The evolution of diverse biological responses to DNA damage: insights from yeast and p53

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The cellular response to ionizing radiation provides a conceptual framework for understanding how a yeast check-point system, designed to make binary decisions between arrest and cycling, evolved in a way as to allow reversible arrest, senescence or apoptosis in mammals. We propose that the diversity of responses to ionizing radiation in mammalian cells is possible because of the addition of a new regulatory control module involving the tumour-suppressor gene p53. We review the complex mechanisms controlling p53 activity and discuss how the p53 regulatory module enables cells to grow, arrest or die by integrating DNA damage checkpoint signals with the response to normal mitogenic signalling and the aberrant signalling engendered by oncogene activation.

The outcome of checkpoint-dependent arrest of the cell cycle depends on the type of organism involved. In simple eukaryotes, such as yeast, extrinsic DNA damage causes a transient cell cycle arrest during which DNA damage is repaired. However, cells with irreparable damage may ultimately continue to cycle, which can lead to cell death or the fixation and subsequent inheritance of mutations. The probability of an individual cell remaining viable while incurring harmful mutations may be low, but when multiplied millions of times in a multicellular organism, the threat to survival through generation of variants capable of growing under inappropriate conditions becomes significant. Metazoans have overcome this problem, in part, by increasing the complexity of their checkpoint pathways to insure that somatic genomes that sustain potentially irreparable damage do not give rise to viable variant progeny (Fig. 1).

Damage sensing, signalling, and cell cycle responses in yeast

An exquisitely sensitive damage response system evolved in yeast that detects and responds to a single double-strand break[1] (Fig. 1a). The purpose of the response is to repair DNA damage without fixing a mutation. To this end, yeast cells have integrated DNA repair systems with mechanisms for delaying the cell cycle delay, or 'checkpoints'. The initial definition of the term checkpoint was a "control mechanism enforcing dependency in the cell cycle"[2], but it has become clear that DNA damage checkpoint pathways also control DNA repair and replication processes in addition to imposing cell cycle delay. In yeast, cell cycle delays allow time for DNA repair. Certain mutants (such as *chk1⁻* in *Schizosaccharomyces pombe* and *rad9* in *Saccharomyces cerevisiae*) abrogate this delay and cause radiation sensitivity that can be largely restored by re-imposing a delay by other means[3,4]. However, other mutants such as *rad3⁻* (*S. pombe*) and *mec1* (*S. cerevisiae*) are far more sensitive to damage, because they orchestrate damage responses other than cell cycle delay.

It is unclear exactly how DNA damage is initially sensed. Two large members of the phosphatidylinositol-3-OH kinase (PI(3)K) family, Mec1/Rad3 (*S. cerevisiae*/ *S. pombe*) and Tel1 (*S. cerevisiae* and *S. pombe)* are activated in response to DNA lesions (Fig. 1a). It remains to be seen whether these signalling kinases are activated directly by interaction with damaged DNA/chromatin, indirectly through affiliated repair complexes, or whether a combination of such mechanisms operate. It may be that a combination of activating signals (that is, direct lesion binding and the detection of active repair proteins) is required for an appropriate checkpoint response. In support of this, several repair protein complexes have been implicated in DNA damage sensing and signalling, but not in the activation of the PI(3)-like kinases in response to all types of DNA damage[5]. These include Rad50–Mre11–Xrs2, and the Rad17–Rfc2–5 putative clamp loading complex in association with Hus1–Rad1–Rad9, a proposed checkpoint sliding clamp (Fig. 1a). Although supporting biochemical data has yet to be gathered, it is proposed that the Rad17–Rfc2–5 complex loads PCNA-like Hus1–Rad1–Rad9 complexes onto damaged DNA in much the same way that the Rfc1–Rfc2–5 complex loads PCNA homotrimers during DNA replication[1].

Once DNA damage is detected, the PI(3)-like kinases are capable of phosphorylating multiple replication, repair and checkpoint proteins. Amongst these are two 'downstream' serine-threonine protein kinases that influence cell cycle progression in both yeasts. It is important to note that, although one or more of the PI(3)-like kinases are involved whenever the checkpoint pathways are activated, the downstream target kinase receiving the signal can depend on the point in the cell cycle when damage is induced. For example, in *S. pombe*, damage in S phase leads to activation of the Cds1 kinase, whereas damage induced in late S/G2 activates Chk1(refs 6, 7). However, in *S. cerevisiae*, Rad53 (the Cds1 homologue) is activated by DNA damage at all stages of the cell cycle, although perhaps by different mechanisms.

Irradiation of *S. cerevisiae* cells in G1 activates Rad53, which delays progression through 'start' (equivalent to the restriction point in mammalia n cells) and entry into S phase. This may be achieved, at least in part, by Rad53-mediated phosphorylation of Swi6 (ref. 8). Swi6 is a component of both the SCB binding factor (SBF) and MCB binding factor (MBF) sequence-specific transcription factors. MBF is required to transcribe a range of genes required for S phase entry and DNA replication. Although Swi6 phosphorylation is correlated with downregulation of the G1 cyclins *CLN1* and *CLN2*, mechanistic links to the checkpoint have not been explored. In both yeasts, DNA damage in G2 activates downstream
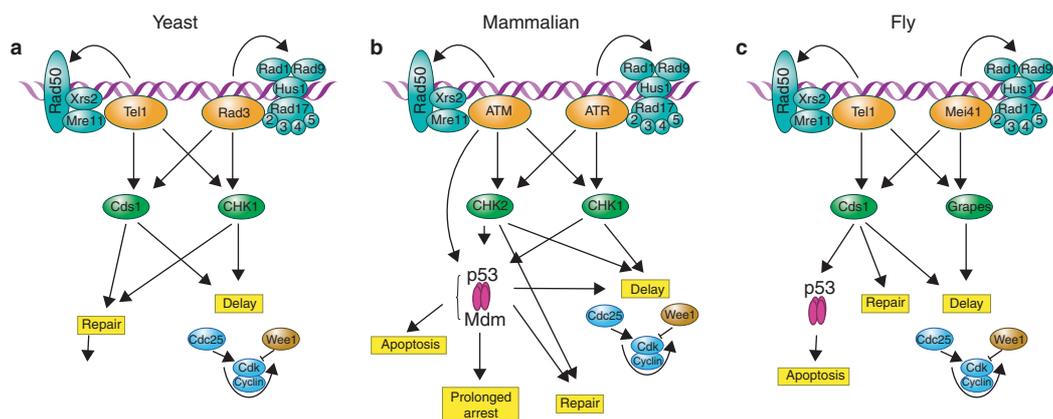
Figure 1 **A schematic representation of the checkpoint pathways in a, yeast, b, *Drosophila* and c, mammalian cells.** For simplicity, the nomenclature for yeast is based on *S. pombe*. The main components of the DNA damage checkpoint are conserved in all three systems. The addition of a p53 module in *Drosophila* seems to have been significantly elaborated during evolution and provides mammalian cells with additional cell fates in response to DNA damage.

kinases (Chk1 in *S. pombe*; Rad53 and Chk1 in *S. cerevisiae*) to induce a mitotic delay. This is achieved by phosphorylating, and thus regulating, key effectors of mitotic onset. The precise targets vary between the two organisms. In *S. pombe*, the key targets of mitotic onset are the regulators of $p34^{cdc2}$. Some controversy still exists over the relative importance of individual proteins, but the data are consistent with a model in which Chk1 regulates the phosphorylation status of $p34^{cdc2}$ Tyr 15 by activating the Wee1/Mik1 kinases and inhibiting Cdc25 phosphatase. In *S. cerevisiae*, the regulation of the $p34^{cdc2}$ homologue, Cdc28, is not required for mitotic delay. This probably reflects the evolutionary changes necessary for the mechanism of cytokinesis associated with budding, but provides us with the opportunity to study alternative mechanisms that may well influence cell cycle delay in other systems, albeit cryptic to $p34^{cdc2}$ phosphorylation. Interestingly, two parallel systems operate in *S. cerevisiae*: Rad53 phosphorylates Cdc5 (a polo-like kinase that regulates the anaphase-promoting complex), whereas Chk1 phosphorylates Pds1, an inhibitor of sister chromosome separation and anaphase[9].

DNA damage response in multicellular eukaryotes involves p53

In metazoans, the DNA damage response pathway involving the p53 tumour-suppressor protein seems to elicit similar consequences to SBF and MBF regulation in yeast. We wondered why the complex regulatory module involving p53 is needed in mammalian cells when the system in yeast, components of which are highly conserved, does such an admirable job of responding to so little DNA damage.

It has been known for almost 40 years that, in contrast to yeast, ionizing radiation can induce both a transient and a prolonged G1 delay in human and rodent fibroblasts[10]. Over the past decade, it has become clear that p53 is absolutely required for prolonged arrest induced by ionizing radiation, but that the transient delay is p53 independent[11–14] (see ref. 15 for a review and references). It is often stated that this lengthy p53-induced G1 arrest allows time for repair, as demonstrated in the yeasts. However, the absence of a sister chromatid to provide a perfect repair template for homologous recombination (HR) would require that less accurate pathways, such as non-homologous end joining (NHEJ), mediate the repair of double-strand breaks (DSBS)[16]. Circumstantial evidence supports the idea that allowing a prolonged time for repair carries risks: DSBS induced by ionizing radiation are repaired by both fast and slow mechanisms, depending on the initial structure of the break. Defects in the slow-repair component correlate with increased chromosomal aberrations, presumably because the slow repair of complex damage is more error prone, and could lead to elevated misrepair rates (see refs 17–19 for reviews). Thus, were p53 to increase the length of time available for repair in G1, it might actually increase the probability of generating variants with structural chromosome aberrations derived from the misrepair of complex lesions. This interpretation is consistent with data showing that p53 does not significantly contribute to repair of DSBS[11] (P. Jeggo, personal communication).

We speculated on the use of p53-induced G1 arrest. Loss of checkpoint-dependent delay generally correlates with radiation sensitivity in yeast. By contrast, primary mammalian cells lacking p53 are typically more radiation-resistant than isogenic cells expressing p53, most likely because p53 activation initiates changes in gene expression that can result in cell elimination[20]. Multicellular organisms can afford to sacrifice cells, and benefit from the reduced chance of accumulating mutations and undergoing uncontrolled proliferation[21,22]. Perhaps one reason the p53 arm of the DNA damage response evolved in metazoans is because it allows cells that have incurred irreparable damage to be taken out of cycle.

The overriding function of p53 is transcriptional induction

p53 is a sequence-specific transcriptional regulator[20,23,24], although whether this is its primary biological function is the subject of debate. Transfection studies with p53 mutants that disable the transactivation domain, and studies with transcription inhibitors, suggest that p53 could induce apoptosis by both transcription-dependent and transcription-independent mechanisms[25–27]. A transcription-independent function might derive from the binding of p53 to mitochondrial proteins, a process that has recently been implicated in the induction of apoptosis under some circumstances[28,29]. On the other hand, p53 clearly induces the transcription of a variety of genes involved in apoptosis, such as: those encoding Bcl2 family members; Bax, PUMA and Noxa, death domain proteins; Fas, DR5 and PIDD, proteins that induce reactive oxygen species; the plasma membrane protein p53 apoptosis effector related to PMP–22 (PERP); the survival factor antagonist insulin-like growth factor binding protein 3 (IGF-BP3); and the apoptosis protease activator apoptotic activating factor 1 (APAF1)[23,30–36]. The complete deficiency of thymic apoptosis after irradiation of transgenic mice[37], or recombinase-activation gene (RAG)-reconstituted mice[38] engineered to express transcriptionally
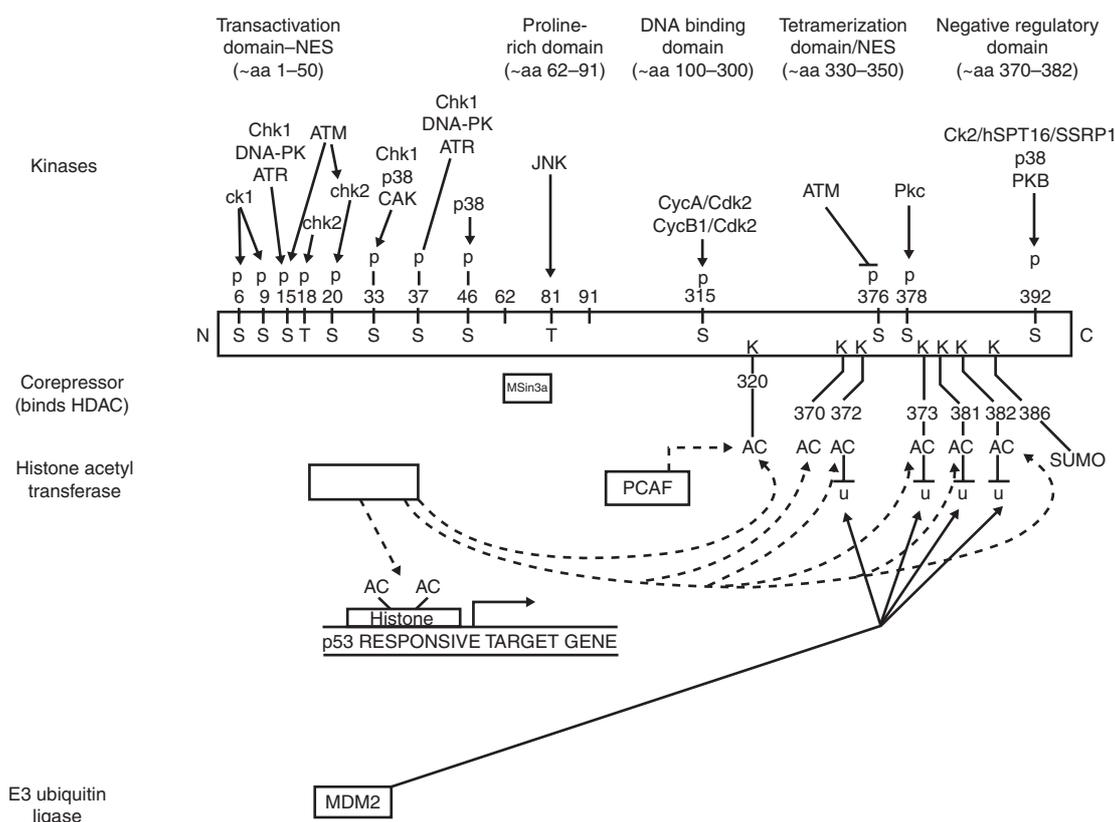
Figure 2 Abbreviated diagram of p53 structural domains, modification sites and coregulators. Structural domains, some modification sites, and coregulators are shown for human p53. This is a simplified version of sites at which p53 is modified by phosphorylation, acetylation, ubiquitination and sumoylation, as described in the text. Some of the enzymes reported to induce modification of the indicated residues *in vivo* and *in vitro* are shown. Residues 373 and 382 are targeted by the histone acetyl transferase p300/CBP (ref. 51), and residues 370, 373, and 381 may also be targets as they are acetylated by p300/CBP *in vitro* (T.P. Yao, personal communication). On the basis of mutagenesis studies, and *in vitro* and *in vivo* transcription analyses, we infer that the major regulatory role of acetylation may be to block MDM2-mediated ubiquitination, and thereby to stabilize p53. Note also that p53 has been reported to bind a corepressor in the proline-rich domain, and this has been reported to be central in p53-mediated repression[81], which seems to be important for responses to stresses, such as hypoxia[81,145]. Some of the many sites of N-terminal phosphorylation are shown, and some reported functional consequences are summarized in Table 1. Recently, JNK has was reported to phosphorylate human p53 at Thr 81 in response to ultraviolet damage, resulting in stabilization and increased transactivation[162].

inactivated *p53* genes, demonstrates that p53-dependent transcriptional regulation is critical for this process. However, as no single gene has emerged to be critical for p53-induced apoptosis, there may be functional redundancy between genes in the apoptotic pathway, or different gene combinations may be required in different tissues. The p53 transactivation domain is also of paramount importance for tumour suppression, as mice containing the transactivation-deficient mutant allele (*p53*QS) developed the same spectrum of tumours, with the same latency, as isogenic mice with a p53-null allele[37] (M. Nister, X.-Q. Sheng, M. Beeche, T. van Dyke, C. Yin and G. Wahl, personal communication). Further evidence of the importance of transcriptional regulation in p53 function comes from observations that diverse p53-dependent cellular responses to various forms of DNA damage require transcriptional targets of p53 (refs 24, 28, 29, 37, 39–46). In conclusion, the data strongly suggest that p53 mediates the majority of its biological functions through its transactivation domain.

Controlling p53 abundance, localization and binding

Much research on p53 has centred around the mechanisms that regulate its transcriptional activity[20,23,24,47–50]. As p53 is capable of dealing a deathblow to cells, it is not surprising that sophisticated pathways have evolved to limit the abundance of p53, and to prevent its conversion into a nuclear species capable of binding DNA and activating or repressing target genes (Fig. 2). However, as a key stress-response protein, rapid activation of p53 is essential. Both of these goals are achieved by rapid turnover of the protein. Furthermore, the abundance and capacity of p53 to bind specific target sequences seems to be regulated predominantly, if not exclusively, by post-transcriptional mechanisms[12]. Four important p53 regulators have emerged thus far: mouse double minute 2 (MDM2), a ring-finger ubiquitin ligase that is both a p53 target gene and the main determinant of p53 stability; alternative reading frame product (ARF), an inhibitor of MDM2 ubiquitin ligase function; Ataxia-telangiectasia mutated (ATM) and AT- and Rad3- related (ATR) large PI(3)-like kinases whose relatives are involved in the DNA damage response in all eukaryotes analysed thus far, as well as Chk2, a kinase that is a target for ATM; and histone acetyl transferases such as p300/CBP[51,52].

p53 binds to its response elements best as a tetramer[53,54], suggesting that its intranuclear concentration may profoundly affect binding kinetics. One factor controlling nuclear abundance involves the export of p53, mediated by a nuclear export sequence (NES) located in its tetramerization domain[55]. A second putative NES in the amino-terminal transactivation domain was identified

Table 1 Some modifications implicated in p53 activation and inactivation

Site	Modifcation	Inducers*	Possible consequences
Ser 6, 9	P	Chemotherapy	–
Ser 15	P	IR, UV, Taxol	Reduce MDM2 binding. Increase co-activator binding. C-terminal acetylation. Histone acetylation of p53RE, open chromatin. Reduce MDM2 binding, increase stability
Ser 20	P	IR, UV	Reduce MDM2 binding. Increase co-activator binding. C-terminal acetylation. Histone acetylation of p53RE, open chromatin. Increase stability
Ser 33, 37	P	IR, UV	Increase transcriptional activation
Ser 46	P	UV (severe damage)	Increase apoptosis
Thr 81	P	UV	Stabilization, increased transactivation
Ser 315	P	–	Nuclear localization. Oligomerization. Modulate target gene selection
Lys 320	Ac	IR, UV	?
Lys 370, 372, 373, 381, 382	Ac	Many stresses	Block ubiquination. Stabilize p53
Lys 370, 372, 373, 381, 382	Ub	–	Required for p53 degradation
Ser 376	P	IR	14-3-3 binding. Increase p53RE binding
Ser 378	De-P'n	IR	14-3-3 binding. Increase p53RE binding
Ser 392	P	UV	Increase tetramerization. Increase p53RE binding and transactivation

* IR, ionizing radiation; UV, ultraviolet radiation; P, phosphorylation; Ac, acetylation; Ub, ubiquitination; De-P'n, Dephosphorylation.

recently[35], but it seems weaker than the NES in the tetramerization domain, as mutation of the latter leads to a predominantly nuclear localization[55–57]. The crystal structure of the p53 tetramerization domain predicts that its NES should be exposed to the export receptor (probably CRM1) in monomers and dimers, but should be occluded in p53 tetramers[55]. This provides a mechanism for tight linkage between p53 structure and function: factors that increase p53 abundance and enhance tetramer formation should increase both p53 nuclear concentration and engagement with p53 response elements.

MDM2 is a key regulator of p53 stability[58–62]. The early embryonic lethality of *MDM2*-null mice, and the complete rescue of this phenotype by p53 deficiency[63,64], demonstrates the importance of MDM2 in p53 regulation. MDM2 binds to p53 in a defined region of the N terminus, which also comprises the p53 transactivation domain[65–68]. A mouse strain expressing the transactivation-deficient mutant allele *p53QS*, which does not bind MDM2, exhibits high levels of p53QS protein in unstressed cells, and is not elevated further by DNA damage[37,38]. This confirms MDM2 as the key regulator of basal and induced levels of p53 *in vivo*, but does not preclude contributions from other pathways, such as JNK kinase or calpain-like proteases[69,70], under specific conditions[71].

It has been proposed that MDM2 inactivates p53 by mechanisms other than degradation. For example, because MDM2 binding occludes the transactivation domain[65,66,72], it may physically prevent association with co-activators. Mutation of Lys 372, 373, 381, 382 to Ala, or Lys 370, 372, 373, 381, 382 and 386 to Arg, in the p53 carboxy-terminal negative regulatory domain, produced p53 that bound MDM2 but showed little, if any, ubiquitination, was refractory to degradation, and retained full transcriptional activity even in the presence of high amounts of MDM2 (refs 73, 74). These observations suggest that MDM2 may act primarily as a cofactor for p53 degradation. However, as some MDM2 mutants ubiquitinate p53 and down-regulate its function without inducing degradation, it is possible that MDM2 may antagonize p53 by additional mechanisms[75].

The N terminus of p53 binds the histone acetylases p300/CBP and pCAF, which acetylate the C terminus of p53 on lysines target-ed by MDM2 for ubiquitination[76–78] (Fig. 2; T.P. Yao, personal communication). This sets up potential competition between p300/CBP and MDM2. Acetylation was initially proposed to enhance DNA binding and transactivation; however, a p53 mutant in which Lys 370, 372, 373, 381 and 382 were changed to arginine was not detectably acetylated *in vitro*[52] (T.P. Yao, personal communication). Other studies demonstrated that similar mutants bound p53 response elements and transactivated target genes with an efficiency similar or identical to that of wild-type p53 (refs 73, 74). Mouse p53QS is also not acetylated, yet it binds p53 response elements constitutively[37,38]. Furthermore, *in vitro* analyses of p53 binding to chromatin showed that C-terminal acetylation is not required for p53 to bind its response element[52], although p300/CBP acetyl transferase activity is required for p53-mediated transactivation. Taken together, these data suggest a model in which recruitment of p300/CBP to the N terminus of p53 leads to C-terminal acetylation, which negatively affects MDM2-mediated ubiquitination. This should increase p53 stability, and generate more of the active nuclear-restricted tetramer. Furthermore, p300/CBP bound to p53 acetylates histones in p53 response elements, leading to chromatin modifications needed for p53 transactivation[52].

There will be more to come concerning the regulation of p53 stability and function by MDM2. For example, MDM2 mono-ubiquitinates p53 at multiple lysines, but polyubiquitination is generally required for optimal proteasome-dependent degradation[79]. Thus, it is likely that another factor, perhaps an E4 (which promotes poly-ubiquitination), will be required for polyubiquitination of p53, or, alternatively, something may induce a conformational change in MDM2 to enable it to add multiple ubiquitins. Perhaps this relates to recent studies showing that residues between the N-terminal MDM2 binding domain and the central DNA binding domain also contribute to MDM2-mediated p53 degradation[80], and that binding of the corepressor mSin3a, to the PXXP domain, adjacent to the N-terminal transactivation domain, in some way interferes with the ability of MDM2 to induce p53 degradation[81]. It is also possible that MDM2 requires a cofactor for degradation, as mutants in the PXXP region of p53 can be ubiqui-

a

Keeping the p53 pathway inactive during
normal mitogenic signalling

Ras

Raf → JunD

MAPK → ARF

???

AP1/Ets 1 — MDM2

↑MDM2 → p53

p53^{UbUbUb}

p53

Two models for oncogene-mediated
activation of p53

b

Hyperproliferative growth stimulus
without DNA damage

Activated Ras, Raf, Myc, etc

CycD, CycE kinase   DAP kinase

Rb^{ppp}

Rb E2F →
+
E2F → APAF1 and other
apoptosis genes

↑ARF   ↑p53 → Senescence genes

MDM2

c

Oncogenic signalling generates
DNA damage

Activated Ras, Raf, Myc, etc.

ROS
(high or sustained levels)

DNA damage

ATM, ATR, other?

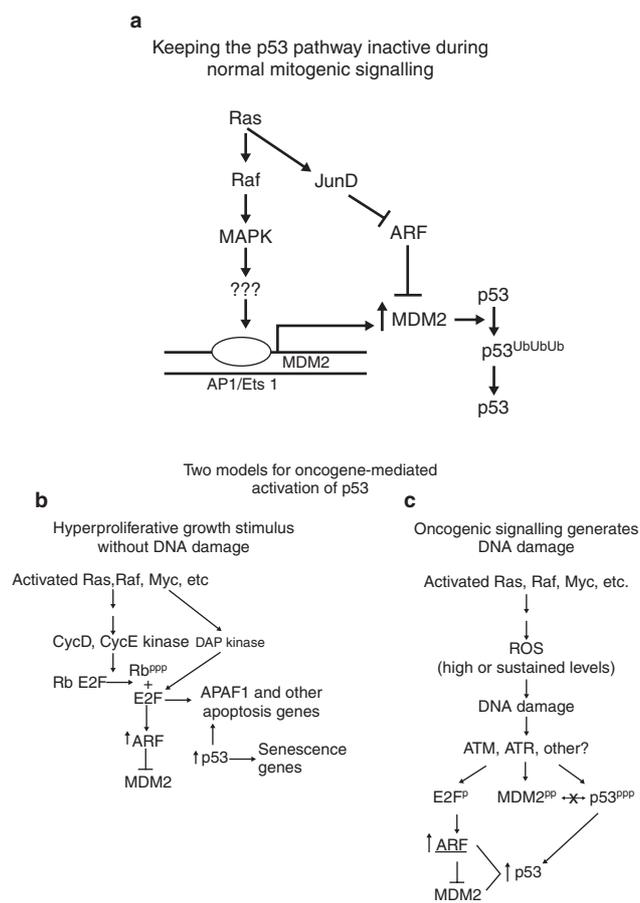E2F^p MDM2^{pp} ⊷×⊷ p53^{ppp}

↑ARF   ↑p53

MDM2

Figure 3 **Mechanisms for p53 activation by activated oncogenes. a,** During normal mitogenic signalling, p53 remains inactive. In response to oncogene activation, the available data suggest two mechanisms by which oncogenes might activate p53. The first, **(b)** proposes that oncogenes activate p53 independently of DNA damage, while the second, **(c)** suggests that high-level oncogene expression, such as occurs in tumours, can generate reactive oxygen species (ROS), which induce DNA damage and the activation of checkpoint signalling pathways (see text for more details).

tinated but are not degraded[80], and deletion of the acidic domain of MDM2 enables p53 ubiquitination, but not degradation[82]. Another interesting possibility is that the MDM2-related protein MDM4 (also called MDMX) participates with MDM2 in p53 degradation, as an MDM4-knockout mouse produced early embryonic lethality, which, like MDM2 deficiency, was rescued by deletion of p53 (ref. 83). Furthermore, MDM2 can bind p300/CBP[84] which reportedly inhibits its acetyltransferase activity. Finally, some splice variants of the p53-related protein p63, may compromise p53 function by acting as dominant negative mutants[85–87]. It is conceivable that their expression in certain tissues may titrate p53 function.

Regulation of the ubiquitin ligase function of MDM2

MDM2-mediated ubiquitination of p53 is controlled by ATM-dependent mechanisms that regulate the ability of MDM2 to bind p53, and also by mechanisms that control the ubiquitin ligase function of MDM2. ATM-dependent phosphorylation of human MDM2 on Ser 395 is one of the most rapid responses to ionizing radiation, and may compromise p53 degradation[88]. An important inhibitor of MDM2 ubiquitin ligase is ARF, a small protein generated from the CDKN4A locus, the same region that encodes the

CDK4 inhibitor p16 (ref. 89). It was initially proposed that ARF inactivates MDM2 by translocating it to the nucleolus, to physically separate it from p53 (ref. 90). However, a recent study shows that an ARF mutant that cannot induce MDM2 nucleolar localization still prevents MDM2 from inducing p53 degradation[91]. As ARF can inhibit the ubiquitin ligase function of MDM2 *in vitro*, inhibition may be sufficient *in vivo* to account for its ability to antagonize MDM2-mediated p53 degradation[92]. Another way that ARF may activate p53 is by reducing the capacity of MDM2 to inhibit the acetyltransferase activity of p300/CBP, thereby enabling increased acetylation of p53 and the chromatin in its response elements, resulting in enhanced p53 activation.

ARF is clearly an important component of the p53 regulatory mechanism as ARF-null mice exhibit almost the same tumour predisposition as *p53*-null mice[93,94]. Furthermore, ARF deficiency, like p53 deficiency, rescues ATM-deficient mouse fibroblasts from premature senescence, and *in vitro* immortalization of mouse fibroblasts can be achieved by loss of p53 or ARF[95]. The ability of ARF deficiency to overcome premature senescence in ATM-deficient fibroblasts is noteworthy. Premature senescence is thought to result from intrinsic DNA damage caused by ATM-associated defects in DNA metabolism, including telomere shortening, or from excessive production of reactive oxygen species[93,94,96]. Thus, just as p53 deficiency mitigates the premature senescence resulting from ATM deficiency through the inability of doubly deficient cells to activate the senescence programme, ARF deficiency may similarly compromise p53 induction in response to DNA damage. Consistent with this model, mouse and human ARF are induced by ionizing radiation (ref. 97, and S. Khan and D. Parry, personal communication), although it is not known whether this is process is ATM dependent. Whether ARF is important in the DNA damage response has not been resolved, however, because one analysis showed that ARF-null mouse embryonic fibroblasts (MEFs) exhibit an arrest deficiency almost equivalent to that of *p21*-null MEFs[97], whereas a second study suggested that *ARF*-null MEFs have a normal radiation-induced arrest response[98].

The balance between ATM (see below), ARF, MDM2 and p53 is likely to be critical in preventing genomic instability during tumour progression. Thus, breeding of telomerase-deficient mice to have shortened telomeres reduced tumorigenicity, presumably because the eroded telomeres in hyperproliferative clones trigger a p53-dependent cell elimination programme. Breeding telomerase-deficient short-telomere mice with p53-deficient mice generates animals that exhibit a spectrum of epithelial tumours with genomic instability[99–101]. In part, this instability derives from the fusion of eroded telomeres, and subsequent bridge-breakage-fusion cycles of the resulting dicentric chromosomes. In contrast, breeding the short-telomere mice with ARF-null mice actually seems to suppress tumorigenicity. Because the ARF-null mice contain functional p53 and ATM, it is likely that the damaged chromosomes can readily activate p53 by this route. In this context, it is important to recall that ARF-null MEFs have a residual or intact p53-mediated DNA damage response (depending on the data set quoted), which is apparently sufficient to eliminate genetically aberrant cells *in vivo*. As aberrant telomeres can signal through ATM to p53 to induce senescence or apoptosis[102], it may be that eroded or dysfunctional telomeres deliver such a robust signal through ATM or ATR, that p53 can be activated even in the absence of ARF. Alternatively, telomere activation of p53 may work independently of ARF.

ATM kinase function regulates p53 abundance by multiple mechanisms

It is now clear that homologues of the large PI(3)-like kinase characterized in yeast, one of which is mutated in patients with ataxia-telangiectasia, are critical in p53 activation[103]. Ataxia-telangiectasia is a progressive neurodegenerative disease. Patients with the disease also exhibit a predisposition to cancer and extreme cellular

radiosensitivity. ATM kinase affects the p53–MDM2 interaction in several ways.

First, ATM directly phosphorylates p53 Ser 15 (refs 103–105) (also note that Ser 15 is phosphorylated by DNA-PK[106] and ATR[107]), which is within the N-terminal transactivation domain. Phosphorylation of Ser 15 enables the recruitment of co-activators, such as the histone acetylases p300 and pCAF[108] and interferes with MDM2 binding[109]. p300 can modify the C-terminal lysine residues targeted by MDM2, but a mouse mutant with a Ser 18→Ala substitution (equivalent to human Ser 15) underwent normal C-terminal acetylation[110]. This suggests that either Ser 18 phosphorylation does not recruit acetylases that modify the C terminus, or that the mouse and human systems are significantly different.

Second, DNA damage induced by ionizing radiation also results in ATM-dependent activation of Chk2, the human orthologue of the yeast Rad53/Cds1 kinase[111–114]. Activated Chk2 phosphorylates Ser 20 of p53, which is within the MDM2-binding region. Phosphorylation of Ser 20 reduces the binding of MDM2 in cultured cells, and a Ser 20→Ala mutation generates less-stable p53 (refs 115, 116). The importance of Chk2 in p53 function is indicated by the finding of a Li-Fraumeni-like cancer predisposition in humans with wild-type p53 but a defective CHK2, and of defective p53-dependent cell cycle and apoptotic responses in mouse cells deficient in Chk2 (ref. 117). Interestingly, p53 stability and transactivation were not altered in a mutant in which most serines known to be targets for phosphorylation were mutated to alanine[118]. Importantly, Ser 20 was not among those residues mutated, consistent with its probable importance in activation by ionizing radiation.

Third, ATM-dependent dephosphorylation of p53 Ser 376 has also been reported[119]. This creates a binding site for a 14-3-3 protein, and has been correlated with p53 activation.

Fourth, a recent study provides yet another way by which ATM kinase function contributes to p53 activation. ATM was shown to phosphorylate and stabilize E2F-1 in response to DNA damage, and this stabilization was required for p53 to induce apoptosis in thymocytes[120]. Although the precise mechanism has not been elucidated, a clue is provided by the observation that E2F-1 can activate the ARF promoter[121–123]. It is possible that damage-induced E2F-1 stabilization could increase p53 levels through ARF-mediated inhibition of MDM2 ubiquitin ligase. This would be consistent with the ability of DNA damage to increase ARF abundance, and of the efficiency of the arrest induced by ionizing radiation being dependent on ARF[97]. However, as Chk2- and p53-deficient thymocytes are equally defective in apoptosis, mechanisms controlling Chk2 function (ATM, and perhaps others) may predominate in the activation of p53-dependent apoptotic responses in the thymus, and perhaps other tissues. Given the myriad ways that ATM interacts with p53, it is surprising that cells devoid of ATM function still accumulate p53, although with delayed kinetics[124,125]. It may be that the related ATR kinase, which is primarily involved in the response to ultraviolet radiation, can partially complement ATM. It is also unclear why the late accumulation of p53 in G1 is insufficient to induce a prolonged G1 arrest. However, previous studies have shown that p53 must be activated before the restriction point to induce an arrest in G1 (refs 11, 126). It may be that the delay to p53 activation in ATM-deficient cells comes too late in G1 for it to have an effect on inactivation of Rb, the most probable downstream target to affect progression from G1 into S phase.

Arrest or apoptose: how to choose?

The addition of p53 and its group of regulatory factors to the DNA damage response pathway allows multiple cell fate decisions. The importance of cell fate choices in a metazoan is likely to be fundamental. For example, stromal fibroblast–epithelial cell interactions are required to enable epithelial cells to proliferate and to generate the correct tissue architecture. Removal of epithelial cells from the extracellular matrix induces anoikis, a form of cell death[127]. It is

possible, therefore, that eliminating fibroblasts would induce anoikis in the overlying epithelium. It is tempting to speculate that the higher threshold for induction of apoptosis in human fibroblasts in cell culture might reflect a tissue-protective effect *in vivo*. Thus, in human fibroblasts in cell culture, ionizing radiation does not induce apoptosis, but rather a senescence-like arrest, which is most efficient when cells are irradiated in G0/G1, and may be mitigated in part by growth conditions[11,128–130]. Interestingly, tissue injury induced by balloon catheterization has been linked to the induction of premature senescence, predominantly in vascular smooth muscle cells *in vivo*[131]. This suggests that in some settings, maintaining cells rather than eliminating them by apoptosis may be advantageous. Clearly, additional studies are required to determine whether radiation induces premature senescence in particular cell types *in vivo*, and are necessary to infer a biologic advantage to this response. By contrast, it is clear that DNA damage induces apoptosis *in vivo* by a mechanism that requires p53 (refs 21, 22). This may have a developmental function in thymocytes, as the programmed DNA breaks that arise during V(D)J recombination in T cell development trigger a p53 response which eliminates thymocytes that fail to generate a functional pre-T receptor[132,133]. Signalling from this receptor through CD3 is reported to block p53-dependent apoptosis, contributing to the survival of cells that have completed successful rearrangement[132,133].

How does p53 activation effect apoptosis in some cell types and premature senescence in others? This important question remains unanswered, but we will raise several possibilities. First, p53 activation could generate apoptotic and cell cycle arrest responses in all tissues, with the outcome being determined by the intensity of survival signalling in the target cell[134]. Second, expression analysis suggests that p53 regulates a core set of genes ubiquitously, but also regulates a significant subset in a manner specific to cell type. It is possible that different chromatin structures are established in each p53 target gene during development, thereby predetermining those capable of responding in the mature tissue. It is also possible that p53 may bind with different affinities to target genes in different cells, either because of the presence of competing transcriptional regulators, or because of cell-type-specific co-activators. Recent analyses by chromatin immune precipitation (ChIP) demonstrated that p53 bound with similar affinities to the p21 and MDM2 promoters (ubiquitous targets), but with significantly less affinity to the pro-apoptotic PIG3 promoter[135]. Activation of the *PIG3* gene seemed to occur after significant dissociation of p53, suggesting that recruitment of other factors, or modification of the promoter by p53 binding, may underlie activation of this gene. *In vitro* studies also reveal that different p53 response elements in the same upstream control region may exhibit different requirements for p53 binding and activation[52].

An additional layer of control over transcriptional profiles may result from the effects of different post-translational modifications on p53–DNA interactions, and/or the regulation of p53 stability. p53 has many potential phosphorylation and acetylation sites, and modification by different kinases can influence which target genes are activated[32,136,137]. Although it remains to be determined how promoter binding and chromatin modification will be affected by p53 modification, ChIP analysis revealed that at least a fraction of the p53 bound to each of the promoters analysed was phosphorylated at Ser 15, and acetylated at Lys 382, and that acetylation seemed to differ depending on the way in which DNA damage was induced[135]. *In vitro* studies demonstrated that recruitment of p300 to a promoter by p53 resulted in histone acetylation over an extended region of the promoter, which correlated with transcriptional activation by p53 (ref. 52).

p53 instigates a time course of transcriptional activation and repression after DNA damage. Indeed, some genes are unaffected until many hours after the damaging event. It is likely that the length of time for which p53 is in its stable, active form, coupled with its differential affinity for particular gene promoters, may

affect the time course of p53-dependent expression in a particular cell, and help determine the final outcome. Similarly, the biological effects of p53 may depend on the balance of p53-dependent gene activation and repression. It will also be important to establish how the network of p53-regulated genes affect the regulation of the response itself, and how they interact with other transcription response pathways in a cell-type-dependent manner. More sophisticated microarray and ChIP analyses are required to provide significant insight into these issues.

One hint of how the regulation of p53 may impact on the types of responses that it can generate comes from analyses of p53 in *Drosophila melanogaster*. *Drosophila* is the most ancient eukaryote to date to be identified with a p53 homologue[138–140]. *Drosophila* p53 lacks an N-terminal regulatory domain, and a C-terminal NES, both of which are conserved in other metazoans as distantly related as squids and humans. Unlike mammalian p53, which, in some cell types can induce permanent arrest or apoptosis in response to DSBS, *Drosophila* p53 seems capable of inducing only apoptosis[138–140] (Fig. 1c). Furthermore, although *Drosophila* contains an ATM-related gene (*Mei41*), thus far they have not been reported to contain homologues for MDM2 or the MDM2 inhibitor ARF[141,142].

One intriguing possibility is that the limited DNA damage response range of *Drosophila* derives from the ability to effect only binary on–off changes in the functional status of p53. By contrast, in mammalian cells, p53 function is controlled at multiple levels, which we speculate may allow more finely tuned and diverse responses to be generated and controlled in accordance with the cellular environment. Thus, activation of p53 often results in collateral activation of MDM2, but their ability to interact can be affected by post-translational modifications. Furthermore, in response to some stresses, like extreme ultraviolet damage, MDM2 is induced only after a long lag period[48]. This enables p53 to attain higher levels for longer times, which may be required for activation of an apoptotic programme. Other stress conditions seem to induce MDM2 activation soon after p53 itself is activated, resulting in a 'bait and prey' negative feedback loop[143]. This may limit the amount of functional p53, or the time that it is active, resulting in a gene expression spectrum that elicits a less drastic biological response, such as transient arrest.

Hypoxia and the genesis of repression through corepressor recruitment

We have focused on the activation of p53 by DNA damage, but it is also clear that other stresses increase p53 activity by a variety of mechanisms that interfere with MDM2–p53 interactions, or that affect MDM2 abundance[144,145], or ubiquitin ligase activity. Stresses like microtubule depletion and ribonucleotide pool depletion activate p53, to induce an arrest that is readily reversed when the offending condition is eliminated[50]. Hypoxia also activates p53, and can produce reversible G1 arrest, presumably to prevent cells from entering S phase, when they are more sensitive to low oxygen[146]. Understanding the response to hypoxia is of great clinical significance, as tumours with high hypoxic fractions are more refractory to radiotherapy[147]. Hypoxia activates p53 by a mechanism that involves transcriptional downregulation of MDM2 and other p53-responsive genes[145]. Importantly, hypoxia does not result in C-terminal acetylation[145]. In contrast to DNA damage and many other stresses that activate p53, hypoxia seems to reduce the association between p53 and p300 and promote the association of p53 with the corepressor mSin3A[81,145]. Thus, hypoxia provides an example of how p53 accumulation is achieved by a mechanism that involves neither activation of kinases nor association with histone acetylases, and results in a different downstream transcriptional programme and subsequent biological responses, through the recruitment of corepressors. Interestingly, hypoxia can make some oncogenically transformed cells sensitive to p53-dependent apoptosis. Consequently, cells that have lost p53 are highly selected, as they resist hypoxia-induced apoptosis[146].

Conflicts between oncogenic signalling and p53

Oncogenes can also trigger the p53 pathway, and this creates a potential problem for normal mitogenic signalling, as persistent high-level activation of genes such as *Ras*, *Raf*, and c-*Myc*, can all induce p53-dependent senescence or apoptosis, depending on the cell type and precise conditions employed[148–151]. By contrast, expression of the same genes in p53-deficient cells promotes unregulated growth and can lead to genetic instability[152–155].

How can p53 produce an irreversible arrest in the presence of excessive oncogene signalling, whereas the same genes activated by normal mitogenic cues promote cell cycle progression (Fig. 3)? It has been proposed that high-level oncogene signalling generates hyperproliferative signals that activate ARF, resulting in MDM2 ubiquitin ligase inhibition, p53 increase, nuclear accumulation and target gene activation. Although this response has been proposed to occur in the absence of DNA damage, the permanent removal of cells from the cell cycle would seems to be inconsistent with other damage-independent consequences of p53 activation (such as reversible arrest). One observation used to justify the conclusion that oncogenic signalling occurs in the absence of DNA damage is the apparent absence of p53 Ser 15 phosphorylation in response to adenovirus E1a expression[149]. However, under certain conditions, DNA damage can also fail to trigger phosphorylation of p53 at Ser 15, and may induce dephosphorylation at Ser 20 (ref. 156). The ability of E2F-1 to transcriptionally activate ARF, and of some oncogenes to activate E2F-1, provides one way by which oncogenes could activate p53 in the absence of overt DNA damage. Furthermore, a recent study demonstrated that activation of c-Myc or E2F-1 could induce DAP kinase to produce an apoptotic response that requires ARF-dependent activation of p53, and that this response suppresses oncogene-induced transformation[157]. It is noteworthy that p53 and ARF were still induced by c-Myc and E2F-1 in the absence of DAP kinase (albeit to lower levels) suggesting that additional means exist by which c-Myc and E2F-1 can activate them.

Why should oncogene activation incite a response that results in cell removal if DNA damage has not been inflicted? Perhaps this provides a way of ensuring that variant cells with aberrant mitogenic programmes do not enter the cycle when the normal developmental programme requires differentiation. A more conservative response to limit the emergence of such variants would be to activate an arrest that would be reversed, pending mitigation of the oncogenic signal. On the other hand, if the supraphysiologic levels of oncogene expression, induced experimentally or resulting from amplification and other chromosomal changes in human cancers, induced DNA damage, such draconian consequences would be consistent with other damage-induced p53 responses. The DNA damage-induced phosphorylation and stabilization of E2F-1 by ATM[120] could explain the aforementioned involvement of ARF in the DNA damage response[97]. However, this still leaves open the question of whether oncogenes can induce DNA damage, and if so, by what mechanism.

One intriguing possibility is that normal physiologic processes involved in mitogenic responses can generate DNA damage, when carried to the extremes encountered during signalling by activated oncogenes. Consistent with this proposal, activated Ras has been reported to induce reactive oxygen species[158], and over-expressed Ras, c-Myc, and c-Mos can generate chromosomal damage[152–154]. In contrast to the consequences of persistent, high-level oncogene signalling, recent data have shown that a conditionally activated Raf mutant actually led to MDM2 induction, mitigation of the p53 response, and cell cycle progression[159]. Ras can also activate JunD, which in turn can inhibit ARF expression[160]. Thus, the complex regulatory circuitry that impinges on p53 function provides opportunities to integrate signals from diverse growth regulatory and DNA damage pathways. The result is a type of 'molecular rheostat' with the potential to activate

different downstream responses depending on the types, duration and amplitude of the input signals.

Summary and Perspective

The evolution in unicellular eukaryotes of the prevention of inappropriate cell cycle progression through the activation of checkpoints in response to genotoxic stress allows 'time for repair' before the initiation of S phase and mitosis, and thus prevents cell death. Cells damaged within S phase also slow replication to co-ordinate it with repair[161]. Multicellular systems have different priorities. A dead cell can be tolerated, but one proliferating inappropriately may kill the organism. In fact, too much time for repair of complex lesions, which are repaired with an intrinsically low fidelity, may well be suppressed. Therefore, checkpoints in multicellular organisms cannot be automatically equated with 'arrest for repair'. Instead, they may allow additional cell fate decisions, such as apoptosis or senescent-like G1 arrest, to be made.

The basic DNA damage sensing and signal transduction mechanism (that is, ATM, ATR, Chk1 and Chk2) is conserved between uni- and multicellular organisms, and is adequate to mediate 'time for repair' delays. The capacity for cell elimination in response to DNA damage apparently involves insertion of a control module involving p53, to transactivate downstream apoptotic and/or cell cycle regulators. To date, the most rudimentary version of this module examined is found in *Drosophila*, where a stripped-down p53 is present that lacks intrinsic control sequences such as the NES in the tetramerization domain, as well as extrinsic regulators such as MDM2 and ARF. Mammalian cells employ p53 to respond to DNA damage, as well as stresses and the presence of regulatory proteins that may or may not induce DNA damage, such as oncogenes that drive normal cell division but also have the potential to fuel malignant progression. This requires judgements to be made concerning whether an arrest should be reversible or senescent-like, or whether a cell death programme should be activated.

Understanding the mechanisms that regulate p53, and identifying the diverse target genes it regulates, will enable us to begin to formulate hypotheses to answer a number of long-standing fundamental questions. Do irreparable DNA damage, eroded or abnormally structured telomeres, or oncogenes activate p53 in unique ways to enable the regulation of a subset of target genes that induce apoptosis or senescence? On the other hand, does the clue to the phenotypic consequences of p53 activation lie not in p53 itself, but in its coregulators, ATM, Chk2, MDM2 and ARF, and its ability to associate with co-activators or corepressors? Could it be that their contributions affect the amount of p53 present, the length of time it spends in the nucleus, or the precise ways in which the chromatin of the target gene is modified? Clearly, the impact of these factors could profoundly influence the array of target genes that p53 binds and regulates. Is it possible that the degeneracy of the p53 response elements that control target gene expression determine the kinetics with which they are activated? It is tempting to speculate that all of these factors generate a complex regulatory circuitry that enables this single protein to produce different responses to the same stress in different tissues. Sophisticated genetic manipulations of yeast, flies, mice and other model systems, combined with microarray analyses, bioinformatics and proteomics are likely to provide insight into some of these important issues. In the end, studies initiated to understand the cellular responses to ionizing radiation and the molecular underpinnings of the DNA damage response may reveal how p53 evolved, as well as providing a robust model for understanding how complex regulatory networks are controlled. □

1. Lowndes, N. F. & Murguia, J. R. Sensing and responding to DNA damage. *Curr. Opin. Genet. Dev.* **10**, 17–25 (2000).
2. Hartwell, L. H. & Weinert, T. A. Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629–634 (1989).
3. Al-Khodairy, F. *et al.* Identification and characterisation of new elements involved in checkpoints and feedback controls in fission yeast. *Mol. Biol. Cell* **5**, 147–160 (1994).
4. Weinert, T. A. & Hartwell, L. H. The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae. Science* **241**, 317–322 (1988).
5. Edwards, R. J., Bentley, N. J. & Carr, A. M. A Rad3–Rad26 complex responds to DNA damage independently of other checkpoint proteins. *Nature Cell Biol.* **1**, 393–398 (1999).
6. Lindsay, H. D. *et al.* S-phase-specific activation of Cds1 kinase defines a subpathway of the checkpoint response in *Schizosaccharomyces pombe. Genes Dev.* **12**, 382–395 (1998).
7. Martinho, R. G. *et al.* Analysis of Rad3 and Chk1 protein kinases defines different checkpoint responses. *EMBO J.* **17**, 7239–7249 (1998).
8. Sidorova, J. M. & Breeden, L. L. Rad53-dependent phosphorylation of Swi6 and down-regulation of CLN1 and CLN2 transcription occur in response to DNA damage in *Saccharomyces cerevisiae. Genes Dev.* **11**, 3032–3045 (1997).
9. Sanchez, Y. *et al.* Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science* **286**, 1166–1171 (1999).
10. Little, J. B. Delayed initiation of DNA synthesis in irradiated human diploid cells. *Nature* **146**, 1064–1065 (1968).
11. Di Leonardo, A., Linke, S. P., Clarkin, K. & Wahl, G. M. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev.* **8**, 2540–2551 (1994).
12. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* **51**, 6304–6311 (1991).
13. Little, J. B. & Nove, J. Sensitivity of human diploid fibroblast cell strains from various genetic disorders to acute and protracted radiation exposure. *Radiat. Res.* **123**, 87–92 (1990).
14. Weichselbaum, R. R., Epstein, J. & Little, J. B. *In vitro* radiosensitivity of human diploid fibroblasts derived from patients with unusual clinical responses to radiation. *Radiology* **121**, 479–82 (1976).
15. Wahl, G. M., Linke, S. P., Paulson, T. G. & Huang, L. C. Maintaining genetic stability through TP53 mediated checkpoint control. *Cancer Surv.* **29**, 183–219 (1997).
16. Johnson, R. D. & Jasin, M. Double-strand-break-induced homologous recombination in mammalian cells. *Biochem. Soc. Trans.* **29**, 196–201 (2001).
17. Maity, A., McKenna, W. G. & Muschel, R. J. The molecular basis for cell cycle delays following ionizing radiation: a review. *Radiother. Oncol.* **31**, 1–13 (1994).
18. Ward, J. F. Biochemistry of DNA lesions. *Radiat. Res. Suppl.* **8**, S103–S111 (1985).
19. Little, J. B. Radiation carcinogenesis. *Carcinogenesis* **21**, 397–404 (2000).
20. Giaccia, A. J. & Kastan, M. B. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.* **12**, 2973–2983 (1998).
21. Clarke, A. R. *et al.* Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* **362**, 849–852 (1993).
22. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A. & Jacks, T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* **362**, 847–849 (1993).
23. Stewart, Z. A. & Pietenpol, J. A. p53 signaling and cell cycle checkpoints. *Chem. Res. Toxicol.* **14**, 243–263 (2001).
24. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
25. Caelles, C., Helmberg, A. & Karin, M. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature* **370**, 220–223 (1994).
26. Haupt, Y., Rowan, S., Shaulian, E., Vousden, K. H. & Oren, M. Induction of apoptosis in HeLa cells by trans-activation-deficient p53. *Genes Dev.* **9**, 2170–2183 (1995).
27. Sabbatini, P., Lin, J., Levine, A. J. & White, E. Essential role for p53-mediated transcription in E1A-induced apoptosis. *Genes Dev.* **9**, 2184–2192 (1995).
28. Sansome, C., Zaika, A., Marchenko, N. D. & Moll, U. M. Hypoxia death stimulus induces translocation of p53 protein to mitochondria. Detection by immunofluorescence on whole cells. *FEBS Lett.* **488**, 110–115 (2001).
29. Marchenko, N. D., Zaika, A. & Moll, U. M. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. *J. Biol. Chem.* **275**, 16202–16212 (2000).
30. Rich, T., Allen, R. L. & Wyllie, A. H. Defying death after DNA damage. *Nature* **407**, 777–783. (2000).
31. Shen, Y. & White, E. p53-dependent apoptosis pathways. *Adv. Cancer Res.* **82**, 55–84 (2001).
32. 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).
33. Attardi, L. D. *et al.* PERP, an apoptosis-associated target of p53, is a novel member of the PMP-22/gas3 family. *Genes Dev.* **14**, 704–718 (2000).
34. Nakano, K. & Vousden, K. H. PUMA, a Novel Proapoptotic Gene, Is Induced by p53. *Mol. Cell* **7**, 683–694 (2001).
35. Yu, J., Zhang, L., Hwang, P. M., Kinzler, K. W. & Vogelstein, B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol. Cell* **7**, 673–682 (2001).
36. Moroni, M. C. *et al.* Apaf-1 is a transcriptional target for E2F and p53. *Nature Cell Biol.* **3**, 552–558 (2001).
37. Jimenez, G. S. *et al.* A transactivation-deficient mouse model provides insights into Trp53 regulation and function. *Nature Genet.* **26**, 37–43 (2000).
38. Chao, C. *et al.* p53 transcriptional activity is essential for p53-dependent apoptosis following DNA damage. *EMBO J.* **19**, 4967–4975 (2000).
39. el-Deiry, W. S. *et al.* WAF1, a potential mediator of p53 tumor suppression. *Cell* **75**, 817–825 (1993).
40. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J. & Leder, P. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* **82**, 675–684 (1995).
41. Hermeking, H. *et al.* 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol. Cell* **1**, 3–11 (1997).
42. Chan, T. A., Hermeking, H., Lengauer, C., Kinzler, K. W. & Vogelstein, B. 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. *Nature* **401**, 616–620 (1999).
43. Zhao, R. *et al.* Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev.* **14**, 981–993 (2000).
44. Murphy, M. *et al.* Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. *Genes Dev.* **13**, 2490–2501 (1999).
45. Zhou, J., Ahn, J., Wilson, S. H. & Prives, C. A role for p53 in base excision repair. *EMBO J.* **20**, 914–923 (2001).

46. Offer, H. *et al.* Structural and functional involvement of p53 in BER *in vitro* and *in vivo. Oncogene* **20**, 581–589 (2001).

47. Prives, C. & Hall, P. A. The p53 pathway. *J. Pathol.* **187**, 112–126 (1999).

48. Ljungman, M. Dial 9-1-1 for p53: mechanisms of p53 activation by cellular stress. *Neoplasia* **2**, 208–225 (2000).

49. Oren, M. Regulation of the p53 tumor suppressor protein. *J. Biol. Chem.* **274**, 36031–36034 (1999).

50. Jimenez, G. S., Khan, S. H., Stommel, J. M. & Wahl, G. M. p53 regulation by post-translational modification and nuclear retention in response to diverse stresses. *Oncogene* **18**, 7656–7665 (1999).

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

52. 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).

53. McLure, K. G. & Lee, P. W. How p53 binds DNA as a tetramer. *EMBO J.* **17**, 3342–3350 (1998).

54. Friedman, P. N., Chen, X., Bargonetti, J. & Prives, C. The p53 protein is an unusually shaped tetramer that binds directly to DNA. *Proc. Natl Acad. Sci. USA* **90**, 3319–3323 (1993).

55. 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).

56. Geyer, R. K., Yu, Z. K. & Maki, C. G. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nature Cell Biol.* **2**, 569–573 (2000).

57. Boyd, S. D., Tsai, K. Y. & Jacks, T. An intact HDM2 RING-finger domain is required for nuclear exclusion of p53. *Nature Cell Biol.* **2**, 563–568 (2000).

58. Honda, R., Tanaka, H. & Yasuda, H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.* **420**, 25–27 (1997).

59. Honda, R. & Yasuda, H. Activity of MDM2, a ubiquitin ligase, toward p53 or itself is dependent on the RING finger domain of the ligase. *Oncogene* **19**, 1473–1476 (2000).

60. Fang, S., Jensen, J. P., Ludwig, R. L., Vousden, K. H. & Weissman, A. M. Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *J. Biol. Chem.* **275**, 8945–8951 (2000).

61. Haupt, Y., Maya, R., Kazaz, A. & Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* **387**, 296–299 (1997).

62. Kubbutat, M. H., Jones, S. N. & Vousden, K. H. Regulation of p53 stability by Mdm2 *Nature* **387**, 299–303 (1997).

63. 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).

64. 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).

65. Kussie, P. H. *et al.* Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **274**, 948–953 (1996).

66. Oliner, J. D. *et al.* Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* **362**, 857–860 (1993).

67. Momand, J., Zambetti, G. P., Olson, D. C., George, D. & Levine, A. J. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **69**, 1237–1245 (1992).

68. Chen, J., Marechal, V. & Levine, A. J. Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell. Biol.* **13**, 4107–4114 (1993).

69. Fuchs, S. Y. *et al.* JNK targets p53 ubiquitination and degradation in nonstressed cells. *Genes Dev.* **12**, 2658–2663 (1998).

70. Kubbutat, M. H. & Vousden, K. H. Proteolytic cleavage of human p53 by calpain: a potential regulator of protein stability. *Mol. Cell. Biol.* **17**, 460–468 (1997).

71. Maki, C. G. Oligomerization is required for p53 to be efficiently ubiquitinated by MDM2. *J. Biol. Chem.* **274**, 16531–16535 (1999).

72. Zauberman, A., Barak, Y., Ragimov, N., Levy, N. & Oren, M. Sequence-specific DNA binding by p53: identification of target sites and lack of binding to p53 - MDM2 complexes. *EMBO J.* **12**, 2799–2808 (1993).

73. 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).

74. Nakamura, S., Roth, J. A. & Mukhopadhyay, T. Multiple lysine mutations in the C-terminal domain of p53 interfere with MDM2-dependent protein degradation and ubiquitination. *Mol. Cell. Biol.* **20**, 9391–9398 (2000).

75. Zhu, Q., Yao, J., Wani, G., Wani, M. A. & Wani, A. A. Mdm2 mutant defective in binding p300 promotes ubiquitination but not degradation of p53: evidence for the role of p300 in integrating ubiquitination and proteolysis. *J. Biol. Chem.* **276**, 29695–29701 (2001).

76. Vassilev, A. *et al.* The 400 kDa subunit of the PCAF histone acetylase complex belongs to the ATM superfamily. *Mol. Cell* **2**, 869–875 (1998).

77. Liu, L. *et al.* p53 sites acetylated *in vitro* by PCAF and p300 are acetylated *in vivo* in response to DNA damage. *Mol. Cell. Biol.* **19**, 1202–1209 (1999).

78. Gu, W., Shi, X. L. & Roeder, R. G. Synergistic activation of transcription by CBP and p53. *Nature* **387**, 819–823 (1997).

79. Lai, Z. *et al.* Human mdm2 Mediates Multiple Mono-ubiquitination of p53 by a Mechanism Requiring Enzyme Isomerization. *J. Biol. Chem.* **276**, 31357–31367 (2001).

80. Gu, J., Chen, D., Rosenblum, J., Rubin, R. M. & Yuan, Z. M. Identification of a sequence element from p53 that signals for Mdm2- targeted degradation. *Mol. Cell. Biol.* **20**, 1243–1253 (2000).

81. Zilfou, J. T., Hoffman, W. H., Sank, M., George, D. L. & Murphy, M. The Corepressor mSin3a Interacts with the Proline-Rich Domain of p53 and Protects p53 from Proteasome-Mediated Degradation. *Mol. Cell. Biol.* **21**, 3974–3985 (2001).

82. Argentini, M., Barboule, N. & Wasylyk, B. The contribution of the acidic domain of MDM2 to p53 and MDM2 stability. *Oncogene* **20**, 1267–1275 (2001).

83. 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).

84. Grossman, S. R. *et al.* p300/MDM2 complexes participate in MDM2-mediated p53 degradation. *Mol. Cell* **2**, 405–415 (1998).

85. Crook, T., Nicholls, J. M., Brooks, L., O'Nions, J. & Allday, M. J. High level expression of deltaN-p63: a mechanism for the inactivation of p53 in undifferentiated nasopharyngeal carcinoma

86. De Laurenzi, V. & Melino, G. Evolution of functions within the p53/p63/p73 family. *Ann. NY Acad. Sci.* **926**, 90–100 (2000).

87. Lohrum, M. A. & Vousden, K. H. Regulation and activation of p53 and its family members. *Cell Death Differ.* **6**, 1162–1168 (1999).

88. Maya, R. *et al.* ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage. *Genes Dev.* **15**, 1067–1077 (2001).

89. Sherr, C. J. & Weber, J. D. The ARF/p53 pathway. *Curr. Opin. Genet. Dev.* **10**, 94–99 (2000).

90. Weber, J. D., Taylor, L. J., Roussel, M. F., Sherr, C. J. & Bar-Sagi, D. Nucleolar Arf sequesters Mdm2 and activates p53. *Nature Cell Biol.* **1**, 20–26 (1999).

91. Llanos, S., Clark, P. A., Rowe, J. & Peters, G. Stabilization of p53 by p14ARF without relocation of MDM2 to the nucleolus. *Nature Cell Biol.* **3**, 445–452 (2001).

92. Honda, R. & Yasuda, H. Association of p19(ARF) with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53. *EMBO J.* **18**, 22–27 (1999).

93. Kamsler, A. *et al.* Increased oxidative stress in ataxia telangiectasia evidenced by alterations in redox state of brains from Atm-deficient mice. *Cancer Res.* **61**, 1849–1854 (2001).

94. Rotman, G. & Shiloh, Y. Ataxia-telangiectasia: is ATM a sensor of oxidative damage and stress? *Bioessays* **19**, 911–917 (1997).

95. Kamijo, T. *et al.* Loss of the ARF tumor suppressor reverses premature replicative arrest but not radiation hypersensitivity arising from disabled atm function. *Cancer Res.* **59**, 2464–2469 (1999).

96. Gatei, M. *et al.* Ataxia-telangiectasia: chronic activation of damage-responsive functions is reduced by alpha-lipoic acid. *Oncogene* **20**, 289–294 (2001).

97. Khan, S. H., Moritsugu, J. & Wahl, G. M. Differential requirement for p19ARF in the p53-dependent arrest induced by DNA damage, microtubule disruption, and ribonucleotide depletion. *Proc. Natl Acad. Sci. USA* **97**, 3266–3271 (2000).

98. Kamijo, T. *et al.* Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* **91**, 649–659 (1997).

99. Artandi, S. E. *et al.* Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* **406**, 641–645 (2000).

100. Artandi, S. E. & DePinho, R. A. A critical role for telomeres in suppressing and facilitating carcinogenesis. *Curr. Opin. Genet. Dev.* **10**, 39–46 (2000).

101. Chin, L. *et al.* p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**, 527–538 (1999).

102. Karlseder, J., Broccoli, D., Dai, Y., Hardy, S. & de Lange, T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science* **283**, 1321–1325 (1999).

103. Shiloh, Y. ATM and ATR: networking cellular responses to DNA damage. *Curr. Opin. Genet. Dev.* **11**, 71–77 (2001).

104. Canman, C. E. *et al.* Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* **281**, 1677–1679 (1998).

105. Banin, S. *et al.* Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* **281**, 1674–1677 (1998).

106. Lees-Miller, S. P., Sakaguchi, K., Ullrich, S. J., Appella, E. & Anderson, C. W. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. *Mol. Cell. Biol.* **12**, 5041–5049. (1992).

107. Tibbetts, R. S. *et al.* A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes Dev.* **13**, 152–157 (1999).

108. Lambert, P. F., Kashanchi, F., Radonovich, M. F., Shiekhattar, R. & Brady, J. N. Phosphorylation of p53 serine 15 increases interaction with CBP. *J. Biol. Chem.* **273**, 33048–33053 (1998).

109. Shieh, S. Y., Ikeda, M., Taya, Y. & Prives, C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell* **91**, 325–334 (1997).

110. Chao, C., Saito, S., Anderson, C. W., Appella, E. & Xu, Y. Phosphorylation of murine p53 at ser-18 regulates the p53 responses to DNA damage. *Proc. Natl Acad. Sci. USA* **97**, 11936–11941 (2000).

111. Matsuoka, S. *et al.* Ataxia telangiectasia-mutated phosphorylates Chk2 *in vivo* and *in vitro. Proc. Natl Acad. Sci. USA* **97**, 10389–10394 (2000).

112. Shieh, S. Y., Ahn, J., Tamai, K., Taya, Y. & Prives, C. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev.* **14**, 289–300 (2000).

113. Hirao, A. *et al.* DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science* **287**, 1824–1827 (2000).

114. Chehab, N. H., Malikzay, A., Appel, M. & Halazonetis, T. D. Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. *Genes Dev.* **14**, 278–288 (2000).

115. Unger, T. *et al.* Critical role for Ser20 of human p53 in the negative regulation of p53 by Mdm2. *EMBO J.* **18**, 1805–1814 (1999).

116. Chehab, N. H., Malikzay, A., Stavridi, E. S. & Halazonetis, T. D. Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. *Proc. Natl Acad. Sci. USA* **96**, 13777–13782 (1999).

117. Bell, D. W. *et al.* Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science* **286**, 2528–2531 (1999).

118. Ashcroft, M., Kubbutat, M. H. & Vousden, K. H. Regulation of p53 function and stability by phosphorylation. *Mol. Cell. Biol.* **19**, 1751–1758 (1999).

119. Waterman, M. J., Stavridi, E. S., Waterman, J. L. & Halazonetis, T. D. ATM-dependent activation of p53 involves dephosphorylation and association with 14-3-3 proteins. *Nature Genet.* **19**, 175–178 (1998).

120. Lin, W. C., Lin, F. T. & Nevins, J. R. Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. *Genes Dev.* **15**, 1833–1844 (2001).

121. Bates, S. *et al.* p14ARF links the tumour suppressors RB and p53. *Nature* **395**, 124–125 (1998).

122. Dimri, G. P., Itahana, K., Acosta, M. & Campisi, J. Regulation of a senescence checkpoint response by the E2F1 transcription factor and p14(ARF) tumor suppressor. *Mol. Cell. Biol.* **20**, 273–285 (2000).

123. Zhu, J. W., DeRyckere, D., Li, F. X., Wan, Y. Y. & DeGregori, J. A role for E2F1 in the induction of ARF, p53, and apoptosis during thymic negative selection. *Cell Growth Differ.* **10**, 829–838 (1999).

124. Kastan, M. B. *et al.* A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* **71**, 587–597 (1992).

125. 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).

126. Linke, S. P. *et al.* p53-mediated accumulation of hypophosphorylated pRb after the G1 restriction point fails to halt cell cycle progression. *Oncogene* **15**, 337–345 (1997).

127. Frisch, S. M. & Francis, H. Disruption of epithelial cell-matrix interactions induces apoptosis. *J. Cell Biol.* **124**, 619–626 (1994).

128. Suzuki, K. *et al.* Radiation-induced senescence-like growth arrest requires TP53 function but not telomere shortening. *Radiat. Res.* **155**, 248–253 (2001).

129. Gadbois, D. M., Bradbury, E. M. & Lehnert, B. E. Control of radiation-induced G1 arrest by cell-substratum interactions. *Cancer Res.* **57**, 1151–1156 (1997).

130. DeSimone, J. N., Dolezalova, H., Redpath, J. L. & Stanbridge, E. J. Prolonged cell cycle arrest in irradiated human diploid skin fibroblasts: the role of nutrient deprivation. *Radiat. Res.* **153**, 131–143 (2000).

131. Fenton, M., Barker, S., Kurz, D. J. & Erusalimsky, J. D. Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries. *Arterioscler. Thromb. Vasc. Biol.* **21**, 220–226 (2001).

132. Haks, M. C., Krimpenfort, P., van den Brakel, J. H. & Kruisbeek, A. M. Pre-TCR signaling and inactivation of p53 induces crucial cell survival pathways in pre-T cells. *Immunity* **11**, 91–101 (1999).

133. Guidos, C. J. *et al.* V(D)J recombination activates a p53-dependent DNA damage checkpoint in scid lymphocyte precursors. *Genes Dev.* **10**, 2038–2054 (1996).

134. Vousden, K. H. p53: death star. *Cell* **103**, 691–694 (2000).

135. Szak, S. T., Mays, D. & Pietenpol, J. A. Kinetics of p53 Binding to Promoter Sites *in vivo*. *Mol. Cell. Biol.* **21**, 3375–3386 (2001).

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

137. Kobayashi, T. *et al.* Differential p53 phosphorylation and activation of apoptosis-promoting genes Bax and Fas/APO-1 by irradiation and ara-C treatment. *Cell Death Differ.* **5**, 584–591 (1998).

138. Ollmann, M. *et al. Drosophila* p53 is a structural and functional homolog of the tumor suppressor p53. *Cell* **101**, 91–101 (2000).

139. Brodsky, M. H. *et al. Drosophila* p53 binds a damage response element at the reaper locus. *Cell* **101**, 103–113 (2000).

140. Jin, S. *et al.* Identification and characterization of a p53 homologue in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **97**, 7301–7306 (2000).

141. Brodsky, M. H., Sekelsky, J. J., Tsang, G., Hawley, R. S. & Rubin, G. M. mus304 encodes a novel DNA damage checkpoint protein required during *Drosophila* development. *Genes Dev.* **14**, 666–678 (2000).

142. Nordstrom, W. & Abrams, J. M. Guardian ancestry: fly p53 and damage-inducible apoptosis. *Cell Death Differ.* **7**, 1035–1038 (2000).

143. Lev Bar-Or, R. *et al.* Generation of oscillations by the p53-Mdm2 feedback loop: a theoretical and experimental study. *Proc. Natl Acad. Sci. USA.* **97**, 11250–11255 (2000).

144. Alarcon, R., Koumenis, C., Geyer, R. K., Maki, C. G. & Giaccia, A. J. Hypoxia induces p53 accumulation through MDM2 down-regulation and inhibition of E6-mediated degradation. *Cancer Res.* **59**, 6046–6051 (1999).

145. Koumenis, C. *et al.* Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. *Mol. Cell. Biol.* **21**, 1297–1310 (2001).

146. 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).

147. Moulder, J. E. & Rockwell, S. Tumor hypoxia: its impact on cancer therapy. *Cancer Metastasis Rev.* **5**, 313–341 (1987).

148. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D. & Lowe, S. W. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88**, 593–602 (1997).

149. de Stanchina, E. *et al.* E1A signaling to p53 involves the p19(ARF) tumor suppressor. *Genes Dev.* **12**, 2434–2442 (1998).

150. Evan, G. I. *et al.* Induction of apoptosis in fibroblasts by c-myc protein. *Cell* **69**, 119–128 (1992).

151. Hermeking, H. & Eick, D. Mediation of c-Myc-induced apoptosis by p53. *Science* **265**, 2091–2093 (1994).

152. Denko, N. C., Giaccia, A. J., Stringer, J. R. & Stambrook, P. J. The human Ha-ras oncogene induces genomic instability in murine fibroblasts within one cell cycle. *Proc. Natl Acad. Sci. USA* **91**, 5124–5128 (1994).

153. Felsher, D. W. & Bishop, J. M. Transient excess of MYC activity can elicit genomic instability and tumorigenesis. *Proc. Natl Acad. Sci. USA* **96**, 3940–3944 (1999).

154. Fukasawa, K. & Vande Woude, G. F. Synergy between the Mos/mitogen-activated protein kinase pathway and loss of p53 function in transformation and chromosome instability. *Mol. Cell. Biol.* **17**, 506–518 (1997).

155. Mai, S. Overexpression of c-myc precedes amplification of the gene encoding dihydrofolate reductase. *Gene* **148**, 253–260 (1994).

156. Craig, A. L., Blaydes, J. P., Burch, L. R., Thompson, A. M. & Hupp, T. R. Dephosphorylation of p53 at Ser20 after cellular exposure to low levels of non-ionizing radiation. *Oncogene* **18**, 6305–6312 (1999).

157. Raveh, T., Droguett, G., Horwitz, M. S., DePinho, R. A. & Kimchi, A. DAP kinase activates a p19ARF/p53-mediated apoptotic checkpoint to suppress oncogenic transformation. *Nature Cell Biol.* **3**, 1–7 (2001).

158. Finkel, T. Redox-dependent signal transduction. *FEBS Lett.* **476**, 52–54 (2000).

159. Ries, S. *et al.* Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. *Cell* **103**, 321–330 (2000).

160. Weitzman, J. B., Fiette, L., Matsuo, K. & Yaniv, M. JunD protects cells from p53-dependent senescence and apoptosis. *Mol. Cell* **6**, 1109–1119 (2000).

161. Rothstein, R., Michel, B. & Gangloff, S. Replication fork pausing and recombination or "gimme a break". *Genes Dev.* **14**, 1–10 (2000).

162. Buschmann, T. *et al.* Jun NH2-terminal kinase phosphorylation of p53 on Thr-81 is important for p53 stabilization and transcriptional activities in response to stress. *Mol. Cell. Biol.* **21**, 2743–2754 (2001).