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Review

Mouse bites dogma: how mouse models are changing our views of how P53 is regulated *in vivo*

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Abstract

P53 is a transcription factor that can cause cells to be eliminated by apoptosis or senescent-like arrest upon its activation by irreparable genetic damage, excessively expressed oncogenes, or a broad spectrum of other stresses. As P53 executes life and death decisions, its activity must be stringently regulated, which implies that it is not likely to be controlled by a simple regulatory mechanism involving a binary on–off switch. This brief review will summarize a subset of the new information presented at the 10th P53 workshop in Dunedin, New Zealand in November 2004 as well as very recent publications that provide new insights into the molecular regulators of P53. Data emerging from mouse models provide a fundamentally different view of how P53 is regulated than suggested by more traditional *in vitro* approaches. The differences between cell culture and mouse models demonstrate the importance of preserving stoichiometric relationships between P53 and its various regulators to obtain an accurate view of the relevant molecular mechanisms that control P53 activity.

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Introduction

Twenty-five years ago, a 53–54 kDa cellular protein now referred to as P53 was reported to interact with viral oncoproteins.[1–5] Initial studies suggested that P53 functioned as an oncogenic protein. Interestingly, recent studies that modeled two such mutations in the mouse suggested that they 'gained function' by binding to and attenuating the functions of the P53-related proteins P63 and P73.[6] This provides one mechanism by which some P53 mutants function as oncogenes. However, structural gene mutations that disable P53 function, or that could engender dominant-negative properties, have been reported in about 50% of human cancers, including those that contribute most to morbidity and mortality (e.g., see http://www-p53.iarc.fr/index.html). Clear loss of both p53 alleles has been reported in a significant fraction of human cancers.[7] Together, these data demonstrate that p53 is an important tumor suppressor.[8,9] Importantly, a substantial fraction of human tumors express wild-type *p53*, but its function is compromised by aberrant expression of proteins that negatively regulate it, such as Hdm2 and HdmX (Hdm2 or HdmX designate the human proteins, and Mdm2 and MdmX the mouse; the corresponding genes are *hdm2*, *hdmx*, *mdm2*, *mdmx*).[10–13] (Below, for simplicity, these regulators are collectively referred to as Mdm2 or MdmX.) Together, the data show that the P53 pathway is disabled in the majority, if not all, human cancers.

P53 is an unstable transcription factor that can regulate numerous downstream targets to induce permanent or reversible cell cycle arrest, apoptosis, and DNA repair.[14,15] While P53 has also been reported to induce apoptosis by nontranscriptional mechanisms,[16–18] substantial evidence indicates that its transcriptional functions are critical for effective tumor suppression.[19]

Signals elicited by stresses including DNA damage (e.g., see, see, Huang *et al.*,[20] Kastan *et al.*,[21] and Wahl and Carr[22]), short or abnormally structured telomeres,[23,24] high-level oncogene signaling,[25–28] hypoxia,[29] and glucose availability[30] activate P53. Cells that encounter such conditions either arrest cell division or die if they possess a functional P53 pathway. By contrast, if P53 is dysfunctional, cells proliferate when exposed to oncogenic stimuli, unrepaired DNA damage, or metabolic perturbations that induce chromosome instability.[31,32] Thus, by eliminating P53 function, key controls to prevent cell cycle entry under inappropriate, potentially genome destabilizing conditions are lifted, allowing outgrowth of the genetically unstable variants that fuel tumor progression.

Since P53 output has the potential to kill cells, stringent regulatory mechanisms have evolved to prevent its errant activation, as well as to allow for rapid activation when appropriate. However, the regulatory mechanisms that enable the P53 pathway to generate different transcriptional responses to different stimuli in different tissues remain to be defined. There is significant medical importance to having a clear idea of P53 regulatory mechanisms as 3 million human cancers per year are estimated to contain a wild-type P53 protein whose function is attenuated. Understanding the mechanisms underlying such negative regulation affords a substantial opportunity for therapeutic intervention. This possibility has been realized with the development of agents such as *cis*-imidazolines (e.g., Nutlin 3A) that interfere with Mdm2–P53 interactions to activate P53 in cells with overexpressed Mdm2.[33] Below, I review data from cell

culture and mouse models that provide a current view of mechanisms that keep P53 off in unstressed cells, allow its activation in response to conditions that produce double-strand breaks, and then turn off P53 upon stress abatement.

Integration of P53 Structure with Regulation

P53 structure suggests many potential levels at which its transcription function could be regulated (Figure 1). Transfection studies suggest that P53 contains one N-terminal transactivation domain (TAD1) comprising the first 40 amino acids,[34] and a second (TAD2) consisting of amino acids 43–63 that is revealed upon inactivation of TAD1.[35] A potential conformational switch may be contained within a domain comprising proline-X-X-proline motifs between the TAD and the large central DNA-binding domain.[36–39] A C-terminal oligomerization domain is necessary to form tetramers.[40] These tetramers not only constitute the most active transcriptional form of P53[40–47] but also conceal the dominant nuclear export signal that lies within the oligomerization domain.[48] The lysine-rich C-terminus constitutes a domain implicated in both positive regulation by post-translational modifications involving acetylation,[49–52] methylation,[53,54] and sumoylation,[55,56] and negative regulation by ubiquitylation[57] or neddylation.[58] The C-terminus has also been proposed to function as an allosteric regulator.[59–61] However, recent data make it more likely that this domain enables P53 to bind nonspecifically to DNA to increase the rate at which specific P53 response elements are detected through linear diffusion.[62,63]

The N-Terminal TAD

Post-translational P53 modifications have been proposed to induce structural alterations to stabilize the protein and to effect the chromatin modifications needed for transcriptional activation. The extreme N-terminal TAD has residues critical for the binding of both the coactivators needed for transactivation, and the E3 ubiquitin ligase MDM2 that contributes most significantly to determining the abundance and short half-life of P53 in unstressed cells.[19] Indeed, mutation of just two amino acids within this domain (L22Q, W23S in human, and L25Q, W26S in mouse; referred to as P53QS below) generates a stable but largely inactive P53 as the mutant can neither interact with Mdm2 (and presumably MdmX, which has a similar P53-binding domain) nor with the required coactivators.

One attractive model is that DNA damage activates the ATM kinase to induce a phosphorylation cascade beginning with human ser15 (i.e., h-ser15), equivalent to mouse ser18 (m-ser18), which triggers the subsequent phosphorylation of h-thr18 (m-thr21) culminating in phosphorylation of h-ser20 (m-ser23).[64] These residues constitute part of a helical domain that interacts with a hydrophobic pocket in the Mdm2 N-terminus.[65] *In vitro* studies with peptides from this region indicate that thr18 phosphorylation can significantly destabilize this structure, leading to Mdm2 dissociation.[66] Transfection studies suggest that h-ser20 phosphorylation significantly destabilizes the Mdm2/P53 interaction,[67] while others indicate that h-ser15 phosphorylation increases the affinity for p300/CBP.[68,69] These events together have been suggested to stimulate P53 transactivation function. By contrast, other studies showed that P53 mutants in which all serine residues in the entire protein were changed to alanine

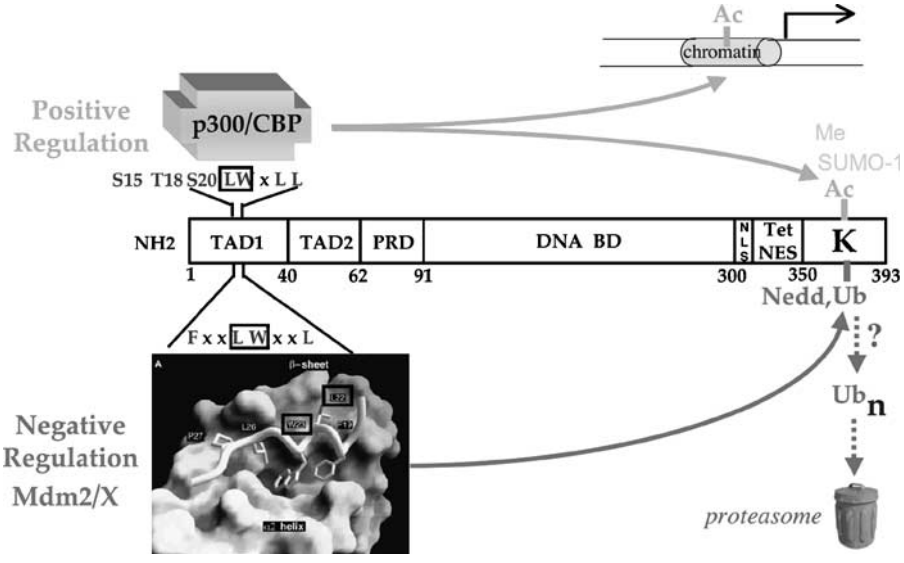

Figure 1 A competition model for positive and negative regulation. This is a very simplified view of p53, the N-terminal binding region for Mdm2 and MdmX, as well as for p300/CBP. The structure of the p53 alpha-helical region binding within the hydrophobic cleft of Mdm2 is shown. The N-terminal region of MdmX that binds p53 is very similar. The positions of S15, T18, and S20 are shown as they have been implicated in altering the N-terminal structure when phosphorylated to either decrease Mdm2 (MdmX?) binding or increase association with p300/CBP. Also shown in simplified form is the introduction of either positive or negative post-translational modifications to multiple C-terminal lysines by either the HATs or by the E3 Mdm2. The N-terminal residues L22W23 that have been shown to prevent Mdm2 binding and severely reduce transactivation when mutated to Q22 and S23 are shown in the primary structure and in their locations deduced from crystallographic analyses. See the text for more details and references

displayed wild-type stability and transactivation.[19] Of great significance, while mouse mutants generated by homologous recombination to encode m-ser18ala appeared to exhibited tissue-specific defects in p53 target gene activation, its capacity to suppress spontaneous tumor formation remained intact.[70–72] Mice expressing the ser23ala mutation also exhibited modest, tissue-specific deficiencies, but not the substantial destabilization and functional inactivation that would have been predicted were phosphorylation of this residue critical for reducing Mdm2 binding.[73,74] These data are most consistent with N-terminal phosphorylations contributing to modulating tissue-specific responses rather than serving as an on–off switch as occurs in *Drosophila*.[75,76]

The interpretation of the significance of N-terminal phosphorylation on P53 function also depends on whether P53 contains two TADs. If it does, then mutations in m-ser18 and m-thr21 might only inactivate the first one, but preserve the function of the second to allow some level of transactivation. The question of whether P53 contains more than one functional TAD was raised at the Dunedin meeting. The human L22QW23S mutant was found by transfection analysis to retain some residual function, and residues 43–63 were subsequently identified as a second potential TAD (see above). Recent data indicate that the second TAD mediates activation of the proapoptotic IGFBP3 gene, and that the basic C-terminus inhibits this function.[77] This interpretation is based on transfection studies, and it is important to emphasize that the function of the second TAD is revealed only in the context of P53 mutants deleted for TAD1, which contains the Mdm2 binding site. Thus, TAD1 mutants should encode a very stable P53 variant capable of binding chromatin constitutively (see below). The idea that the C-terminus is inhibitory to P53 function is inconsistent with other studies indicating that it mediates linear diffusion to augment the ability of P53 to find its response elements (see below). Therefore, it remains to be determined whether the second putative TAD can contribute to P53 functions in the context of a structurally wild-type P53 with compromised function for TAD1 while preserving stability control by Mdm2.

A second approach to define the importance of transactivation for P53-mediated tumor suppression, and to assess the relative contribution of each P53 TAD *in vivo*, uses homologous recombination to generate mice with inactivating mutations in TAD1. Mice expressing a *p53* with the mouse equivalent of the L22QW23S mutations (i.e., L25QW26S, referred to as p53QS) that largely inactivate human TAD1 have been generated by three groups.[78–80] Two previous studies showed that the p53QS mutant appeared to be completely devoid of function.[78,79] However, we now know that the targeting construct we used to generate this mutant contained an additional alanine to valine mutation at codon 135 in the DNA-binding domain (Ala135 to Val135). The Val135 mutation also generates a temperature-sensitive allele.[81] Unfortunately, a second study did not attempt to produce mice expressing *p53QS*, but did generate differentiated ES cells and reconstituted thymus expressing this allele.[78] The data from both our studies and those of Chao *et al.*,[78] as well as our more recent comparisons of the *p53QS* alleles with Ala135 (p53QSA) or Val135 (p53QSV),[81] show that the p53QS mutations themselves generate p53 molecules devoid of detectable transcriptional activity, ability to induce cell cycle arrest or apoptosis, or suppress tumor formation under the conditions tested. In stark contrast to this conclusion, data presented at the Dunedin meeting showed that an independently generated p53QS with Ala135 is embryonic lethal in heterozygotes.[80] As no homozygous *p53QSA* embryos were obtained, the source of the lethality remains to be determined. It is possible that the P53QSA protein could stabilize and activate the remaining wild-type P53, that the mutant allele could retain transcription function in the proposed second TAD, or that another mechanism such as that described below could underlie the observed lethality.

Our recent studies provide another way of envisioning lethality generated by a stable, but transcriptionally inert transcriptional regulator.[81] We carefully compared the properties of endogenous p53QSV with exogenously expressed p53QSA (introduced into *p53*-null MEFs by lentiviral infection, and clones expressing the same levels of P53QSV and P53QSA proteins were identified and analyzed). We detected no transactivation activity of either mutant on genes such as *p21*, *mdm2*, and *Puma*, but, consistent with the results from the Attardi lab, we did detect bax transactivation by our *p53QSA*. However, *bax* activation by either our p53QSV or QSA MEFs or those from the Attardi lab was only twice that observed in *p53*-null cells. We have yet to observe induction of cell cycle arrest, apoptosis, or suppression of xenograft formation by p53QSA or p53QSV.[81] p53QSA and p53QSV are, as expected, extremely stable proteins ($T_{1/2} > 8$ h). There is, however, one very important difference between P53QSA and P53QSV proteins. While p53QSA binds *p21*, *mdm2*, *Puma*, and *Noxa* promoters constitutively and at levels equal to that of activated wild-type p53, p53QSV binds these promoters far less efficiently. Interestingly, we found that the p53QSA generated in the Attardi lab is at least five times as abundant in MEFs as p53QSV in MEFs derived from the mice we generated, even though both proteins are equivalently stable and p53 mRNA synthesis is similar for each allele.[82]

Based on all the available data, we propose the following explanations for the embryonic lethality exhibited by the mice produced by Johnson *et al.*[80] First, the very high level of the p53QSA protein in their mice could exhibit low activity in particular cell types under specific conditions *in vivo* due to its constitutive chromatin binding and weak transactivation mediated by the second TAD. Another interpretation suggested by our recent data suggests a transactivation-independent mechanism of lethality. That is, although p53QSA lacks measurable transactivation function, we speculate that its accumulation to high levels, combined with its tight and constitutive binding to chromatin, could interfere with cellular transcription, replication, or other DNA-associated molecular events to engender lethality.

The C-Terminus and p53 Regulation

The data summarized above lead us to suggest that there is one dominant TAD in the N-terminus, and that, as deduced from *in vitro* studies, there are residues within it that are essential for the control of both P53 stability and transactivation. A model that is accepted by most of the field has emerged

in which the binding of coactivators competes for binding of the negative regulators Mdm2 and MdmX to the N-terminus. This sets up a competition for competing modifications such as acetylation and ubiquitylation of key highly conserved lysines (six in human, seven in mouse) in the extreme C-terminus.[51]

As described above, the C-terminus is likely to contribute to P53 function by increasing the efficiency with which it finds its specific response elements. Indeed, elegant studies presented at Dunedin by C Prives showed that the C-terminus enables p53 to engage circular DNA molecules, regardless of whether they have a p53 response element. Deletion of the C-terminus reduces association with random DNA, as found earlier in studies of chromatin association.[83] Once P53 engages random DNA, the rate at which it detects specific P53 response elements is increased by linear diffusion.[63] However, neither acetylation nor phosphorylation of the C-terminus affected linear diffusion or target gene transactivation.[63,83]

These *in vitro* observations raise the question of whether C-terminal modifications affect P53 function *in vivo*. Human P53 mutants in which all six highly conserved C-terminal lysine residues were mutated to arginine to prevent post-translational modifications including ubiquitylation and acetylation proved to be stable and more active than wild-type P53.[84,85] However, it is difficult to recreate the normal stoichiometric relationships between P53, Mdm2, and MdmX using cotransfection of P53 with Mdm2 into human cancer cell lines that contain other alterations that could affect p53 function.

I presented data at the Dunedin meeting obtained from mice generated by homologous recombination to encode *p53* with arginine substituted for the seven C-terminal lysines that are analogous to the corresponding residues in human p53 (i.e., $p53^{7KR}$;[86] also see[87] for a study that generated a *p53* allele in which six lys were changed to arg but mice were not produced). We developed this model to determine the impact of C-terminal modifications on P53 regulation. While the *in vitro* data suggested this mutation should be lethal if homozygous, we observed normal Mendelian transmission and no evidence of early mortality, sex bias, or developmental abnormalities.[86] Surprisingly, mouse $P53^{7KR}$ and wild-type P53 exhibit the same half-life, and like wild-type P53, $p53^{7KR}$ is degraded by Mdm2-mediated proteasome-dependent proteolysis. *In vitro* analyses showed that $P53^{7KR}$ can be ubiquitylated by Mdm2 after transfection, but the pattern was not the same as that of wild-type P53. As other studies indicate that lysines other than in the C-terminus may control P53 stability,[88] it is formally possible that alternative lysine(s) are targeted for ubiquitylation when those in the C-terminus are not available. However, as $P53^{7KR}$ has precisely the same half-life as wild-type P53, it seems unlikely that Mdm2 could engage widely separated lysines with equal efficiency. Furthermore, while Mdm2 can polyubiquitylate itself, it can only polyubiquitylate P53 at multiple lysines. This means that if P53 needs to be polyubiquitylated for degradation, this must require the action of an E4 ubiquitin ligase, such as p300.[89] It is difficult to reconcile the identical half-lives of these two proteins with the observation that Mdm2 is sufficient to meditate its own polyubiquitylation, while P53 requires both Mdm2 and an E4. Thus, our data raise the intriguing possibility that P53 degradation may merely require association with Mdm2, and it is the self-ubiquitylation of Mdm2 that drives the degradation of both proteins. However, this model seems simplistic as Mdm2 can also bind to the P53-related proteins P63 and P73, yet it does not mediate their degradation.[90,91] Consequently, there must be other critical steps, such as correct presentation of the substrate to the proteasome, or engagement of linking molecules such as hHR23 to effect substrate degradation.[92,93] Clearly, more research is needed to resolve the precise mechanism by which Mdm2 induces P53 degradation, and to reveal how Mdm2 selectively degrades p53.

We also investigated the activity of $P53^{7KR}$ in cell culture and in thymocytes *in vivo*. $P53^{7KR}$ and wild-type P53 proved to be experimentally indistinguishable in terms of their activation kinetics, ability to induce target genes, cell cycle arrest, and apoptosis in MEFs. However, $P53^{7KR}$ was activated at lower doses of ionizing radiation in mice when freshly explanted thymocytes were analyzed. Importantly, this apparently increased activity of $P53^{7KR}$ was also observed *in vitro* when MEFs were grown according to a 3T3 passage protocol. We observed identical initial growth rates of MEFs expressing wild type and $P53^{7KR}$, but MEFs encoding $P53^{7KR}$ entered a senescent state from which immortal variants did not arise. This contrasts with MEFs encoding wild-type P53 that entered crisis, but emerged as immortal variants. These data suggest that while the conserved C-terminal lysines and associated modifications are not essential for P53 control, they are likely to fine-tune P53 activity, and to ensure that the magnitude of a stress responses is appropriate. This fine-tuning is likely to be critical for optimal lifespan of metazoans to guarantee that cells that undergo numerous divisions, or at risk of stress exposure, do not experience errant P53 activation.

The Proline-Rich Domain (PRD) and Mdm2 Interaction

P53 is exquisitely sensitive to Mdm2 expression levels. For example, mice expressing a hypomorphic *mdm2* allele produced 30–50% of the normal Mdm2 protein level.[94] This resulted in precocious P53 activation in lymphoid tissue and in some epithelial cells. Recent studies showed that *mdm2*+/− heterozygous mice were far more resistant to the development of lymphoid tumors induced by expression of the *Eu-Myc* transgene.[95] Finally, earlier onset and increased cancer predisposition has been reported in premenopausal women in whom a single-nucleotide polymorphism changes a T to a G in the *mdm2* promoter.[96] This base change creates a binding site for the ubiquitous transcription factor Sp1 adjacent to a response element for the estrogen receptor, which apparently together contribute to two- to four-fold elevated Mdm2 levels that attenuate P53 function. Together, these data clearly show that Mdm2 abundance is an important determinant of the output of the P53 stress response pathway.

One way to modulate the impact of Mdm2 on P53 function is to regulate the efficiency with which it binds P53. As stated above, N-terminal phosphorylation may participate in this, but its importance appears more significant in specific cell types. Another region that may affect Mdm2 binding consists of a

loosely conserved PRD between the TAD and the DNA-binding domain. This region contains a variable number of PXXP motifs, where P = proline and X = any amino acid. PXXP motifs provide potential interaction sites with proteins containing src homology 3 motifs, and the PXXP domains of P53 have been reported to interact with proteins including Mdm2,[38,39,97] p300,[98] WWOX1,[99] and the corepressor mSin3a.[100] Early studies showed that deletion of the PRD in human P53 alter P53 transactivation in such a way as to preserve the apoptotic function, while compromising cell cycle arrest.[101] Initial studies suggested that PRD deletion reduced the affinity of P53 for apoptotic target genes,[102,103] but analyses performed at lower, more natural expression levels, revealed reduced regulation of a broader spectrum of target genes.[104]

Recent analyses have provided a potential molecular mechanism for the compromised functionality of P53 lacking the PRD. DNA damage can induce phosphorylation of multiple threonine residues in threonine–proline motifs. This creates sites that can be bound by the prolyl isomerase Pin1, which can then induce *cis–trans* isomerization of the adjacent proline. Prolyl isomerization has been reported to reduce Mdm2 binding, and to stabilize and activate P53.[36,37,39] Deleting the prolines, or mutating the threonine to nonphosphorylatable alanine, prevents the required prolyl isomerization, and P53 damage responses appear to be compromised in Pin1-deficient MEFs. A recent twist on this story was added by Ygal Haupt at the Dunedin meeting, when he reported that phosphorylation of thr81 is required to recruit Pin1, and that Pin1 recruitment is required for efficient association with the damage-activated kinase Chk2.[39] Chk2 recruitment induces phosphorylation of ser20, which then reduces affinity for Mdm2, allowing P53 activation. Importantly, some humans with the Li–Fraumeni cancer predisposition syndrome have been reported to have chk2 mutations,[105,106] while others encode mutant *p53* with proline 82 replaced by leucine (i.e., pro82leu).[107] The latter data suggest the importance of pro82 as an important contributor to P53 function *in vivo.* Consistent with this interpretation, Haupt described transfection studies showing that P53 Pro82Leu accumulated less efficiently after ionizing radiation, and exhibited reduced ability to activate a p21 promoter-driven luciferase reporter.[39] These data suggest that the PRD may be important for regulating P53 output by fine-tuning Mdm2 binding. One note of caution: earlier studies by Dumaz and Meek showed that the impact of deleting the PRD on P53 activation was critically dependent on the ratio of Mdm2 to P53.[97] Therefore, firm assessment of the contribution of the PRD to P53 control will await the generation and analysis of mice expressing *p53* with a PRD deletion, and others in which the prolines and threonines have been mutated to rigorously evaluate the validity of the conformational hypothesis advanced above.

New Thoughts on an Old Dogma

Over 36 000 papers addressing p53 function have been published since its discovery 25 years ago. By contrast, a fraction of this number has dealt with Mdm2 and MdmX, which partly reflects their more recent discoveries.[19,57,108,109] However, Mdm2 and MdmX are clearly critical negative regulators of P53, and their functions are nonoverlapping since deletion of either engenders early embryonic lethality.[110–114] This raises the possibility that important aspects of P53 control remain to be elucidated due to the paucity of attention focused on Mdm2 and MdmX and proteins that control their functionality, such as Arf,[115,116] gankyrin,[117,118] and the ubiquitin-specific protease HAUSP (herpes virus-associated ubiquitin-specific protease) (Li *et al.*,[119,120] and Meulmeester *et al.*[121]; and see below). Another puzzle is why this system requires the two similar RING domain proteins Mdm2 and MdmX to each serve as a P53-negative regulator. Below, I present a model I proposed at the Dunedin meeting in which I divided p53 regulation into four phases, and accounts for the kinetic relationships between p53, Mdm2, and MdmX we had observed at that time. I will review recent unpublished and published data relevant to this model (Figure 2).

Phase 1: Homeostasis in unstressed cells

P53 is maintained at low levels and in an inactive state in unstressed cells. This can be achieved in several ways. First, Mdm2 can bind to P53 to mediate proteasomal degradation.[122,123] Second, Mdm2 can bind to the P53 TAD to prevent recruitment of coactivators that access the same site.[124] Third, as described by Moshe Oren[125] in Dunedin, Mdm2 can monoubiquitinate the histones located in the vicinity of the P53 response element when a P53–Mdm2 complex engages chromatin, and Mdm2 can also monoubiquitinate histones in a P53-independent manner when it is overexpressed. Less appreciated is the contribution of MdmX to attenuating P53 function in unstressed cells. This could be substantial as MdmX is produced constitutively and is very stable (see Marine and Jochemsen[108,109] for reviews). The molecular abundance of Mdm2 relative to MdmX in nonstressed and stressed cells remains to be determined. Thus, P53 is held at bay in unstressed cells by interactions with the two negative regulators, Mdm2 and MdmX.

What then controls the levels of Mdm2 and MdmX? The Mdm2 level in unstressed cells is determined by a number of factors including, but not limited to the following: (1) P53-dependent transactivation of the Mdm2 gene (see Lahav *et al.*[125] for recent kinetic modeling); (2) mitogen-dependent activation of factors such as Erk that also transactivate Mdm2;[126] (3) mitogen-dependent post-translational modifications that modulate Mdm2 stability (e.g., see Ashcroft *et al.*[127] and Gottlieb *et al.*[128]); and (4) interaction with HAUSP.[119–121,129] The factors that regulate MdmX abundance have not been widely studied, but one that appears increasingly important involves interaction with HAUSP.[121] HAUSP was first identified to participate in P53 control by Gu and coworkers as a P53-associated protein.[120] Inactivating HAUSP by siRNA or by homologous recombination in HCT116 cells resulted in Mdm2 and MdmX destabilization.[120,121,130] The importance of HAUSP in regulating MdmX level is demonstrated by the lack of detectable MdmX in HAUSP-deficient cells.[121] As eliminating HAUSP greatly destabilized both Mdm2 and MdmX, P53 became stabilized and activated.[130] These observations imply that HAUSP interaction with, and deubiquitination of, Mdm2 and MdmX contributes significantly

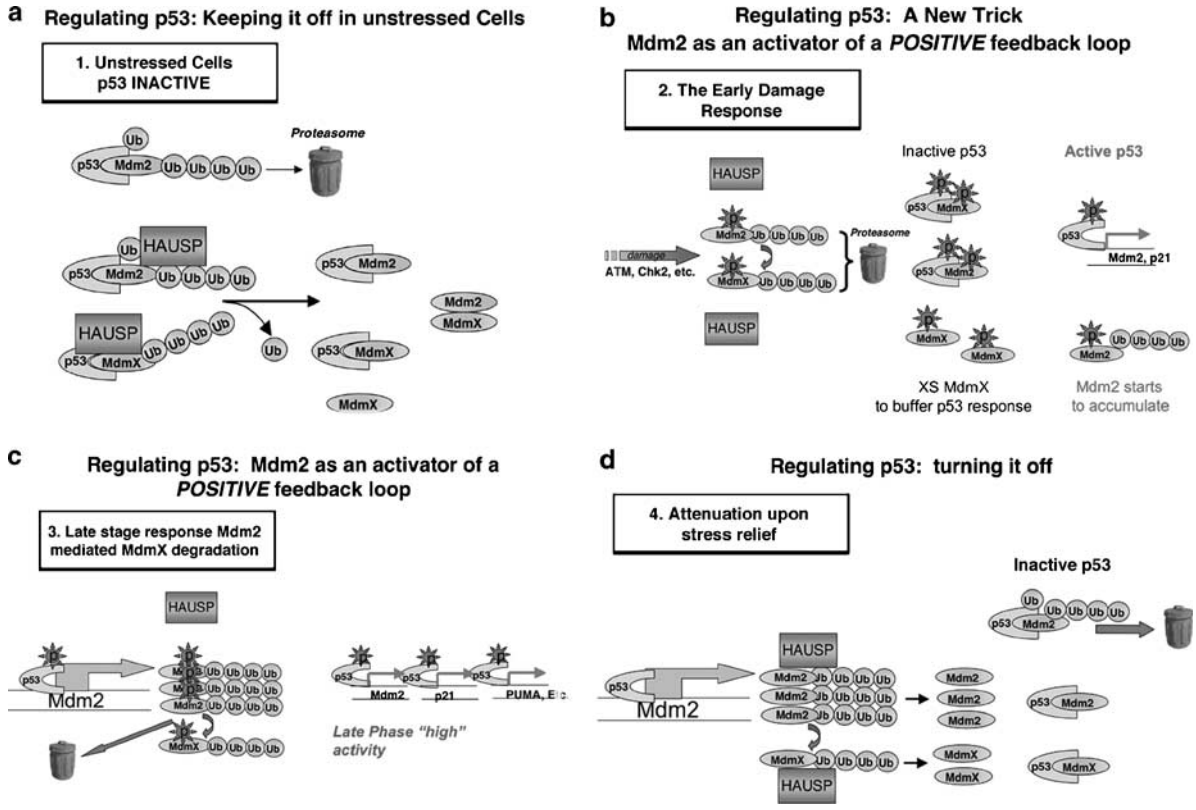

Figure 2 Models for attenuating and destabilizing p53, and for activating and stabilizing it in response to stress. P53 regulation is presented in four phases as described in detail in the text. It is important to note that the relative numbers of molecules of P53, Mdm2, MdmX, and HAUSP are not known. Neither is the extent to which Mdm2 and MdmX exist as homo- and heterodimers, and whether this changes as Mdm2 and MdmX levels increase or decline during a stress response. *Indicate damage-kinase phosphorylations that prevent interaction with HAUSP. See the text for a detailed explanation of the model and additional limitations

to their steady-state levels in unstressed cells. As MdmX has a longer half-life than either Mdm2 or P53, it is reasonable to speculate that MdmX may be the preferred substrate for HAUSP-mediated deubiquitination.

To summarize regulation of Phase 1: Mdm2 accumulates as a consequence of transcriptional activation by P53 and mitogen-activated factors, as well as by 'stabilization' through HAUSP interaction. While Mdm2 can ubiquitinate MdmX, MdmX is deubiquitinated and stabilized via interaction with HAUSP. The aggregate levels of Mdm2 and MdmX determine both P53 abundance and transcriptional activity. Mdm2-mediated ubiquitination of histones may provide an additional means of controlling P53 transcription functions.

Phase 2: Early activation

DNA damage leads to the activation of ATM and other kinases within minutes, as well as phosphorylation of N-terminal serines that may modulate association with Mdm2/X and coactivators. However, of equal importance, these kinases also rapidly phosphorylate Mdm2 and MdmX.[131–134] We showed that phosphorylation of MDM2 by damage kinases mediates its accelerated degradation early in the damage response.[135] Studies from the Yuan and Chen labs showed that DNA damage also leads to MdmX degradation,[132,136] and we now know this requires the RING and acidic domains and ubiquitin ligase activity of Mdm2.[131,137–139]

A picture of what triggers the accelerated degradation of Mdm2 early in the damage response, and what converts MdmX from a stable to an unstable protein is now emerging. Recent work from the Shiloh and Jochemsen labs show that the damage-induced phosphorylations on Mdm2 and MdmX destabilize both proteins by preventing them from interacting with HAUSP.[121,134] Another study provided a somewhat different explanation that phosphorylation increased association between Mdm2 and MdmX.[140] Regardless of which mechanism is correct, the data taken together imply that DNA damage triggers accelerated degradation of both negative regulators. However, early in the DNA damage response in normal fibroblasts and epithelial cells, it appears that MdmX levels do not decline immediately, and that P53 target genes are activated weakly. Indeed, full activation takes between 1 and 2 h.[135] The next section provides one explanation of this delay.

Phase 3. Full activation

It is clear that full P53 activation occurs only after a lag of about 2 h.[135] Damage kinases are active over this time, P53 remains phosphorylated at h-ser15, and presumably Mdm2 and Mdmx are also phosphorylated over this interval. One of the first genes activated by P53 is Mdm2, but early in the damage response, the Mdm2 that is made is unstable, likely because it is phosphorylated and cannot interact with HAUSP. Mdm2

does accumulate over time, however, and this correlates with progressively decreasing MdmX levels. This raises the possibility that P53 activates Mdm2 so that its increased abundance ensures MdmX degradation. This model predicts that conditions that lead to P53 activation in the absence of activation of damage kinases should also lead to MdmX degradation due to the elevated Mdm2 abundance. As shown by Lubo Vassilev at the Dunedin meeting, P53 can be fully activated by Nutlin3a, which prevents Mdm2 from binding to P53.[141] Indeed, we found that Nutlin3a results in substantial increases in Mdm2 abundance, and that MdmX levels decline in parallel (J Stommel, M Wade, M Tang, and G Wahl, unpublished data). Importantly, we also showed that proteasome inhibitors prevent P53 activation after DNA damage, even though P53 was phosphorylated in its N-terminus.[135] By contrast, Nutlin addition during proteasome inhibitor treatment enabled P53 activation by reducing Mdm2 (and presumably)–MdmX interaction with P53.[135] Together, the data indicate that P53 activation requires Mdm2 and Mdmx phosphorylation to decrease HAUSP interaction with both proteins, the net effect of which is to increase Mdm2-mediated self-ubiquitylation and ubiquitylation of MdmX. This decreases the levels of Mdm2 at early time points and as a consequence activates P53, resulting in increased Mdm2 transactivation. The increased levels of Hdm2 can then degrade MdmX, resulting in full activation of P53. In this model, P53 activation requires a *positive feedback loop* in which increasing Mdm2 abundance titrates the amount of MdmX degradation to assure the fine-tuning of the timing and magnitude of the P53 transcriptional response.

Phase 4: Attenuation

The components of this system also provide the potential to attenuate P53 signaling should a stress dissipate or DNA damage be repaired. I suggest this could occur in the following way. ATM and P53 ser15 phosphorylation return to background levels within 4–6 h of induction of DNA damage with a low dose of the radiomimetic agent neocarzinostatin.[135] In normal human fibroblasts, Mdm2 levels are high at 4 h, and start to decline thereafter, along with a parallel decrease in P53 transactivation. In light of the data summarized above, it is reasonable to propose that the damage-phosphorylated Mdm2 pool is replaced with a nonphosphorylated pool as a consequence of new synthesis and diminished abundance of activated ATM. The elevated levels of Mdm2 should be able to interact with and inactivate P53 by the mechanisms described above. Furthermore, in the absence of activated damage kinases, MdmX will be allowed to interact with HAUSP, leading to deubiquitination and stabilization. This provides a second barrier to continued P53 activation.

Perspectives

The integration of recent data described above provides a new way of conceptualizing P53 regulation. Homeostasis is maintained by the well-known negative feedback loop. A new twist to the loop is provided by the idea that the activation of Mdm2 during a stress response actually creates a positive feedback loop in which Mdm2 mediates MdmX degradation to achieve full P53 activation. This elegant system enables gradual and tightly controlled increases in P53 activity rather than an all-or-none response that might be created were the system solely controlled solely by phosphorylation. Finally, the reservoir of Mdm2 created by P53-mediated activation enables attenuation of the P53 response since most of the MdmX is eliminated in the activation phase.

This model accounts for most of the major regulators known to operate in this system, and it is consistent with the kinetics of the response in the normal fibroblasts and epithelial cells we have analyzed. It is clear that the system is very sensitive to small changes in the stoichiometric relationships between HAUSP, Mdm2, MdmX, and P53, and it is likely that proteins that regulate Mdm2 function, such as Arf or Gankyrin, may enable finer tuning of the system, or be required for responses to stressors other than DNA damage. Clearly, an important goal for the near future will be to determine the precise numbers of each of these molecules in normal and neoplastic cells, and to determine how differences in stoichiometry affect the dynamics and magnitude of stress-activated responses. We have begun a cursory analysis of cell lines with wild-type P53, and have found substantial variations in the relative abundance of each molecule. This may well explain the attenuation of the P53 response in the numerous cancers expressing wild-type P53.

An important unresolved question concerns the molecular mechanism that switches Mdm2 target specificity from P53 to MdmX. Clearly, one component of the mechanism is determined by whether HAUSP interacts with and deubiquitinates Mdm2 and MdmX. However, as described above, the binding of Mdm2 to P53 may be all that is needed for P53 to be degraded. As N-terminal phosphorylation of P53 seems unlikely to prevent Mdm2 from binding to P53, we need to determine whether P53 stabilization involves preventing Mdm2 from interacting with P53, or whether additional factors are involved. As one example, perhaps proteins that connect Mdm2 to the proteasome, such as hHR23, comprise part of the switch.[92,93]

The excitement in this field has grown with the successful isolation of a variety of compounds such as the Nutlins that can activate wild-type P53 in some tumor lines. What we now need to determine is whether the different expression patterns of the key proteins that control P53 function will impact on the utility of these agents, or if the molecular signatures of cancer cells can be used to define subsets of patients most likely to respond to P53 activator therapies. Of significance, as MdmX has emerged to play an increasingly important role in P53 activation, the optimal drugs for p53 activation clearly need to target both Mdm2 and MdmX to prevent both from interacting with and inhibiting P53.

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at the problem very differently. The work of Crystal Lee and Franck Toledo, who have also left recently, pointed out the importance of MdmX as a critical negative regulator of P53 transactivation in an *in vivo* setting. Finally, current members Kurt Krummel, Mark Wade, Vivian Wang, Ee-Tsin Wong, Leo Li, Stephanie Kinkel, and Mengjia Tang either provided data or suggestions that improved or clarified the manuscript. This work has been supported by grants from the NCI (CA61449, CA48405) to GMW, MT, KK, and MW.

References

1. Kress M, May E, Cassingena R and May P (1979) Simian virus 40-transformed cells express new species of proteins precipitable by anti-Simian virus 40 tumor serum. J. Virol. 31: 472–483.
2. Melero JA, Stitt DT, Mangel WF and Carroll RB (1979) Identification of new polypeptide species (48–55K) immunoprecipitable by antiserum to purified large T antigen and present in SV40-infected and -transformed cells. Virology 93: 466–480.
3. DeLeo AB, Jay G, Appella E, Dubois GC, Law LW and Old LJ (1979) Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc. Natl. Acad. Sci. USA 76: 2420–2424.
4. Lane DP and Crawford LV (1979) T antigen is bound to a host protein in SV40-transformed cells. Nature 278: 261–263.
5. Linzer DI and Levine AJ (1979) Characterization of a 54K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell 17: 43–52.
6. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D and Jacks T (2004) Mutant p53 gain of function in two mouse models of Li–Fraumeni syndrome. Cell 119: 847–860.
7. Hollstein M, Sidransky D, Vogelstein B and Harris CC (1991) p53 mutations in human cancers. Science 253: 49–53.
8. Malkin D, Li FP, Strong LC, Fraumeni Jr JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA and Friend SH (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250: 1233–1238.
9. Srivastava S, Zou ZQ, Pirollo K, Blattner W and Chang EH (1990) Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li–Fraumeni syndrome. Nature 348: 747–749.
10. Danovi D, Meulmeester E, Pasini D, Migliorini D, Capra M, Frenk R, de Graaf P, Francoz S, Gasparini P, Gobbi A, Helin K, Pelicci PG, Jochemsen AG and Marine JC (2004) Amplification of Mdmx (or Mdm4) directly contributes to tumor formation by inhibiting p53 tumor suppressor activity. Mol. Cell. Biol. 24: 5835–5843.
11. Momand J, Jung D, Wilczynski S and Niland J (1998) The MDM2 gene amplification database. Nucleic Acids Res. 26: 3453–3459.
12. Reifenberger G, Liu L, Ichimura K, Schmidt EE and Collins VP (1993) Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res. 53: 2736–2739.
13. Riemenschneider MJ, Buschges R, Wolter M, Reifenberger J, Bostrom J, Kraus JA, Schlegel U and Reifenberger G (1999) Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification. Cancer Res. 59: 6091–6096.
14. Hoh J, Jin S, Parrado T, Edington J, Levine AJ and Ott J (2002) The p53MH algorithm and its application in detecting p53-responsive genes. Proc. Natl. Acad. Sci. USA 99: 8467–8472.
15. Vogelstein B, Lane D and Levine AJ (2000) Surfing the p53 network. Nature 408: 307–310.
16. Caelles C, Helmberg A and Karin M (1994) p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature 370: 220–223.
17. Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M and Green DR (2004) Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 303: 1010–1014.
18. Marchenko ND, Zaika A and Moll UM (2000) Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J. Biol. Chem. 275: 16202–16212.
19. Wahl GM, Stommel JM, Krummel KA and Wade M (2005) Gatekeepers of the guardian: p53 regulation by post-translational modification, mdm2 and mdmx. In *25 Years of p53 Research*, Wiman K, Hainaut P (eds) (Netherlands: Springer) pp. 73–113.
20. Huang L-C, Clarkin KC and Wahl GM (1996) Sensitivity and selectivity of the p53-mediated DNA damage sensor. Proc. Natl. Acad. Sci. USA 93: 4827–4832.
21. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B and Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51 (Part 1): 6304–6311.
22. Wahl GM and Carr AM (2001) The evolution of diverse biological responses to DNA damage: insights from yeast and p53. Nat. Cell Biol. 3: E277–E286.
23. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, Greider CW and DePinho RA (1999) p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 97: 527–538.
24. Karlseder J, Broccoli D, Dai Y, Hardy S and de Lange T (1999) p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 283: 1321–1325.
25. Denko NC, Giaccia AJ, Stringer JR and Stambrook PJ (1994) The human Ha-ras oncogene induces genomic instability in murine fibroblasts within one cell cycle. Proc. Natl. Acad. Sci. USA 91: 5124–5128.
26. Felsher DW and Bishop JM (1999) Transient excess of MYC activity can elicit genomic instability and tumorigenesis. Proc. Natl. Acad. Sci. USA 96: 3940–3944.
27. Sherr CJ (2001) The INK4a/ARF network in tumour suppression. Nat. Rev. Mol. Cell. Biol. 2: 731–737.
28. Vafa O, Wade M, Kern S, Beeche M, Pandita TK, Hampton GM and Wahl GM (2002) c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. Mol. Cell 9: 1031–1044.
29. Alarcon R, Koumenis C, Geyer RK, Maki CG and Giaccia AJ (1999) Hypoxia induces p53 accumulation through MDM2 down-regulation and inhibition of E6-mediated degradation. Cancer Res. 59: 6046–6051.
30. Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ and Thompson CB (2005) AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol. Cell 18: 283–293.
31. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T and Tlsty TD (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70: 923–935.
32. Yin Y, Tainsky MA, Bischoff FZ, Strong LC and Wahl GM (1992) Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell 70: 937–948.
33. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N and Liu EA (2004) *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. Science 303: 844–848.
34. Lin J, Chen J, Elenbaas B and Levine AJ (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.
35. Zhu J, Zhou W, Jiang J and Chen X (1998) Identification of a novel p53 functional domain that is necessary for mediating apoptosis. J. Biol. Chem. 273: 13030–13036.
36. Zacchi P, Gostissa M, Uchida T, Salvagno C, Avolio F, Volinia S, Ronai Z, Blandino G, Schneider C and Del Sal G (2002) The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insults. Nature 419: 853–857.
37. Zheng H, You H, Zhou XZ, Murray SA, Uchida T, Wulf G, Gu L, Tang X, Lu KP and Xiao ZX (2002) The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. Nature 419: 849–853.
38. Berger M, Vogt Sionov R, Levine AJ and Haupt Y (2001) A role for the polyproline domain of p53 in its regulation by Mdm2. J. Biol. Chem. 276: 3785–3790.
39. Berger M, Stahl N, Del Sal G and Haupt Y (2005) Mutations in proline 82 of p53 impair its activation by Pin1 and Chk2 in response to DNA damage. Mol. Cell. Biol. 25: 5380–5388.

40. Jeffrey PD, Gorina S and Pavletich NP (1995) Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. Science 267: 1498–1502.
41. Arrowsmith CH and Morin P (1996) New insights into p53 function from structural studies. Oncogene 12: 1379–1385.
42. Clore GM, Ernst J, Clubb R, Omichinski JG, Kennedy WM, Sakaguchi K, Appella E and Gronenborn AM (1995) Refined solution structure of the oligomerization domain of the tumour suppressor p53. Nat. Struct. Biol. 2: 321–333.
43. Miller M, Lubkowski J, Rao JK, Danishefsky AT, Omichinski JG, Sakaguchi K, Sakamoto H, Appella E, Gronenborn AM and Clore GM (1996) The oligomerization domain of p53: crystal structure of the trigonal form. FEBS Lett. 399: 166–170.
44. Maki CG (1999) Oligomerization is required for p53 to be efficiently ubiquitinated by MDM2. J. Biol. Chem. 274: 16531–16535.
45. Clore GM, Omichinski JG, Sakaguchi K, Zambrano N, Sakamoto H, Appella E and Gronenborn AM (1994) High-resolution structure of the oligomerization domain of p53 by multidimensional NMR [see comments] [published erratum appears in *Science* 1995 Mar 10;**267**(5203):1515]. Science 265: 386–391.
46. Wang P, Reed M, Wang Y, Mayr G, Stenger JE, Anderson ME, Schwedes JF and Tegtmeyer P (1994) p53 domains: structure, oligomerization, and transformation. Mol. Cell. Biol. 14: 5182–5191.
47. McLure KG and Lee PW (1998) How p53 binds DNA as a tetramer. EMBO J. 17: 3342–3350.
48. Stommel JM, Marchenko ND, Jimenez GS, Moll UM, Hope TJ and Wahl GM (1999) A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. EMBO J. 18: 1660–1672.
49. Gu W and Roeder RG (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 90: 595–606.
50. Gu W, Shi XL and Roeder RG (1997) Synergistic activation of transcription by CBP and p53. Nature 387: 819–823.
51. Li M, Luo J, Brooks CL and Gu W (2002) Acetylation of p53 inhibits its ubiquitination by Mdm2. J. Biol. Chem. 277: 50607–50611.
52. Prives C and Manley JL (2001) Why is p53 acetylated? Cell 107: 815–818.
53. An W, Kim J and Roeder RG (2004) Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. Cell 117: 735–748.
54. Chuikov S, Kurash JK, Wilson JR, Xiao B, Justin N, Ivanov GS, McKinney K, Tempst P, Prives C, Gamblin SJ, Barlev NA and Reinberg D (2004) Regulation of p53 activity through lysine methylation. Nature 432: 353–360.
55. Gostissa M, Hengstermann A, Fogal V, Sandy P, Schwarz SE, Scheffner M and Del Sal G (1999) Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1. EMBO J. 18: 6462–6471.
56. Rodriguez MS, Desterro JM, Lain S, Midgley CA, Lane DP and Hay RT (1999) SUMO-1 modification activates the transcriptional response of p53. EMBO J. 18: 6455–6461.
57. Michael D and Oren M (2003) The p53–Mdm2 module and the ubiquitin system. Semin. Cancer Biol. 13: 49–58.
58. Xirodimas DP, Saville MK, Bourdon JC, Hay RT and Lane DP (2004) Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. Cell 118: 83–97.
59. Hupp TR and Lane DP (1994) Allosteric activation of latent p53 tetramers. Curr. Biol. 4: 865–875.
60. Hupp TR, Meek DW, Midgley CA and Lane DP (1993) Activation of the cryptic DNA binding function of mutant forms of p53. Nucleic Acids Res. 21: 3167–3174.
61. Waterman JL, Shenk JL and Halazonetis TD (1995) The dihedral symmetry of the p53 tetramerization domain mandates a conformational switch upon DNA binding. EMBO J. 14: 512–519.
62. Ahn J and Prives C (2001) The C-terminus of p53: the more you learn the less you know. Nat. Struct. Biol. 8: 730–732.
63. McKinney K, Mattia M, Gottifredi V and Prives C (2004) p53 linear diffusion along DNA requires its C terminus. Mol. Cell 16: 413–424.
64. Sakaguchi K, Saito S, Higashimoto Y, Roy S, Anderson CW and Appella E (2000) Damage-mediated phosphorylation of human p53 threonine 18 through a cascade mediated by a casein 1-like kinase. Effect on Mdm2 binding. J. Biol. Chem. 275: 9278–9283.
65. Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ and Pavletich NP (1996) Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science 274: 948–953.
66. Schon O, Friedler A, Bycroft M, Freund SM and Fersht AR (2002) Molecular mechanism of the interaction between MDM2 and p53. J. Mol. Biol. 323: 491–501.
67. Unger T, Juven-Gershon T, Moallem E, Berger M, Vogt Sionov R, Lozano G, Oren M and Haupt Y (1999) Critical role for Ser20 of human p53 in the negative regulation of p53 by Mdm2. EMBO J. 18: 1805–1814.
68. Dumaz N and Meek DW (1999) Serine15 phosphorylation stimulates p53 transactivation but does not directly influence interaction with HDM2. EMBO J. 18: 7002–7010.
69. Lambert PF, Kashanchi F, Radonovich MF, Shiekhattar R and Brady JN (1998) Phosphorylation of p53 serine 15 increases interaction with CBP. J. Biol. Chem. 273: 33048–33053.
70. Borges HL, Chao C, Xu Y, Linden R and Wang JY (2004) Radiation-induced apoptosis in developing mouse retina exhibits dose-dependent requirement for ATM phosphorylation of p53. Cell Death Differ. 11: 494–502.
71. Chao C, Hergenhahn M, Kaeser MD, Wu Z, Saito S, Iggo R, Hollstein M, Appella E and Xu Y (2003) Cell type- and promoter-specific roles of Ser18 phosphorylation in regulating p53 responses. J. Biol. Chem. 278: 41028–41033.
72. Sluss HK, Armata H, Gallant J and Jones SN (2004) Phosphorylation of serine 18 regulates distinct p53 functions in mice. Mol. Cell. Biol. 24: 976–984.
73. MacPherson D, Kim J, Kim T, Rhee BK, Van Oostrom CT, DiTullio RA, Venere M, Halazonetis TD, Bronson R, De Vries A, Fleming M and Jacks T (2004) Defective apoptosis and B-cell lymphomas in mice with p53 point mutation at Ser 23. EMBO J. 23: 3689–3699.
74. Wu Z, Earle J, Saito S, Anderson CW, Appella E and Xu Y (2002) Mutation of mouse p53 Ser23 and the response to DNA damage. Mol. Cell. Biol. 22: 2441–2449.
75. Brodsky MH, Weinert BT, Tsang G, Rong YS, McGinnis NM, Golic KG, Rio DC and Rubin GM (2004) *Drosophila melanogaster* MNK/Chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. Mol. Cell. Biol. 24: 1219–1231.
76. Peters M, DeLuca C, Hirao A, Stambolic V, Potter J, Zhou L, Liepa J, Snow B, Arya S, Wong J, Bouchard D, Binari R, Manoukian AS and Mak TW (2002) Chk2 regulates irradiation-induced, p53-mediated apoptosis in *Drosophila*. Proc. Natl. Acad. Sci. USA 99: 11305–11310.
77. Harms KL and Chen X (2005) The C terminus of p53 family proteins is a cell fate determinant. Mol. Cell. Biol. 25: 2014–2030.
78. Chao C, Saito S, Kang J, Anderson CW, Appella E and Xu Y (2000) p53 transcriptional activity is essential for p53-dependent apoptosis following DNA damage. EMBO J. 19: 4967–4975.
79. Jimenez GS, Nister M, Stommel JM, Beeche M, Barcarse EA, Zhang XQ, O'Gorman S and Wahl GM (2000) A transactivation-deficient mouse model provides insights into Trp53 regulation and function. Nat. Genet. 26: 37–43.
80. Johnson TM, Hammond EM, Giaccia A and Attardi LD (2005) The p53QS transactivation-deficient mutant shows stress-specific apoptotic activity and induces embryonic lethality. Nat. Genet. 37: 145–152.
81. Nister M, Tang M, Zhang XQ, Yin C, Beeche M, Hu X, Enblad G, van Dyke T and Wahl GM (2005) p53 must be competent for transcriptional regulation to suppress tumor formation. Oncogene 24: 3563–3573.
82. Tang M, Wahl GM and Nister M (2006) Explaining the biological activity of transactivation-deficient p53 variants. Nat. Genet. 38: 395–396.
83. Espinosa JM and Emerson BM (2001) Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. Mol. Cell 8: 57–69.
84. Nakamura S, Roth JA and Mukhopadhyay T (2000) Multiple lysine mutations in the C-terminal domain of p53 interfere with MDM2-dependent protein degradation and ubiquitination. Mol. Cell. Biol. 20: 9391–9398.
85. Rodriguez MS, Desterro JM, Lain S, Lane DP and Hay RT (2000) Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. Mol. Cell. Biol. 20: 8458–8467.
86. Krummel KA, Toledo F, Lee CJ and Wahl GM (2005) The C-terminal lysines fine-tune p53 stress responses in a mouse model, but are not required for stability control or transactivation. Proc. Natl. Acad. Sci. USA 102: 10188–10193.

87. Feng L, Lin T, Uranishi H, Gu W and Xu Y (2005) Functional analysis of the roles of posttranslational modifications at the p53 C terminus in regulating p53 stability and activity. Mol. Cell. Biol. 25: 5389–5395.
88. Gu J, Nie L, Wiederschain D and Yuan ZM (2001) Identification of p53 sequence elements that are required for MDM2-mediated nuclear export. Mol. Cell. Biol. 21: 8533–8546.
89. Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y and Livingston DM (2003) Polyubiquitination of p53 by a ubiquitin ligase activity of p300. Science 300: 342–344.
90. Balint E, Bates S and Vousden KH (1999) Mdm2 binds p73 alpha without targeting degradation. Oncogene 18: 3923–3929.
91. Zeng X, Chen L, Jost CA, Maya R, Keller D, Wang X, Kaelin Jr WG, Oren M, Chen J and Lu H (1999) MDM2 suppresses p73 function without promoting p73 degradation. Mol. Cell. Biol. 19: 3257–3266.
92. Brignone C, Bradley KE, Kisselev AF and Grossman SR (2004) A post-ubiquitination role for MDM2 and hHR23A in the p53 degradation pathway. Oncogene 23: 4121–4129.
93. Glockzin S, Ogi FX, Hengstermann A, Scheffner M and Blattner C (2003) Involvement of the DNA repair protein hHR23 in p53 degradation. Mol. Cell. Biol. 23: 8960–8969.
94. Mendrysa SM, McElwee MK, Michalowski J, O'Leary KA, Young KM and Perry ME (2003) Mdm2 is critical for inhibition of p53 during lymphopoiesis and the response to ionizing irradiation. Mol. Cell. Biol. 23: 462–472.
95. Alt JR, Greiner TC, Cleveland JL and Eischen CM (2003) Mdm2 haplo-insufficiency profoundly inhibits Myc-induced lymphomagenesis. EMBO J. 22: 1442–1450.
96. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G and Levine AJ (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591–602.
97. Dumaz N, Milne DM, Jardine LJ and Meek DW (2001) Critical roles for the serine 20, but not the serine 15, phosphorylation site and for the polyproline domain in regulating p53 turnover. Biochem. J. 359 (Part 2): 459–464.
98. Liu G, Xia T and Chen X (2003) The activation domains, the proline-rich domain, and the C-terminal basic domain in p53 are necessary for acetylation of histones on the proximal p21 promoter and interaction with p300/CBP. J. Biol. Chem. 278: 17557–17565.
99. Chang NS, Pratt N, Heath J, Schultz L, Sleve D, Carey GB and Zevotek N (2001) Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity. J. Biol. Chem. 276: 3361–3370.
100. Zilfou JT, Hoffman WH, Sank M, George DL and Murphy M (2001) The corepressor mSin3a interacts with the proline-rich domain of p53 and protects p53 from proteasome-mediated degradation. Mol. Cell. Biol. 21: 3974–3985.
101. Walker KK and Levine AJ (1996) Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc. Natl. Acad. Sci. USA 93: 15335–15340.
102. Sionov RV and Haupt Y (1999) The cellular response to p53: the decision between life and death. Oncogene 18: 6145–6157.
103. Venot C, Maratrat M, Dureuil C, Conseiller E, Bracco L and Debussche L (1998) The requirement for the p53 proline-rich functional domain for mediation of apoptosis is correlated with specific PIG3 gene transactivation and with transcriptional repression. EMBO J. 17: 4668–4679.
104. Zhu J, Jiang J, Zhou W, Zhu K and Chen X (1999) Differential regulation of cellular target genes by p53 devoid of the PXXP motifs with impaired apoptotic activity. Oncogene 18: 2149–2155.
105. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE and Haber DA (1999) Heterozygous germ line hCHK2 mutations in Li–Fraumeni syndrome. Science 286: 2528–2531.
106. Takai H, Naka K, Okada Y, Watanabe M, Harada N, Saito S, Anderson CW, Appella E, Nakanishi M, Suzuki H, Nagashima K, Sawa H, Ikeda K and Motoyama N (2002) Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. EMBO J. 21: 5195–5205.
107. Sun XF, Johannsson O, Hakansson S, Sellberg G, Nordenskjold B, Olsson H and Borg A (1996) A novel p53 germline alteration identified in a late onset breast cancer kindred. Oncogene 13: 407–411.
108. Marine JC and Jochemsen AG (2004) Mdmx and Mdm2: brothers in arms? Cell Cycle 3: 900–904.
109. Marine JC and Jochemsen AG (2005) Mdmx as an essential regulator of p53 activity. Biochem. Biophys. Res. Commun. 331: 750–760.
110. Jones SN, Roe AE, Donehower LA and Bradley A (1995) Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. Nature 378: 206–208.
111. Montes de Oca Luna R, Wagner DS and Lozano G (1995) Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. Nature 378: 203–206.
112. Parant J, Chavez-Reyes A, Little NA, Yan W, Reinke V, Jochemsen AG and Lozano G (2001) Rescue of embryonic lethality in Mdm4-null mice by loss of Trp53 suggests a nonoverlapping pathway with MDM2 to regulate p53. Nat. Genet. 29: 92–95.
113. Finch RA, Donoviel DB, Potter D, Shi M, Fan A, Freed DD, Wang CY, Zambrowicz BP, Ramirez-Solis R, Sands AT and Zhang N (2002) mdmx is a negative regulator of p53 activity *in vivo*. Cancer Res. 62: 3221–3225.
114. Migliorini D, Denchi EL, Danovi D, Jochemsen A, Capillo M, Gobbi A, Helin K, Pelicci PG and Marine JC (2002) Mdm4 (Mdmx) regulates p53-induced growth arrest and neuronal cell death during early embryonic mouse development. Mol. Cell. Biol. 22: 5527–5538.
115. Pomerantz J, Schreiber-Agus N, Liegeois NJ, Silverman A, Alland L, Chin L, Potes J, Chen K, Orlow I, Lee HW, Cordon-Cardo C and DePinho RA (1998) The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. Cell 92: 713–723.
116. Xirodimas D, Saville MK, Edling C, Lane DP and Lain S (2001) Different effects of p14ARF on the levels of ubiquitinated p53 and Mdm2 *in vivo*. Oncogene 20: 4972–4983.
117. Higashitsuji H, Higashitsuji H, Itoh K, Sakurai T, Nagao T, Sumitomo Y, Masuda T, Dawson S, Shimada Y, Mayer RJ and Fujita J (2005) The oncoprotein gankyrin binds to MDM2/HDM2, enhancing ubiquitylation and degradation of p53. Cancer Cell 8: 75–87.
118. Higashitsuji H, Liu Y, Mayer RJ and Fujita J (2005) The oncoprotein gankyrin negatively regulates both p53 and RB by enhancing proteasomal degradation. Cell Cycle 4: 1335–1337.
119. Li M, Brooks CL, Kon N and Gu W (2004) A dynamic role of HAUSP in the p53–Mdm2 pathway. Mol. Cell 13: 879–886.
120. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J and Gu W (2002) Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature 416: 648–653.
121. Meulmeester E, Maurice MM, Boutell C, Teunisse AF, Ovaa H, Abraham TE, Dirks RW and Jochemsen AG (2005) Loss of HAUSP-mediated deubiquitination contributes to DNA damage-induced destabilization of Hdmx and Hdm2. Mol. Cell 18: 565–576.
122. Haupt Y, Maya R, Kazaz A and Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387: 296–299.
123. Kubbutat MH, Jones SN and Vousden KH (1997) Regulation of p53 stability by Mdm2. Nature 387: 299–303.
124. Momand J, Zambetti GP, Olson DC, George D and Levine AJ (1992) The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 69: 1237–1245.
125. Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB and Alon U (2004) Dynamics of the p53–Mdm2 feedback loop in individual cells. Nat. Genet 36: 147–150.
126. Ries S, Biederer C, Woods D, Shifman O, Shirasawa S, Sasazuki T, McMahon M, Oren M and McCormick F (2000) Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. Cell 103: 321–330.
127. Ashcroft M, Ludwig RL, Woods DB, Copeland TD, Weber HO, MacRae EJ and Vousden KH (2002) Phosphorylation of HDM2 by Akt. Oncogene 21: 1955–1962.
128. Gottlieb TM, Leal JF, Seger R, Taya Y and Oren M (2002) Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis. Oncogene 21: 1299–1303.
129. Wood SA (2002) Dubble or nothing? Is HAUSP deubiquitylating enzyme the final arbiter of p53 levels? Sci. STKE 2002 (143): pe34.
130. Cummings JM, Rago C, Kohli M, Kinzler KW, Lengauer C and Vogelstein B (2004) Tumour suppression: disruption of HAUSP gene stabilizes p53. Nature 428: 486–487.

131. de Graaf P, Little NA, Ramos YF, Meulmeester E, Letteboer SJ and Jochemsen AG (2003) Hdmx protein stability is regulated by the ubiquitin ligase activity of Mdm2. J. Biol. Chem. 278: 38315–38324.
132. Kawai H, Wiederschain D, Kitao H, Stuart J, Tsai KK and Yuan ZM (2003) DNA damage-induced MDMX degradation is mediated by MDM2. J. Biol. Chem. 278: 45946–45953.
133. Meek DW and Knippschild U (2003) Posttranslational modification of MDM2. Mol. Cancer Res. 1: 1017–1026.
134. Pereg Y, Shkedy D, de Graaf P, Meulmeester E, Edelson-Averbukh M, Salek M, Biton S, Teunisse AF, Lehmann WD, Jochemsen AG and Shiloh Y (2005) Phosphorylation of Hdmx mediates its Hdm2- and ATM-dependent degradation in response to DNA damage. Proc. Natl. Acad. Sci. USA 102: 5056–5061.
135. Stommel JM and Wahl GM (2004) Accelerated MDM2 auto-degradation induced by DNA-damage kinases is required for p53 activation. EMBO J. 23: 1547–1556.
136. Pan Y and Chen J (2003) MDM2 promotes ubiquitination and degradation of MDMX. Mol. Cell. Biol. 23: 5113–5121.
137. Kawai H, Wiederschain D and Yuan ZM (2003) Critical contribution of the MDM2 acidic domain to p53 ubiquitination. Mol. Cell. Biol. 23: 4939–4947.
138. Meulmeester E, Frenk R, Stad R, de Graaf P, Marine JC, Vousden KH and Jochemsen AG (2003) Critical role for a central part of Mdm2 in the ubiquitylation of p53. Mol. Cell. Biol. 23: 4929–4938.
139. Tanimura S, Ohtsuka S, Mitsui K, Shirouzu K, Yoshimura A and Ohtsubo M (1999) MDM2 interacts with MDMX through their RING finger domains. FEBS Lett. 447: 5–9.
140. Chen L, Gilkes DM, Pan Y, Lane WS and Chen J (2005) ATM and Chk2-dependent phosphorylation of MDMX contribute to p53 activation after DNA damage. EMBO J. 24: 3411–3422.
141. Thompson T, Tovar C, Yang H, Carvajal D, Vu BT, Xu Q, Wahl GM, Heimbrook DC and Vassilev LT (2004) Phosphorylation of p53 on key serines is dispensable for transcriptional activation and apoptosis. J. Biol. Chem. 279: 53015–53022.