A complex barcode underlies the heterogeneous response of p53 to stress

Fiona Murray-Zmijewski, Elizabeth A. Slee and Xin Lu

Abstract | The tumour suppressor p53 is activated following stress and initiates a heterogeneous response in a cell-, tissue- and stress-dependent manner. This heterogeneity is reflected in the different physiological outcomes that follow p53 activation. One mechanism that may contribute to this variability is the promoter selectivity of p53 target genes. p53 is at the hub of numerous signalling pathways that are triggered in response to particular stresses, all of which can leave their mark on p53 by way of post-translational modifications and interactions with cofactors. The precise combination of these marks, much like the bars in a barcode, dictates the behaviour of p53 in any given situation.

Senescence
The irreversible arrest of cell growth. This process limits the lifespan of mammalian cells and prevents the growth of cells that are at risk of neoplastic transformation.

Missense mutation
A genetic mutation whereby a single nucleotide is substituted, which changes a codon so that it codes for a different amino acid. This change in one amino acid can alter the activity of the protein.

Telomere erosion
The shortening of the ends of telomeres due to incomplete replication of the lagging strand of DNA. The shortening of telomeres can happen in the early stages of cancer, leading to short dysfunctional telomeres.

Ludwig Institute for Cancer Research Ltd, University of Oxford, Nuffield Department of Clinical Medicine, Old Road Campus Research Building, Off Roosevelt Drive, Oxford, OX3 7DQ, United Kingdom. Correspondence to X.L.
e-mail: xin.lu@licr.org
doi:10.1038/nrm2451

The tumour suppressor *p53* was discovered in 1979[1–3], and has since become one of the most studied genes in human diseases, primarily because the majority of human tumours have defects in the p53 pathway. Many cancer therapies, such as radiotherapy and chemotherapy, exploit the p53 pathway to suppress cancer-cell growth by stimulating p53-mediated cell-cycle arrest, apoptosis and senescence. Conversely, cancer cells can escape the tumour suppression function of p53 through missense mutation of the *p53* gene or deregulation of p53 activity. Gene mutations inactivate p53 in around 50% of human cancers, although the mutation rate of p53 varies dramatically, from 10–12% in leukaemias, to 38–70% in lung cancers and 43–60% in colon cancers (see the IARC TP53 Mutation Database and The p53 Web Site; details in Further information).

The tumour-suppression function of p53 can also be impaired by the elevated activities of its inhibitors, such as MDM2, MDMX and iASPP, or by the reduced activities of its activators, such as Arf, ASPP1, ASPP2, ATM and p300. p53 is a transcription factor and can transactivate and transrepress many hundreds of genes in response to a large number of stress signals, including those caused by DNA damage, telomere erosion, hypoxia, temperature change, nucleolar disruption and dNTP depletion[4,5]. Furthermore, p53 interacts with over 100 cellular proteins and influences the cellular response to stress signals through transcription-dependent and -independent pathways. Thus, p53 functions as a key integrator that translates diverse stress signals into different cellular outcomes — ranging from cell-cycle arrest, DNA repair, genome stability, apoptosis, induction of autophagy[6,7], cell migration[8] and senescence, to differentiation, embryo implantation[9], regulation of metabolism[10–13] and angiogenesis[14].

The manner in which p53 responds to different stresses and decides between its many biological functions is of paramount importance when considering p53-targeted therapies for the treatment of cancer[15]. Here we review our current understanding of how p53 integrates particular stress signals into a range of cellular responses. We emphasize that the p53 response is heterogeneous and dependent on both the incoming stress signals and the environment of the cell. The physiological outcome of p53 activation is, therefore, tissue and cell-type dependent, and the heterogeneous response is likely to be caused by the presence of protein cofactors and modifying enzymes, which can induce alterations in the stability, subcellular localization and DNA-binding properties of p53. Thus, the cofactors that associate with p53 and the post-translational modifications that are imposed on p53 are the bars of a 'barcode' that governs p53 activity, thereby forming the underlying basis of the heterogeneous p53 response.

Cell-type-dependent p53 response

Levels of p53 protein increase in response to stress signals, resulting in cell-cycle arrest or apoptosis, principally through the ability of p53 to transcriptionally modulate target genes that mediate these processes. Many elegant studies in transgenic mouse models demonstrate, however, that the response to p53 activation is dependent on

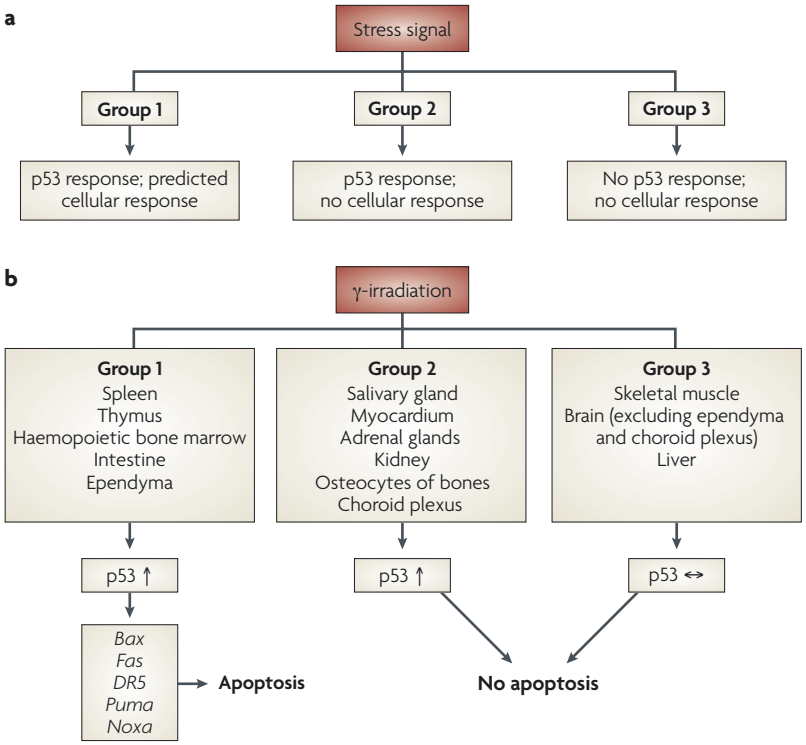

Figure 1 | **Classification of tissues based on the heterogeneous p53 response.** **a** | Following a particular stress, tissues can be classified into one of three groups on the basis of their p53 response and their cellular response. Group 1 tissues exert a p53 response and a cellular response; group 2 tissues show a p53 response but not a cellular response; and group 3 tissues have neither a p53 response nor a cellular response. **b** | Tissues from γ-irradiated adult mice can be split into three broad categories on the basis of the accumulation of p53 protein levels and the induction of apoptosis. p53 protein levels are induced in group 1 and group 2 tissues, but changes in the p53 level are not detected in group 3 tissues. Apoptotic target genes, such as *Bax*, *Puma* and *Noxa*, are induced and apoptosis is detected in group 1 tissues, whereas apoptosis is not induced in group 2 and 3 tissues. It is likely that the tissue in each group varies depending on which stress is encountered. Data compiled from REFS 16,18,19,24.

both tissue and cell type. MacCallum *et al.*[16] compared the cellular responses of wild-type and p53-deficient mice to γ-irradiation and observed that different tissues respond differently to γ-irradiation *in vivo*. The ability of γ-irradiation to cause the accumulation of p53 and to induce apoptosis divides various tissues into three groups[16] (summarized in FIG. 1). Group 1 tissues retain the classical p53 response and exhibit an increase in *p53* expression and apoptosis upon γ-irradiation; group 2 tissues show γ-irradiation-induced p53 accumulation without detectable increases in apoptosis; and group 3 tissues fail to induce both p53 accumulation and apoptosis upon γ-irradiation. Classification of tissues in this way is not absolute, and depends on the method, and the sensitivity of the method, used. For example, liver is classified as a group 3 tissue because induction of p53 is not observed by immunohistochemistry; however, p53 protein levels are clearly induced when analysed by western blotting[16].

These studies showed that the p53 response to stress signals is heterogeneous *in vivo* and raised many questions about how the heterogeneous response of p53 is achieved. For example, why does the same stress signal fail to induce p53 in group 3 tissues? The underlying mechanisms are poorly understood, although an association between high levels of *p53* mRNA and rapid apoptosis was postulated[17]. However, the ability of p53 to induce apoptosis is not strictly associated with its mRNA levels. In lung and kidney, both of which are group 2 tissues, p53 mRNA levels are similar to that seen in the small intestine, a group 1 tissue. However, being group 2 tissues, γ-irradiation fails to induce apoptosis in lung and kidney, despite inducing an increase in the p53 protein levels[16,17].

So why does induced p53 fail to initiate apoptosis in group 2 tissues? By examining the ability of p53 to induce various genes in different tissues, it was revealed that induction of certain sets of target genes is one mechanism by which p53 integrates stress signals into a cellular response. In thymus, spleen and colon, for example, γ-irradiation-induced p53 accumulation induces the expression of pro-apoptotic genes such as *Bax*, *Fas*, *DR5*, *Puma* and *Noxa*[18,19]. The importance of Puma and Noxa as apoptotic mediators of p53-induced apoptosis is confirmed in Puma- and Noxa-deficient mice models, in which γ-irradiation-induced apoptosis is impaired[20,21]. It remains unclear as to why γ-irradiation-induced p53 accumulation can induce the expression of *Puma*, *Noxa*, *Bax* and *Fas* in some tissues, such as thymus and spleen, but not in the other tissues, such as lung and kidney[16–19]. Perhaps the ability of p53 to respond to stimuli is coordinated by its cellular regulators, which are likely to be expressed in a tissue- and cell-type-specific manner, thus contributing to the heterogeneous p53 response *in vivo*. It is also possible that the post-translational modifications of p53 differ between the group 1, 2 and 3 tissues.

The classification of tissues based on the p53 response and the induction of apoptosis following γ-irradiation by MacCallum *et al.* might be applied to other stress signals *in vivo*, although the actual tissues in each group would probably vary depending on the type of stress. On this basis, a defined stress signal would induce a particular p53 response and cellular response in group 1 tissues; induce a p53 response but not a cellular response in group 2 tissues; and fail to generate both a p53 and a cellular response in group 3 tissues (FIG. 1). Therefore, the activity of p53 would vary between different tissues, resulting in different cellular responses to the same stress signal.

Stimulus-dependent p53 response

The variation in the p53 response is not only cell-type and tissue dependent, it is also affected by the type of stress that is experienced. Initially, this is reflected in the timing and duration of the elevation in p53 levels. For example, in mouse prostate cells, ultraviolet (UV) radiation does not cause an increase in p53 until 4 hours post exposure, after which time the p53 levels continue to rise. In the same cells, however, γ-irradiation causes an acute elevation in p53 levels 1 hour post exposure, after which time the p53 levels decline (FIG. 2; REF. 22). Hypoxia causes an increase in p53 levels that is proportional to the duration of oxygen deprivation, with p53 levels returning to normal levels after re-oxygenation[23].

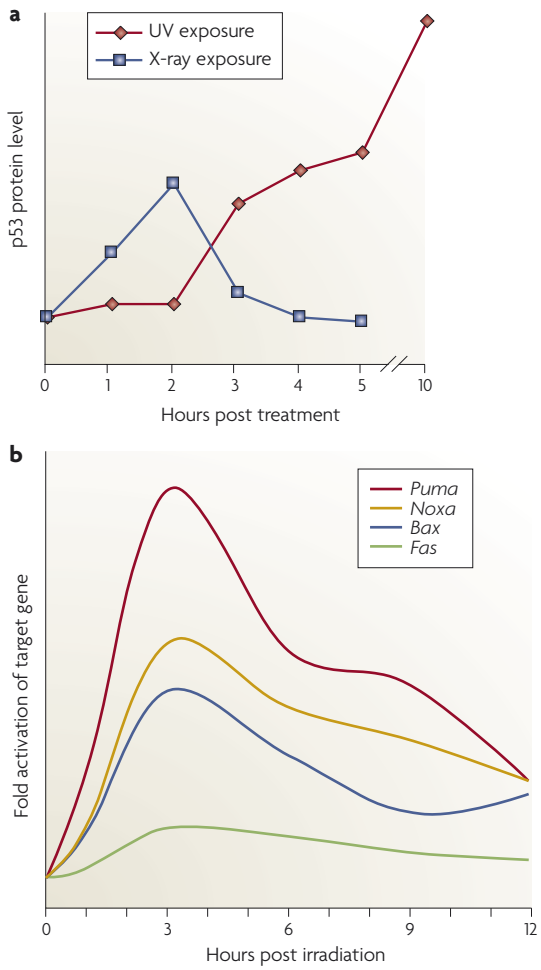

Figure 2 | **Kinetics of the p53 response. a** | Induction of p53 protein levels following ultraviolet (UV) or X-ray irradiation in mouse prostate cells. The time course of the p53 response to the same doses of UV and X-ray was analysed by ELISA (enzyme-linked immunosorbent assay). p53 protein levels are differentially induced in the same cells following UV or X-ray, indicating that the way in which p53 responds depends on the stress signal. Data compiled from REF. 22. **b** | The kinetics of induction of p53 pro-apoptotic target-gene mRNAs from the thymus of whole-body γ-irradiated mice. Induction of p53 apoptotic target genes is observed after just 1 hour following p53 activation, with maximal activity observed at 3 hours. Comparison of panels **a** and **b** indicate that the kinetics of target-gene induction reflect the kinetics of p53 protein induction, with peak mRNA levels occurring after maximal p53 protein levels have been reached. Data compiled from REF. 26.

Similarly, the duration of the p53 response following γ-irradiation is affected by the rate at which the DNA lesions are repaired, as p53 elevation can be prolonged by concurrent exposure to an inhibitor of the DNA-repair enzyme poly(ADP-ribose) polymerase[22].

Analysis of the timing of the p53 response in the organs of γ-irradiated mice has revealed that two waves of p53-dependent apoptotic activity occur in radiosensitive tissues. Interestingly, the initial wave of apoptosis was transcription independent and occurred in response to the rapid translocation of p53 to the mitochondria, which was detected as early as 30 minutes post irradiation in thymus and spleen[24]. p53 transcription-dependent apoptosis had a longer lag phase, with detection of the first apoptotic gene target, *Puma*, occurring in the thymus 2 hours post irradiation. Induction of *Noxa* and *Bax* occurred after 4 and 8 hours, respectively. The temporal variation of the p53 response is also reflected in the timing of the expression of different categories of p53-responsive genes. A detailed study by Levine and colleagues, which analysed p53-mediated gene expression by oligonucleotide array, found that the genes expressed using a zinc-inducible wild-type p53 could be separated into a number of groups, largely depending on the time at which they were elevated[25]. Those genes that are involved in cell-cycle arrest were found to be the first to have their expression elevated, and the pro-apoptotic genes were expressed at intermediate and later stages[25].

The array of genes that are activated or repressed by p53 also varies depending on the stimulus. In Levine's study, cell lines that expressed wild-type p53 that were exposed to either γ-radiation or UV-radiation elevated a different spectra of genes compared with cells that expressed the zinc-inducible wild-type p53. In fact, the altered expression of only a few individual genes was common to all three circumstances. This strongly suggests that distinct stress signals result in the expression of a different set of genes, and this is also probably cell-type dependent[25].

The complexity of the p53 response is also reflected by the fact that gene-expression profiles can be affected by the intensity of a particular stress signal. A recent *in vivo* study showed that even in the same tissues and in response to the same stimulus, pro-apoptotic target genes exhibit different sensitivities to p53 induction. The *Puma* gene is induced in the thymus and spleen following a 0.1-Gy irradiation dose, whereas the *Bax* and *Killer* (also known as *DR5*) genes are induced after 0.2 Gy and *Noxa* is induced after 0.5 Gy[26]. Interestingly, these observations agree with work carried out in knockout mice, which show different degrees of apoptosis in tissues depending on whether *Puma*, *Bax* or *Noxa* has been deleted. Loss of Noxa results in only a slightly reduced level of apoptosis in the thymus of mice, whereas the loss of Bax results in a reduction in apoptosis in both the thymus and the central nervous system (CNS)[20]. Strikingly, loss of Puma shows a dramatic decrease in apoptosis in both thymus and the CNS, which, together with the kinetic data from the May group, suggest that Puma is perhaps one of the most sensitive apoptotic targets of p53 activation[20,21,26]. As yet, it is unclear what regulates the sensitivity of different p53 targets. Detailed *in situ* hybridization studies may help to determine which different pro-apoptotic targets of p53 are induced in different cells.

Therefore, the exact nature of the actions, and cellular outcomes, of p53 is determined by cell and tissue type, the nature of the stress to which the cell has been exposed and the dose or severity of this stress. This specific set of circumstances governs whether the p53 response is dependent or independent of transcription, the timing and range of genes the expression of which is altered,

γ-radiation
A type of electromagnetic radiation that is generally characterized by having high frequency and energy, but short wavelength. γ-radiation is often used to kill living cells, such as in the sterilization of medical equipment, in a process called irradiation.

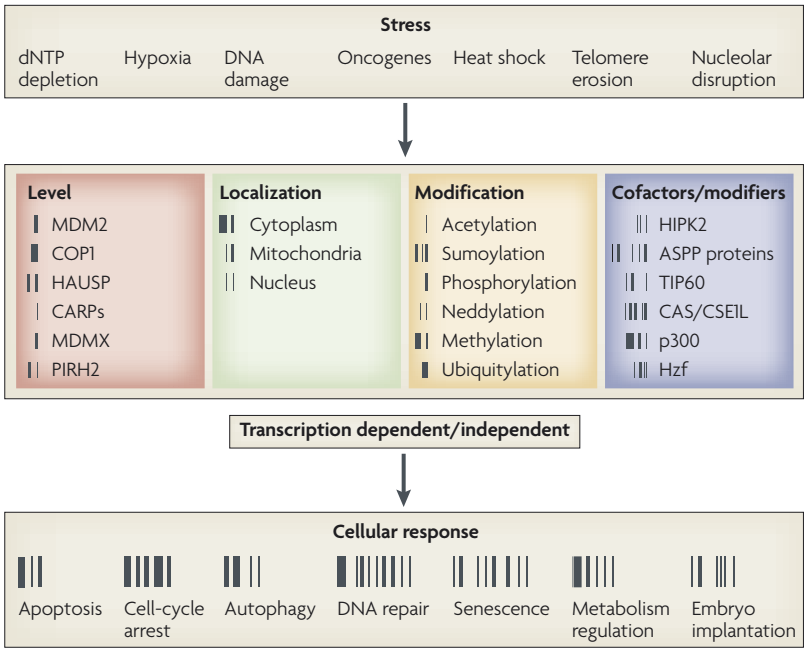

Figure 3 | **The p53 barcode.** p53 is activated by an array of cellular stresses and responds by activating various signalling pathways that are involved in diverse cellular mechanisms, from apoptosis to DNA repair. The protein level, localization, post-translational modifications and the cofactors of p53 are crucial to the function and regulation of p53. We propose that each individual aspect of p53 regulation represents a bar from a barcode that directs p53 activity. Different combinations of bars form different barcodes, and the barcode dictates which response p53 induces, be it apoptosis, cell-cycle arrest or senescence. Importantly, this allows p53 to be activated in a manner that is stress and cell-type dependent. The diagram shows a range of p53 regulations that control p53 activity and, ultimately, determine the cellular response. This response may be transcription dependent or independent. Each regulator is illustrated with its own bars. The number and width of the bars was assigned arbitrarily and has no relevance to the importance of each aspect in the regulation of p53 activity.

and so governs the fate of the cell. How can one protein integrate all these variables? Numerous pathways are triggered in response to a particular stress, and each one has the potential to leave their mark on p53 by inducing post-translational modifications and influencing its interactions with cofactors. Thus, p53 is at the hub of the stress response, and the way it responds in any particular situation is dictated by the precise combination of these marks. These marks, we propose, can be thought of like the bars in a barcode. While this is an extension of the 'histone code' analogy that was proposed by Toledo and Wahl[27], the countless potential combinations of the 'bars' in this model reflects the variety of cellular environments, triggers and consequences that are mediated by p53 (FIG. 3).

Regulation at the protein level

p53 levels in a cell can be controlled at the transcriptional and the translational levels[28–31]. For example, in response to γ-irradiation, increased translation of *p53* mRNA occurs, which contributes to the induction of p53 levels[29,32]. However, the principal way in which p53 levels are controlled is through the stability and degradation of the protein. p53 degradation by the proteasome requires poly-ubiquitylation. Several comprehensive reviews have recently discussed the complexities of regulating p53 stability; therefore, we will only briefly discuss some of the key observations[27,33,34].

Regulation of p53 stability by ubiquitin. The main mediator of endogenous p53 ubiquitylation is the MDM2 E3 ligase. MDM2 binds the N terminus of p53 and recruits E2 ligases, which directly transfer ubiquitin molecules to Lys residues in the C terminus of p53. MDM2 is also a transcriptional target of p53, which creates an autoregulatory loop whereby p53 controls the expression of its own negative regulator (FIG. 4).

The importance of effective negative regulation of p53 activity is highlighted by the embryonic lethality of *Mdm2*-knockout mice, which die due to aberrant p53-induced apoptosis. This phenotype is completely rescued following deletion of the *p53* gene, demonstrating that it is the uncontrolled activity of the p53 protein that prevents development of the mice[35,36]. Interestingly, a genetic deletion of MDMX, another negative regulator of p53, also caused embryonic lethality, which was also completely rescued by p53 deletion[37]. In contrast to MDM2, MDMX contains no intrinsic E3 ligase activity. The precise mechanisms by which MDMX inhibits p53 function remains unclear, although it can negatively regulate p53 function by directly binding to p53 and inhibiting its transcriptional activity (reviewed in REF. 34), and MDMX has also been shown to complex with MDM2 and positively influence the E3 ligase activity of MDM2 (REF. 38). Mice expressing an MDM2 mutant that binds p53, but that does not act as an E3 ligase, exhibit a similar p53-dependent embryonic lethality to the *Mdm2*-knockout mice[39]. However, endogenous MDMX is not sufficient to alleviate the embryonic lethality of mice in which MDM2 has been mutated so that it no longer has E3 ligase activity[39]. Collectively, the data from the mouse models raise the question of whether MDM2 can inhibit p53 via mechanisms that are independent of its E3 ligase activity.

The counterbalances to MDM2 in regulating p53 stability are de-ubiquitylating enzymes, such as HAUSP[40]. De-ubiquitylation of p53 leads to its stabilization and induction of cell-cycle arrest[41]. However, HAUSP can also de-ubiquitylate MDM2, thereby adding another level of complexity to the MDM2–MDMX feedback loop[42] (FIG. 4). The regulation of p53 by MDM2 and HAUSP has been extensively reviewed elsewhere[33].

Several other E3 ligases antagonize p53, including PIRH2 and COP1, which, like MDM2, are transcriptional targets of p53 (REFS 43,44). Recently, a family of ubiquitin ligases called caspase 8/10-associated RING proteins (CARPs) were described[45]. CARPs target either unmodified p53 or p53 that has been phosphorylated on Ser20 for degradation, independently of MDM2 (REF. 45). It remains to be seen whether CARPs are as crucial as MDM2 and MDMX in counteracting the actions of p53 during development. Certainly in the *Mdm2*-knockout mouse model their collective E3 ligase activities are not sufficient to compensate for the loss of MDM2 activity, at least during development, and they may only function in specific tissues or in particular circumstances[35,36].

Poly-ubiquitylation
The addition of multiple ubiquitin molecules to a target protein. The process usually involves the addition of a chain of ubiquitin to a target Lys residue (or residues) on the target protein.

E3 ligase
The third enzyme in a series (after E1 and E2) of enzymes that mediate the ubiquitylation of target proteins. E3 ligases recruit E2 ligases and the specific substrate, and aid in the transfer of ubiquitin to the target protein.

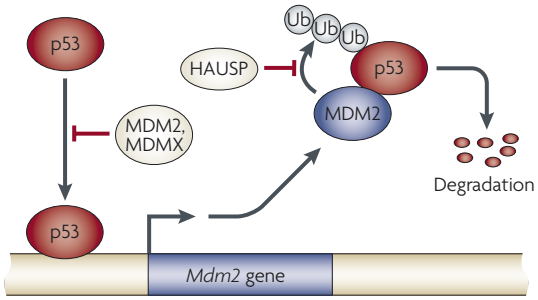

Figure 4 | **The p53–MDM2 feedback loop.** The *Mdm2* gene is a transcriptional target of p53 and, following induction by stress, p53 induces *Mdm2* expression. MDM2 is a negative regulator of p53 and induces its ubiquitylation and subsequent degradation, thus, preventing further gene transactivation. The MDM2-related protein MDMX can also inhibit p53 transactivation, although the precise mechanism involved has yet to be uncovered. HAUSP is a deubiquitylating enzyme and functions as a negative regulator of MDM2 and, therefore, as an activator of p53.

Regulation of p53 stability by small modifications. In addition to ubiquitylation, p53 is heavily modified by other small modifications such as phosphorylation, acetylation, methylation, ribosylation and glycosylation. Over 30 residues of p53 have been reported to be modified by these small modifications, and over 20 of these are Ser or Thr residues that are phosphorylated.

Despite a wealth of information about the effects of these post-translational modifications on p53 stability in cell-culture systems, the biological significance of these modifications remains uncertain. Knock-in mouse genetic models have largely failed to offer any clarification, as most of the phosphorylation- or acetylation-site knock-in mice exhibit minimal phenotypes (reviewed in detail in REFS 27,46). Currently, genetic evidence does not resolve whether other post-translational modifications, such as methylation, ribosylation and glycosylation, have profound effects on p53 stability. Nevertheless, some of the methylation sites (Lys370, Lys372 and Lys382) have been tested in knock-in mouse models where the six C-terminal Lys residues (Lys370, Lys372, Lys373, Lys381, Lys382 and Lys386), which can also be acetylated, sumoylated, ubiquitylated and neddylated, have been mutated to Arg residues (6KR mice)[47,48]. In these models only minimal differences in p53-dependent gene activation were observed in thymocytes and ES cells from the 6KR mice in response to irradiation[47]. One possible reason for these subtle phenotypes is that the coordinated regulation of p53 by ubiquitylation and by other post-translational modifications may regulate the stability and/or activity of p53 in highly specific situations. Thus, a particular barcode combination may be needed to instruct p53 under these circumstances; however, the specific stimuli that characterize a specific situation have yet to be identified.

Furthermore, we cannot exclude the possibility that many of these residue-specific modifications occur in combination with other modifications and protein interactions. This may result in some overlap between different mechanisms of p53 activation, although different modifications may activate p53 in different ways. This is particularly relevant in the case of residues that are modified in multiple ways, in a mutually exclusive manner. For example, Lys370 can be methylated, ubiquitylated and neddylated, with each having a different effect on p53 activity[49–51]. Also, even the same type of modification can result in opposite effects on p53 activity. Mono-methylation of Lys370 represses p53 transcription and helps to maintain low concentrations of promoter-associated p53, whereas dimethylation promotes the interaction of p53 with the co-activator 53BP1 (REF. 52). Mutation of Lys370 in mouse knock-in models may not yield a phenotype because p53 can neither be activated nor repressed following modification. Lys370 is not the only residue in p53 that is modified by multiple modifications; many other p53 C-terminal Lys residues are modified by ubiquitylation, sumoylation, neddylation and acetylation. So, residues that can be modified in more than one way, which may have opposite effects, can result in a neutral phenotype in mouse models because the opposite effects can cancel each other out.

Regulating p53 localization

Because p53 functions as a transcriptional regulator, it must be localized in the cell nucleus. However, p53 can also function outside the nucleus, notably by stimulating apoptosis directly at mitochondria. Thus, regulating the subcellular localization of p53 is important for controlling its activity. Whereas the steady-state levels of p53 are predominantly controlled by poly-ubiquitylation, localization of p53 is often regulated by mono-ubiquitylation and other ubiquitin-like modifications (FIG. 5).

Nuclear localization. In order to function as a transcription factor, p53 must localize to the nucleus and bind DNA. Many cellular factors affect the nuclear localization of p53, and the list of cofactors that retain p53 in the nucleus is growing (reviewed in REF. 53). For example, ubiquitylation by the E3 ubiquitin ligase E4F1, which poly-ubiquitylates p53 on Lys320, a residue distinct to those used by MDM2, affects the location, rather than the abundance, of p53. Instead of targeting it for degradation, ubiquitylation of p53 by E4F1 results in the enhanced localization of p53 to chromatin, although it does not increase the levels of p53 in the nucleus. p53 that has been ubiquitylated by E4F1 is enriched on the p21 promoter and specifically enhances the expression of cell-cycle-arrest genes such as *p21, cyclin G1* and *GADD45*, which results in growth arrest and inhibition of apoptosis[54]. Thus, ubiquitin ligases have a diverse role in controlling p53 by influencing its stability, location and activity. Again, the regulation of p53 localization by these modifications also probably depends on the tissue, environment, timing and/or the presence of other protein cofactors that regulate p53 activity.

In contrast to sumoylation and mono-ubiquitylation (see below), poly(ADP-ribose) polymerase-1 (PARP1)-mediated poly(ADP-ribosyl)ation of p53 leads to its nuclear accumulation. Following DNA damage, PARP1 becomes activated and poly(ADP-ribosyl)ates p53, preventing it from interacting with the nuclear export receptor CRM1

Co-activator
A protein that enhances gene expression by binding (directly or indirectly) to a transcription factor.

Mono-ubiquitylation
The addition of a single ubiquitin molecule to a target Lys residue on the substrate protein.

Poly(ADP-ribosyl)ation
The covalent or non-covalent attachment of polymers of ADP-ribose units to proteins. Poly(ADP-ribose) polymerase-1 (PARP1) catalyses the covalent poly(ADP-ribosyl)ation of p53

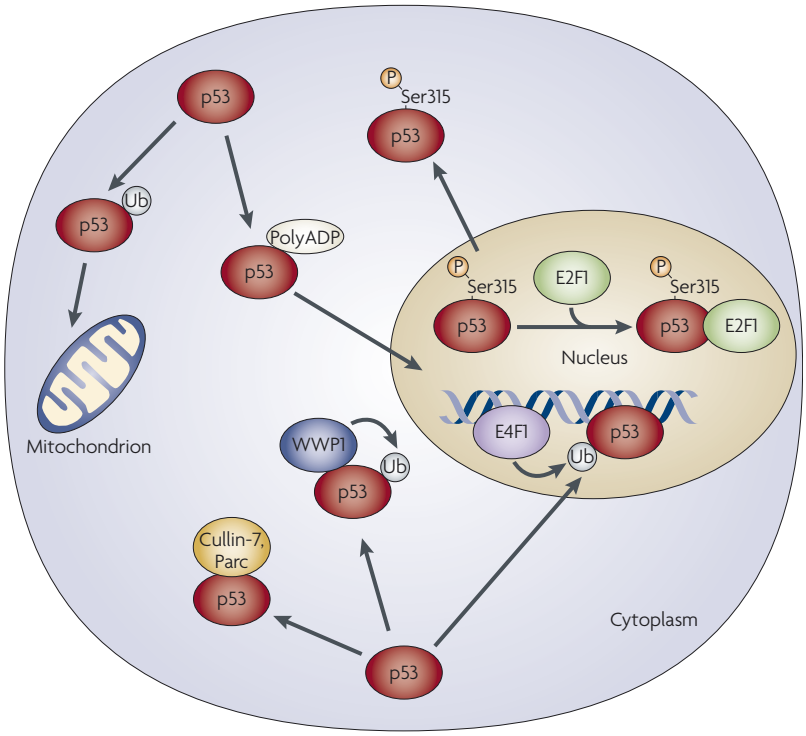

Figure 5 | **Post-translational modifications and interactions with protein cofactors can regulate the subcellular localization of p53.** Mono-ubiquitylation by MDM2 targets p53 to the mitochondria, whereas poly(ADP-ribosyl)ation retains p53 in the nucleus. Phosphorylation on Ser315 of p53 is thought to retain p53 in the nucleus in a manner that is dependent on the interaction of p53 with the transcription factor E2F1. The interaction of p53 with several E3 ligases also influences p53 localization. Parc and Cullin-7 anchor p53 in the cytoplasm, without targeting it for ubiquitylation. By contrast, WWP1 and E4F1 mediate the ubiquitylation of p53, but without subsequent degradation. WWP1 holds p53 in an inactive state in the cytoplasm, whereas E4F1 targets p53 to the chromatin, where it enhances transactivation of cell-cycle-arrest genes.

(REF. 55). This modification, therefore, provides a means by which p53 levels can accumulate in the nucleus following stress. Phosphorylation of p53 on Ser315 in its C terminus can result in increased export of p53 from the nucleus, which may be because the nuclear-export signal in this region becomes unmasked. However, phosphorylation at this site also enhances the interaction of p53 with the nuclear transcription factor E2F1, thereby retaining p53 in the nucleus[56–58].

Cytoplasmic localization. Retention of p53 in the cytoplasm is another way to regulate p53 function. This was first illustrated with viral proteins, including the X protein of hepatitis B virus and the E1B 55kDa protein of adenovirus, which negatively regulate p53 activity by retaining p53 in the cytoplasm[59–62]. Recent studies have shown that, in addition to catalysing poly-ubiquitylation of p53, MDM2 induces p53 mono-ubiquitylation, which occurs when MDM2 levels in the cell are low[63]. The addition of a single ubiquitin molecule to p53 induces the translocation of the protein from the nucleus to the cytoplasm, suggesting that in unstressed cells (when MDM2 levels are low) p53 is located in the cytoplasm. A recent quantitative analysis of p53 subcellullar localization indicated, however, that p53 was distributed evenly between the nucleus and cytoplasm before DNA damage[64]. Analysis of specific modified forms of p53 and their subcellular localizations may increase our understanding of these apparent discrepancies.

Recent work has shown that MDM2 can enhance the sumoylation of mono-ubiquitylated p53 by promoting the interaction of p53 with the SUMO E3 ligase PIASy[65]. The resultant sumoylated p53 promotes the release of MDM2 and is more effectively exported to the cytoplasm. Cytoplasmic localization of p53 can also be mediated by the ubiquitin ligases Cullin-7, Parc and WWP1 (REFS 66–68). All three ligases accumulate transcriptionally inactive p53 in the cytoplasm, without targeting it for degradation. Parc and Cullin-7 are members of a family of E3 ligases, but fail to directly ubiquitylate p53. By contrast, WWP1 directly binds p53 and mediates its ubiquitylation, and modified p53 then remains inactive in the cytoplasm. These ligases all inhibit the transactivation activity of p53 by retaining p53 in the cytoplasm.

Although cytoplasmic p53 is transcriptionally inactive, p53 can nevertheless induce cellular responses such as apoptosis through transcriptionally independent pathways. Mono-ubiquitylated p53 accumulates at the mitochondria and directly induces apoptosis[69]. A recent study also showed that cytoplasmic, but not nuclear, p53 can repress autophagy independently of the transcriptional activity of p53 (REF. 70). This raises the question of how the conditions of the cell affect which role cytoplasmically retained p53 performs.

Mitochondrial localization. p53 functions in the cytoplasm and can directly activate apoptosis at the mitochondria through its direct interaction with the pro-apoptotic proteins Bak and Bax, as well as with the anti-apoptotic protein Bcl-xL[71–73]. The *in vivo* significance of p53 transcription-independent apoptosis has recently been demonstrated in mouse models[24]. Targeting of p53 to the outer membrane of mitochondria induced apoptosis and suppressed tumorigenesis of primary lymphomas in Eμ-Myc transgenic mice, in which *Myc* overexpression is driven by the Ig heavy-chain enhancer[74]. Furthermore, insulin-like growth-factor-binding protein-1 (IGFBP1) negatively regulates the interaction between p53 and Bak, resulting in impaired apoptosis. This regulation was confirmed in IGFBP1-deficient mice, which show spontaneous apoptosis and accumulation of p53 at the mitochondria[75].

Therefore, one crucial role of the p53 barcode is to ensure that p53 is in the correct cellular compartment so that it can instigate the correct outcome, be it cell-cycle arrest, apoptosis, autophagy or survival.

Regulating target-gene selection

The function of p53 as a transcription factor is its best characterized mode of action, and the role of modifications and cofactors in ensuring its presence in the nucleus is just one aspect of the barcode's function. When p53 is in the nucleus, the barcode also acts in more subtle ways to direct target-gene selection and, therefore, the biological outcome. This can occur by post-translational modification, the actions of cofactors or a combination of both.

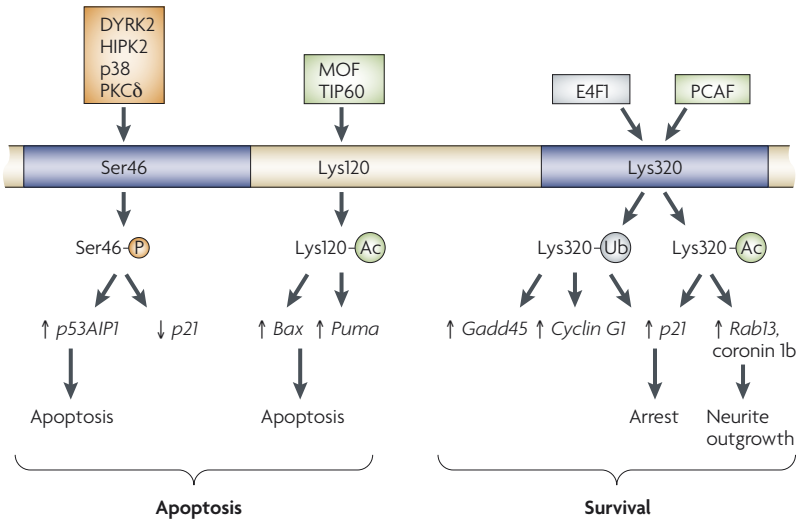

Figure 6 | **Residues that directly influence p53 promoter selectivity following post-translational modification.** The modifying enzymes that can effect the modification of Ser46, Lys120 and Lys320 of p53 are indicated above the particular residues, and the modifications that they induce are shown directly below the residues. Modification of these residues results in the selective activation or repression of particular p53 target genes, resulting in cell survival or apoptosis.

Modifications that affect promoter selectivity. Initial cell-culture studies indicated that p53 selects which genes to activate based on its abundance and affinity for a particular promoter. In other words, when p53 levels are low, it activates genes with high-affinity promoters that tend to be associated with cell-cycle arrest, and when p53 levels are high, it activates low-affinity promoters that tend to be involved in the apoptotic response[76,77]. However, the situation is not this clear cut.

The modification of specific residues in p53 can also directly influence the promoter to which p53 will bind (FIG. 6). In response to UV and genotoxic stress, p53 is phosphorylated on Ser46 by the kinases homeodomain-interacting protein kinase-2 (HIPK2) and the dual-specificity tyrosine-phosphorylation-regulated kinase-2 (DYRK2), respectively[78–80]. This occurs in the later stages of p53 activation and influences the response by specifically promoting the induction of the apoptotic gene *p53AIP1* (REF. 81). This is accompanied by down-regulation of p21 expression, ultimately resulting in p53-dependent apoptosis.

p53-dependent apoptosis can also be specifically enhanced following DNA damage through the acetylation of Lys120 by the MYST family acetyltransferases MOF and TIP60. Lys120 lies in the DNA-binding domain of p53, and its acetylation leads to increased recruitment of p53 specifically to pro-apoptotic target genes, such as *Puma* and *Bax*, suggesting that this modification alone can influence how p53 responds to DNA-damage signals. This modification appears to be required for p53-dependent apoptosis, as mutants that can no longer be modified in this way exhibit impaired apoptotic activity while maintaining the proper regulation of *Mdm2* and cell-cycle-arrest genes[82,83]. These data show that a defined p53 modification can be linked to a specific cellular outcome.

By contrast, Lys320 of p53 can be modified independently by both acetylation and ubiquitylation to influence promoter selectivity. The E3 ligase E4F1 ubiquitylates p53 at Lys320, and specifically increases the activation of cell-cycle-arrest genes, such as *p21*, *Gadd45* and *cyclin G1* while the expression of apoptotic targets remains unchanged[54]. Chromatin immunoprecipitation experiments demonstrated that Lys320-ubiquitylated p53 was bound to the *p21* gene promoter, but not to that of the apoptotic target gene *Noxa*. Similarly, acetylation of Lys320 following drug-induced DNA-damage promotes cell survival, with Lys320-acetylated p53 binding more efficiently to the *p21* promoter than does non-acetylated p53 (REF. 84). Despite their apparent overlapping functions, the modification of Lys320 by these two distinct mechanisms appears to be mutually exclusive, as ubiquitylation abolishes acetylation, and vice versa[54]. Thus, the two modifications may represent a way that p53 can selectively target cell-cycle arrest genes in response to different incoming signals. Furthermore, the same modification on the same residue can also result in different cellular outcomes. Acetylation of Lys320 in neuronal cells does not cause cell-cycle arrest, but it is specifically involved in promoting neurite outgrowth by elevating the expression of two p53 target genes: coronin 1b, which encodes an actin-binding protein, and *Rab13*, which encodes a GTPase[85]. Thus, even the same modification can cause markedly different outcomes, depending on the tissue in which activation occurs (FIG. 6).

Ultimately, these differences must be due to the presence of cofactors in the cell that are differentially expressed from one cell type to another. Analysis of p53 cofactors must, therefore, be taken into consideration when trying to predict the p53 response.

Cofactors that regulate promoter selectivity. The availability of p53 cofactors in different tissues is crucial for generating the barcode and for its interpretation, because the modification of p53 can affect its interaction with factors that may affect both transcription-dependent and -independent activities of p53. The proteins that interact with p53, therefore, play an important role in directing its heterogeneous response[27,48].

A number of proteins cooperate directly with p53 to influence the expression of target genes and regulate cell fate. Many of these bind to the DNA-binding domain of p53, a region that is conserved among all species. The first example of this group of proteins is the evolutionarily conserved ASPP family, which specifically regulate the apoptotic, but not the cell-cycle-arrest, function of p53 and its family members p63 and p73. The family consists of three members: ASPP1, ASPP2 and iASPP. Whereas ASPP1 and ASPP2 bind and enhance p53-dependent apoptosis by stimulating the binding of p53 to pro-apoptotic gene promoters such as *Bax* and *Pig3*, iASPP inhibits p53-mediated apoptosis by antagonizing the elevation in pro-apoptotic gene expression[86,87]. Importantly, p63 and p73 are required for p53-mediated apoptosis in some cell types following DNA damage[88]; thus, the ASPP family may direct this process by orchestrating promoter binding and gene activation.

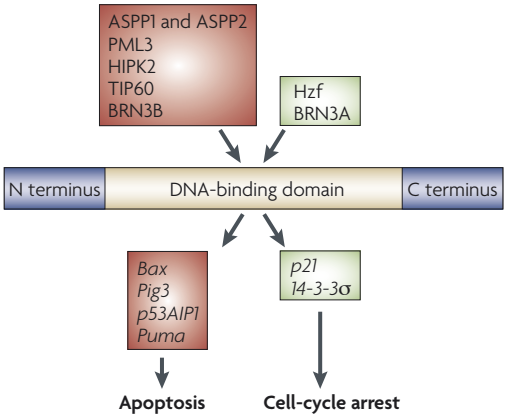

Figure 7 | **Protein interactions that directly affect p53 promoter selectivity.** Many of the cofactors that directly influence the gene-promoter selectivity of p53 bind its DNA-binding domain. This region makes direct contact with the promoters of p53 target genes; therefore, in principle this would be the best place for proteins to influence the target specificity of p53. Cofactors are listed above p53, and the target genes they activate are listed below. Genes and proteins that promote apoptosis are boxed in red, whereas those that promote cell-cycle arrest are boxed in green.

Like the ASPP family, the haematopoietic zinc finger (Hzf) protein binds to the DNA-binding domain of p53. However, rather than inducing the expression of apoptotic genes, Hzf preferentially transactivates pro-arrest target genes[89]. *Hzf* is itself a p53 target gene, creating an autoregulatory feedback loop and favouring a cell-cycle-arrest response. However, upon prolonged stress, Hzf is targeted for degradation, allowing the release of p53 and the strong activation of apoptotic genes. The BRN3A and BRN3B proteins also bind to the DNA-binding region of p53 and contribute to p53 activity, although they appear to regulate p53 with opposing outcomes. BRN3A activates p21 and represses Bax expression, whereas BRN3B activates Bax and represses p21 (REFS 90,91). Additionally, the DNA-binding domain is also important for targeting p53 to nuclear bodies by an isoform of the promyelocytic leukaemia protein (PML3), which can then alter the promoter selectivity of p53 (REF. 92). Upon relocalization into nuclear bodies, p53-dependent transcription is enhanced in a promoter-specific manner, with strong activation of PIG3 and weak activation of p21.

The promoter selectivity of p53 can also be influenced by post-translational modifications of p53, which in turn influence cofactor binding. For example, phosphorylation of p53 on Ser33, Thr81 and Ser315 following genotoxic stress dramatically increases its binding to the peptidyl-prolyl isomerase PIN1 (REFS 93,94). This changes the conformation of p53, thereby enhancing its activity by stabilizing p53 and altering its interaction with DNA. Furthermore, PIN1 can stimulate binding of other transcriptional cofactors, such as p300, resulting in increased p53 acetylation and activation[95]. The binding of ASPP family proteins to p53 can also be regulated by post-translational modification. Following Ser46 phosphorylation, induced in response to cytotoxic stimuli, p53 dissociates from iASPP in a PIN1-dependent manner, thereby enabling p53 to promote apoptosis[95]. The *in vivo* significance of the interaction between p53 and PIN1, and of the isomerization of Pro residues, has been tested in two *p53* knock-in mice strains that lack either one of the PIN1-binding sites (Thr81) or the crucial Pro residues in p53 (REF. 96). In agreement with the *in vitro* data, PIN1 stabilizes p53; however, there was no evidence that PIN1 regulates the transcriptional activity of p53. Future studies are needed to clarify the biological significance of the interaction between PIN1 and the residues Ser33 and Ser315 on p53 in regulating the transcriptional activity of p53 *in vivo*.

Taken together, the existing evidence supports a hypothesis that the DNA-binding domain of p53 may function as the region of the protein where the appropriate response to a particular stress is encoded, either through post-translational modifications and/or through interaction with protein cofactors (FIG. 7). It is also the region of p53 that houses the majority of tumour-derived mutations. Importantly, a number of tumour-derived p53 mutants that house mutations in this region alter promoter selectivity. For example, mutants R181L and R181C have selectively lost the ability to transcriptionally activate pro-apoptotic genes even though they retain wild-type p53 activity towards the transcriptional activation of p21 and MDM2 (REFS 97,98). This may, at least in part, be explained by the fact that this mutation prevents binding to ASPP1 and ASPP2 (REF. 86). Another p53 mutant with similar properties is p53 R175P. This mutant can induce cell-cycle arrest, but fails to induce apoptosis[99]. Collectively, these data support the notion that the expression of appropriate cofactors in a particular tissue can signal through the DNA-binding domain of p53 to coordinate the cellular response in a manner that fits both the environment of the cell and the type of stress that has been inflicted.

Cofactors that indirectly influence p53. Many cofactors of p53 can indirectly affect p53 function by regulating the proteins that are directly involved in its activation and repression, thus affecting promoter specificity (FIG. 8). As a ubiquitous transcriptional co-activator, p300/CBP activity is tightly regulated by an array of associated proteins. Acetylation of p53 by p300/CBP and the p300/CBP-associated factor (PCAF) occurs in response to DNA-damaging agents, such as UV and ionizing radiation, and is associated with the induction of sequence-specific DNA binding and transactivation by p53. During the DNA-damage response, p300 interacts with its cofactor, junction-mediating and regulatory protein (JMY), resulting in increased p53 acetylation and subsequently enhancing p53-dependent transcription and apoptosis[100]. This interaction between JMY and p300 is facilitated by the recruitment of a second p300 cofactor, Strap[101]. Strap also increases the levels and half-life of p53 by preventing

Prolyl isomerase
A molecule that mediates the interconversion of Pro residues in specific amino-acid motifs from the *cis* to the *trans* conformation, and vice versa.

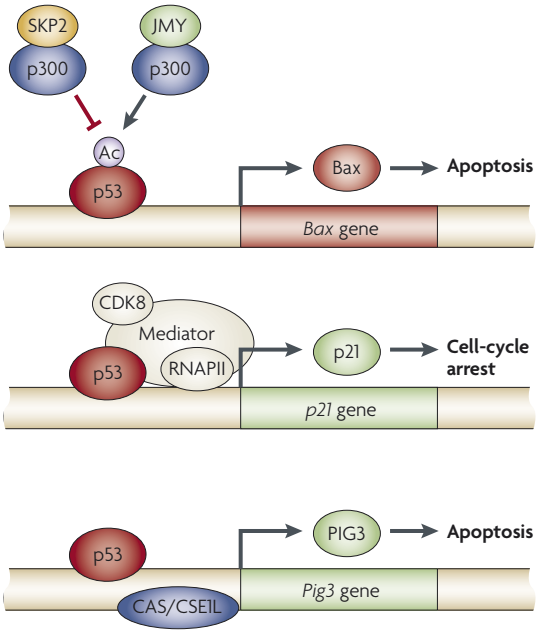

Figure 8 | **Cofactors that indirectly affect p53 activity.** Activation of the *Bax* gene promoter can be enhanced by p300-mediated acetylation of p53. The p300 cofactor JMY can enhance this acetylation, whereas SKP2 can bind p300 and antagonize acetylation of p53. p53-dependent transactivation of the *p21* gene promoter can be enhanced through the recruitment of CDK8 to the Mediator complex. Independent binding of the long-range chromatin modifier CAS/CSE1L, which is the human orthologue of yeast Cse1, to promoters, such as the *Pig3* gene promoter, can also enhance p53-dependent transcription.

MDM2-mediated downregulation[102]. More recently, a third p300 cofactor, SKP2, was identified and shown to affect p53 activity by preventing the interaction between p300 and p53, thereby preventing acetylation of p53 and suppressing p53 transactivation and apoptosis[103]. Although some factors, such as JMY, enhance transactivation while other factors, such as SKP2, repress apoptosis, such factors do not appear to cause p53 to discriminate between pro-apoptotic or cell-cycle-arrest promoters. Human cellular apoptosis susceptibility protein (CAS/CSE1L) suppresses p53-mediated transcription by binding directly to the promoters of certain p53 target genes in a p53-independent manner. Again, the promoters bound by CAS/CSE1L do not appear to correlate with a particular physiological outcome (that is, arrest or apoptosis), although silencing of CAS/CSE1L inhibits UV-induced death[104]. Components of the basal transcription machinery have also been implicated in specifically regulating p53 activity. The CDK8 subunit of the CDK module of the Mediator complex can function as a p53 co-activator and is recruited to the p21 promoter in a proportional manner only under conditions of strong p21 activation by p53, so that there is stronger transcriptional activation as more CDK8 binds to the promoter[105].

Post-translational modifications and p53-interacting proteins influence the promoter selectivity and outcome of p53 activation[106]. Many of these modifiers and cofactors are expressed in a tissue- and cell-dependent manner. Consequently, they function in certain cellular contexts, in response to a particular stress or in association with a single gene; they function in response to a specific p53 barcode. The identification of cofactors that indirectly influence p53 activity is a new and emerging field. Future studies will no doubt identify more proteins that can regulate p53 in this way, adding to the complexity of the p53 response.

Summary and future prospects

Nearly 30 years of research on the p53 protein has shown that p53 is a major regulator of cellular stress and an important tumour suppressor. p53 is activated following different forms of cellular stress and integrates the incoming signals so that an appropriate cellular response is made. An increasing number of p53 target genes, with an ever-varied function in the cell, are being identified, and many transcription-independent functions of p53 are also now being identified.

The new challenge in p53 research will be to examine the vast heterogeneity of the p53 response and to unravel the extreme complexities of p53 activity. We need to understand how p53 can integrate a particular stress signal into a cellular response. The classification of the tissue response *in vivo* will apply to all stress signals, so that in response to a defined stress signal, any given tissue will always fall into one of three groups: group 1, group 2 or group 3. Understanding the heterogeneous p53 response to stress signals will ultimately allow us to understand the factors that govern this classification, such as p53 cofactors that bind and/or modify p53. A detailed understanding of the significance of modifications of specific residues will also be needed to fully appreciate the multiple outcomes and effects that modifications can have on p53 regulation.

A comprehensive understanding of the heterogeneity of the p53 response will be important in defining which cellular functions of p53 are crucial for its tumour suppression function in specific tissues in response to carcinogenic signals. For example, γ-irradiation induction of p53 activity failed to cause apoptosis in lung, raising the question as to whether apoptosis forms part of the tumour-suppression function of p53 in lung cancer. Alternatively, apoptosis may be induced in the lung in response to signals other than γ-irradiation. An elegant study by Gerard Evan's group showed that pathological apoptosis induced by γ-irradiation is not crucial for the tumour suppression function of p53 in a transgenic mouse model system of lymphoma; rather, the ability of p53 to suppress the oncogenic signal is crucial[107]. The true response of p53 might only be revealed if the cells are stressed and the effects monitored in a way that allows subtle changes to be detected. Thus, a new era of p53 research is likely to emerge during which we will begin to unravel the response in each particular setting, defining a barcode for each particular p53 response.

1. Lane, D. P. & Crawford, L. V. T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261–263 (1979).
2. Linzer, D. I. & Levine, A. J. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **17**, 43–52 (1979).
3. DeLeo, A. B. *et al.* 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 (1979).
4. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
5. Rubbi, C. P. & Milner, J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* **22**, 6068–6077 (2003).
6. Crighton, D. *et al.* DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* **126**, 121–134 (2006).
7. Amaravadi, R. K. *et al.* Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J. Clin. Invest.* **117**, 326–336 (2007).
8. Roger, L., Gadea, G. & *Roux.*, P. Control of cell migration: a tumour suppressor function for p53? *Biol. Cell* **98**, 141–152 (2006).
9. Hu, W., Feng, Z., Teresky, A. K. & Levine, A. J. p53 regulates maternal reproduction through LIF. *Nature* **450**, 721–724 (2007).
10. Bensaad, K. *et al.* TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* **126**, 107–120 (2006).
11. Jones, R. G. *et al.* AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol. Cell* **18**, 283–293 (2005).
12. Matoba, S. *et al.* p53 regulates mitochondrial respiration. *Science* **312**, 1650–1653 (2006).
13. Bensaad, K. & Vousden, K. H. p53: new roles in metabolism. *Trends Cell Biol.* **17**, 286–291 (2007).
14. Teodoro, J. G., Parker, A. E., Zhu, X. & Green, M. R. p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase. *Science* **313**, 968–971 (2006).
15. Woods, Y. L. & Lane, D. P. Exploiting the p53 pathway for cancer diagnosis and therapy. *Hematol. J.* **4**, 233–247 (2003).
16. MacCallum, D. E. *et al.* The p53 response to ionising radiation in adult and developing murine tissues. *Oncogene* **13**, 2575–2587 (1996).
One of the first reports that showed that the p53 response *in vivo* is tissue dependent.
17. Komarova, E. A., Christov, K., Faerman, A. I. & Gudkov, A. V. Different impact of p53 and p21 on the radiation response of mouse tissues. *Oncogene* **19**, 3791–3798 (2000).
18. Fei, P., Bernhard, E. J. & El-Deiry, W. S. Tissue-specific induction of p53 targets *in vivo*. *Cancer Res.* **62**, 7316–7327 (2002).
One of the first reports to examine the differential induction of p53 target genes *in vivo* and link selective target-gene induction to differences in tissue response.
19. Bouvard, V. *et al.* Tissue and cell-specific expression of the p53-target genes: *Bax*, *Fas*, *Mdm2* and *Waf1/p21*, before and following ionising irradiation in mice. *Oncogene* **19**, 649–660 (2000).
20. Villunger, A. *et al.* p53- and drug-induced apoptotic responses mediated by BH3-only proteins Puma and Noxa. *Science* **302**, 1036–1038 (2003).
21. Jeffers, J. R. *et al.* Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. *Cancer Cell* **4**, 321–328 (2003).
22. Lu, X. & Lane, D. P. Differential induction of transcriptionally active p53 following UV or ionizing radiation: defects in chromosome instability syndromes? *Cell* **75**, 765–778 (1993).
One of the first reports that determined the kinetics of p53 induction and found that the kinetics of the response of p53 will be different depending on the activating stress.
23. Graeber, T. G. *et al.* Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol. Cell. Biol.* **14**, 6264–6277 (1994).
24. Erster, S., Mihara, M., Kim, R. H., Petrenko, O. & Moll, U. M. *In vivo* mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. *Mol. Cell. Biol.* **24**, 6728–6741 (2004).
The first article to show *in vivo* that, in response to γ-irradiation, the first wave of p53-dependent apoptosis is independent of transcription.
25. Zhao, R. *et al.* Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev.* **14**, 981–993 (2000).
A comprehensive comparison of the kinetics of p53 target-gene induction in response to three different ways of activating p53.
26. Alvarez, S. *et al.* A comprehensive study of p53 transcriptional activity in thymus and spleen of γ-irradiated mouse: high sensitivity of genes involved in the two main apoptotic pathways. *Int. J. Radiat. Biol.* **82**, 761–770 (2006).
Showed the different sensitivities and kinetics of p53 target genes *in vivo* in response to γ-irradiation.
27. Toledo, F. & Wahl, G. M. Regulating the p53 pathway: *in vitro* hypotheses, *in vivo* veritas. *Nature Rev. Cancer* **6**, 909–923 (2006).
28. Takaoka, A. *et al.* Integration of interferon-α /β signalling to p53 responses in tumour suppression and antiviral defence. *Nature* **424**, 516–523 (2003).
29. Takagi, M., Absalon, M. J., McLure, K. G. & Kastan, M. B. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. *Cell* **123**, 49–63 (2005).
30. Galban, S. *et al.* Influence of the RNA-binding protein HuR in pVHL-regulated p53 expression in renal carcinoma cells. *Mol. Cell. Biol.* **23**, 7083–7095 (2003).
31. Mazan-Mamczarz, K. *et al.* RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc. Natl Acad. Sci. USA* **100**, 8354–8359 (2003).
32. Fu, L. & Benchimol, S. Participation of the human p53 3′ UTR in translational repression and activation following γ-irradiation. *EMBO J.* **16**, 4117–4125 (1997).
33. Brooks, C. L. & Gu, W. p53 ubiquitination: Mdm2 and beyond. *Mol. Cell* **21**, 307–315 (2006).
34. Marine, J. C. *et al.* Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. *Cell Death Differ.* **13**, 927–934 (2006).
35. Jones, S. N., Roe, A. E., Donehower, L. A. & Bradley, A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* **378**, 206–208 (1995).
36. Montes de Oca Luna, R., Wagner, D. S. & Lozano, G. Rescue of early embryonic lethality in Mdm2-deficient mice by deletion of p53. *Nature* **378**, 203–206 (1995).
37. Parant, J. *et al.* Rescue of embryonic lethality in *Mdm4*-null mice by loss of Trp53 suggests a nonoverlapping pathway with MDM2 to regulate p53. *Nature Genet.* **29**, 92–95 (2001).
38. Uldrijan, S., Pannekoek, W. J. & Vousden, K. H. An essential function of the extreme C-terminus of MDM2 can be provided by MDMX. *EMBO J.* **26**, 102–112 (2007).
39. Itahana, K. *et al.* Targeted inactivation of Mdm2 RING finger E3 ubiquitin ligase activity in the mouse reveals mechanistic insights into p53 regulation. *Cancer Cell* **12**, 355–366 (2007).
40. Li, M. *et al.* Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature* **416**, 648–653 (2002).
41. Cummins, J. M. *et al.* Tumour suppression: disruption of HAUSP gene stabilizes p53. *Nature* **428**, (2004).
42. Li, M., Brooks, C. L., Kon, N. & Gu, W. A dynamic role of HAUSP in the p53–Mdm2 pathway. *Mol. Cell* **13**, 879–886 (2004).
43. Leng, R. P. *et al.* Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. *Cell* **112**, 779–791 (2003).
44. Dornan, D. *et al.* The ubiquitin ligase COP1 is a critical negative regulator of p53. *Nature* **429**, 86–92 (2004).
45. Yang, W. *et al.* CARPs are ubiquitin ligases that promote MDM2-independent p53 and phospho-p53ser20 degradation. *J. Biol. Chem.* **282**, 3273–3281 (2007).
46. Olsson, A., Manzl, C., Strasser, A. & Villunger, A. How important are post-translational modifications in p53 for selectivity in target-gene transcription and tumour suppression? *Cell Death Differ.* **14**, 1561–1575 (2007).
47. Feng, L., Lin, T., Uranishi, H., Gu, W. & Xu, Y. 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 (2005).
48. Krummel, K. A., Lee, C. J., Toledo, F. & Wahl, G. M. 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 (2005).
49. Huang, J. *et al.* Repression of p53 activity by Smyd2-mediated methylation. *Nature* **444**, 629–632 (2006).
50. Xirodimas, D. P., Saville, M. K., Bourdon, J. C., Hay, R. T. & Lane, D. P. MDM2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. *Cell* **118**, 83–97 (2004).
51. Rodriguez, M. S., Desterro, J. M., Lain, S., Lane, D. P. & Hay, R. T. Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. *Mol. Cell. Biol.* **20**, 8458–8467 (2000).
52. Huang, J. *et al.* p53 is regulated by the lysine demethylase LSD1. *Nature* **449**, 105–108 (2007).
53. O'Brate, A. & Giannakakou, P. The importance of p53 location: nuclear or cytoplasmic zip code? *Drug Resist. Update* **6**, 313–322 (2003).
54. Le Cam, L. *et al.* E4F1 is an atypical ubiquitin ligase that modulates p53 effector functions independently of degradation. *Cell* **127**, 775–788 (2006).
55. Kanai, M. *et al.* Inhibition of Crm1–p53 interaction and nuclear export of p53 by poly(ADP-ribosyl)ation. *Nature Cell Biol.* **9**, 1175–1183 (2007).
56. Stommel, J. M. *et al.* 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 (1999).
57. Fogal, V. *et al.* ASPP1 and ASPP2 are new transcriptional targets of E2F. *Cell Death Differ.* **12**, 369–376 (2005).
58. Hsieh, J. K. *et al.* Novel function of the cyclin A binding site of E2F in regulating p53-induced apoptosis in response to DNA damage. *Mol. Cell. Biol.* **22**, 78–93 (2002).
59. Sarnow, P., Ho, Y. S., Williams, J. & Levine, A. J. Adenovirus E1b-58kd tumor antigen and SV40 large tumor antigen are physically associated with the same 54 kd cellular protein in transformed cells. *Cell* **28**, 387–394 (1982).
60. Zantema, A. *et al.* Localization of the E1B proteins of adenovirus 5 in transformed cells, as revealed by interaction with monoclonal antibodies. *Virology* **142**, 44–58 (1985).
61. Elmore, L. W. *et al.* Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc. Natl Acad. Sci. USA* **94**, 14707–14712 (1997).
62. Takada, S., Kaneniwa, N., Tsuchida, N. & Koike, K. Cytoplasmic retention of the p53 tumor suppressor gene product is observed in the hepatitis B virus X gene-transfected cells. *Oncogene* **15**, 1895–1901 (1997).
63. Li, M. *et al.* Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. *Science* **302**, 1972–1975 (2003).
64. Wang, Y. V. *et al.* Quantitative analyses reveal the importance of regulated Hdmx degradation for p53 activation. *Proc. Natl Acad. Sci. USA* **104**, 12365–12370 (2007).
65. Carter, S., Bischof, O., Dejean, A. & Vousden, K. H. C-terminal modifications regulate MDM2 dissociation and nuclear export of p53. *Nature Cell Biol.* **9**, 428–435 (2007).
66. Andrews, P., He, Y. J. & Xiong, Y. Cytoplasmic localized ubiquitin ligase cullin 7 binds to p53 and promotes cell growth by antagonizing p53 function. *Oncogene* **25**, 4534–4548 (2006).
67. Nikolaev, A. Y., Li, M., Puskas, N., Qin, J. & Gu, W. Parc: a cytoplasmic anchor for p53. *Cell* **112**, 29–40 (2003).
68. Laine, A. & Ronai, Z. Regulation of p53 localization and transcription by the HECT domain E3 ligase WWP1. *Oncogene* **26**, 1477–1483 (2007).
69. Marchenko, N. D., Wolff, S., Erster, S., Becker, K. & Moll, U. M. Monoubiquitylation promotes mitochondrial p53 translocation. *EMBO J.* **26**, 923–934 (2007).
70. Tasdemir, E. *et al.* Regulation of autophagy by cytoplasmic p53. *Nature Cell Biol.* **10**, 676–687 (2008).
71. Mihara, M. *et al.* p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell* **11**, 577–590 (2003).
72. Leu, J. I., Dumont, P., Hafey, M., Murphy, M. E. & George, D. L. Mitochondrial p53 activates Bak and causes disruption of a Bak–Mcl1 complex. *Nature Cell Biol.* **6**, 443–450 (2004).
73. Chipuk, J. E. *et al.* Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* **303**, 1010–1014 (2004).

74. Talos, F., Petrenko, O., Mena, P. & Moll, U. M. Mitochondrially targeted p53 has tumor suppressor activities *in vivo*. *Cancer Res.* **65**, 9971–9981 (2005).
75. Leu, J. I. & George, D. L. Hepatic IGFBP1 is a prosurvival factor that binds to BAK, protects the liver from apoptosis, and antagonizes the proapoptotic actions of p53 at mitochondria. *Genes Dev.* **21**, 3095–3109 (2007).
76. Chen, X., Ko, L. J., Jayaraman, L. & Prives, C. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev.* **10**, 2438–2451 (1996).
77. Weinberg, R. L., Veprintsev, D. B., Bycroft, M. & Fersht, A. R. Comparative binding of p53 to its promoter and DNA recognition elements. *J. Mol. Biol.* **348**, 589–596 (2005).
78. Hofmann, T. G. *et al.* Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. *Nature Cell Biol.* **4**, 1–10 (2002).
79. D'Orazi, G. *et al.* Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser46 and mediates apoptosis. *Nature Cell Biol.* **4**, 11–19 (2002).
80. Taira, N., Nihira, K., Yamaguchi, T., Miki, Y. & Yoshida, K. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. *Mol. Cell* **25**, 725–738 (2007).
81. Oda, K. *et al.* p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell* **102**, 849–862 (2000).
One of the first reports to identify that post-translational modification of a single residue in p53 can direct promoter selectivity.
82. Sykes, S. M. *et al.* Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol. Cell* **24**, 841–851 (2006).
83. Tang, Y., Luo, J., Zhang, W. & Gu, W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol. Cell* **24**, 827–839 (2006).
This article, together with reference 82, recently identified a single residue in the binding domain of p53 that is acetylated, resulting in promoter selectivity and induction of apoptosis.
84. Knights, C. D. *et al.* Distinct p53 acetylation cassettes differentially influence gene-expression patterns and cell fate. *J. Cell Biol.* **173**, 533–544 (2006).
85. Di Giovanni, S. *et al.* The tumor suppressor protein p53 is required for neurite outgrowth and axon regeneration. *EMBO J.* **25**, 4084–4096 (2006).
86. Samuels-Lev, Y. *et al.* ASPP proteins specifically stimulate the apoptotic function of p53. *Mol. Cell* **8**, 781–794 (2001).
One of the first articles to identify a family of cofactors that can regulate the promoter selectivity of p53.
87. Bergamaschi, D. *et al.* iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. *Nature Genet.* **33**, 162–167 (2003).
88. Flores, E. R. *et al.* p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature* **416**, 560–564 (2002).
89. Das, S. *et al.* Hzf determines cell survival upon genotoxic stress by modulating p53 transactivation. *Cell* **130**, 624–637 (2007).
Identifies a new cofactor of p53 that specifically enhances p53 promoter selectivity of cell-cycle-arrest genes.
90. Budhram-Mahadeo, V. S. *et al.* Brn-3b enhances the pro-apoptotic effects of p53 but not its induction of cell cycle arrest by cooperating in trans-activation of *Bax* expression. *Nucleic Acids Res.* **34**, 6640–6652 (2006).
91. Budram-Mahadeo, V., Morris, P. J. & Latchman, D. S. The Brn-3a transcription factor inhibits the pro-apoptotic effect of p53 and enhances cell cycle arrest by differentially regulating the activity of the p53 target genes encoding Bax and p21(CIP1/Waf1). *Oncogene* **21**, 6123–6131 (2002).
92. Fogal, V. *et al.* Regulation of p53 activity in nuclear bodies by a specific PML isoform. *EMBO J.* **19**, 6185–6195 (2000).
93. Zacchi, P. *et al.* The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insults. *Nature* **419**, 853–857 (2002).
94. Zheng, H. *et al.* The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. *Nature* **419**, 849–853 (2002).
95. Mantovani, F. *et al.* The prolyl isomerase Pin1 orchestrates p53 acetylation and dissociation from the apoptosis inhibitor iASPP. *Nature Struct. Mol. Biol.* **14**, 912–920 (2007).
96. Toledo, F. *et al.* Mouse mutants reveal that putative protein interaction sites in the p53 proline-rich domain are dispensable for tumor suppression. *Mol. Cell. Biol.* **27**, 1425–1432 (2007).
97. Ludwig, R. L., Bates, S. & Vousden, K. H. Differential activation of target cellular promoters by p53 mutants with impaired apoptotic function. *Mol. Cell. Biol.* **16**, 4952–4960 (1996).
98. Crook, T., Marston, N. J., Sara, E. A. & Vousden, K. H. Transcriptional activation by p53 correlates with suppression of growth but not transformation. *Cell* **79**, 817–827 (1994).
99. Rowan, S. *et al.* Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. *EMBO J.* **15**, 827–838 (1996).
100. Shikama, N. *et al.* A novel cofactor for p300 that regulates the p53 response. *Mol. Cell* **4**, 365–376 (1999).
Identifies a novel p53 cofactor, which was one of the first to be identified that specifically enhances the apoptotic function of p53.
101. Demonacos, C. *et al.* A new effector pathway links ATM kinase with the DNA damage response. *Nature Cell Biol.* **6**, 968–976 (2004).
102. Demonacos, C., Krstic-Demonacos, M. & La Thangue, N. B. A TPR motif cofactor contributes to p300 activity in the p53 response. *Mol. Cell* **8**, 71–84 (2001).
103. Kitagawa, M., Lee, S. H. & McCormick, F. Skp2 suppresses p53-dependent apoptosis by inhibiting p300. *Mol. Cell* **29**, 217–231 (2008).
104. Tanaka, T., Ohkubo, S., Tatsuno, I. & Prives, C. hCAS/CSE1L associates with chromatin and regulates expression of select p53 target genes. *Cell* **130**, 638–650 (2007).
Identifies a new p53 cofactor that enhances p53-dependent transactivation, even though it binds chromatin independently of p53.
105. Donner, A. J., Szostek, S., Hoover, J. M. & Espinosa, J. M. CDK8 is a stimulus-specific positive coregulator of p53 target genes. *Mol. Cell* **27**, 121–133 (2007).
106. Espinosa, J. M. & Emerson, B. M. Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. *Mol. Cell* **8**, 57–69 (2001).
107. Christophorou, M. A., Ringshausen, I., Finch, A. J., Swigart, L. B. & Evan, G. I. The pathological response to DNA damage does not contribute to p53-mediated tumour suppression. *Nature* **443**, 214–217 (2006).

Acknowledgements
We thank the Ludwig Institute for Cancer Research, the Association for International Cancer Research and the European Union for funding, and G. Bond for critical reading of the manuscript.

Competing financial interests
The authors declare competing financial interests: see web version for details.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Bax | *Fas* | *p53* | *Puma* | *Noxa*
UniProtKB: http://ca.expasy.org/sprot
ASPP1 | ASPP2 | BRN3A | BRN3B | HAUSP | HIPK2 | MDM2 | MDMX | SKP2 | TIP60

FURTHER INFORMATION
Xin Lu's homepage:
http://www.ludwig.ox.ac.uk/research_x_lu.htm
IARC TP53 Mutation Database: http://www-p53.iarc.fr
The p53 Web Site: http://p53.free.fr

ALL LINKS ARE ACTIVE IN THE ONLINE PDF