed in ref. 21). The early embryonic lethal phenotype of MDM2 knockout mice is rescued when crossed into a p53 null background [22, 23]. MDM2 plays an important role in the regulation of p53 levels in cells by targeting it for degradation [24–26]. MDM-2 mediated degradation of p53 requires the function of the proteasome [24, 25] and, indeed, it was reported that MDM2 is an E3 ubiquitin ligase [27].

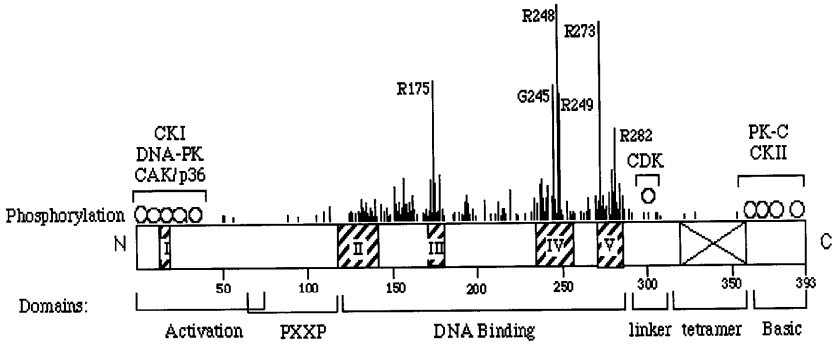

Figure 1. Landmarks of the p53 protein with phosphorylation sites, kinases and location on tumor derived mutations indicated above, and functional domains indicated below.

p53 can associate with several polypeptides that participate in the general transcription machinery, including TATA box binding protein (TBP), TBP associated factor (TAF) (*Drosophila* TAFS 42 and 62, and human TAF31) and the p62 component of TFIIH (1–3). p300/CBP has more recently been shown to interact with p53 [28–30], and while the region of interaction on p53 has not been finely mapped, Gu et al. [28] reported that the association between p53 and CBP is disrupted by mutating two N-terminal residues, L226Q and W236S (generating the double mutant p53[22/23]), which were previously identified by Lin et al. [31] as playing critical roles in transactivation by p53. It is assumed that the various interactions of p53 with the transcriptional machinery are required for its function as a transcriptional activator, and the fact that the transcriptionally impaired p53[22/23] cannot bind to several of these transcription factors supports this notion.

The interactions of p53 with MDM2 are part of a negative feedback loop between the two proteins: overexpressed or induced p53 transactivates the MDM2 promoter [32, 33], and when MDM2 accumulates and binds to p53, it represses the function of its activation domain possibly by more than a simple masking effect: MDM2 itself possesses a transcriptional repression domain that may come into play when tethered by p53 to the template [34].

N-terminal effects on DNA binding

There are a myriad of studies showing that proteins which interact with or modify the C-terminal region can cause dramatic effects on the ability of the core domain to bind to DNA (see below). However, effects of N-terminal interactors and modifiers on DNA binding have also been noted. Indeed, in some cases results from different studies have not always been fully consistent. Zauberman et al. [35] using 'McKay' assays with cell extracts observed that whereas DNA was detected in immunoprecipitates containing p53 alone, the amount of bound DNA in p53-MDM2 complexes was greatly diminished. By contrast, however, Bottger et al. [36] showed, by electrophoretic mobility shift assay (EMSA) with purified recombinant proteins, that MDM-2-p53-DNA complexes can form, and no significant quantitative differences were seen when comparing p53 bound to DNA in the presence and absence of MDM2. Thus, two different DNA-binding assays, using different sources of p53 and MDM2, provided markedly different results with respect to assessing the eff ects of MDM2 on p53 DNA binding. Dutta et al. [37] showed that p53 can inhibit the interaction of RP-A with single strands. However, according to Miller et al. [38], RP-A could inhibit the ability of p53 to bind to its cognate site, unless RP-A was bound to single strands. Further experiments will hopefully resolve these discrepancies.

Several proteins when bound to the N-terminus stimulate or stabilize p53 DNA binding: Using both DNaseI footprinting and EMSA, Chen et al. [39] showed that both TBP and TFIID can augment the ability of p53 to bind to DNA encompassing a p53-binding site, whether or not the DNA also contains a TATA box. Martin and Berk [40] have reported that the adenovirus E1B 55 kDa product increases p53 protection from DNaseI cutting on a cognate site, suggesting the possibility that TFIID and E1B may interact in a similar manner with p53. Additional insight into how N-terminal interacting proteins might affect DNA binding came from reports showing that DNA binding by p53 is inherently thermally sensitive. Temperature-sensitive DNA binding by wild-type [41] or mutant [42] forms of p53 can be stabilized when bound by anti-p53 monoclonal antibodies that react with epitopes within the N-terminus. Those binding to residues 46–55 provided the greatest protection from thermal inactivation [42]. Although N-terminal protein interactions can quantitatively increase DNA binding, phosphorylation of N-terminal sites in p53 (by DNA-PK) does not augment DNA binding [15].

N-terminal regulation of p53 turnover

The increase in p53 protein after stress signals results in good part from a posttranslational mechanism in which the normally rapidly degraded protein becomes greatly stabilized with a dramatically increased half-life. Given that MDM2 can strongly promote degradation of p53, it follows that regulation of this effect is related to the stabilization of p53. At least two different mechanisms have been recently proposed to explain how p53:MDM2 interaction might be controlled. The first of these has derived from observations that p53 becomes inducibly phosphorylated at a residue, Ser 15, that is within the vicinity of the MDM2 interaction region [15, 16]. Phosphorylation of p53 at Ser 15 (and Ser 37) by DNA-PK markedly reduces its ability to bind to MDM2 [15]. While it is thus possible that phosphorylation of p53 at this site is involved in regulation of p53 stability after DNA damage, mutational analysis of p53 has shown that the mechanism of stabilization of p53 is considerably more complicated [25, and unpublished]. Additional phosphorylation sites at the N-terminus may also be involved in regulating p53 stabilization. It should be noted that CK I Δ is also a DNA damage-inducible kinase that can phosphorylate p53 [43]. The second line of evidence implicating MDM2 in p53 stabilization comes from the observation

that the alternatively spliced product of the murine p16INK4A locus, p19ARF, can stabilize p53 most likely through its interaction with MDM2 [44, 45].

Regulation of p53 through its central core domain

We first discuss how the core domain of p53 binds to DNA as an autonomous entity. p53 has five evolutionarily conserved regions (I–V) and four of these, regions II–V, map within the central region (reviewed in ref. 46). Moreover, the great majority of missense mutations that are identified in human tumours are located within this conserved central portion of p53 [47]. Taken together, as indicated above, the implications are that sequence-specific DNA binding is central to p53's role as a tumour suppressor. After the identification of the central sequence-specific binding domain, a number of studies were performed that defined features of core p53 interaction with DNA. The central domain of p53 binds a motif containing two copies of 5′ R R R C A/T T/A G Y Y Y 3′, thus consisting of two pairs of head-to-tail dimers [48]. The crystal structure of the core domain has provided invaluable information as to how the core binds its cognate site [49]. Conserved regions III and IV form two large loops, and these along with the loop-sheet-helix specified by regions II and V form the DNA-binding surface of p53, responsible for major and minor groove contacts within the p53 consensus site. The less conserved regions contribute to a β-sheet sandwich that forms a scaffold supporting the DNA-binding element. Importantly, codons previously identified as 'hot spots' for mutation in tumours were shown to encode amino acids that make critical contributions to DNA binding, either directly or by affecting the conformation of the domain. Informative as it is, however, the crystal structure cannot fully explain how full-length p53 binds to DNA, or even how four core monomers contact the four half sites.

The p53 cognate site implies that p53 binds to DNA as a tetramer. Since purified p53 in solution is largely tetrameric, this suggests that a function of the tetramerization domain is to present four molecules of p53 to its binding site under conditions where, as monomers, such binding would be predicted to be very weak. The strongly cooperative binding of core monomers [50, 51], then, was somewhat surprising, and poses the question of the function of the tetramerization region. Indeed, an internal deletion of the tetramerization domain generates a p53 protein (p53ΔDD) that is incapable of binding to DNA [52, 53]. Missense mutations in the p53 tetramerization domain [54], however, yield protein that displays partial, selective DNA binding, and p53 protein lacking the entire COOH terminus exhibits strong binding [55]. Furthermore, Jayaraman et al. [53] made the perplexing observation that p53 that has deleted both the tetramerization domain and the N-terminal 22 amino acids (p53ΔDDΔN22] can now bind well to DNA. Thus it is not clear why p53 lacking only its tetramerization domain cannot bind to DNA. p53ΔDD apparently has an altered tertiary structure, as determined by its altered protease cleavage patterns [53]. Deletion of the tetramerization region in the context of an otherwise intact molecule may produce a protein so disordered that access of the core domain to DNA is prevented.

The roles of redox and metal binding in DNA binding by p53

The central core of p53 contains a zinc atom, and binding to zinc is essential for its ability to bind to DNA. Human p53 protein has 10 cysteine residues all of which are localized in the central DNA-binding domain. The importance of cysteine residues in p53 function is underscored by the facts that 8 of the 10 cysteines are evolutionarily conserved, mutations in many of these cysteines completely or partially block the transactivation and tumour suppressor functions of p53, and 9 of these cysteines have been found mutated in naturally occurring human cancers (reviewed in ref. 56). Furthermore, the co-crystal structure of the central domain of p53 complexed with cognate DNA showed that the loops that form the DNA-binding surface of p53 are connected by a tetrahedrally coordinated zinc atom, bound by residues Cys 176, His 179, Cys 238 and Cys 242 [49].

The importance of metal ions/metal chelators and reducing agents as well as the individual contributions of each of the cysteines has been extensively analysed. Milner et al. have sought to establish the importance of cysteines and zinc in stabilizing p53 DNA binding (57–59). Their results demonstrated that agents which reversibly perturb the wild-type conformation of p53 also modulate its DNA-binding ability. Exposure to metal chelators such as 1,10-phenanthroline (OP) caused p53 to adopt a conformation similar to that of an oncogenic p53 mutant as detected by conformation-specific p53 monoclonal antibodies (i.e., reactive to PAb240, nonreactive to PAb246). Similarly, exposure to OP prior to incubation with DNA also abolished p53 DNA-binding ability, as assessed by electrophoretic mobility shift assays, and this could be partially overcome by addition of Zn (II). These experiments thus suggested a crucial role for zinc in stabilizing the tertiary structure of p53 since OP has a strong affinity for zinc and much higher concentrations of other chelating agents, such as EDTA or EGTA [which preferentially bind Ca (II) and Mg (II)], were required to elicit the same effect. Note, however, that Bullock et al. [60] showed that zinc re-

mains bound to the core even after thermal denaturation, suggesting that its presence is not a prerequisite for the native conformation of the domain.

In addition to binding to zinc, p53 also exhibits binding to copper (Cu^{2+}) in vitro [59]. In order to test whether binding to copper has any in vivo significance, Verhaegh et al. [61] tested the effect of pyrrolidinedithiocarbamate (PDTC), an antioxidant capable of binding and transporting copper, on the DNA-binding function of p53. Their data showed that treatment of wild-type p53 expressing cells with PDTC resulted in the abrogation of the specific DNA-binding ability of p53, concomitant with the loss of p53 'wild-type' conformation as assessed by reactivity to conformation-specific antibodies. Furthermore, manipulation of the redox state of copper and its entry into the cell showed that reagents that promote the conversion of copper into a more oxidized state (Cu^{2+}) also enhance the DNA-binding capacity of p53. Here, in contrast to the negative effects of 1, 10 OP described above, treatment with 1, 10 OP in this context actually upregulated p53 activity since it increased the redox activity of copper. Thus, binding to and interaction with metal ions plays a crucial role in the physiological regulation of p53.

Since cysteine sulphydryl residues in p53 contribute to metal liganding, and removal of metal ions results in oxidation of thiols, Milner's group then directly investigated the effect of redox conditions on p53 conformation and DNA-binding activity [58]. Interestingly, while addition of reducing agent 1,4-dithio-1-threitol (DTT) favoured p53 folding into wild-type conformation and restored DNA binding, treatment with oxidant diamide induced p53 to acquire a mutant phenotype with concomitant loss of DNA binding. Using metal chelators OP and EDTA, the alkylating agent *N*-ethyl maleimide (NEM), diamide, DTT and cellular redox protein Ref-1, other groups have also arrived at similar conclusions, that is, that p53 DNA binding is governed by redox [62–65]. Removal of reducing agents by dialysis drastically reduced p53 DNA binding, reiterating the need for a reducing cellular environment for active p53 [64, 65]. Experiments assessing the relative contributions of Zn binding and protein reduction to p53 DNA binding showed that while Zn remains tightly bound to p53 during purification in Chelex-treated buffers, it is still not sufficient for optimal binding; both bound Zn and reduction of p53 are necessary to achieve optimal binding [64]. Rainwater et al. [64] reported that substitution of the three critical murine p53 cysteines implicated in Zn binding by crystallography studies (residues 173, 235 and 239) resulted in a striking loss of DNA binding and transcriptional activation and a dramatic increase in oncogenic function. Substitutions at positions 40, 179, 274, 293 and 308 had no effect, whereas those at 121, 132, 138 and 272 (located within the loop-sheet-helix and therefore possibly contributing to structure of DNA-binding domain) partially abrogated transcription and transformation suppression functions. They showed that the cys mutants retained the ability to form tetramers and higher-order oligomers.

What is the biological significance of regulating p53 function by redox? Unfortunately, in the case of normal cells, little is known about the changes in redox status or their possible link with the cell cycle or apoptosis. What is known, however, is that the concentration of redox molecules such as glutathione (and Ref-1) are higher in the nucleus than in the cytoplasm. Interestingly, a mutant fission yeast strain in which p53 transcriptional activity and growth suppression are defective, lacks thioredoxin reductase (trr1) [66]. Furthermore, in *Saccharomyces cerevisiae*, deletion of the TRR1 gene causes reduced p53 transactivation function [67]. Jayaraman et al. [65] have shown that the redox/repair protein Ref-1 can enhance DNA binding and transcriptional activation by p53 both in vitro and in vivo, although the contribution of the redox function of Ref-1 to its ability to stimulate p53 has not been fully clarified. Significantly, gene knockout experiments with mice carrying mutations in the p53, Ref-1 and XPC genes in varying combinations showed that not only does Ref-1 lie upstream of p53 in the DNA damage repair pathway, but also that defects in Ref-1 functions synergize with inactivating mutations in the repair pathway in leading to predisposition to cancer [68]. It is worth noting here that hypoxia, a hallmark of solid tumours, elevates p53 protein levels [69] as well as levels of Ref-1 [70].

Ref-1 notwithstanding, given the fact that a reducing environment drives p53 to assume a wild-type DNA-binding conformation, the very entry of p53 into the nucleus of normal cells would favour its reduction and ensure its activity. In the case of cancer cells, a more prooxidant atmosphere resulting from increased generation of oxygen radicals has been reported. Reactive oxygen radicals also accumulate in a host of cellular stress conditions such as in response to DNA damage by ionizing radiation. Indeed, there appears to be a paradox which must be considered: although in vitro evidence is compelling that reduction of p53 is required for its DNA-binding function, and oxidation inactivates it, in cells p53 can be induced by agents of oxidative damage. For example, it is well documented that treating cells with H_2O_2 leads to increased p53 levels in cells, most likely due to ensuing DNA strand breaks. Interestingly, it has been reported that the apoptotic response to H_2O_2 is transmitted by p85, a regulator of PI3K (PI3 kinase) in a p53-dependent manner [70a]. To reconcile these discrepancies we might speculate that even under conditions which lead to a more oxidative environment there might be a way to maintain p53 protein in a reduced

state: perhaps Ref-1 might directly interact with p53, and so engender its reduction. It can be further suggested that the oxidative environment within a tumour cell might actually drive p53 activation and increase the selective pressure for p53 mutation.

DNA bending

The core domain also induces a significant bend in the DNA to which it is bound, and this may represent yet another level at which the function of p53 can be controlled. Balagurumoorthy et al. [51] reported that DNA binding by the core DNA-binding domain of p53 results in bending of the target DNA. Nagaich et al. [71] subsequently showed that the stability of the p53-DNA complex is greatly enhanced by the DNA-bending angle. Their data, in fact, suggest that the sequence of the p53 cognate site influences the bend angle, which in turn correlates with affinity of p53 for DNA. Molecular models of core-DNA interactions suggest that DNA bending is necessary to accommodate four p53 core monomers without steric clashes [72]. The discovery by Jayaraman et al. [73] that HMG-1 is a potent stimulator of p53 may be relevant to these observations, as it has been previously shown that HMG-1 box-containing proteins can bend DNA [74]. The unexpected observation that HMG-1 stimulates both full-length and C-terminally deleted p53 (as well as the core alone) showed that HMG-1's effect on p53 is distinct from other known regulators of p53 which require an intact C-terminus for activation of p53 DNA binding. Although the mechanism by which HMG-1 stimulates p53 DNA binding is not fully established, the fact that HMG-1 promotes higher-order p53:DNA complexes is consistent with its ability to promote DNA bending and the stabilization of p53 on bent DNA [73]. Thus it is likely that HMG-1/2 proteins can recognize specific bases within a p53 binding site [74], and while they do not form a stable interaction with this DNA, they can induce a bend, thus making it a better binding target for p53.

Regulation of p53 through its C-terminus

The C-terminus of p53 can be viewed both as a regulatory region that controls sequence-specific DNA binding and also as a separate functional domain. It has been shown to possess autonomous DNA binding [75, 76] and strand reassociation ability [77–79]; however, in contrast to the core domain, DNA binding by the C-terminus is not sequence-specific. p53 protein exists in solution predominantly as tetramers, and indeed it is the tetrameric form that binds DNA most efficiently [80]. The structure of the oligomerization domain of human p53 was deciphered by three groups using three-dimensional nuclear magnetic resonance (NMR) [81, 82] and X-ray crystallography [83] and found to contain a β-sheet-turn-α-helix motif that forms dimers such that the fully tetramerized domain consists of a dimer of dimers. The oligomerization domain has also been shown in earlier studies to be sufficient for cellular transformation [84], presumably through its ability to form inactive heterotetramers with full-length p53. The tetramerization region can be substituted with another tetramerization domain, and such a chimeric protein retains at least some of the activities of wild-type p53 [85]. Substitution of p53 tetramerization region residues with alanine [86] or hydrophobic amino acids [87] leads to altered oligomerization properties. It is interesting that disruption or loss of oligomerization function is associated with loss of cell-cycle arrest but not growth suppression as measured by colony formation assays [88, 89].

The C-terminal basic region [363–393] has been shown to be an element that negatively regulates DNA binding by the central core domain. This supposition is built upon observations that both covalent and noncovalent modifiers of p53 which bind to or modify this region can stimulate its sequence-specific binding. A p53 monoclonal antibody, PAb421, which binds residues 371–380, or other proteins such as Dna K which can stably associate with the C-terminus of p53, stimulate binding by the central core. Additionally, phosphorylation of CKII sites (Ser 392) or PK-C sites (Sers 371, 376, 378) within this region result in stimulation of binding. Finally, deletion of C-terminal residues 363–393 yields a protein which is extremely active in binding to DNA (reviewed in refs 1 and 2). Additional modifiers of p53 DNA binding which are likely to work through the C-terminus include short single strands of DNA [90], p53-derived peptides spanning the basic PAb421 epitope [91, 92], or Ref-1 [65]. The mechanism by which the C-terminus regulates DNA binding is not yet fully understood. Two models have been proposed: in the first, it has been hypothesized that the C-terminus allosterically regulates conversion of p53 from an inert 'latent' form to one which is active for DNA binding [93, 94]. Evidence for this model is based upon the observation that p53 can be isolated from baculovirus-infected insect cells or synthesized in vitro in a form that binds DNA only weakly but which, upon treatments as outlined above, becomes activated [65, 93, 94]. The second model postulates that binding by the central domain is hindered by the interactions of the C-terminus with longer DNA molecules [95, 96]. It is not clear at this point which of these two models (or both) is correct. Validation of the allosteric model will require physical evidence for a conformational alteration in p53 protein upon one or more of the modifications noted previously.

Effects of phosphorylation of the C-terminus

Whatever the mode by which the C-terminus regulates the functions of the central core domain, there are a number of more recent observations that bear discussion and review. We first consider current developments in studying how phosphorylation regulates p53 DNA binding. It should be noted that attempts to discern phenotypes of phosphorylation site mutants in p53 have been confusing at best and disappointing at worst. Mutation of murine phosphorylation sites at N- [97–99] and C-termini [100–102, and unpublished] has yielded p53 proteins which are roughly equivalent to wild-type protein in standard transient transfection assays of transcriptional activation of p53 responsive reporters (reviewed in ref. 1). However, it is noteworthy that Hao et al. [103] found that wild-type p53 in G1-arrested murine cells is inactive, whereas mutation of the murine CKII site to a charged residues (Glu) rescued p53 function. Additionally, whereas mutation of the murine CKII site to alanine had little effect on transactivation of p21 and ribosomal gene cluster (RGC) reporters, it markedly inhibited the ability of p53 to repress the SV40 promoter [104]. Although it is not yet well understood, we might speculate that an interaction of the C-terminus with a protein component of the general transcription machinery such as TBP [105, 106] or p300 [30], both of which have been implicated in the repression function of p53, might either require p53 phosphorylated at that site, or might be affected by mutating Ser 392. There have been attempts to pursue the mechanism by which p53 is stimulated by CKII: p53 is bound stably by the β subunit of CKII, and after phosphorylation, p53 no longer can reanneal DNA [107]. The region of p53 required for the interaction with CKII was mapped between residues 287 and 340 [108]. This interaction as well as phosphorylation may contribute to the effect of the protein kinase on p53 DNA binding. That inhibition of p53 nonspecific interaction with DNA, as measured by strand reassociation activity, correlates with stimulation of specific DNA binding is consistent with the proposal of Anderson et al. [96] that the C-terminus, when bound to DNA, interferes with specific binding by the central region. Sakaguchi et al. [109], however, have noted another effect of phosphorylation of the CKII site in human p53: peptides spanning the C-terminal part of the protein (residues 303–393) display a 10-fold greater association constant for reversible tetramer formation after phosphorylation by CKII. Whether this striking observation is true as well of full-length p53 awaits experimentation. Importantly, Kapoor and Lozano [110], using an antibody specific for p53 phosphorylated at the CKII site, showed that ultraviolet (UV) (but not γ) irradiation induces phosphorylation at the rat p53 CKII site.

The initial finding that PK-C can phosphorylate human p53 within the C-terminal basic regulatory region was met with excitement because of the potential that this might represent the end-point of a signal transduction pathway leading to activation of the protein [111, 112]. Murine p53 is also phosphorylated on comparable sites within the C-terminal 30 amino acids [113], and an interaction between murine p53 residues 320–346 and PK-C was detected [114]. Milne et al. [113], however, have found that in SV3T3 cells p53 may not be inducibly phosphorylated at PK-C sites after 12-O-tetradecanoyl phorbol-13-acetate (TPA) treatment. To gain further insight into this protein kinase, one approach has been to exploit the use of PK-C inhibitors [115, 116]. Chernov et al. [115] found that H7 or bisindolymaleimide I treatment lead to accumulation of p53 protein, and, reciprocally, phorbol ester inhibits the accumulation of p53 after DNA damage. How this is related to actual phosphorylation of p53 at PKC sites has not been firmly established. Another group found that although staurosporine inhibits p53 accumulation, other PK-C inhibitors did not affect p53 in their system [116].

Another region of the C-terminus which is also regulatory in vitro spans the CDK site within the linker region: it was observed that phosphorylation of Ser 315 selectively stimulates p53 DNA binding to a subset of p53 sites [117]. Furthermore, a mutant p53, S315A, binds DNA poorly, when compared with wild-type p53 (unpublished). Whether Ser 315 phosphorylation operates mechanistically in the same fashion as modification of residues within the C-terminal basic region [363–393] is not yet established. However, mutation of the CDK site reduces activation by Dna K, and a monoclonal antibody (PAb 241] which binds in the vicinity of the site can stimulate DNA binding, analogous to the effects of PAb421 at the extreme C-terminus [118]. Furthermore, while a truncated p53 containing residues 1–363 (p53ΔC30) is capable of being phosphorylated at Ser 315 by CDKs to the same extent as full-length p53, DNA binding by p53ΔC30 is not stimulated by phosphorylation at Ser 315 (unpublished). As was shown for CKII, a binding site for CDK on human p53 has been identified within residues 330–339 [119]. Interestingly, substitution of Ser 392, but not Ser 315, with the acidic residue Asp led to reduced interaction of CDK with p53. Reciprocally, whereas phosphorylation of Ser 392 increases tetramerization, this effect is reversed when p53 is phosphorylated at Ser 315 [109]. Taken together, these data suggest an interdependent relationship between the CKII and CDK sites on p53.

p53 phosphorylation appears to be carried out by a number of different protein kinases, each of which, as described above, appear to phosphorylate residues exclusively either within the N- or the C-termini of the protein. One possible exception to this is the TFIIH-as-

sociated CAK complex. While Ko et al. [11] demonstrated that CAK phosphorylates p53 at Ser 33, Levine's laboratory reported that the same trimeric complex can phosphorylate site(s) within the C-terminus [120]. Whether CAK is the only kinase that can phosphorylate both N- and C-terminal sites is not yet known. However, since p53 was shown previously to interact with other constituents of TFIIH, namely, the XP-B, XP-D [121] and p62 [122] polypeptides, it is attractive to speculate that these interactions with, and modifications of, p53 serve important regulatory roles in vivo.

Other covalent modifications

Phosphorylation is not the only modification of p53. Another example of a covalently attached group is derived from the observation that 5.8S ribosomal RNA (rRNA) is attached to the penultimate serine of murine p53 [123]. p53 has also been shown to be O-glycosylated within the PAb421 epitope region in a cell-type-specific manner [92]. Such glycosylation renders p53 incapable of recognition by PAb421. Recently Gu and Roeder [124] discovered that p53 can be acetylated by p300/CBP predominantly at lysines 373 and 382, and that such modification leads to increased DNA binding. Glycosylation and acetylation of p53 may account for observations that subpopulations of p53 from different sources in some cases lack PAb421 reactivity.

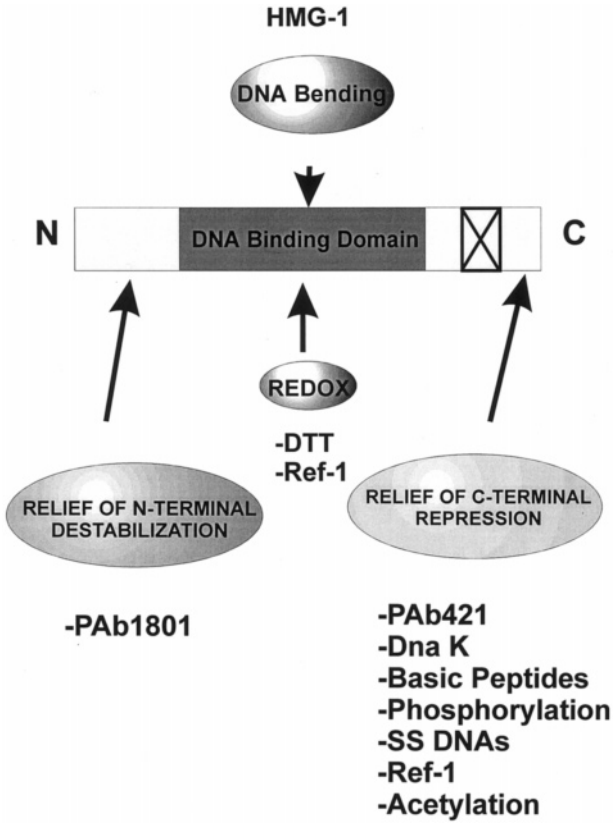

Figure 2. Multiple levels of regulation of p53 sequence-specific DNA binding.

Noncovalent modifiers

Covalent modifications are clearly a mode by which cells can signal to and regulate p53. But noncovalent regulators of p53 are also likely to become increasingly important. In this respect it is interesting to consider the relationship between p53 and DNA damage and repair. In addition to its possible role in regulation of the central core domain, the C-terminus of p53 has been postulated to be a damage recognition region, based on its ability to bind DNA ends and single strands [38, 125], mismatches [126], Holliday junctions [127] and irradiated DNA [128]. Although the significance of these interactions awaits further study, it is worth considering that they may perform a regulatory function in a manner analogous to proteins that interact with the C-terminus. Moreover, a number of DNA repair proteins have been identified that interact with or regulate p53, presumably through its C-terminus. These include the XP-B and XP-D components of TFIIH [121] and Rad51 [129]. The impact of these interactions on the sequence-specific transactivation function of p53 has not yet been fully explored. Moreover, as described above the redox/repair protein Ref-1 was discovered to be a potent activator of p53 DNA binding and transactivation [65]. Ref-1, functioning as an apurinic/apyrimidinic (A/P) endonuclease, plays a central role in base excision repair. Although it works directly on the central domain by providing critical redox function, Ref-1 also has a redox-independent role in activating p53, as evidenced by the fact that it stimulates p53 in the presence of high concentrations of reducing agents, and this stimulation requires an intact C-terminus [65]. Thus, p53 appears to be intimately connected to DNA repair processes both through signalling after DNA damage, as well as through its multiple interactions with components of the DNA repair machinery.

Conclusion

As detailed above, it is clear that there are a host of ways that p53 can be controlled. With respect to one of its most critical functions, namely, sequence-specific DNA binding, we can postulate at least four levels of regulation, which are diagrammed in figure 2. These include interactions at the N-terminus as evidenced by antibodies such as PAb 1801 which provide thermosta-

bilization, direct regulation of the core by both redox/metal binding and facilitation of DNA bending, and finally manipulation of the C-terminus by covalent and noncovalent modifiers leading to relief of repression by this region.

Thus, regulation of p53 is complex and still not well understood. There remain a plethora of questions to be answered and paradoxes to be solved. How does p53 become stabilized after DNA damage, and are there multiple mechanisms for this stabilization? How is p53 DNA binding regulated in cells? Is such regulation promoter-selective? How is it that phosphorylation of sites within the C-terminus can dramatically stimulate DNA binding in cells, and yet phosphorylation site mutants do not appear to yield p53 protein that is defective in transactivation, as measured by transient transfection assays?

With respect to the last question several possibilities for the discrepancies can be suggested:

1) DNA binding by p53 is regulated by redundant mechanisms: phosphorylation at several sites, and noncovalent activators such as Ref-1 and HMG-1, themselves abundant proteins in cells, may serve to ensure that, once stabilized, p53 is activated.

2) Transactivation assays or other overexpression systems for measuring p53 activity yield such high concentrations of p53 protein that regulation is overridden by sheer quantity of p53 protein.

3) The DNA-binding assays used to assess the effects of phosphorylation and other stimulators have usually been gel mobility shift assays, employing as ligand short duplex oligonucleotides. These assays may not mimic faithfully the interactions of p53 with DNA in cells.

Yet, providing some measure of confidence that observations made in vitro have physiological relevance, are the striking observations that introduction of C-terminal antibodies or peptides into cells can markedly stimulate the transactivation [130–132] or even proapoptotic function [132] of the resident wild-type or even mutant forms of p53. These experiments support cautious optimism that by delving further through ways into which p53 protein is regulated, we may eventually be able to develop small molecules which can, when introduced into cells, lead to stabilization of the wild-type tumour suppressor function of mutant or inactive forms of p53. Moreover, insight into regulation of p53 will be important not only for studying this important gene product but also for gaining understanding as to how other transcriptional regulators are controlled. Whatever the answers, the questions are as fascinating as the p53 protein itself.

1 Ko L. and Prives C. (1996) p53: puzzle and paradigm. Genes Dev. **10:** 1054–1072
2 Gottlieb M. T. and Oren M. (1996) p53 in growth control and neoplasia. Biochim. Biophys. Acta **1287:** 77–102
3 Levine A. J. (1997) p53, the cellular gatekeeper for growth and division. Cell **88:** 323–331
4 Unger T., Mietz J. A., Scheffner M., Yee C. L. and Howley P. M. (1993) Functional domains of wild-type and mutant p53 proteins involved in transcriptional regulation, transdominant inhibition and transformation suppression. Mol. Cell. Biol. **13:** 5186–5194
5 Chang J., Kim D.-H., Lee S. W., Choi K. Y. and Sung Y. C. (1995) Transactivation ability of p53 transcriptional activation domain is directly related to the binding affinity to TATA-binding protein. J. Biol. Chem. **270:** 25014–25019
6 Candau R, Scolnick D. M., Darpino P., Ying C. Y., Halazonetis T. D. and Berger S. L. (1997) Two tandem and independent sub-activation domains in the amino terminus of p53 require the adaptor complex for activity. Oncogene **15:** 807–816
7 Walker K. K. and Levine A. J. (1996) Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc. Natl. Acad. Sci. USA **93:** 15335–15340
8 Ruaro E. M., Collavin L., Del Sal G., Haffner R., Oren M., Levine A. J. et al. (1997) A proline-rich motif in p53 is required for transactivation-independent growth arrest as induced by Gas1. Proc. Natl. Acad. Sci. USA **94:** 4675–4680
9 Sakamuro D., Sabbatini P., White E. and Prendergast G. C. (1997) The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene **15:** 887–898
10 Meek D. (1994) Post-translational modification of p53. Sem. Cancer Biol. **5:** 203–207
11 Ko L. J., Chen X., Shieh S.-Y., Jayaraman L., Tamai K., Taya Y. et al. (1997) p53 is phosphorylated by CDK7/cyclin H in a p36/MAT1 dependent manner. Mol. Cell. Biol. **17:** 7220–7229
12 Milne D. M., Campbell L. E., Campbell D. G. and Meek D. W. (1995) p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1. J. Biol. Chem. **270:** 5511–5518
13 Adler V., Pincus M. R., Minamoto T., Fuchs S. Y., Bluth M. J., Brandt-Rauf P. W. et al. (1997) Conformation-dependent phosphorylation of p53. Proc. Natl. Acad. Sci. USA **94**, 1686–1691
14 Milne D. M., Campbell D. G., Caudwell F. B. and Meek D. W. (1994) Phosphorylation of the tumour suppressor protein p53 by mitogen-activated protein kinases. J. Biol. Chem. **269:** 9253–9260
15 Shieh S.-Y., Ikeda M., Taya Y. and Prives C. (1997) DNA damage-induced phosphorylation of p53 alleviates inhibition by mdm2. Cell **91:** 325–334
16 Siliciano J. D., Canman C. E., Taya Y., Sakaguchi K., Appella E. and Kastan M. B. (1997) DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev. **11:** 3471–3481
17 Chen J., Marechal V. and Levine A. J. (1993) Mapping of the p53 and mdm-2 interaction domains. Mol. Cell. Biol. **13:** 4107–4114
18 Oliner J. D., Pietenpol J. A., Thiagalingam S., Gyuris J., Kinzler K. W. and Vogelstein B. (1993) Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. Nature **362:** 857–860
19 Picksley S. M., Vojtesek B., Sparks A. and Lane D. P. (1994) Immunochemical analysis of the interaction of p53 with MDM2; fine mapping of the MDM2 binding site on p53 using synthetic peptides. Oncogene **9:** 2523–2529
20 Kussie P. H., Gorina S., Marechal V., Elenbaas B., Moreau J., Levine A. J. et al. (1996) Structure of the MDM2 oncoprotein bound to the p53 tumour suppressor transactivation domain. Science **274:** 948–953
21 Lane D. P. and Hall P. A. (1997) MDM2-arbiter of p53's destruction. Trends Biochem. Sci. **22:** 372–374
22 de Oca Luna R. M., Wagner D. S. and Lozano G. (1995) Rescue of early embryonic lethality in *mdm2*-deficient mice by deletion of p53. Nature **378:** 203–206

23 Jones S. N., Roe A. E., Donehower L. A. and Bradley A. (1995) Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. Nature **378:** 206–208
24 Haupt Y., Maya R., Kazaz A. and Oren M. (1997) Mdm2 promotes the rapid degradation of p53. Nature **387:** 296–299
25 Kubbutat M. H. G., Jones S. N. and Vousden K. H. (1997) Regulation of p53 stability by Mdm2. Nature **387:** 299–303
26 Bottger A., Bottger V., Sparks A., Liu W. L., Howard S. F. and Lane D. P. (1997) Design of a synthetic Mdm2-binding mini protein that activates the p53 response in vivo. Curr. Biol. **7:** 860–869
27 Honda R., Tanaka H. and Yasuda H. (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumour suppressor p53. FEBS Lett. **420:** 25–27
28 Gu W., Shi X. L. and Roeder R. G. (1997) Synergistic activation of transcription by CBP and p53. Nature **387:** 819–823
29 Lill N. L., Grossman S. R., Ginsberg D., DeCaprio J. and Livingston D. M. (1997) Binding and modulation of p53 by p300/CBP coactivators. Nature **387:** 823–827
30 Avantaggiati M. L., Ogryzko V., Gardner K., Giordano A., Levine A. S. and Kelly K. (1997) Recruitment of p300/CBP in p53-dependent signal pathways. Cell **89:** 1175–1184
31 Lin J., Chen J., Elenbaas B. and Levine A. J. (1994) Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. Genes Dev. **8:** 1235–1246
32 Wu X., Bayle J. H., Olson D. and Levine A. J. (1993) The p53-mdm-2 autoregulatory feedback loop. Genes Dev. **7:** 1126–1132
33 Barak Y., Juven T., Haffner R. and Oren M. (1993) mdm2 expression is induced by wild type p53 activity. EMBO J. **12:** 461–468
34 Thut C. J., Goodrich J. A. and Tjian R. (1997) Repression of p53-mediated transcription by MDM2: a dual mechanism. Genes Dev. **11:** 1974–1986
35 Zauberman A., Barak Y., Ragimov N., Levy N. and Oren M. (1993) Sequence-specific DNA binding by p53: identification of target sites and lack of binding to p53-MDM2 complexes. EMBO J. **12:** 2799–2808
36 Bottger A., Bottger V., Garcia-Echeverria C., Chene P., Hochkeppel H. K., Sampson W. et al. (1997) Molecular characterization of the hdm2-p53 interaction. J. Mol. Biol. **269:** 744–756
37 Dutta A., Ruppert J. M., Aster J. C. and Winchester E. (1993) Inhibition of DNA replication factor RPA by p53. Nature **365:** 79–82
38 Miller S. D., Moses K., Jayaraman L. and Prives C. (1997) Complex formation between p53 and RP-A inhibits the sequence specific DNA binding of p53 and is regulated by single stranded DNA. Mol. Cell. Biol. **17:** 2194–2201
39 Chen X., Zhu H., Farmer G., Prywes R. and Prives C. (1993) p53 and TATA binding protein cooperate to bind to DNA. Genes Dev. **7:** 1837–1849
40 Martin M. E. and Berk A. J. (1998) Adenovirus E1B 55K represses p53 activation in vitro. J. Virol. **72:** 3146–3154
41 Hansen S., Hupp T. R. and Lane D. P. (1996) Allosteric regulation of the thermosensitivty and DNA binding activity of human p53 by specific interacting proteins. J. Biol. Chem. **271:** 3917–3924
42 Friedlander P., Legros Y., Soussi T. and Prives C. (1996) Regulation of mutant p53 temperature sensitive DNA binding. J. Biol. Chem. **271:** 25468–25478
43 Knippschild U., Milne D. M., Campbell L. E., DeMaggio A. J., Christenson E., Hoekstra M. F. et al. (1997) p53 is phosphorylated in vitro and in vivo by the delta and epsilon isoforms of casein kinase 1 and enhances the level of casein kinase 1 delta in response to topoisomerase-directed drugs. Oncogene **15:** 1727–1736
44 Pomerantz J., Schreiber-Agus N., Liegeois N. J., Silverman A., Alland L., Chin L. et al. (1998) The Ink4a tumour suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell **92:** 713–723
45 Zhang Y., Xiong Y. and Yarbrough W. (1998) GARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumour suppression pathways. Cell **92:** 725–734
46 Soussi T. and May P. (1996) Structural aspects of the p53 protein in relation to gene evolution: a second look. J. Mol. Biol. **260:** 623–637
47 Hainaut P., Soussi T., Shomer B., Hollstein M., Greenblatt M., Hovig E. et al. (1997) Database of p53 gene somatic mutations in human tumours and cell lines: updated compilation and future prospects. Nucleic Acids Res. **25:** 151–157
48 Vogelstein B. and Kinzler K. W. (1992) p53 function and disfunction. Cell **70:** 5236–5238
49 Cho Y., Gorina S., Jeffrey P. D. and Pavletich N. P. (1994) Crystal structure of a p53 tumour suppressor-DNA complex: understanding tumourigenic mutations. Science **265:** 346–355
50 Wang Y., Schwedes J. F., Parks D., Mann K. and Tegtmeyer P. (1995) Interaction of p53 with its consensus DNA-binding site. Mol. Cell. Biol. **15:** 2157–2165
51 Balagurumoorthy P., Sakamoto H., Lewis M. S., Zambrano N., Clore G. M., Gronenborn A. M. et al. (1995) Four p53 DNA binding domain peptides bind natural p53 response elements and bend the DNA. Proc. Natl. Acad. Sci. USA **92:** 8591–8595
52 Shaulian E., Zauberman A., Milner J., Davies E. A. and Oren M. (1993) Tight DNA binding and oligomerization are dispensable for the ability of p53 to transactivate target genes and suppress transformation. EMBO J. **12:** 2789–2797
53 Jayaraman L., Freulich E. and Prives C. (1997) Functional dissection of the p53 tumour suppressor protein. Methods Enzymol. **283:** 245–256
54 Tarunina M. and Jenkins J. R. (1993) Human p53 binds DNA as a protein homodimer but monomeric variants retain full transcription transactivation activity. Oncogene **8:** 3165–3173
55 Wang Y., Reed M., Wang P., Stenger J. E., Mayr G., Anderson M. E. et al. (1993) p53 domains: identification and characterization of two autonomous DNA-binding regions. Genes Dev. **7:** 2575–2586
56 Sun Y. and Oberley L. W. (1996) Redox regulation of transcriptional activators. Free Radical Biol. Med. **21:** 335–348
57 Hainaut P. and Milner J. (1992) Interaction of heat-shock protein 70 with p53 translated in vitro: evidence for interaction with dimeric p53 and for a role in the regulation of p53 conformation. EMBO J. **10:** 3513–3520
58 Hainaut P. and Milner J. (1993) Redox modulation of p53 conformation and sequence-specific DNA binding in vitro. Cancer Res. **53:** 4469–4473
59 Hainaut P., Rolley N., Davies M. and Milner J. (1995) Modulation by copper of p53 conformation and sequence specific DNA binding: Role for Cu(II)/Cu(I) redox mechanism. Oncogene **10:** 27–32
60 Bullock A. N., Henckel J., DeDecker B. S., Johnson C. M., Nikolova P. V., Proctor M. R. et al. (1997) Thermodynamic stability of wild-type and mutant p53 core domain. Proc. Natl. Acad. Sci. USA **94:** 14338–14342
61 Verhaegh G. W., Richard M. J. and Hainaut P. (1997) Regulation of p53 by metal ions and by antioxidants: dithiocarbamate down-regulates p53 DNA-binding activity by increasing the intracellular level of copper. Mol. Cell. Biol. **17:** 5699–5706
62 Hupp T. R., Meek C. A., Midgley C. A. and Lane D. P. (1993) Activation of the cryptic DNA binding function of mutant forms of p53. Nucleic Acids Res. **21:** 3167–3174
63 Delphin C., Cahen P., Lawrence J. J. and Baudier J. (1994) Characterization of baculovirus recombinant wild-type p53. Dimerization of p53 is required for high-affinity DNA binding and cysteine oxidation inhibits p53 DNA binding. Eur. J. Biochem. **223:** 683–692
64 Rainwater R., Parks D., Anderson M. E., Tegtmeyer P. and Mann K. (1995) Role of cysteine residues in regulation of p53 function. Mol. Cell. Biol. **15:** 3892–3903

65 Jayaraman L., Murthy K. G. K., Curran T., Xanthoudakis S. and Prives C. (1997) Identification of redox/repair protein Ref-1 as an activator of p53. Genes Dev. **11:** 558–570
66 Casso D. and Beach D. A. (1996) Mutation in a thioredoxin reductase homolog suppresses p53-induced growth inhibition in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **252:** 518–529
67 Pearson G. D. and Merrill G. F. (1998) Deletion of the *Saccharomyces cerevisiae* TRR1 gene encoding thioredoxin reductase inhibits p53-dependent reporter gene expression. J. Biol. Chem. **273:** 5431–5434
68 Meira L. B., Cheo D. L., Hammer R. E., Burns D. K., Reis A. and Friedberg E. C. (1997) Genetic interaction between HAP1/REF-1 and p53. Nature Genet. **17:** 145
69 Graeber T. G., Osmanian C., Jacks T., Housman D. E., Koch C. J., Lowe S. W. et al. (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature **379:** 88–91
70 Yao K.-S., Xanthoudakis S., Curran T. and O'Dwyer P. J. (1994) Activation of AP-1 and of a nuclear redox factor, Ref-1, in the response of HT29 colon cancer cells to hypoxia. Mol. Cell. Biol. **14:** 5997–6003
70a Yin Y., Terauchi Y., Solomon G. G., Aizawa S., Rangarajan P. N., Yazaki Y. et al. (1998) Involvement of p85 in p53-dependent response to oxidative stress. Nature **391:** 707–710
71 Nagaich A. K., Zhurkin V. B., Sakamoto H., Gorin A. A., Clore G. M., Gronenborn A. M. et al. (1997) Architectural accommodation in the complex of four p53 DNA binding domain peptides with the p21/waf1/cip1 DNA response element. J. Biol. Chem. **272:** 14830–14841
72 Nagaich A. K., Appella E. and Harrington R. E. (1997) DNA bending is essential for the site-specific recognition of DNA response elements by the DNA binding domain of the tumour suppressor protein p53. J. Biol. Chem. **272:** 14842–14849
73 Jayaraman L., Murthy K. G. K., Manley J. L., Bustin M. and Prives C. (1998) Identification of HMG-1 as a unique activator of p53. Genes Dev. **12:** 462–472
74 Bustin M. and Reeves R. (1996) High mobility group proteins. Progress Nucleic Acid Res. Mol. Biol. **54:** 35–100
75 Pavletich N. P., Chambers K. A. and Pabo C. O. (1993) The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots. Genes Dev. **7:** 2556–2564
76 Wang Y., Reed M., Wang P., Stenger J. E., Mayr G., Anderson M. E. et al. (1993) p53 domains: identification and characterization of two autonomous DNA-binding regions. Genes Dev. **7:** 2575–2586
77 Brain R. and Jenkins J. R. (1994) Human p53 directs DNA strand reassociation and is photolabelled by 8-azido ATP. Oncogene **9:** 1775–1780
78 Wu L., Bayle J. H., Elenbaas B., Pavletich N. P. and Levine A. J. (1995) Alternatively spliced forms in the carboxy-terminal domain of the p53 protein regulate its ability to promote annealing of complementary single strands of nucleic acids. Mol. Cell. Biol. **15:** 497–504
79 Balkalkin G., Yakovleva T., Selivanova G., Magnusson K. P., Szekely L., Kiseleva E. et al. (1994) p53 binds single-stranded DNA ends and catalyzes DNA renaturation and strand transfer. Proc. Natl. Acad. Sci. USA **91:** 413–417
80 Waterman J. L., Shenk J. L. and Halazonetis T. D. (1995) The dihedral symmetry of the p53 tetramerization domain mandates a conformational switch upon DNA binding. EMBO J. **14:** 512–519
81 Clore G. M., Ernst J., Clubb R., Omichinski J. G., Kennedy W. M. P., Sakaguchi K. et al. (1995) Refined solution structure of the oligomerization domain of the tumour suppressor p53. Struct. Biol. **2:** 321–332
82 Lee W., Harvey T. S., Yin Y., Yau P., Litchfield D. and Arrowsmith C. H. (1994) Solution structure of the tetrameric minimum transforming domain of p53. Nature Struct. Biol. **1:** 877–90
83 Jeffrey P. D., Gorina S. and Pavletich N. P. (1995) Crystal structure of the tetramerization domain of the p53 tumour suppressor at 1.7 angstroms. Science **267:** 1498–1502
84 Shaulian E., Zauberman A., Milner J., Davies E. A. and Oren M. (1993) Tight DNA binding and oligomerization are dispensable for the ability of p53 to transactivate target genes and suppress transformation. EMBO J. **12:** 2789–2797
85 Waterman M. J., Waterman J. L. and Halazonetis T. D. (1996) An engineered four-stranded coiled coil substitutes for the tetramerization domain of wild-type p53 and alleviates transdominant inhibition by tumour-derived p53 mutants. Cancer Res. **56:** 158–163
86 Chene P., Mittl P. and Grutter M. (1997) In vitro structure-function analysis of the beta-strand 326–333 of human p53. J. Mol. Biol. **273:** 873–881
87 McCoy M., Stavridi E. S., Waterman J. L., Wieczorek A. M., Opella S. J. and Halazonetis T. D. (1997) Hydrophobic side-chain size is a determinant of the three-dimensional structure of the p53 oligomerization domain. EMBO J. **16:** 6230–6236
88 Ishioka C., Englert C., Winge P., Yan Y. X., Engelstein M. and Friend S. H. (1995) Mutational analysis of the carboxy-terminal portion of p53 using both yeast and mammalian cell assays in vivo. Oncogene **10:** 1485–1492
89 Pellegata N. S., Cajot J. F. and Stanbridge E. J. (1995) The basic carboxy-terminal domain of human p53 is dispensable for both transcriptional regulation and inhibition of tumour cell growth. Oncogene **11:** 337–349
90 Jayaraman L. and Prives C. (1995) Activation of p53 sequence-specific DNA binding by short single strands of DNA requires the p53 C-terminus. Cell **81:** 1021–1029
91 Hupp T. R., Sparks A. and Lane D. P. (1995) Small peptides activated the latent sequence-specific DNA binding function of p53. Cell **83:** 237–245
92 Shaw P., Freeman J., Bovey R. and Iggo R. (1996) Regulation of specific DNA binding by p53: evidence for a role for O-glycosylation and charged residues at the carboxy-terminus. Oncogene **12:** 20797–20802
93 Halazonetis T. D., Davis L. J. and Kandil A. N. (1993) Wild-type p53 adopts a 'mutant'-like conformation when bound to DNA. EMBO J. **12:** 1021–1028
94 Hupp T. R. and Lane D. P. (1994) Allosteric activation of latent p53 tetramers. Curr. Biol. **4:** 865–875
95 Bayle J. H., Elenbaas B. and Levine A. J. (1995) The carboxyl-terminal domain of the p53 protein regulates sequence-specific DNA binding through its non-specific nucleic acid binding activity. Proc. Natl. Acad. Sci. USA **92:** 5729–5733
96 Anderson M. E., Woelker B., Reed M., Wang P. and Tegtmeyer P. (1997) Reciprocal interference between the sequence-specific core and nonspecific C-terminal DNA binding domains of p53: implications for regulation. Mol. Cell. Biol. **17:** 6255–6264
97 Fiscella M., Ullrich S. J., Zambrano N., Shields M. T., Lin D., Lees-Miller S. P. et al. (1993) Mutation of the serine 15 phosphorylation site of human p53 reduces the ability of p53 to inhibit cell cycle progression. Oncogene **8:** 1519–1528
98 Fuchs B., O'Connor D., Fallis L., Scheidtmann K. H. and Lu X. (1995) p53 phosphorylation mutants retain transcription activity. Oncogene **10:** 789–793
99 Mayr G. A., Reed M., Wang P., Wang Y., Schwedes J. F. and Tegtmeyer P. (1995) Serine phosphorylation in the NH2 terminus of p53 facilitates transactivation. Cancer Res. **55:** 2410–2417
100 Fiscella M., Zambrano N., Ullrich S. J., Unger T., Lin D., Cho B. et al. (1994) The carboxy-terminal serine 392 phosphorylation site of human p53 is not required for wild-type activities. Oncogene **9:** 3249–3257
101 Marston N. J., Crook T. and Vousden K. H. (1994) Interaction of p53 with MDM2 is independent of E6 and does not mediate wild type transformation suppressor function. Oncogene **9:** 2707–2716

102 Slingerland J. M., Jenkins J. R. and Benchimol S. (1993) The transforming and suppressor functions of p53 alleles: effects of mutations that disrupt phosphorylation, oligomerization and nuclear translocation. EMBO J. **12:** 1029–1037

103 Hao M., Lowy A. M., Kapoor M., Deffie A., Liu G. and Lozano G. (1996) Mutation of phosphoserine 389 affects p53 function in vivo. J. Biol. Chem. **271:** 29380–29385

104 Hall S. R., Campbell L. E. and Meek D. W. (1996) Phosphorylation of p53 at the casein kinase II site selectively regulates p53-dependent transcriptional repression but not transactivation. Nucleic Acids Res. **24:** 1119–1126

105 Horikoshi N., Usheva A., Chen J., Levine A. J., Weinmann R. and Shenk T. (1995) Two domains of p53 interact with the TATA-binding protein, and the adenovirus 13S E1A protein disrupts the association, relieving p53-mediated transcriptional repression. Mol. Cell. Biol. **15:** 227–234

106 Shaulian E., Haviv I., Shaul Y. and Oren M. (1995) Transcriptional repression by the C-terminal domain of p53. Oncogene **10:** 671–680

107 Filhol O., Baudier J., Chambaz E. M. and Cochet C. (1996) Casein kinase 2 inhibits the renaturation of complementary DNA strands mediated by p53 protein. Biochem. J. **316:** 331–335

108 Appel K., Wagner P., Boldyreff B., Issinger O. G. and Montenarh M. (1995) Mapping of the interaction sites of the growth suppressor protein p53 with the regulatory beta-subunit of protein kinase CK2. Oncogene **11:** 1971–1978

109 Sakaguchi K., Sakamoto H., Lewis M. S., Anderson C. W., Erickson J. W., Appella E. et al. (1997) Phosphorylation of serine 392 stabilizes the tetramer formation of tumour suppressor protein p53. Biochemistry **36:** 10117–10124

110 Kapoor M. and Lozano G. (1998) Functional activation of p53 via phosphorylation following DNA damage by UV but not gamma radiation. Proc. Natl. Acad. Sci. USA **95:** 2834–2837

111 Takenaka I., Morin F., Seizinger B. R. and Kley N. (1995) Regulation of the sequence-specific DNA binding function of p53 by protein kinase C and protein phosphatases. J. Biol. Chem. **270:** 5405–5411

112 Baudier J., Delphin C., Grunwald D., Khochbin S. and Lawrence J. J. (1992) Characterization of the tumour suppressor protein p53 as a protein kinase C substrate and a S100b-binding protein. Proc. Natl. Acad. Sci. USA **89:** 11627–11631

113 Milne D. M., McKendrick L., Jardine L. J., Deacon E., Lord J. M. and Meek D. W. (1996) Murine p53 is phosphorylated within the PAb421 epitope by protein kinase C in vitro, but not in vivo, even after stimulation with the phorbol ester *o*-tetradecanoylphorbol 13-acetate. Oncogene **13:** 205–211

114 Delphin C., Huang K. P., Scotto C., Chapel A., Vincon M., Chambaz E. et al. (1997) The in vitro phosphorylation of p53 by calcium-dependent protein kinase C-characterization of a protein-kinase-C-binding site on p53. Eur. J. Biochem. **245:** 684–692

115 Chernov M. V., Ramana C. V., Adler V. V. and Stark G. R. (1998) Stabilization and activation of p53 are regulated independently by different phosphorylation events. Proc. Natl. Acad. Sci. USA **95:** 2284–2289

116 Pitkanen K., Haapajarvi T. and Laiho M. (1998) U.V.C.-induction of p53 activation and accumulation is dependent on cell cycle and pathways involving protein synthesis and phosphorylation. Oncogene **16:** 459–469

117 Wang Y. and Prives C. (1995) Increased and altered DNA binding of human p53 by S and G2/M but not G1 cyclin-dependent kinases. Nature **376:** 88–91

118 Hansen S., Midgley C. A., Lane D. P., Freeman B. C., Morimoto R. I. and Hupp T. R. (1996) Modification of two distinct COOH-terminal domains is required for murine p53 activation by bacterial Hsp70. J. Biol. Chem. **271:** 30922–30928

119 Wagner P., Fuchs A., Gotz C., Nastainczyk W. and Montenarh M. (1998) Fine mapping and regulation of the association of p53 with p34cdc2. Oncogene **16:** 105–111

120 Lu H., Fisher R. P., Bailey P. and Levine A. J. (1997) The CDK7-cycH-p36 complex of transcription factor IIH phosphorylates p53, enhancing its sequence-specific DNA binding activity in vitro. Mol. Cell. Biol. **17:** 5923–5934

121 Wang X., Vermeulen W., Coursen J. D., Gibson M., Lupold S. E., Forrester K. et al. (1996) The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway. Genes Dev. **10:** 1219–1232

122 Xiao H., Pearson A., Coulombe B., Truant R., Zhang S., Regier J. L. et al. (1994) Binding of basal transcription factor TFIIH to the acidic activation domains of VP16 and p53. Mol. Cell. Biol. **14:** 7013–7024

123 Fontoura B. M., Sorokina E. A., David E. and Carroll R. B. (1992) p53 is covalently linked to 5.8S rRNA. Mol. Cell. Biol. **12:** 5145–5151

124 Gu W. and Roeder R. G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:** 595–606

125 Balkalkin G., Selivanova G., Yakovleva T., Kiseleva E., Kashuba E., Magnusson K. P. et al. (1995) p53 binds single-stranded DNA ends through the C-terminal domain and internal DNA segments via the middle domain. Nucleic Acids Res. **23:** 362–369

126 Lee S., Elenbaas B., Levine A. and Griffith J. (1995) p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell **81:** 1013–1020

127 Lee S., Cavallo L. and Griffith J. (1997) Human p53 binds Holliday junctions strongly and facilitates their cleavage. J. Biol. Chem. **272:** 7532–7539

128 Reed M., Woelker B., Wang P., Wang Y., Anderson M. E. and Tegtmeyer P. (1995) The C-terminal domain of p53 recognizes DNA damaged by ionizing radiation. Proc. Natl. Acad. Sci. USA **92:** 9455–9459

129 Buchhop S., Gibson M. K., Wang X. W., Wagner P., Sturzbecher H. W. and Harris C. C. (1997) Interaction of p53 with the human Rad51 protein. Nucleic Acids Res. **25:** 3868–3874

130 Abarzua P., LoSardo J. E., Gubler M. L., Spathis R., Lu Y. A., Felix A. et al. (1996) Restoration of the transcription activation function to mutant p53 in human cancer cells. Oncogene **13:** 2477–2482

131 Abarzua P., LoSardo J. E., Gubler M. L. and Neri A. (1995) Microinjection of monoclonal antibody Pab421 into human SW480 colorectal carcinoma cells restores the transcription activation function to mutant p53. Cancer Res. **55:** 3490–3494

132 Selivanova G., Iotsova V., Okan I., Fritsche M., Strom M., Groner B. et al. (1997) Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nature Med. **3:** 632–638