REVIEW

p53: puzzle and paradigm

Linda J. Ko and Carol Prives¹

Department of Biological Sciences, Columbia University, New York, New York 10027 USA

As the tale of p53 unfolds, it becomes ever more intriguing. Although our understanding of the critical and complex roles played by p53 is progressing rapidly, new findings continue to pose new paradoxes. Here we present some of these recent advances in p53 research and discuss how they either shed light on or add to the complexities of p53. Therefore, we only briefly summarize some of the key developments leading to our current state of knowledge. For further information, the reader is referred to several excellent reviews that have focused on p53 research (see Donehower and Bradley 1993; Levine 1993; Greenblatt et al. 1994; Oren 1994; Prives 1994; Kinzler and Vogelstein 1996).

Historic landmarks in p53 research

After the identification of the p53 protein and the subsequent cloning of *p53* genes from several species, early observations suggested that *p53* might function as an oncogene, because overexpression of *p53* appeared to cause oncogenic transformation of cells. In the late 1980s, however, several critical discoveries defined the normal function of *p53* to be anti-oncogenic. Wild-type *p53* genes, when introduced into cells, were found to be growth suppressive. The screening of DNA from colon cancer patients revealed that *p53* mutations occur with unusually high frequency in tumor tissue, an observation that was extended to most of the other major forms of human cancer. Indeed, members of Li-Fraumeni cancer-prone families were shown to carry germ-line *p53* mutations. The importance of these observations was underscored by the finding that mice that are homozygous null for *p53*, although developmentally competent, are highly predisposed to tumors.

The functional character of the p53 protein was determined by experiments showing that p53 contains a strong transcriptional activation domain within its amino terminus and that it is a tetrameric, sequence-specific DNA-binding protein with a defined cognate binding site containing two copies of the 10-mer (5'-RRRCA/TT/AGYYY-3'). Although the p53 protein acts as a transcriptional activator of genes containing p53-binding sites, it is also capable of strongly inhibiting transcription from many genes lacking p53-binding sites. Several oncogenic DNA viruses express viral gene prod-

ucts that associate with and inhibit the *trans*-activation function of p53, notably SV40 large T antigen, the adenovirus E1B 55-kD protein, and the E6 protein of oncogenic forms of human papillomavirus (HPV E6). In cells, p53 can associate with a 90-kD protein, identified as the product of the *mdm-2* oncogene, which is amplified in some types of tumors. When bound to mdm-2, p53 can no longer function as an activator of transcription.

p53 plays multiple roles in cells. Expression of high levels of wild-type (but not mutant) p53 has two outcomes: cell cycle arrest, or apoptosis. The observation that DNA-damaging agents induce levels of p53 in cells led to the definition of p53 as a checkpoint factor, akin, perhaps, to the product of the *rad9* gene in yeast. While dispensable for viability, in response to genotoxic stress, p53 acts as an "emergency brake" inducing either arrest or apoptosis, protecting the genome from accumulating excess mutations. Consistent with this notion, cells lacking p53 were shown to be more genetically unstable and thus more prone to tumors.

p53 research today

Literature on p53 during the past few years has been massive, making it impossible for us to refer to every recent publication. We therefore apologize to those whose work we may have overlooked. In the interest of brevity, we focus on discoveries made in the past 3 years centered on several general themes in p53 research.

p53 domains: structure and function

The p53 protein can be divided roughly into thirds, encompassing the amino-terminal domain containing the activation domain, the central core containing its sequence-specific DNA-binding domain, and the multifunctional carboxy-terminal domain (Fig. 1). The acidic activation domain lies within residues 1–43 (Unger et al. 1992), although neighboring sequences are also likely to contribute to the transcriptional activity of p53 (Chang et al. 1995). The central core of p53 lies within residues 100–300, and the carboxyl terminus of p53 lies within residues 300–393.

Like the acidic activator VP16, p53 binds in vitro to several proteins through its activation domain (see Fig. 1 and Table 1). The amino terminus of p53 interacts with many general transcription factors such as the TATA box-binding protein (TBP) component of the general transcription factor TFIID (Horikoshi et al. 1995, and

¹Corresponding author.

Figure 1. Landmarks of the human p53 protein. (A) p53 mutations found in human cancer. Hatched boxes represent evolutionarily conserved regions. Vertical lines above represent the frequency at which mutations are found at each particular residue and are clustered in conserved regions II–V. Several hot spots for mutations R175, G245, R248, R249, R273, and R282 are also indicated. (B) Structural organization of the p53 protein. Hatched boxes represent conserved regions. (Basic) The extreme carboxyl terminus, which contains several basic residues. Nuclear localization sequences (NLSs) and phosphorylation sites (circled P's) are shown below. Additionally, analysis of murine p53 has also identified phosphorylation sites for JNK kinase at amino acid 34 and MAP kinase at amino acids 73 and 83 (see text). However, corresponding residues in the human protein are not conserved acceptor sites for Ser/Thr phosphorylation. Of the many proteins demonstrated to interact with p53 [see Table 1], a subset of them is known to interact with particular regions of p53; these are shown at the bottom.

references therein), several TBP-associated factors (TAFs), including *Drosophila* TAF-40 and TAF-60 (Thut et al. 1995), the human TAF31 (Lu and Levine 1995), and the p62 subunit of the dual transcription/repair factor, TFIIH (Xiao et al. 1994; X. Wang et al. 1995; Leveillard et al. 1996). p53 also recognizes the eukaryotic single-stranded DNA-binding protein RP-A (Dutta et al. 1993; He et al. 1993; Li and Botchan 1993).

p53 has several unique properties, such as the ability to cooperate with TBP (or TFIID) in binding to DNA (X. Chen et al. 1993). Furthermore, TBP interacts with the carboxyl terminus of p53 as well as with its activation domain (Horikoshi et al. 1995). The association of p53 with the product of the *mdm-2* gene (see Fig. 1 and Table 1) is also unique to p53. These interactions may be im-

portant for transcriptional regulation, as mutation of residues 22 and 23 renders p53 transcriptionally inactive (Lin et al. 1994), and disrupts its interactions with TAFs and mdm-2 (Lin et al. 1994; Thut et al. 1995).

The central core region of p53 contains the sequence-specific DNA-binding domain (Bargonetti et al. 1993; Halazonetis and Kandil 1993; Pavletich et al. 1993; Wang et al. 1993). The vast majority of p53 missense mutations in tumors are clustered within this central portion, particularly within the four highly conserved regions (Hollstein et al. 1994). These mutations affect properties of p53 whose alteration leads to increased tumorigenic potential of cells such as its ability to bind DNA.

The purified core DNA-binding domain can bind co-

Table 1. *p53-interacting proteins*

Cellular	Viral	
<i>General transcription factors</i>	<i>Transcriptional activators</i>	SV40 TAg AdE1B55 kD
TFIID components	WT-1	HPV E6
TBP	Sp1	HBV HBx
dTAF40/hTAF31	CBF	EBV BZLF1
dTAF60		EBV EBNA-5
TFIIH components	<i>Kinases</i>	
hERCC2/hXPD/yRAD3	casein kinase II	
hERCC3/hXPB/ySSL2	c-abl	
hp62/yTFB1		
	<i>Calcium-binding proteins</i>	
	S100b	
<i>Replication and repair proteins</i>	<i>Ubiquitin-system proteins</i>	
RP-A (p70)		
TFIIH components	E6-AP	
XPD		
XPB	<i>Uncharacterized</i>	
p62	53BP1	
CSB	53BP2	
<i>Oncogene products</i>		
mdm-2		

Proteins interacting with p53 are divided between cellular and viral proteins. (See text for description of functional relevance of interactions, where known. Also see Fig. 1 and text for regions of p53 with which the proteins interact, where known.) [Ad] Adenovirus, (HPV) human papillomavirus, (HBV) hepatitis B virus, (EBV) Epstein-Barr virus.

References are as follows: (TBP) Horikoshi et al. (1995) and references therein; (TAFs) Lu and Levine (1995), Thut et al. (1995); (TFIIH) Xiao et al. (1994), X. Wang et al. (1995); (WT-1) Maheswaran et al. (1995); (SP1) Borellini and Glazer (1993), Gualberto and Baldwin et al. (1995); (CBF) Agoff et al. (1993); (RP-A) Dutta et al. (1993), He et al. (1993), Li and Botchan (1993); (CSB) Wang et al. (1995); (mdm-2) Oliner et al. (1993), Wu and Levine (1994); (CKII) Filhol et al. (1992); (c-abl) Goga et al. (1995); (S100b) Baudier et al. (1992); (E6-AP) Huibregtse et al. (1993); (53BP1 and 53BP2) Iwabuchi et al. (1994); (SV40 T, AdE1B 55 kD, HPV E6) see Levine (1993) and references therein; (HBV HBx) X. Wang et al. (1994), Truant et al. (1995); (EBV BZLF1) Q. Zhang et al. (1994); (EBV EBNA-5) Szekely et al. (1993).

operatively to DNA (Bargonetti et al. 1993; Pavletich et al. 1993; Wang et al. 1993; Balagurumoorthy et al. 1995; Y. Wang et al. 1995). In a manner that may be mediated by DNA, core domains can form strong interactions with each other, which may facilitate DNA bending (Balagurumoorthy et al. 1995) as well as looping (Stenger et al. 1994). These properties may be relevant in promoters that contain two p53-binding sites spaced at a distance from each other, for example, in the p53 target genes *p21/WAF1/Cip1* and *cyclin G* (see Zauberman et al. 1995, see below).

An important advance in understanding p53 was the solution of the three-dimensional co-crystal structure of the DNA-binding domain bound to its cognate site (Cho et al. 1994). The structure bears resemblance to the

DNA-binding domain of the NF- κ B p50 homodimer (Muller et al. 1995). The core structure led to several important observations: (1) The four conserved regions within the core comprise the DNA-binding element that is responsible for contacting the major and minor grooves of the p53-binding site, whereas the less conserved regions make up a β -sandwich that forms a scaffold to support the DNA-binding element; (2) each of the residues that are mutated most frequently in cancer patients makes critical contributions to sequence-specific DNA binding, and (3) the two classes of tumor-derived mutants identified previously as differing in antibody and heat shock protein binding, as well as protease resistance, bear mutations in either the scaffold or the structural element of the DNA-binding domain, and thus can now be classified as conformational or contact mutants, respectively (see Cho et al. 1994 and references therein).

The carboxyl terminus of p53 can function as an autonomous domain capable of binding nonspecifically to different forms of DNA, including damaged DNA (Wang et al. 1993; Balkalkin et al. 1994; Bayle et al. 1995; Lee et al. 1995; Reed et al. 1995) and reannealing complementary single strands of DNA or RNA (Brain and Jenkins 1994; Prives et al. 1994; Balkalkin et al. 1995; Wu et al. 1995). The carboxyl terminus can be subdivided further into three regions, a flexible linker (residues 300–320) that connects the DNA-binding domain to the tetramerization domain, the tetramerization domain itself (residues 320–360), and, at the extreme carboxyl terminus, a stretch of 30 amino acids that is rich in basic residues (residues 363–393; see Fig. 1). The three groups that have reported the structure of the tetramerization domain, using three-dimensional nuclear magnetic resonance (NMR) (W. Lee et al. 1994; Clore et al. 1995) and X-ray crystallography (Jeffrey et al. 1995), agree that the tetramerization region contains a β -sheet–turn– α -helix motif that can homodimerize, and that the p53 tetramer contains a pair of such dimers. However, differences in the relative orientation of the dimers were reported. The unusual elliptical shape of the full-length p53 tetramer (Friedman et al. 1993), as derived from estimation of the Stokes radius and sedimentation coefficient, is attributable to the carboxy-terminal region of p53 (P. Wang et al. 1994).

Although it is known that the minimal region of p53 necessary for cellular transformation localizes to the oligomerization domain (Shaulian et al. 1992), the normal role of this domain is not entirely clear. Experiments demonstrating the requirement for p53 oligomerization for DNA binding (Halazonetis and Kandil 1993; Shaulian et al. 1993; Pietenpol et al. 1994) are contradicted by the observation that the central core alone binds to DNA (see above). In some cases, the oligomerization domain appears dispensable for sequence-specific *trans*-activation (Shaulian et al. 1993; Slingerland et al. 1993; Tarunina and Jenkins 1993), but not in others (Halazonetis and Kandil 1993; Pietenpol et al. 1994). It is possible that the presence of this domain may be required for binding to

some p53 cognate sites but not others. There is likely to be complex communication between the different domains of p53, and removal or alteration of one domain may affect the function of the others.

Identification of the regions of p53 required for growth suppression and transformation suppression has suggested that the two properties are quite distinct. Mutant versions of p53 lacking either the activation domain (Unger et al. 1993) or the carboxy-terminal region (Shaulian et al. 1995) are still capable of suppressing transformation, albeit at lower efficiencies than wild type. In contrast, growth suppression requires both the amino- and carboxy-terminal regions of p53 (Pietenpol et al. 1994). This domain analysis is consistent with other observations noting a correlation between the transcriptional activity of various mutants of p53 and the ability to suppress growth but not the ability to suppress transformation (Crook et al. 1994; Pietenpol et al. 1994). The ability to suppress transformation may involve apoptotic mechanisms as well as those that arrest growth, and thus properties beyond the transcriptional activating functions of p53 may be required (see below).

Exercising self-restraint: regulation by the carboxyl terminus

The potency of p53 necessitates tight regulation in cells, and the last 30 amino acids of p53 appear to be important in this regard. p53 is strongly stimulated to bind specifically to DNA when this carboxy-terminal portion is either (1) deleted (Hupp et al. 1992); (2) bound by antibody or dnaK (Hupp et al. 1992; Halazonetis et al. 1993); or (3) phosphorylated by protein kinases casein kinase II (CKII) (Hupp et al. 1992) or protein kinase C (PKC) (Takenaka et al. 1995). Additionally, peptides spanning a region within the last 30 amino acids of the carboxyl terminus can strongly stimulate DNA binding by full-length p53 *in vitro* (Hupp et al. 1995). These data have led to the postulate that the carboxyl terminus functions to allosterically regulate the conversion of p53 between forms that are inactive or active for DNA binding (Halazonetis et al. 1993; Hupp and Lane 1994; Waterman et al. 1995). The presence of an autoinhibitory region is not unique to p53; this has also been noted in other DNA-binding proteins such as the Ets protein (Petersen et al. 1995 and references therein).

It is not yet fully understood how the carboxyl terminus of p53 regulates the specific DNA-binding central core. The observation that the p53 carboxy-terminal monoclonal antibody pAb 421 stimulates sequence-specific DNA binding but also inhibits nonspecific interactions and reannealing by p53 (Balkalkin et al. 1995; Jayaraman and Prives 1995; Wu et al. 1995) suggests a model in which p53 can exist in two conformations with differing properties. As regulated by the carboxyl terminus, p53 in one configuration might be inhibited for DNA binding but might remain active for other activities, whereas conversion to the second conformation might allow sequence-specific DNA binding but preclude, for example, nonspecific interactions with DNA.

Solution of the structure of full-length inactive and active p53 proteins would provide considerable insight into the role(s) of the carboxyl terminus.

It is noteworthy that a form of murine p53, the product of an alternatively spliced mRNA, which is most abundant in the G₂ phase of the cell cycle, lacks this carboxy-terminal portion and has, instead, 17 alternate amino acids (Kulesz-Martin et al. 1994 and references therein). This form of the murine protein is more constitutively active for DNA binding (Wu et al. 1994; Bayle et al. 1995; Wolkowicz et al. 1995) but cannot anneal single strands of nucleic acids (Wu et al. 1995).

Getting some input: signaling to p53

What is upstream of p53, and how are signals transmitted from damaged DNA to activate the p53 pathway? Given the complexity and importance of the cellular response to p53, it is likely that there are multiple ways by which p53 can be induced, potentially resulting in both an increase in the levels of p53 and a conversion of p53 from an inactive to an activated form for DNA binding. Although evidence for the former is abundant, evidence for the latter is just beginning to accumulate.

Wild-type p53 is present in extremely small quantities in most cells and displays a rapid turnover rate that is on the order of minutes. It is generally agreed that the inductive response is post-transcriptional (Kastan et al. 1991) and appears to be cell-type dependent (Midgley et al. 1995). Irradiation of cells with either ionizing radiation (IR) or UV light induces p53, and the presence of DNA strand breaks is critical for this induction (Nelson and Kastan 1994; see Figure 2). It has been predicted that even a single double-stranded break is enough to induce p53 (DiLeonardo et al. 1994).

In addition to its response to genotoxic stress, p53 has been proposed to mediate a more general stress response to suboptimal growth conditions (see Donehower and Bradley 1993). Both hypoxia and heat induce p53, as does starvation (Zhan et al. 1993; Graeber et al. 1994). In fact, many common experimental protocols measuring p53 effects in cells, such as radiolabeling (YeARGIN and Haas 1995) and DNA transfection (Renzing and Lane 1995), may themselves perturb the very outcomes that they purport to measure.

The accumulation of p53 in virally transformed cells was anticipated to be caused by complex formation with viral proteins known to interact with p53 (see above). This is not always the case, however, and SV40 T antigen has been shown to stabilize p53 without physically associating with it (for review, see Maxwell and Roth 1994). Moreover, the stabilization of p53 has been observed with the expression of either adenovirus E1A or the E7 protein of oncogenic forms of human papillomavirus (HPV E7), both of which bind retinoblastoma (Rb) protein but not p53. (Lowe and Ruley 1993; Demers et al. 1994b). Interestingly, the cellular product of the Wilms' tumor gene, WT1, another tumor suppressor, also stabilizes p53 (Maheswaran et al. 1995).

Although the normal mechanism of rapid turnover of

p53 is not clear, studies of HPV have revealed that it has evolved a mechanism to inactivate p53 by degradation mediated by the virally encoded E6 protein. Formation of a tripartite complex of E6, p53, and a cellular protein, E6-AP, targets p53 for ubiquitin-dependent proteolysis (Scheffner et al. 1993). The demonstration that p53 accumulates in a cell line with a defect in the ubiquitin pathway (Chowdary et al. 1994), suggests that this pathway may normally degrade p53 protein in cells. If ubiquitination is the mode by which p53 turnover is regulated, it will be of great interest to determine how DNA damage affects this process.

p53 levels may also be controlled, at least partly, at the level of translation. Mosner et al. (1995) have intriguing data suggesting that p53 is negatively autoregulated by specifically inhibiting translation of its own mRNA in vitro.

An interesting possibility exists that the specific activity of p53 may also be increased after DNA damage. Support for this hypothesis can be found in the observation that p53 can be isolated from cells in a form inactive for DNA binding but can be converted to an active state by various conditions: (1) antibody binding, (2) redox conditions, (3) the presence of short single strands of DNA, and (4) phosphorylation.

Treatment of cell extracts with antibodies that recognize the carboxyl terminus can cause a strong stimulation of p53 DNA binding (Hupp and Lane 1994). Furthermore, microinjection of the carboxy-terminal-specific antibody pAb 421 into cells stimulates expression from a p53-responsive reporter construct (Abarzua et al. 1995; Hupp et al. 1995). This result may explain the earlier observation of Mercer and colleagues (1982) that injection of the p53 carboxy-terminal-specific antibody pAb 122 leads to inhibition of growth in cells. It has not yet been determined, however, whether the antibody is not simply stabilizing the p53 protein levels in microinjected cells.

The DNA-binding ability of p53 is subject to redox regulation such that oxidation inhibits DNA binding, whereas reduction favors it (Hainaut and Milner 1993; Hupp et al. 1993; Rainwater et al. 1995). Several cysteine residues in the core DNA-binding domain have been implicated in zinc coordination, and mutational analysis has identified cysteines at positions 173, 235, and 239, which contribute to DNA binding and are also essential for transcriptional activation and suppression of transformation by p53 (Rainwater et al. 1995). These results may be of great interest with respect to the induction of p53 after genotoxic stress. Oxygen radicals are produced in response to stress conditions, including ionizing radiation, and p53 may be regulated by both the presence of oxygen intermediates and the counterbalancing cellular reducing response (discussed in Hainaut and Milner 1993 and references therein).

Another effector of p53 may be short single DNA strands, because single-stranded DNA of 16–40 nucleotides, within the size range generated during excision repair, were shown to stimulate p53 DNA binding in vitro (Jayaraman and Prives 1995). Clearly, the demon-

stration of activation of p53 in vivo by single-stranded DNA would provide an interesting link between p53 and DNA repair (see below).

Phosphorylation may also induce inactive p53 activity in cells. p53 is multiply phosphorylated at serines and threonines within its amino- and carboxy-terminal regions in vivo and in vitro (for review, see Meek 1994; Fig. 1). The following protein kinases have been shown to phosphorylate p53: cyclin-dependent kinases (CDKs), CKI and CKII, double-stranded DNA-activated protein kinase (DNA-PK), and PKC (for review, see Meek 1994). More recently, other protein kinases have been included in this roster: mitogen activated protein kinase (MAP) (Milne et al. 1994), Jun amino-terminal kinase (JNK) (Milne et al. 1995), and Raf kinase (Jamal and Ziff 1995). In addition, a yeast gene, *PAK1*, whose encoded protein is predicted to be a Ser/Thr protein kinase, was identified by its ability to up-regulate p53 activity in vivo when p53 is exogenously expressed in yeast (Thiagalagam et al. 1995).

How might these kinases affect p53? Phosphorylation could alter turnover rate or activity. Indeed, treatment of cells with the serine phosphatase inhibitor okadaic acid induces hyperphosphorylation of wild-type p53 and was found to increase the steady-state level of p53 protein (W. Zhang et al. 1994). A number of studies have also documented dramatic stimulatory effects of phosphorylation of p53 on DNA binding in vitro by CKII (Hupp et al. 1992), PKC (Hupp and Lane 1994; Takenaka et al. 1995), and CDKs (Wang and Prives 1995).

Although some studies have demonstrated alterations in p53 function as a result of mutation of phosphorylation sites, others have shown no effect. For example, altered growth suppression function of p53 was observed when mutations were introduced in the DNA-PK site at residue 15 (Fiscella et al. 1993) or the CKII site (Milne et al. 1992). Other studies, however, have reported no effect of mutations at either the CDK site or the CKII site (Slingerland et al. 1993; Crook et al. 1994; Fiscella et al. 1994; Marston et al. 1994). Mutation of multiple phosphorylation sites within the p53 activation domain caused significant loss of transcriptional activation and suppression activities in one case (Mayr et al. 1995) but only minor effects on transcriptional activation in another case (Fuchs et al. 1995). Differences in cell lines or cell growth states may yield different results with phosphorylation site mutants. Moreover, the overexpression of large quantities of protein in transfection assays may mask the effects of mutations at phosphorylation sites and thus account for the discrepancy between results concerning phosphorylation of p53 in vitro and in vivo. The fact that p53 is phosphorylated at several sites in vivo by multiple protein kinases may further complicate interpretation of experimental data. Clearly, p53 phosphorylation needs considerable attention before its significance is understood.

Direct hits: transcriptional target genes

Although we do not yet fully understand how p53 elicits

its effects upon cells, it is clear that the transcriptional activating function of p53 is a major component of its biological effects (Crook et al. 1994; Pietenpol et al. 1994). Identification of transcriptional targets of p53 has been critical in discerning pathways by which p53 affects global cellular outcomes such as growth, arrest, and death (Fig. 2).

A substantial number of genes have been claimed to contain p53-binding sites and/or response elements and thus to have the potential to be target genes. Not all of these, however, fit the following criteria of a bona fide p53 response gene: (1) the existence of p53-binding sites that can be specifically recognized by p53; (2) the ability of these sites to act as a p53 response element, activating basal transcription (generally in a reporter gene construct) in a wild-type p53-dependent manner; (3) the response of the element to p53 in the context of the endogenous genomic promoter; and (4) the induction of the target gene after cellular stress, such as DNA damage, in cells containing wild-type but not mutant forms of p53. The following good candidates for p53 response genes have functions that are particularly relevant to the biological functions of p53:

p21/WAF1/Cip1

The *p21/WAF1/Cip1* gene (El-Diery et al. 1993; Harper et al. 1993; Xiong et al. 1993b) is the most well studied p53 response gene (El-Diery et al. 1993), and its encoded protein forms part of a quaternary complex found in normal cells along with cyclin/CDKs and the DNA polymerase processivity factor PCNA (Xiong et al. 1993b). At high protein concentrations, p21/WAF1/Cip1 inhibits the function of CDKs, particularly those that function during the G₁ phase of the cell cycle (Gu et al. 1993; Harper et al. 1993; Xiong et al. 1993a). In response to irradiation, p53-dependent G₁ arrest is mediated, at least in part, through p53's induction of p21/WAF1/Cip1 (El-Diery et al. 1994) (Fig. 2 and see below). It was therefore somewhat unexpected that fibroblasts homozygous null for p21 are only partially defective in their response to DNA damage (Brugarolas et al. 1995; Deng et al. 1995). The expression of p21/WAF1/Cip1 in a variety of tissues from p53 null mice suggests that it is also regulated by p53-independent mechanisms (Michieli et al. 1994; Macleod et al. 1995; Parker et al. 1995).

mdm-2

The *mdm-2* gene (Wu et al. 1993; Barak et al. 1993) is amplified in 30%–40% of human sarcomas (Oliner et al. 1992) and encodes a protein that complexes with p53 and inhibits its transcriptional activation ability (Momand et al. 1992; Oliner et al. 1993). The *mdm-2* gene itself is a transcriptional target of p53 and is activated in response to UV irradiation, thus implying an autoregulatory feedback loop between p53 and mdm-2 (Barak et al. 1993; Perry et al. 1993; Wu et al. 1993) (Fig. 2). The physiological consequence of this regulatory loop has been shown in the ability of mdm-2 overexpression to inhibit p53-

dependent G₁ arrest in response to irradiation (C.-Y. Chen et al. 1994).

GADD45

The *GADD45* gene is induced when cells are subjected to DNA damage leading to arrest in the G₁ phase of the cell cycle (Kastan et al. 1992 and references therein). The GADD45 protein was reported to interact with the replication and repair factor PCNA and to inhibit the entry of cells into S phase (Smith et al. 1994). In many (but not all) types of cells, DNA damage induces GADD45 in a p53-dependent manner (Kastan et al. 1992; Lu and Lane 1993).

cyclin G

The *cyclin G* gene encodes a novel cyclin that is strongly induced in a p53-dependent manner in cells subjected to DNA damage (Okamoto and Beach 1994; Zauberman et al. 1995). It has not yet been shown to associate with or activate any known cyclin dependent kinase.

bax

The *bax* gene, which is upregulated in response to expression of p53 (Miyashita and Reed 1995) encodes a protein with homology to the survival factor Bcl-2 (Miyashita and Reed 1995). These two proteins, which can heterodimerize, are critical to the regulation of the apoptotic process: Bcl-2 enhances cell survival while Bax promotes cell death (for review, see White 1996). Induction of Bax in response to IR appears to correlate with p53 status in human cells (Zhan et al. 1994).

IGF-BP3

The insulin-like growth factor binding protein 3 gene (*IGF-BP3*) has recently been identified as a p53 target that can be induced in cells after DNA damage (Buckbinder et al. 1995). Because IGF-BP3 protein inhibits signaling by insulin-like growth factor and therefore is antimitogenic, this represents yet another possible way in which p53 can suppress growth.

Other candidates for p53 response genes include genes encoding transforming growth factor- α (TGF- α) (Shin et al. 1995), thrombospondin-1 (Dameron et al. 1994), fas/APO-1 (Owen-Schaub et al. 1995), Rb (Osifchin et al. 1994), PCNA (Shivakumar et al. 1995), the epidermal growth factor (EGF) receptor (Deb et al. 1994), cyclin D (Chen et al. 1995), creatine kinase (Zhao et al. 1994), and p53 itself (Deffie et al. 1993). These genes have not generally met as many criteria as those described above; therefore, it is not yet clear whether they are true p53 target genes. Additional good candidates for p53 target genes will, no doubt, continue to be identified.

Figure 2. Pathways of the biological effects of p53.

Holding back: transcriptional repression

p53 represses transcription of a number of cellular and viral genes with promoters lacking p53-binding sites, including, among others, *c-fos*, *c-jun*, *IL-6*, *Rb*, and *bcl-2* (see Donehower and Bradley 1993, and references therein; Jackson et al. 1993; Miyashita et al. 1994). The potency of p53 as a broad-range repressor may be an important component of its tumor suppressor function, and it has been proposed to play a role in apoptosis (see below). It will be of considerable interest to identify endogenous, specific target genes that are repressed after p53 induction.

Despite the wide range of promoters that are inhibited by coexpression of p53 in transfected cells, it was reported that only those promoters containing TATA boxes, and not those containing an initiator element, are inhibited by p53 (Mack et al. 1993). This result, coupled with the known interaction of p53 with TBP, suggests that the mechanism by which p53 represses these promoters is by squelching, that is, sequestering TBP and thus inhibiting efficient initiation of transcription (Seto et al. 1992; Mack et al. 1993). The mapping of the p53 transcriptional repression domain to the carboxyl terminus (Subler et al. 1994; Horikoshi et al. 1995; Shaulian et al. 1995), which also interacts with TBP (Horikoshi et al. 1995), suggests further that transcriptional repression by p53 is mediated by its interaction with TBP. Because TBP exists as a multisubunit complex, holoTFIID, however, there is no evidence that p53 repression functions through TBP squelching in cells. Interestingly, the p53_{gln22,ser23} mutant, which retains the ability to interact with TBP but is unable to bind to TAFs, is unable to repress transcription (see Sabbatini et al. 1995b; G. Farmer and C. Prives, unpubl.). These data suggest that not only TBP, but also TAFs, may be necessary for p53-mediated repression of transcription.

In at least one case, transcriptional repression by p53

has been proposed to act through interaction with a transcriptional activator rather than the general transcriptional machinery. Repression of the *hsp70* gene by p53 is mediated by an interaction between p53 and CCAAT binding factor (CBF), a transcriptional activator of the *hsp70* promoter (Agoff et al. 1993).

It is noteworthy that Yew et al. (1994) showed that inhibition of p53 activation function by the adenovirus E1B 55-kD protein is not simply by passive masking of the p53 activation domain but, rather, that the E1B protein contains a potent repression domain that is recruited to the promoter by p53.

Arrest: braking and not entering

It is now well documented that induction of p53 leads to cell growth arrest or cell death (Fig. 2). Both provide mechanisms by which p53 functions to control DNA damage, protecting cellular descendants from accumulating excessive mutations. A number of experiments have provided information as to what is involved in each of these two responses, although there are still many questions to be answered.

The most well-understood component of p53 function is its ability to induce a G₁ arrest (Fig. 2). In response to DNA damage, p53 accumulates and can act on its transcriptional targets (see above). The *p21* gene, encoding a CDK inhibitor (CKI), is a critical target of p53 in facilitating arrest. Up-regulation of p21 expression by p53 in response to radiation can inhibit cyclin E/cdk2 and cyclin A/cdk2 activities (Dulic et al. 1994) allowing the accumulation of hypophosphorylated Rb (Demers et al. 1994a; Slebos et al. 1994), which results in radiation-induced G₁ arrest. Whether there are other p53 targets whose activation is required for G₁ arrest is not yet established. Evidence that overexpression of p21 itself can lead to arrested growth (Harper et al. 1995) and

that *p21* $-/-$ cells show defective cell cycle arrest in response to radiation (Brugarolas et al. 1995; Deng et al. 1995) strongly implicates *p21* as a critical target of p53 in G_1 .

In the original conception of p53 as a checkpoint factor, it was proposed that DNA damage would induce transient G_1 arrest in cells with wild-type but not mutant p53, to allow time for damaged DNA to be repaired before continuation of the cell cycle (see Kastan et al. 1991). Although this proposal was based on experiments with myeloblastic leukemia cells, more recent experiments have shown that DNA damage of normal human diploid fibroblasts results in a prolonged and apparently irreversible G_1 arrest (DiLeonardo et al. 1994). Whether the discrepancy between these results and earlier ones is a function of differences in cell type remains to be addressed. Furthermore, the absence of p53 was demonstrated to affect the cell cycle even without DNA damage. When analyzed by flow cytometry, the percentage of cells in the G_0/G_1 stage of the cell cycle was lower, and the percentages of cells in S phase higher for *p53* $-/-$ fibroblasts than for wild-type fibroblasts; heterozygous cells had intermediate percentages of cells in G_0/G_1 and S as compared to *p53* null and *p53* wild-type cells (Harvey et al. 1993c). These changes in the cell cycle occurred in cells even in the absence of DNA-damaging treatments.

p53 was first shown to mediate cell-cycle arrest primarily in G_1 . However, a significant G_2 arrest function has been reported for p53 (Agarwal et al. 1995; Stewart et al. 1995), and a number of recent reports implicate roles for p53 in G_2 (Ryan et al. 1993; Kulesz-Martin et al. 1994; Aloni-Grinstein et al. 1995; Guillouf et al. 1995; Powell et al. 1995). p53 has been shown to act as a mitotic checkpoint factor as well (Cross et al. 1995). Fibroblasts from *p53* $-/-$ mice, in contrast to those from wild-type mice, did not arrest in response to spindle inhibitors but, rather, would undergo multiple rounds of DNA synthesis without the appropriate chromosome segregation, resulting in formation of tetraploid and octaploid cells. These results are consistent with previous observations identifying a correlation between loss of p53 and aneuploidy (Livingstone et al. 1992; Harvey et al. 1993c).

p53 has also been suggested to participate in the growth arrest resulting from the overexpression of the *gas1* gene, which blocks the $G_0 \rightarrow S$ transition (Del Sal et al. 1995). These results may be related to the observation that contact-inhibited cells lacking p53 transcriptional activity could be stimulated to progress through the cell cycle by expression of cyclin E, which also restored the transcriptional activity of p53 (Deffie et al. 1995).

To die for: p53 and apoptosis

p53 mediates apoptosis in several cell types, particularly those of the hematopoietic lineages (see Oren 1994 and references therein; Eizenberg et al. 1995) (Fig. 2). Several stimuli, including DNA damage (Clarke et al. 1993;

Lowe et al. 1993), adenovirus *E1A* expression (Debbas and White 1993; Lowe and Ruley 1993), *myc* expression (Hermeking and Eick 1994; Wagner et al. 1994), or withdrawal of growth factors (Johnson et al. 1993; Gottlieb et al. 1994) can cause p53-dependent apoptosis. However, apoptosis can also occur by pathways independent of p53, for example, glucocorticoids or treatments that mimic T-cell receptor engagement (Clarke et al. 1993; Lowe et al. 1993).

Expression of the survival factor Bcl-2 or adenovirus E1B 19-kD protein can block p53-mediated apoptosis (Debbas and White 1993; Chiou et al. 1994). Apoptosis in response to p53 expression can also be inhibited by exposure of cells to a variety of growth factors such as IL-3, IL-6, and erythropoietin (Epo) that act as survival factors (Johnson et al. 1993; Yonish-Rouach et al. 1993; Gottlieb et al. 1994; Canman et al. 1995).

p53-mediated apoptosis has been shown to be an important mechanism by which transformation is suppressed in oncogene-expressing cells (Lowe et al. 1994b), and tumor growth and progression is inhibited (Symonds et al. 1994). p53-mediated apoptosis results from exposure to physiologic conditions such as UV irradiation (Ziegler et al. 1994) and hypoxia (Graeber et al. 1996), and can act as a protective mechanism for removing cells with DNA damage from the small intestine (Merritt et al. 1994) and the skin (Ziegler et al. 1994). The p53-dependent apoptotic pathway has not only been demonstrated to be critical to the development of tumors but also in their treatment. Lowe et al. (1994a) demonstrated that the effectiveness of various cancer therapies correlated with the ability to induce a p53-dependent apoptotic response.

Mechanisms of p53-dependent apoptosis

Although the ability of p53 to function as a transcriptional activator is necessary for its function in mediating G_1 arrest (see above), some studies have provided evidence that p53 may have another transcription-independent function in apoptosis. p53-Dependent cell death was shown to occur in the presence of either the transcriptional inhibitor actinomycin D or the translational inhibitor cycloheximide (Caelles et al. 1994; Wagner et al. 1994). The complexity of the situation is best exemplified by two recent papers reporting contradictory results with the mutant p53_{gln22,ser23}, which abrogates the transcriptional activating function of p53. In a transient transfection apoptosis assay (Yonish-Rouach et al. 1994; Haupt et al. 1995a), Oren and colleagues concluded that p53-mediated apoptosis does not require its transcription function in HeLa cells: Two different mutant forms of p53 that cannot activate transcription, including the transcriptionally defective mutant p53_{gln22,ser23}, still acted as potent inducers of apoptosis (Haupt et al. 1995b). In contrast, the White laboratory demonstrated that BRK cells stably expressing E1A plus the temperature-sensitive p53_{val135} underwent apoptosis after shift to 32°C (the wild-type permissive temperature), whereas cells expressing p53_{gln22,ser23} in the background of the

same temperature-sensitive p53, did not (Sabbatini et al. 1995b). Thus, their protocol demonstrated a requirement for the transcriptional activation function of p53 for apoptosis. These and other data imply that p53 may have separate transcription-dependent and -independent modes of inducing cell death. Whether different cell types or experimental protocols are the reason for obtaining these conflicting results is not yet established. Haupt et al. (1996) report that the requirement for the transcriptional activating function of p53 for apoptosis varies with the cell type.

Recent evidence suggests that the transcriptional repression function of p53 may be important in mediating apoptosis. The adenovirus E1B 19-kD protein and the cellular protein, Bcl-2, both of which block p53-dependent apoptosis, were found to suppress transcriptional repression but to have no effect on transcriptional activation by p53 (Shen and Shenk 1994; Sabbatini et al. 1995a).

Several other possibilities exist for the mechanisms by which p53 could exert its effect in a manner independent of transcriptional regulation. A number of cellular proteins have been found to interact with p53 (see Table 1), and interaction with other proteins may mediate apoptosis. Alternatively, the ability of p53 to reanneal single-stranded nucleic acids (see above) might be relevant, given that p53 was shown to interfere with translation of the CDK4 mRNA in cells (Ewen et al. 1995) as well as translation of its own mRNA in vitro (Mosner et al. 1995).

These issues may also be related to the observations that mutants of p53 defective in *trans*-activation retain biological function in other assays such as suppression of oncogene-mediated transformation of primary cells (Unger et al. 1993), and $G_0 \rightarrow S$ transition growth arrest (Del Sal et al. 1995).

Apoptosis or arrest?

How is it decided whether p53 will induce arrest or apoptosis? Cell type appears to be at least one factor (Midgley et al. 1995; Haupt et al. 1996). Several experimental systems have demonstrated that it is possible to manipulate cells to undergo either response dependent on (1) viral protein expression, (2) growth factor availability, or (3) expression of Rb and/or E2F. The G_1 arrest that primary rodent cells normally undergo in response to IR is not observed in the presence of E1A expression, and these cells instead undergo apoptosis (Debbas and White 1993; Lowe and Ruley 1993). Similarly, hematopoietic Baf-3 cells respond to irradiation by arresting in G_1 in the presence of IL-3 but undergo apoptosis in the absence of IL-3 (Canman et al. 1995).

Numerous pieces of evidence point toward a cooperativity between the p53 pathway and the Rb/E2F pathway in determining the outcome of DNA damage. Loss of Rb by expression of viral proteins that inactivate Rb function or by homozygous gene disruption has been correlated with loss of G_1 arrest after DNA damage (Demers et al. 1994a; Hickman et al. 1994; Slebos et al. 1994) and

apoptosis (Howes et al. 1994; Morgenbesser et al. 1994; Pan and Griep et al. 1994). Lack of Rb would be expected to result in an increase in free E2F, which can act in promoting progression of the cell cycle. Therefore, overexpression of E2F would be expected to be the functional equivalent to loss of Rb (see Fig. 2). Indeed, overexpression of E2F results in loss of G_1 arrest and induction of apoptosis (Qin et al. 1994; Wu and Levine 1994) similar to effects described above for the loss of Rb function. Overexpression of Rb has also been noted to block p53-dependent apoptosis (Haupt et al. 1995a). Thus, overexpression of Rb or E2F can have opposing effects in the balance among apoptosis, cell growth and survival, and G_1 arrest. The relationship between p53 and Rb helps to explain why several DNA tumor viruses inactivate both tumor suppressors (for review, see White 1996).

p53 and genomic stability

p53 has been proposed to be involved in maintaining stability of the genome (Livingstone et al. 1992; Yin et al. 1992), and both cell cycle arrest and apoptosis can be considered mechanisms by which this may be accomplished. In the presence of DNA damage, cells will either arrest, presumably to allow DNA repair, or undergo cell death, in a p53-dependent manner. In either case, the propagation of potentially deleterious mutations can thus be averted.

Consistent with a role for p53 in protecting genomic integrity, fibroblasts from p53-deficient mice demonstrate chromosomal abnormalities that appear at early passage in homozygous null fibroblasts and at later passage in heterozygous fibroblasts (Harvey et al. 1993c). Aneuploidy and evidence of chromosomal instability was also found in tumors from *p53* $-/-$ mice and from mice with both a *Wnt-1* transgene and homozygous for the null allele of *p53*. (Purdie et al. 1994; Donehower et al. 1995). Finally, fibroblasts from *p53* $-/-$ mice become tetraploid and octaploid after exposure to spindle inhibitors, in contrast to those from wild-type mice, which undergo arrest (Cross et al. 1995).

p53-deficient cells exhibit a higher tolerance to genetic abnormalities arising from radiation as well as spontaneously. In response to γ -irradiation, cells from mice homozygous for the null allele show increased accumulation of double-stranded DNA damage as compared to heterozygotes or wild-type littermates (J. Lee et al. 1994).

Is there a role for p53 in DNA replication and DNA repair?

p53 induction can block cells in G_1 and thereby prevent them from progressing to S phase. There are experiments, however, suggesting that p53 plays one or more roles regulating processes such as DNA replication and DNA repair. Indeed, p53 may have a direct impact on the ability of a cell to synthesize DNA through its function as a transcriptional activator. The products of two different p53 target genes, *p21/WAF1/Cip1* and *GADD45*, have been shown to interact with PCNA, a factor that is

involved in both DNA repair and replication. p21/WAF1/Cip1 has been shown to directly inhibit the function of PCNA in replication, although its function in repair is relatively unaffected (Flores-Rozas et al. 1994; Li et al. 1994; Waga et al. 1994). GADD45 was also shown to bind to PCNA (Smith et al. 1994), although the implications of this interaction are not yet well understood (see Kazantsev et al. 1995).

The possibility also exists that p53 directly regulates DNA repair and replication in a manner independent of its *trans*-activation function. However, because there are also lines of evidence suggesting that p53 does not directly affect either of these processes at this point, we present both sides of the issue here.

Evidence suggesting a role for p53 in replication and repair

(1) p53 binds to several proteins involved in DNA repair in vitro (see Fig. 1 and Table 1): (a) The 70-kD subunit of the single-stranded DNA-binding protein RP-A, a protein with well-defined roles in both replication and repair (Dutta et al. 1993; He et al. 1993; Li and Botchan 1993); (b) several polypeptides of TFIIH, the dual function transcription-repair factor, including p62, and the XPD (ERCC2) and XPB (ERCC3) DNA helicase polypeptides (X. Wang et al. 1994; Xiao et al. 1994; X. Wang et al. 1995; Leveillard et al. 1996); and (c) the strand-specific repair factor CSB (ERCC6), which may also be a helicase protein (X. Wang et al. 1995). (2) p53 possesses the ability to recognize and bind tightly to both irradiated DNA and mismatched DNA (Lee et al. 1995; Reed et al. 1995). (3) p53 can inhibit viral and cellular DNA helicases (X. Wang et al. 1995 and references therein), possibly because of its potent DNA reannealing activity. (4) p53 can block DNA replication in vitro, in both *Xenopus laevis* egg extracts (Cox et al. 1995) and extracts from murine fibroblasts (Miller et al. 1995). (5) Deficiency of p53 function was shown to result in reduced repair of cellular DNA in some cases (Ford and Hanawalt 1995; Havre et al. 1995; Smith et al. 1995; X. Wang et al. 1995), suggesting a role for p53 in nucleotide excision repair.

Evidence against a role for p53 in replication and repair

(1) Interactions of RP-A and TFIIH with p53 are not unique to p53: Both factors interact with other acidic activators such as VP16 or the Epstein-Barr virus protein EBNA 2 (He et al. 1993; Li and Botchan 1993; Xiao et al. 1994; Tong et al. 1995), suggesting that these interactions relate to p53 as a regulator of transcription rather than DNA replication or repair. (2) Although Miller et al. (1995) found that p53 blocks polyoma DNA replication in vitro, Kanda et al. (1994) using the same p53-binding site-containing polyoma *ori*-DNA constructs, reported the stimulation of DNA replication by p53 in vivo. (3) Li-Fraumeni cells exhibit defective global DNA repair but are normal for transcription-coupled repair (Ford and Hanawalt 1995), and p53^{-/-} mouse fibroblasts display

normal rates of repair as do wild-type p53-containing cells (Ishizaki et al. 1994; Sands et al. 1995). (4) p53 does not influence DNA repair in vitro (Sancar 1995; Leveillard et al. 1996).

Thus, there are good arguments for either side of the interesting question as to whether p53 directly affects DNA replication or repair. It is hoped that further experiments will clarify these issues.

Going without: p53 in development

The ability to generate mice lacking p53 implied that p53 is dispensable for growth, differentiation, and embryonic development (Donehower et al. 1992). Subsequent studies, however, have shown that the genetic background within which p53 gene disruptions are made can significantly influence the phenotype of p53 null mice. Although the initial study yielded numbers of homozygous null mice close to the expected 25% (Donehower et al. 1992), p53-deficient embryos from a different genetic background recovered fewer p53^{-/-} mice than expected. Furthermore, a phenotypic examination revealed that ~16% of 13.5-day p53^{-/-} embryos displayed marked exencephaly, with an overgrowth of brain tissue (Sah et al. 1995). Interestingly, all of these embryos were females. Thus, in contrast to early reports, deficiency of p53 does have a developmental phenotype, although it only shows partial penetrance. Lethality of a subset of p53 null embryos was also observed by Nicol et al. (1995), who additionally noted that the p53 genotype of pregnant mice affected the teratogenicity of their embryos.

One interesting recent finding involves the respective roles of mdm-2 and p53 in development. Homozygous deletion of *mdm-2* results in an early embryonic lethality, sometime between implantation and day 5.5 of gestation (Jones et al. 1995; Montes de Oca Luna et al. 1995). However, this lethality can be rescued in the absence of p53; whereas *mdm-2*^{-/-} p53^{+/+}, and *mdm-2*^{-/-} p53^{+/-} embryos die in utero, *mdm-2*^{-/-} p53^{-/-} mice are viable. These results suggest that the primary role of mdm-2 during development is to negatively regulate p53, with p53 and mdm-2 acting in concert to regulate the cell cycle during early development.

To B or not to B? p53 in differentiation

p53 has also been suggested to play a role in the differentiation of several cell lineages based on the correlation between overexpression of p53 and induction of differentiation markers. The immunoglobulin chains μ and κ are induced in early pre-B and pre-B cell lines, respectively, upon expression of p53; furthermore, the introduction of mutant p53 into pre-B cells was found to block κ chain expression (Rotter et al. 1994 and references therein). The relationship between p53 and B cell differentiation is particularly interesting in view of the fact that DNA rearrangements involving double-stranded DNA breaks occur during the process of B cell development. Hemoglobin expression is also stimulated

in erythroleukemic cells and chronic myelogenous leukemic cells in response to p53 [Johnson et al. 1993]. A role for p53 in spermatogenesis has been suggested as well, based on the highly defined spatial and cyclical expression of the *p53* gene in tetraploid pachytene primary spermatocytes [Schwartz et al. 1993]. That each of these lineages appears normal in *p53* knockout mice might indicate the existence of compensatory mechanisms such that differentiation may proceed even in the absence of p53.

p53 and tumor formation

The initial observation that *p53* mutation occurs with extraordinarily high frequency in diverse types of human cancers has been confirmed and extended with the analysis of >2500 tumors and tumor cell lines [Hollstein et al. 1994]. Approximately half of the major forms of cancer contain *p53* missense mutations, ~40% of which localize to hot spots (see Fig. 1). The impact of altered or loss of p53 function on tumorigenesis may be even greater than predicted initially, as mechanisms other than point mutations can functionally inactivate p53. Earlier observations noted *mdm-2* amplification in tumors containing wild-type p53 [Oliner et al. 1992]. Furthermore, wild-type p53 is localized exclusively in the cytoplasm in some tumors, precluding its ability to act as a transcription factor. This novel mechanism for p53 inactivation was first identified in breast cancer cells and subsequently in a large majority of undifferentiated neuroblastomas [Moll et al. 1995 and references therein].

A vast literature exists concerning the role of p53 in tumorigenesis, and the reader is referred to Greenblatt et al. (1994) for reviews of this topic. We have chosen to focus on two specific areas highlighting recent progress in the analysis of *p53*-deficient mice and in the relationship between hypoxia, angiogenesis, and mutation of p53.

Examination of tumor spectrum and incidence in *p53*-deficient mice has led to several observations [Donehower et al. 1992; Harvey et al. 1993a; Jacks et al. 1994; Purdie et al. 1994]. *p53* $-/-$ mice are highly prone to spontaneous tumor formation and predominantly develop lymphomas. The particular genetic background of the mice, however, affects tumor incidence and spectrum. Mice heterozygous for the inactivated *p53* allele also show increased incidence of spontaneous malignancies as compared to *p53* $+/+$ mice: In this case, the heterozygous mice develop predominantly osteosarcomas and soft tissue sarcomas. In the majority of cases with heterozygotes, the wild-type *p53* allele has been lost in tumors. In addition to susceptibility to spontaneous tumors, p53 deficiency also results in increased sensitivity to induced tumorigenesis, both by the carcinogen dimethylnitrosamine, or γ -irradiation [Harvey et al. 1993b; Kemp et al. 1994].

Mouse embryo fibroblasts derived from *p53* $-/-$ mice have several altered growth characteristics relative to wild-type fibroblasts, such as (1) a significantly shorter doubling time, (2) an increased ability to grow under conditions of low cell density, and (3) a lack of senescence even at high passage [Harvey et al. 1993c], consistent

with previous data correlating loss of p53 with immortalization [for review, see Donehower and Bradley 1993]. Indeed, loss of p53 was found to allow the immortalization of hematopoietic cells by the *myc* and *raf* oncogenes [Metz et al. 1995]. At present, it is unclear whether the loss of p53 per se is sufficient for immortalization or whether the absence of p53 allows other genetic changes to occur, which then result in immortalization.

In a skin model system, the lack of p53 did not enhance tumor initiation or promotion but greatly increased malignant progression [Kemp et al. 1993], and in a prostate cancer model, deficiency of p53 was correlated with a high degree of metastasis [Thompson et al. 1995]. In an examination of tumorigenesis of the brain choroid plexus epithelium, the absence of p53 correlated with aggressive tumor growth and a decrease in apoptosis, suggesting that p53-dependent apoptosis normally acts as a check to tumor growth and progression in this tissue [Symonds et al. 1994].

Mice subjected to a wide variety of genetic manipulations, such as those with targeted inactivation of particular genes or bearing transgenes, are now being crossed and analyzed for the effects of combinations of overexpression and/or lack of expression of several interesting genes. Mice carrying a *Wnt-1* transgene in a *p53* $-/-$ background have an earlier age of tumor onset than *Wnt-1* transgenics in a wild-type *p53* background [Donehower et al. 1995]. Similarly, mice deficient in both *Rb* and p53 show cooperative effects of both tumor suppressor genes, with reduced viability and faster rate of tumor development than in mice deficient in either one [Williams et al. 1994; Harvey et al. 1995a]. Furthermore, mice heterozygous for both *Rb* and *p53* display novel tumor types not observed in mice containing either *Rb* or *p53* mutant alleles [Williams et al. 1994; Harvey et al. 1995a].

Although the loss of p53 function clearly contributes to tumor development, recent observations have elucidated the involvement of mutant p53 in tumor progression as well. The efficient growth of tumors is dependent on their ability to develop new blood supplies, and angiogenesis, the formation of new blood vessels, is controlled by the balance between stimulatory and inhibitory influences. Wild-type p53 expression results in the secretion of inhibitors of angiogenesis [Dameron et al. 1994; Van Meir et al. 1994], and this may be an additional mechanism by which the presence of wild-type p53 inhibits tumor progression. Low oxygen, or hypoxic conditions, such as those in a tumor with inadequate blood supply, induce accumulation of the p53 protein [Graeber et al. 1994]. The ensuing reducing environment, by redox regulation, would be expected to stimulate the DNA-binding ability of p53 [Hainaut and Milner 1993]. Because hypoxia can induce apoptosis in a p53-dependent manner [Graeber et al. 1996], low oxygen conditions can provide a selective advantage for cells carrying mutations in p53, allowing escape from apoptosis. Indeed, cells lacking p53 can overtake wild-type cells after hypoxia treatment. In addition to a resulting cellular survival and growth advantage, mutation of p53 selected by

hypoxic conditions would be expected to favor expansion of the tumor by loss of expression of anti-angiogenic factors as described above, allowing growth of new blood vessels to the tumor. Furthermore, mutant, but not wild-type, p53 has been shown to synergize with PKC in inducing expression of the angiogenic vascular endothelial growth factor (*VEGF*) gene (Kieser et al. 1994). Hypoxia itself is known to induce VEGF (Schweiki et al. 1992), and it is interesting to speculate that this process may be occurring through its selection of mutant p53. In this way, mutation of the *p53* gene may be a critical event in tumor progression not only for allowing cell growth but also for regulating tumor expansion by stimulating vascularization.

Mutant p53: if it's broke, then fix it!

As described above, approximately half of the major forms of cancer contain *p53* missense mutations. There are three modes by which mutation of p53 might affect its function: (1) a loss of wild-type function, (2) a *trans*-dominant effect of mutant over wild-type p53 function (dominant-negative effect), and/or (3) a gain of oncogenic potential. Experiments supporting each have been reported. The fact that *p53* null mice are highly tumor prone argues strongly that the loss of its function is sufficient to contribute to tumorigenesis (see above). The dominant-negative effect of mutant p53 proteins (through oligomerization with wild-type p53) results in an inhibition of the wild-type ability to bind DNA and activate transcription. Indeed, *p53* wild-type mice carrying a dominant-negative transgene show increased tumor incidence and decreased survival compared to non-transgenic animals (Harvey et al. 1995b and references therein). Thus, the presence of mutant p53 proteins in tumors might result from selection for dominant-negative mutants that cause a loss of wild-type function.

Additionally, some p53 mutants are capable of conferring increased tumorigenicity, metastatic potential, and/or tissue invasiveness (Dittmer et al. 1993; Hsiao et al. 1994). The gain-of-function properties of these mutant p53 proteins may be related to the ability of mutant, but not wild-type, p53 proteins to (1) preferentially stimulate the transcription of several cellular and viral promoters, for example, that of the multidrug resistance (*MDR1*) gene (Dittmer et al. 1993 and references therein); (2) associate with cellular proteins p38 and p42 (Y. Chen et al. 1994); or (3) synergize with PKC in the induction of the expression of the angiogenic *VEGF* gene (Kieser et al. 1994). Interestingly, a p53 mutant (Gly-281) in a background of the transcriptionally inactivating mutation at residues 22 and 23, was no longer tumorigenic (Lin et al. 1995), suggesting that the oncogenicity of mutant p53 requires the wild-type activation domain.

The discrimination between dominant-negative and gain-of-function mutations was illustrated in experiments with transgenic mice expressing a murine Val-135 mutant p53 protein in wild-type and p53-deficient mice. Expression of this mutant p53 increased the tumor incidence in mice carrying one or both wild-type alleles of

p53 but not in mice that were homozygous null for p53. This suggests that dominant-negative effects of interference with wild-type function are distinct from the acquisition of new oncogenic properties (Harvey et al. 1995b).

Although it was initially assumed that in contrast to the wild-type form of p53, all tumor-derived mutant p53 proteins would be defective for sequence-specific *trans*-activation, more recent experiments have refined this concept. First, not all mutants can be considered the same; individual hot spot mutants differ in their properties. Whereas some mutants, particularly His-273 and Ala-143, can in some circumstances, show a degree of wild-type DNA-binding and transcriptional functions (see below), other mutants are generally negative in these same assays (J.-Y. Chen et al. 1993; Chumakov et al. 1993; Park et al. 1994; Pietenpol et al. 1994; Zhang et al. 1993, 1994a). Second, the restoration of DNA-binding and/or transcriptional activity to some mutant p53 proteins can be accomplished by (1) stimulation with the antibody 421 (Hupp et al. 1992; Zhang et al. 1993; Abarzua et al. 1995; Niewolik et al. 1995); (2) incubation with the bacterial heat shock protein dnaK (Hupp et al. 1992); (3) using artificial, high-affinity DNA-binding sequences for p53 (J.-Y. Chen et al. 1993; Chumakov et al. 1993; Park et al. 1994; Pietenpol et al. 1994); or (4) temperature shift (J.-Y. Chen et al. 1993; W. Zhang et al. 1994a; P. Friedlander and C. Prives, unpubl.).

These reports confirm that under certain conditions mutant p53 proteins can adopt wild-type properties, one of the "holy grails" of p53 research. Because success in treatment with therapeutic agents is often correlated with the degree of p53 responsiveness (Lowe et al. 1994a), the ability to regain wild-type p53 function in tumor cells containing mutant p53 protein has obvious clinical relevance. Thus, it is with great anticipation that we await further progress in this line of investigation.

Acknowledgments

Thanks are extended to our many colleagues in the p53 field who generously provided us with preprints and reprints of their recent work. We also thank Maria Riley, as well as the other members of the Prives laboratory for their help during the preparation of this manuscript. This paper was supported by grants from the National Institutes of Health (CA58316) and the U.S. Army breast cancer program (DAMD17-94-J-4275). L.J.K. is a Leukemia Society Fellow.

References

- Abarzua, P., J.E. LoSardo, M.L. Gubler, and A. Neri. 1995. Microinjection of monoclonal antibody Pab421 into human SW480 colorectal carcinoma cells restores the transcription activation function to mutant p53. *Cancer Res.* 55: 3490–3494.
- Agarwal, M.L., A. Agarwal, W.R. Taylor, and G.R. Stark. 1995. p53 controls both the G2/M and the G1 cell cycle check-

- points and mediates reversible growth arrest in human fibroblasts. *Proc. Natl. Acad. Sci.* **92**: 8493–8497.
- Agoff, S.N., J.H. Hou, D.I.H. Linzer, and B. Wu. 1993. Regulation of the human hsp70 promoter by p53. *Science* **259**: 84–87.
- Aloni-Grinstein, R., D. Schwartz, and V. Rotter. 1995. Accumulation of wild-type p53 protein upon γ -irradiation induces a G2 arrest-dependent immunoglobulin κ light chain gene expression. *EMBO J.* **14**: 1392–1401.
- Balagurumoorthy, P., H. Sakamoto, M.S. Lewis, N. Zambrano, G.M. Clore, A.M. Gronenborn, E. Appella, and R.E. Harrington. 1995. Four p53 DNA-binding domain peptides bind natural p53 response elements and bend the DNA. *Proc. Natl. Acad. Sci.* **92**: 8591–8595.
- Balkalkin, G., T. Yakovleva, G. Selivanova, K.P. Magnusson, L. Szekely, E. Kiseleva, G. Klein, L. Terenius, and K.G. Wiman. 1994. p53 binds single-stranded DNA ends and catalyzes DNA renaturation and strand transfer. *Proc. Natl. Acad. Sci.* **91**: 413–417.
- Balkalkin, G., G. Selivanova, T. Yakovleva, E. Kiseleva, E. Kashuba, K.P. Magnusson, L. Szekely, G. Klein, L. Terenius, and K.G. Wiman. 1995. p53 binds single-stranded DNA ends through the C-terminal domain and internal DNA segments via the middle domain. *Nucleic Acids Res.* **23**: 362–369.
- Barak, Y., T. Juven, R. Haffner, and M. Oren. 1993. mdm2 expression is induced by wild type p53 activity. *EMBO J.* **12**: 461–468.
- Bargonetti, J., J.J. Manfredi, X. Chen, D.R. Marshak, and C. Prives. 1993. A proteolytic fragment from the central region of p53 has marked sequence-specific DNA-binding activity when generated from wild-type but not from oncogenic mutant p53 protein. *Genes & Dev.* **7**: 2565–2574.
- Baudier, J., C. Delphin, D. Grunwald, S. Khochbin, and J.J. Lawrence. 1992. Characterization of the tumor suppressor protein p53 as a protein kinase C substrate and a S100b-binding protein. *Proc. Natl. Acad. Sci.* **89**: 11627–11631.
- Bayle, J.H., B. Elenbaas, and A.J. Levine. 1995. The carboxyl-terminal domain of the p53 protein regulates sequence-specific DNA binding through its nonspecific nucleic acid-binding activity. *Proc. Natl. Acad. Sci.* **92**: 5729–5733.
- Borellini, F. and R.I. Glazer. 1993. Induction of Sp1-p53 DNA-binding heterocomplexes during granulocyte/macrophage colony-stimulating factor-dependent proliferation in human erythroleukemia cell line TF-1. *J. Biol. Chem.* **268**: 7923–7928.
- Brain, R. and J.R. Jenkins. 1994. Human p53 directs DNA strand reassociation and is photolabelled by 8-azido ATP. *Oncogene* **9**: 1775–1780.
- Brugarolas, J., C. Chandrasekaran, J.I. Gordon, D. Beach, T. Jacks, and G.J. Hannon. 1995. Radiation-induced cell cycle arrest compromised by p21 deficiency. *Nature* **377**: 552–557.
- Buckbinder, L., R. Talbott, S. Velasco-Miguel, I. Takenaka, B. Faha, B.R. Seizinger, and N. Kley. 1995. Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature* **377**: 646–649.
- Caelles, C., A. Helmborg, and M. Karin. 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature* **370**: 220–223.
- Canman, C.E., T.M. Gilmer, S.B. Coutts, and M.B. Kastan. 1995. Growth factor modulation of p53-mediated growth arrest versus apoptosis. *Genes & Dev.* **9**: 600–611.
- Chang, J., D.-H. Kim, S.W. Lee, K.Y. Choi, and Y.C. Sung. 1995. Transactivation ability of p53 transcriptional activation domain is directly related to the binding affinity to TATA-binding protein. *J. Biol. Chem.* **270**: 25014–25019.
- Chen, C.-Y., J.D. Oliner, Q. Zhan, A.J. Fornace, Jr., B. Vogelstein, and M.B. Kastan. 1994. Interactions between p53 and MDM2 in a mammalian cell cycle checkpoint pathway. *Proc. Natl. Acad. Sci.* **91**: 2684–2688.
- Chen, J.-Y., W.D. Funk, W.E. Wright, J.W. Shay, and J.D. Minna. 1993. Heterogeneity of transcriptional activity of mutant p53 proteins and p53 DNA target sequences. *Oncogene* **8**: 2159–2166.
- Chen, X., G. Farmer, H. Zhu, R. Prywes, and C. Prives. 1993. Cooperative DNA binding of p53 with TFIID (TBP): A possible mechanism for transcriptional activation. *Genes & Dev.* **7**: 1837–1849.
- Chen, X., J. Bargonetti, and C. Prives. 1995. p53, through p21 (WAF1/CIP1), induces cyclin D1 synthesis. *Cancer Res.* **55**: 4257–4263.
- Chen, Y., P.L. Chen, and W.H. Lee. 1994. Hot-spot p53 mutants interact specifically with two cellular proteins during progression of the cell cycle. *Mol. Cell. Biol.* **14**: 6764–6772.
- Chiou, S., L. Rao, and E. White. 1994. Bcl-2 blocks p53-dependent apoptosis. *Mol. Cell. Biol.* **14**: 2556–2563.
- Cho, Y., S. Gorina, P.D. Jeffrey, and N.P. Pavletich. 1994. Crystal structure of a p53 tumor suppressor-DNA complex: Understanding tumorigenic mutations. *Science* **265**: 346–355.
- Chowdary, D.R., J.J. Dermody, K.K. Jha, and H.L. Ozer. 1994. Accumulation of p53 in a mutant cell line defective in the ubiquitin pathway. *Mol. Cell. Biol.* **14**: 1997–2003.
- Chumakov, A.M., C.W. Miller, D.L. Chen, and H.P. Koeffler. 1993. Analysis of p53 transactivation through high-affinity binding sites. *Oncogene* **8**: 3005–3011.
- Clarke, A.R., C.A. Purdie, D.J. Harrison, R.G. Morris, C.C. Bird, M.L. Hooper, and A.H. Wyllie. 1993. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* **362**: 849–852.
- Clore, G.M., J. Ernst, R. Clubb, J.G. Omichinski, W.M.P. Kennedy, K. Sakaguchi, E. Appella, and A.M. Gronenborn. 1995. Refined solution structure of the oligomerization domain of the tumor suppressor p53. *Struct. Biol.* **2**: 321–332.
- Cox, L.S., T. Hupp, C.A. Midgley, and D.P. Lane. 1995. A direct effect of activated human p53 on nuclear DNA replication. *EMBO J.* **14**: 2099–2105.
- Crook, T., N.I. Marston, E.A. Sara, and K.H. Vousden. 1994. Transcriptional activation by p53 correlates with suppression of growth but not transformation. *Cell* **79**: 817–827.
- Cross, S.M., C.A. Sanchez, C.A. Morgan, M.K. Schimke, S. Ramel, R.L. Idzerda, W.H. Raskind, and B.J. Reid. 1995. A p53-dependent mouse spindle checkpoint. *Science* **267**: 1353–1356.
- Dameron, K.M., O.V. Volpert, M.A. Tainsky, and N. Bouck. 1994. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* **265**: 1582–1584.
- Deb, S.P., R.M. Munoz, D.R. Brown, M.A. Subler, and S. Deb. 1994. Wild-type human p53 activates the human epidermal growth factor receptor promoter. *Oncogene* **9**: 1341–1349.
- Debbas, M. and E. White. 1993. Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. *Gene & Dev.* **7**: 546–554.
- Deffie, A., H. Wu, V. Reinke, and G. Lozano. 1993. The tumor suppressor p53 regulates its own transcription. *Mol. Cell. Biol.* **13**: 3415–3423.
- Deffie, A., M. Hao, R. Montes de Oca Luna, D.L. Hulboy, and G. Lozano. 1995. Cyclin E restores p53 activity in contact-inhibited cells. *Mol. Cell. Biol.* **15**: 3926–3933.
- Del Sal, G.D., E.M. Ruaro, R. Utrera, C.N. Cole, A.J. Levine, and C. Schneider. 1995. Gas1-induced growth suppression requires a transactivation-independent p53 function. *Mol. Cell. Biol.* **15**: 7152–7160.
- Demers, G.W., S.A. Foster, C.L. Halbert, and D.A. Galloway.

- 1994a. Growth arrest by induction of p53 in DNA damaged keratinocytes is bypassed by human papillomavirus 16 E7. *Proc. Natl. Acad. Sci.* **91**: 4382–4386.
- Demers, G.W., C.L. Halbert, and D.A. Galloway. 1994b. Elevated wild-type p53 protein levels in human epithelial cell lines immortalized by human papillomavirus type 16 E7 gene. *Virology* **198**: 169–174.
- Deng, C., P. Zhang, J.W. Harper, S.J. Elledge, and P. Leder. 1995. Mice lacking p21^{CIP1/WAF1} undergo normal development, but are defective in G1 checkpoint control. *Cell* **82**: 675–684.
- DiLeonardo, A., S.P. Linke, K. Clarkin, and G.M. Wahl. 1994. DNA damage triggers a prolonged p53-dependent G₁ arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes & Dev.* **8**: 2540–2551.
- Dittmer, D., S. Pati, G. Zambetti, S. Chu, A.K. Teresky, M. Moore, C. Finlay, and A.J. Levine. 1993. Gain of function mutations in p53. *Nature Genet.* **4**: 42–45.
- Donehower, L.A. and A. Bradley. 1993. The tumor suppressor p53. *Biochim. Biophys. Acta* **1155**: 181–205.
- Donehower, L.A., M. Harvey, B.L. Slagle, M.J. McArthur, C.A. Montgomery Jr., J.S. Butel, and A. Bradley. 1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**: 215–221.
- Donehower, L.A., L.A. Godley, C.M. Aldaz, R. Pyle, Y.-P. Shi, D. Pinkel, J. Gray, A. Bradley, D. Medina, and H.E. Varmus. 1995. Deficiency of p53 accelerates mammary tumorigenesis in *Wnt-1* transgenic mice and promotes chromosomal instability. *Genes & Dev.* **9**: 882–895.
- Dulic, V., W.K. Kaufmann, S.J. Wilson, T.D. Tlsty, E. Lees, J.W. Harper, S.J. Elledge, and S.I. Reed. 1994. p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. *Cell* **76**: 1013–1023.
- Dutta, A., S.M. Ruppert, J.C. Aster, and E. Winchester. 1993. Inhibition of DNA replication factor RPA by p53. *Nature* **365**: 79–82.
- Eizenberg, O., A. Faber-Elman, E. Gottlieb, M. Oren, V. Rotter, and M. Schwartz. 1995. Direct involvement of p53 in programmed cell death of oligodendrocytes. *EMBO J.* **14**: 1136–1144.
- El-Deiry, W.S., T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, and B. Vogelstein. 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* **75**: 817–825.
- El-Deiry, W.S., J.W. Harper, P.M. O'Connor, V.E. Velculescu, C.E. Canman, J. Jackman, J.A. Pietenpol, M. Burrell, D.E. Hill, Y. Wang, K.G. Wiman, W.E. Mercer, M.B. Kastan, K.W. Kohn, S.J. Elledge, K.W. Kinzler, and B. Vogelstein. 1994. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res.* **54**: 1169–1174.
- Ewen, M.E., C.J. Oliver, H.K. Sluss, S.J. Miller, and D.S. Peeper. 1995. p53-dependent repression of CDK4 translation in TGF- β -induced G₁ cell-cycle arrest. *Genes & Dev.* **9**: 204–217.
- Filhol, O., J. Baudier, C. Delphin, P. Loue-Mackenbach, E.M. Chambaz, and C. Cochet. 1992. Casein kinase II and the tumor suppressor protein p53 associate in a molecular complex that is negatively regulated upon p53 phosphorylation. *J. Biol. Chem.* **267**: 20577–20583.
- Fiscella, M., S.J. Ullrich, N. Zambrano, M.T. Shields, D. Lin, S.P. Lees-Miller, C.W. Anderson, W.E. Mercer, and E. Appella. 1993. Mutation of the serine 15 phosphorylation site of human p53 reduces the ability of p53 to inhibit cell cycle progression. *Oncogene* **8**: 1519–1528.
- Fiscella, M., N. Zambrano, S.J. Ullrich, T. Unger, D. Lin, B. Cho, W.E. Mercer, C.W. Anderson, and E. Appella. 1994. The carboxy-terminal serine 392 phosphorylation site of human p53 is not required for wild-type activities. *Oncogene* **9**: 3249–3257.
- Flores-Rozas, H., Z. Kelman, F.B. Dean, Z.-Q. Pan, J.W. Harper, S.J. Elledge, M. O'Donnell, and J. Hurwitz. 1994. Cdk-interacting protein 1 directly binds with proliferating cell nuclear antigen and inhibits DNA replication catalyzed by the DNA polymerase δ holoenzyme. *Proc. Natl. Acad. Sci.* **91**: 8655–8659.
- Ford, J.M. and P.C. Hanawalt. 1995. Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. *Proc. Natl. Acad. Sci.* **92**: 8876–8880.
- Friedman, P.N., X. Chen, J. Bargonetti, and C. Prives. 1993. The p53 protein is an unusually shaped tetramer that binds directly to DNA. *Proc. Natl. Acad. Sci.* **90**: 3319–3323.
- Fuchs, B., D. O'Connor, L. Fallis, K.H. Scheidtmann, and X. Lu. 1995. p53 phosphorylation mutants retain transcription activity. *Oncogene* **10**: 789–793.
- Goga, A., X. Liu, T.M. Hambuch, K. Senechal, E. Major, A.J. Berk, O. Witte, and C.L. Sawyers. 1995. p53-dependent growth suppression by the c-Abl nuclear tyrosine kinase. *Oncogene* **11**: 791–799.
- Gottlieb, E., R. Haffner, T. von Ruden, E.F. Wagner, and M. Oren. 1994. Down-regulation of wild-type p53 activity interferes with apoptosis of IL-3-dependent hematopoietic cells following IL-3 withdrawal. *EMBO J.* **13**: 1368–1374.
- Graeber, T.G., J.F. Peterson, M. Tsai, K. Monica, A.J. Fornace Jr., and A.J. Giaccia. 1994. 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.
- Graeber, T.G., C. Osmanian, T. Jacks, D.E. Housman, C.J. Koch, S.W. Lowe, and A.J. Giaccia. 1996. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* **379**: 88–91.
- Greenblatt, M.S., W.P. Bennett, M. Hollstein, and C.C. Harris. 1994. Mutations in the p53 tumor suppressor gene: Clues to cancer etiology and molecular pathogenesis. *Cancer Res.* **54**: 4855–4878.
- Gu, Y., C.W. Turck, and D.O. Morgan. 1993. Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. *Nature* **366**: 707–710.
- Gualberto, A. and A.S. Baldwin Jr. 1995. p53 and Sp1 interact and cooperate in the tumor necrosis factor-induced transcriptional activation of the HIV-1 long terminal repeat. *J. Biol. Chem.* **270**: 19680–19683.
- Guillouf, C., F. Rosselli, K. Krishnaraju, E. Moustacchi, B. Hoffman, and D.A. Liebermann. 1995. p53 involvement in control of G2 exit of the cell cycle: role in DNA damage-induced apoptosis. *Oncogene* **10**: 2263–2270.
- Hainaut, P. and J. Milner. 1993. Redox modulation of p53 conformation and sequence-specific DNA binding in vitro. *Cancer Res.* **53**: 4469–4473.
- Halazonetis, T.D. and A.N. Kandil. 1993. Wild-type p53 adopts a “mutant”-like conformation when bound to DNA. *EMBO J.* **12**: 1021–1028.
- Halazonetis, T.D., L.J. Davis, and A.N. Kandil. 1993. Wild-type p53 adopts a “mutant”-like conformation when bound to DNA. *EMBO J.* **12**: 1021–1028.
- Harper, J.W., G.R. Adami, N. Wei, K. Keyomarsi, and S.J. Elledge. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **75**: 805–816.

- Harper, J.W., S.J. Elledge, K. Keyomarse, B. Dynlacht, L.-H. Tsai, P. Zhang, S. Dobrowolski, C. Bai, L. Connell-Crowley, E. Swindell, M.P. Fox, and N. Wei. 1995. Inhibition of cyclin-dependent kinases by p21. *Mol. Biol. Cell* **6**: 387–400.
- Harvey, M., M.J. McArthur, C.A. Montgomery, A. Bradley, and L.A. Donehower. 1993a. Genetic background alters the spectrum of tumors that develop in p53-deficient mice. *FASEB J.* **7**: 938–943.
- Harvey, M., M.J. McArthur, C.A. Montgomery Jr., J.S. Butel, A. Bradley, and L.A. Donehower. 1993b. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. *Nature Genet.* **5**: 225–229.
- Harvey, M., A.T. Sands, R.S. Weiss, M.E. Hegi, R.W. Wiseman, P. Pantazis, B.C. Giovanella, M.A. Tainsky, A. Bradley, and L.A. Donehower. 1993c. In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* **8**: 2457–2467.
- Harvey, M., H. Vogel, E.Y. Lee, A. Bradley, and L.A. Donehower. 1995a. Mice deficient in both p53 and Rb develop tumors primarily of endocrine origin. *Cancer Res.* **55**: 1146–1151.
- Harvey, M., H. Vogel, D. Morris, A. Bradley, A. Bernstein, and L.A. Donehower. 1995b. A mutant p53 transgene accelerates tumour development in heterozygous but not nullizygous p53-deficient mice. *Nature Genet.* **9**: 305–311.
- Haupt, Y., S. Rowan, and M. Oren. 1995a. p53-mediated apoptosis in HeLa cells can be overcome by excess pRB. *Oncogene* **10**: 1563–1571.
- Haupt, Y., S. Rowan, E. Shaulian, K. Vousden, and M. Oren. 1995b. Induction of apoptosis in HeLa cells by transactivation-deficient p53. *Genes & Dev.* **9**: 2170–2183.
- Haupt, T., Y. Barak, and M. Oren. 1996. Cell type specific inhibition of p53-mediated apoptosis by *mdm2*. *EMBO J.* **15**: 1596–1606.
- Havre, P.A., J. Yuan, L. Hedrick, K.R. Cho, and P.M. Glazer. 1995. p53 inactivation by HPV16 results in increased mutagenesis in human cells. *Cancer Res.* **55**: 4420–4424.
- He, Z., B.T. Brinton, J. Greenblatt, J.A. Hassell, and C.J. Ingels. 1993. The transactivator proteins VP16 and GAL4 bind replication factor A. *Cell* **73**: 1223–1232.
- Hermeking, H. and D. Eick. 1994. Mediation of c-Myc-induced apoptosis by p53. *Science* **265**: 2091–2093.
- Hickman, E.S., S.M. Picksley, and K.H. Vousden. 1994. Cells expressing HPV16 E7 continue cell cycle progression following DNA damage induced by p53 activation. *Oncogene* **9**: 2177–2181.
- Hollstein, M., K. Rice, M.S. Greenblatt, T. Soussi, R. Fucks, T. Sorlie, E. Hovig, B. Smith-Sorensen, R. Montesano, and C.C. Harris. 1994. Database of p53 gene somatic mutations in human tumors and cell lines. *Nucleic Acids Res.* **22**: 3551–3555.
- Horikoshi, N., A. Usheva, J. Chen, A.J. Levine, R. Weinmann, and T. Shenk. 1995. Two domains of p53 interact with the TATA-binding protein, and the adenovirus 13S E1A protein disrupts the association, relieving p53-mediated transcriptional repression. *Mol. Cell. Biol.* **15**: 227–234.
- Howes, K.A., N. Ransom, D.S. Papermaster, J.G.H. Lasudry, D.M. Albert, and J.J. Windle. 1994. Apoptosis or retinoblastoma: Alternative fates of photoreceptors expressing the HPV-16 E7 gene in the presence or absence of p53. *Genes & Dev.* **8**: 1300–1310.
- Hsiao, M., J. Low, E. Dorn, D. Ku, P. Pattengale, J. Yeargin, and M. Haas. 1994. Gain-of-function mutations of the p53 gene induce lymphohematopoietic metastatic potential and tissue invasiveness. *Am. J. Pathol.* **145**: 702–714.
- Huibregtse, J.M., J. Scheffner, and P.M. Howley. 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. *Mol. Cell. Biol.* **13**: 775–784.
- Hupp, T.R. and D.P. Lane. 1994. Allosteric activation of latent p53 tetramers. *Curr. Biol.* **4**: 865–875.
- Hupp, T.R., D.W. Meek, C.A. Midgley, and D.P. Lane. 1992. Regulation of the specific DNA binding function of p53. *Cell* **71**: 875–886.
- . 1993. Activation of the cryptic DNA binding function of mutant forms of p53. *Nucleic Acids Res.* **21**: 3167–3174.
- Hupp, T.R., A. Sparks, and D.P. Lane. 1995. Small peptides activated the latent sequence-specific DNA binding function of p53. *Cell* **83**: 237–245.
- Ishizaki, K., Y. Ejima, T. Matsunaga, R. Hara, A. Sakamoto, M. Ikenaga, Y. Ikawa, and S.I. Aizawa. 1994. Increased UV-induced SCEs but normal repair of DNA damage in p53-deficient mouse cells. *Int. J. Cancer* **57**: 254–257.
- Iwabuchi, K., P.L. Bartel, B. Li, R. Marraccino, and S. Fields. 1994. Two cellular proteins that bind to wild-type but not mutant p53. *Proc. Natl. Acad. Sci.* **91**: 6098–102.
- Jacks, T., L. Remington, B.O. Williams, E.M. Schmitt, S. Halachmi, R.T. Bronson, and R.A. Weinberg. 1994. Tumor spectrum analysis in p53-mutant mice. *Curr. Biol.* **4**: 1–7.
- Jackson, P., E. Bos, and A.W. Braithwaite. 1993. Wild-type p53 down-regulates transcription from different virus enhancer/promoters. *Oncogene* **8**: 589–597.
- Jamal, S. and E.B. Ziff. 1995. Raf phosphorylates p53 in vitro and potentiates p53-dependent transcriptional transactivation in vivo. *Oncogene* **10**: 2095–2101.
- Jayaraman, L. and C. Prives. 1995. Activation of p53 sequence-specific DNA binding by short single strands of DNA requires the p53 C-terminus. *Cell* **81**: 1021–1029.
- Jeffrey, P.D., S. Gorina, and N.P. Pavletich. 1995. Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. *Science* **267**: 1498–1502.
- Johnson, P., S. Chung, and S. Benchimol. 1993. Growth suppression of Friend virus-transformed erythroleukemia cells by p53 protein is accompanied by hemoglobin production and is sensitive to erythropoietin. *Mol. Cell. Biol.* **13**: 1456–1463.
- Jones, S.N., A.E. Roe, L.A. Donehower, and A. Bradley. 1995. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* **378**: 206–208.
- Kanda, T., K. Segawa, N. Ohuchi, S. Mori, and Y. Ito. 1994. Stimulation of polyomavirus DNA replication by wild-type p53 through the DNA-binding site. *Mol. Cell. Biol.* **14**: 2651–2663.
- Kastan, M.B., O. Onyekwere, D. Sidransky, B. Vogelstein, and R.W. Craig. 1991. Participation of p53 in the cellular response to DNA damage. *Cancer Res.* **51**: 6304–6311.
- Kastan, M.B., Q. Zhan, W.S. El-Deiry, F. Carrier, T. Jacks, W.V. Walsh, B.S. Plunkett, B. Vogelstein, and A.J. Fornace Jr. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* **71**: 587–597.
- Kazantsev, A., A. Sancar, J.M. Kearsey, M.K.K. Shivji, P.A. Hall, and R. D. Wood. 1995. Does the p53 up-regulated Gadd45 protein have a role in excision repair? (Technical Comments) *Science* **270**: 1003–1005.
- Kelley, L.L., W.F. Green, G.G. Hicks, M.C. Bondurant, M.J. Koury, and H.E. Ruley. 1994. Apoptosis in erythroid progenitors deprived of erythropoietin occurs during the G1 and S phases of the cell cycle without growth arrest or stabilization of wild-type p53. *Mol. Cell. Biol.* **14**: 4183–4192.
- Kemp, C.J., L.A. Donehower, A. Bradley, and A. Balmain. 1993. Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell* **74**: 813–822.

- Kemp, C.J., T. Whelson, and A. Balmain. 1994. p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. *Nature Genet.* **8**: 66–69.
- Kieser, A., H.A. Weich, G. Brandner, D. Marmè, and W. Kolch. 1994. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. *Oncogene* **9**: 963–969.
- Kinzler, K.W. and B. Vogelstein. 1996. Life (and death) in a malignant tumor. *Nature* **379**: 19–20.
- Kulesz-Martin, M.F., B. Lisafeld, H. Huang, N.D. Kisiel, and L. Lee. 1994. Endogenous p53 protein generated from wild-type alternatively spliced p53 RNA in mouse epidermal cells. *Mol. Cell. Biol.* **14**: 1698–1708.
- Lee, J.M., J.L.A. Abrahamson, R. Kandel, L.A. Donchower, and A. Bernstein. 1994. Susceptibility to radiation-carcinogenesis and accumulation of chromosomal breakage in p53 deficient mice. *Oncogene* **9**: 3731–3736.
- Lee, S., B. Elenbaas, A. Levine, and J. Griffith. 1995. p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. *Cell* **81**: 1013–1020.
- Lee, W., T.S. Harvey, Y. Yin, P. Yau, D. Litchfield, and C.H. Arrowsmith. 1994. Solution structure of the tetrameric minimum transforming domain of p53. *Nature Struct. Biol.* **1**: 877–890.
- Leveillard, T., L. Andera, N. Bissonnette, L. Schaeffer, L. Bracco, J.-M. Egly, and B. Wasylyk. 1996. Functional interactions between p53 and the TFIIH complex are affected by tumour-associated mutations. *EMBO J.* **15**: 1615–1624.
- Levine, A. 1993. The tumor suppressor genes. *Annu. Rev. Biochem.* **62**: 623–651.
- Li, R. and M.R. Botchan. 1993. The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate in vitro BPV-1 DNA replication. *Cell* **73**: 1207–1221.
- Li, R., S. Waga, G.J. Hannon, D. Beach, and B. Stillman. 1994. Differential effects by the p21 CDK inhibitor on PCNA-dependent DNA replication and repair. *Nature* **371**: 534–537.
- Lin, J., J. Chen, B. Elenbaas, and A.J. Levine. 1994. Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes & Dev.* **8**: 1235–1246.
- Lin, J.L., A.K. Teresky, and A.J. Levine. 1995. Two critical hydrophobic amino acids in the amino-terminal domain of the p53 protein are required for the gain of function phenotypes of human p53 mutants. *Oncogene* **10**: 2387–2390.
- Livingstone, L.R., A. White, J. Sprouse, E. Livanos, T. Jacks, and T.D. Tlsty. 1992. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* **70**: 923–935.
- Lowe, S. and H.E. Ruley. 1993. Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. *Genes & Dev.* **7**: 535–545.
- Lowe, S.W., E.M. Schmitt, S.W. Smith, B.A. Osborne, and T. Jacks. 1993. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* **362**: 847–852.
- Lowe, S.W., S. Bodis, A. McClatchey, L. Remington, H.E. Ruley, D.E. Fisher, D.E. Housman, and T. Jacks. 1994a. p53 status and the efficacy of cancer therapy in vivo. *Science* **266**: 807–810.
- Lowe, S.W., T. Jacks, D.E. Housman, and H.E. Ruley. 1994b. Abrogation of oncogene-associated apoptosis allows transformation of p53-deficient cells. *Proc. Natl. Acad. Sci.* **91**: 2026–2030.
- Lu, X. and D.P. Lane. 1993. Differential induction of transcriptionally active p53 following UV or ionizing radiation: Defects in chromosome instability syndromes? *Cell* **75**: 765–778.
- Lu, H. and A.J. Levine. 1995. Human TAF31 protein is a transcriptional coactivator of the p53 protein. *Proc. Natl. Acad. Sci.* **92**: 5154–5158.
- Mack, D.H., J. Vartikar, J.M. Pipas, and L.A. Laimins. 1993. Specific repression of TATA-mediated but not initiator-mediated transcription by wild-type p53. *Nature* **363**: 281–283.
- MacLeod, K.F., N. Sherry, G. Hannon, D. Beach, T. Tokino, K. Kinzler, B. Vogelstein, and T. Jacks. 1995. p53-dependent and independent expression of p21 during cell growth, differentiation, and DNA damage. *Genes & Dev.* **9**: 935–944.
- Maheswaran, S., C. Englert, P. Bennett, G. Heinrich, and D.A. Haber. 1995. The WT1 gene product stabilizes p53 and inhibits p53-mediated apoptosis. *Genes & Dev.* **9**: 2143–2156.
- Marston, N.J., T. Crook, and K.H. Vousden. 1994. Interaction of p53 with MDM2 is independent of E6 and does not mediate wild type transformation suppressor function. *Oncogene* **9**: 2707–2716.
- Maxwell, S.A. and J.A. Roth. 1994. Posttranslational regulation of p53 tumor suppressor protein function. *Crit. Rev. Oncol.* **5**: 23–57.
- Mayr, G.A., M. Reed, P. Wang, Y. Wang, J.F. Schwedes, and P. Tegtmeyer. 1995. Serine Phosphorylation in the NH₂ terminus of p53 facilitates transactivation. *Cancer Res.* **55**: 2410–2417.
- Meek, D. 1994. Post-translational modification of p53. *Semin. Cancer Biol.* **5**: 203–210.
- Mercer, W.E., D. Nelson, A.B. DeLeo, L.J. Old, and R. Beserga. 1982. Microinjection of monoclonal antibody to protein p53 inhibits serum-induced DNA synthesis in 3T3 cells. *Proc. Natl. Acad. Sci.* **79**: 6309–6312.
- Merritt, A.J., C.S. Potten, C.J. Kemp, J.A. Hickman, A. Balmain, D.P. Lane, and P.A. Hall. 1994. The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. *Cancer Res.* **54**: 614–617.
- Metz, T., A.W. Harris, and J.M. Adams. 1995. Absence of p53 allows direct immortalization of hematopoietic cells by the myc and raf oncogenes. *Cell* **82**: 29–36.
- Michieli, P., M. Chedid, D. Lin, J.H. Peirce, W.E. Mercer, and D. Givol. 1994. Induction of WAF1/CIP1 by a p53-independent pathway. *Cancer Res.* **54**: 3391–3395.
- Midgley, C.A., B. Owens, C.V. Briscoe, T. Brynmor, and D.P. Lane. 1995. Coupling between gamma irradiation, p53 induction and the apoptotic response depends upon cell type in vivo. *J. Cell Sci.* **108**: 1843–1848.
- Miller, S.D., G. Farmer, and C. Prives. 1995. p53 inhibits DNA replication in vitro in a DNA-binding-dependent manner. *Mol. Cell. Biol.* **15**: 6554–6560.
- Milne, D.M., R.H. Palmer, and D.W. Meek. 1992. Mutation of the casein kinase II phosphorylation site abolishes the antiproliferative activity of p53. *Nucl. Acids Res.* **20**: 5565–5570.
- Milne, D.M., D.G. Campbell, F.B. Caudwell, and D.W. Meek. 1994. Phosphorylation of the tumor suppressor protein p53 by mitogen-activated protein kinases. *J. Biol. Chem.* **269**: 9253–9260.
- Milne, D.M., L.E. Campbell, D.G. Campbell, and D.W. Meek. 1995. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-jun kinase, JNK1. *J. Biol. Chem.* **270**: 5511–5518.
- Miyashita, T. and J.C. Reed. 1995. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* **80**: 293–299.

Ko and Prives

- Miyashita, T., M. Harigai, M. Hanaka, and J.C. Reed. 1994. Identification of a p53-dependent negative response element in the bcl-2 gene. *Cancer Res.* **54**: 3131–3135.
- Moll, U., M. LaQuaglia, J. Benard, and G. Riou. 1995. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc. Natl. Acad. Sci.* **92**: 4407–4411.
- Momand, J., G.P. Zambetti, D.C. Olson, D. George, and A.J. Levine. 1992. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **69**: 1237–1245.
- Montes de Oca Luna, R., D.S. Wagner, and G. Lozano. 1995. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature* **378**: 203–206.
- Morgenbesser, S.D., B.O. Williams, T. Jacks, and R.A. DePinho. 1994. p53-dependent apoptosis produced by Rb-deficiency in the developing mouse lens. *Nature* **371**: 72–74.
- Mosner, J., T. Mummembrauer, C. Bauer, G. Sczakiel, F. Grosse, and W. Deppert. 1995. Negative feedback regulation of wild-type p53 biosynthesis. *EMBO J.* **14**: 4442–4449.
- Muller, C.W., F.A. Rey, M. Sodeoka, G.L. Verdine, and S.C. Harrison. 1995. Structure of the NF- κ B p50 homodimer bound to DNA. *Nature* **373**: 311–317.
- Nelson, W.G. and M.B. Kastan. 1994. DNA strand breaks: The DNA template alterations that trigger p53-dependent DNA damage response pathways. *Mol. Cell. Biol.* **14**: 1815–1823.
- Nicol, C.J., M.L. Harrison, R.R. Laposa, I.L. Gimelshtein, and P.G. Wells. 1995. A teratologic suppressor role for p53 in benzo[a]pyrene-treated transgenic p53-deficient mice. *Nature Genet.* **10**: 181–187.
- Niewollik, D., B. Vojtesek, and J. Kovarik. 1995. p53 derived from human tumour cell lines and containing distinct point mutations can be activated to bind its consensus target sequence. *Oncogene* **10**: 881–890.
- Okamoto, K. and D. Beach. 1994. Cyclin G is a transcriptional target of the p53 tumor suppressor protein. *EMBO J.* **13**: 4816–4822.
- Oliner, J.D., K.W. Kinzler, P.S. Meltzer, D.L. George, and B. Vogelstein. 1992. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* **358**: 80–83.
- Oliner, J.D., J.A. Pietenpol, S. Thiagalingam, J. Gyuris, K.W. Kinzler, and B. Vogelstein. 1993. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* **362**: 857–860.
- Oren, M. 1994. Relationship of p53 to the control of apoptotic cell death. *Semin. Cancer Biol.* **5**: 221–227.
- Osifchin, N.E., D. Jiang, N. Ohtani-Fujita, T. Fujita, M. Carroza, S.-J. Kim, T. Sakai, and P.D. Robbins. 1994. Identification of a p53 binding site in the human retinoblastoma susceptibility gene promoter. *J. Biol. Chem.* **269**: 6383–6389.
- Owen-Schaub, L.B., W. Zhang, J.C. Cusack, L.S. Angelo, S.M. Santee, T. Fujiwara, J.A. Roth, A.B. Deisseroth, W.-W. Zhang, E. Kruzel, and R. Radinsky. 1995. Wild-type human p53 and a temperature-sensitive mutant induce fas/APO-1 expression. *Mol. Cell. Biol.* **15**: 3032–3040.
- Pan, H. and A.E. Griep. 1994. Altered cell cycle regulation in the lens of HPV-16 E6 or E7 transgenic mice: Implications for tumor suppressor gene function in development. *Genes & Dev.* **8**: 1285–1299.
- Park, D. J., H. Nakamura, A.M. Chumakov, J.W. Said, C.W. Miller, D.L. Chen, and H.P. Koeffler. 1994. Transactivational and DNA binding abilities of endogenous p53 in p53 mutant cell lines. *Oncogene* **9**: 1899–1906.
- Parker, S.B., G. Eichele, P. Zhang, A. Rawls, A.T. Sands, A. Bradley, E.N. Olson, J.W. Harper, and S.J. Elledge. 1995. p53-independent expression of p21^{Cip1} in muscle and other terminally differentiating cells. *Science* **267**: 1024–1027.
- Pavletich, N.P., K.A. Chambers, and C.O. Pabo. 1993. The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots. *Genes & Dev.* **7**: 2556–2564.
- Perry, M.E., J. Piette, J.A. Zawadzki, D. Harvey, and A.J. Levine. 1993. The mdm-2 gene is induced in response to UV light in a p53-dependent manner. *Proc. Natl. Acad. Sci.* **90**: 11623–11627.
- Petersen, J.M., J.J. Skalicky, L.W. Donaldson, L.P. McIntosh, T. Alber, and B.J. Graves. 1995. Modulation of transcription factor ets-1 DNA binding: DNA-induced unfolding of an helix. *Science* **269**: 1866–1869.
- Pietenpol, J.A., T. Tokino, S. Thiagalingam, W.S. El-Deiry, K.W. Kinzler, and B.S. Vogelstein. 1994. Sequence-specific transcriptional activation is essential for growth suppression by p53. *Proc. Natl. Acad. Sci.* **91**: 1998–2002.
- Powell, S.N., J.S. DeFrank, P. Connell, M. Eogan, F. Preffer, D. Dombkowski, W. Tang, and S. Friend. 1995. Differential sensitivity of p53 (–) and p53 (+) cells to caffeine-induced radiosensitization and override of G2 delay. *Cancer Res.* **55**: 1643–1648.
- Prives, C. 1994. How loops, β sheets, and α helices help us to understand p53. *Cell* **78**: 543–546.
- Prives, C., J. Bargonetti, G. Farmer, E. Ferrari, P. Friedlander, Y. Wang, L. Jayaraman, N. Pavletich, and U. Hubscher. 1994. DNA-binding properties of the p53 tumor suppressor protein. *Cold Spring Harbor Symp. Quant. Biol.* **59**: 207–213.
- Purdie, C.A., D.J. Harrison, A. Peter, L. Dobbie, S. White, S.E.M. Howie, D.M. Salter, C.C. Bird, A.H. Wyllie, M.L. Hooper, and A.R. Clarke. 1994. Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. *Oncogene* **9**: 603–609.
- Qin, X.-Q., D.M. Livingston, W.G. Kaelin Jr., and P.D. Adams. 1994. Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. *Proc. Natl. Acad. Sci.* **91**: 10918–10922.
- Rainwater, R., D. Parks, M.E. Anderson, P. Tegtmeyer, and K. Mann. 1995. Role of cysteine residues in regulation of p53 function. *Mol. Cell. Biol.* **15**: 3892–3903.
- Reed, M., B. Woelker, P. Wang, Y. Wang, M.E. Anderson, and P. Tegtmeyer. 1995. The C-terminal domain of p53 recognizes DNA damaged by ionizing radiation. *Proc. Natl. Acad. Sci.* **92**: 9455–9459.
- Renning, J. and D.P. Lane. 1995. p53-dependent growth arrest following calcium phosphate-mediated transfection of murine fibroblasts. *Oncogene* **10**: 1865–1868.
- Rotter, V., R. Aloni-Grinstein, D. Schwartz, N.B. Elkind, A. Simons, R. Wolkowicz, M. Lavigne, P. Beserman, A. Kapon, and N. Goldfinger. 1994. Does wild-type p53 play a role in normal cell differentiation? *Semin. Cancer Biol.* **5**: 229–236.
- Ryan, I.J., R. Danish, C.A. Gottlieb, and M.F. Clarke. 1993. Cell cycle analysis of p53-induced cell death in murine erythroleukemia cells. *Mol. Cell. Biol.* **13**: 711–719.
- Sabbatini, P., S.K. Chiou, L. Rao, and E. White. 1995a. Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. *Mol. Cell. Biol.* **15**: 1060–1070.
- Sabbatini, P., J. Lin, A.J. Levine, and E. White. 1995b. Essential role for p53-mediated transcription in E1A-induced apoptosis. *Genes & Dev.* **9**: 2184–2192.
- Sah, V.P., L.D. Attardi, G.J. Mulligan, B.O. Williams, R.T. Bronson, and T. Jacks. 1995. A subset of p53-deficient embryos exhibit exencephaly. *Nature Genet.* **10**: 175–179.
- Sancar, A. 1995. Excision repair in mammalian cells. *J. Biol. Chem.* **270**: 15915–15918.

- Sands, A.T., M.B. Suraokar, A. Sanchez, J.E. Marth, L.A. Donehower, and A. Bradley. 1995. p53 deficiency does not affect the accumulation of point mutations in a transgene target. *Proc. Natl. Acad. Sci.* **92**: 8517–8521.
- Scheffner, M., J.M. Huibregtse, R.D. Vierstra, and P.M. Howley. 1993. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* **75**: 495–505.
- Schwartz, D., N. Goldfinger, and V. Rotter. 1993. Expression of p53 protein in spermatogenesis is confined to the tetraploid pachytene primary spermatocytes. *Oncogene* **8**: 1487–1494.
- Schweiki, D., A. Itin, D. Soffer, and E. Keshet. 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* **359**: 843–845.
- Seto, E., A. Usheva, G.P. Zambetti, J. Momand, N. Horikoshi, R. Weinmann, A.J. Levine, and T. Shenk. 1992. Wild-type p53 binds to the TATA-binding protein and represses transcription. *Proc. Natl. Acad. Sci.* **89**: 12028–12032.
- Shaulian, E., A. Zauberman, D. Ginsberg, and M. Oren. 1992. Identification of a minimal transforming domain of p53: negative dominance through abrogation of sequence-specific DNA binding. *Mol. Cell. Biol.* **12**: 5581–5592.
- Shaulian, E., A. Zauberman, J. Milner, E.A. Davies, and M. Oren. 1993. Tight DNA binding and oligomerization are dispensable for the ability of p53 to transactivate target genes and suppress transformation. *EMBO J.* **12**: 2789–2797.
- Shaulian, E., I. Haviv, Y. Shaul, and M. Oren. 1995. Transcriptional repression by the C-terminal domain of p53. *Oncogene* **10**: 671–680.
- Shen, Y. and T. Shenk. 1994. Relief of p53-mediated transcriptional repression by the adenovirus E1B 19-kDa protein or the cellular Bcl-2 protein. *Proc. Natl. Acad. Sci.* **91**: 8940–8944.
- Shin, T.H., A.J. Paterson, and J.E. Kudlow. 1995. p53 stimulates transcription from the human transforming growth factor α promoter: a potential growth-stimulatory role for p53. *Mol. Cell. Biol.* **15**: 4694–4701.
- Shivakumar, C.V., D.R. Brown, S. Deb, and S.P. Deb. 1995. Wild-type human p53 transactivates the human proliferating cell nuclear antigen promoter. *Mol. Cell. Biol.* **15**: 6785–6793.
- Slebos, R.J.C., M.H. Lee, B.S. Plunkett, T.D. Kessis, B.O. Williams, T. Jacks, L. Hedrick, M.B. Kastan, and K.R. Cho. 1994. p53-dependent G1 arrest involves pRB-related proteins and is disrupted by the human papillomavirus 16 E7 oncoprotein. *Proc. Natl. Acad. Sci.* **91**: 5320–5324.
- Slingerland, J.M., J.R. Jenkins, and S. Benchimol. 1993. The transforming and suppressor functions of p53 alleles: effects of mutations that disrupt phosphorylation, oligomerization and nuclear translocation. *EMBO J.* **12**: 1029–1037.
- Smith, M.L., I.-T. Chen, Q. Zhan, I. Bae, C.-Y. Chen, T.M. Gilmer, M.B. Kastan, P.M. O'Connor, and A.J. Fornace Jr. 1994. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* **266**: 1376–1380.
- Smith, M.L., I.-T. Chen, Q. Zhan, P.M. O'Connor, and A.J. Fornace Jr. 1995. Involvement of the p53 tumor suppressor in repair of u.v.-type DNA damage. *Oncogene* **10**: 1053–1059.
- Stewart, N., G.G. Hicks, F. Paraskevas, and M. Mowat. 1995. Evidence for a second cell cycle block at G2/M by p53. *Oncogene* **10**: 109–115.
- Stenger, J.E., P. Tegtmeyer, G.A. Mayr, M. Reed, Y. Wang, P. Wang, P.V.C. Hough, and I.A. Mastrangelo. 1994. p53 oligomerization and DNA looping are linked with transcriptional activation. *EMBO J.* **13**: 6011–6020.
- Subler, M.A., D.W. Martin, and S. Deb. 1994. Overlapping domains on the p53 protein regulate its transcriptional activation and repression functions. *Oncogene* **9**: 1351–1359.
- Symonds, H., L. Krall, L. Remington, M. Saenz-Robles, S. Lowe, R. Jacks, and T. Van Dyke. 1994. p53-dependent apoptosis suppresses tumor growth and progression in vivo. *Cell* **78**: 703–711.
- Szekely, L., G. Selivanova, K.P. Magnusson, G. Klein, and K.G. Wiman. 1993. EBNA-5, an Epstein-Barr virus-encoded nuclear antigen, binds to the retinoblastoma and p53 proteins. *Proc. Natl. Acad. Sci.* **90**: 5455–5459.
- Takenaka, I., F. Morin, B.R. Seizinger, and N. Kley. 1995. Regulation of the sequence-specific DNA binding function of p53 by protein kinase C and protein phosphatases. *J. Biol. Chem.* **270**: 5405–5411.
- Tarunina, M. and J.R. Jenkins. 1993. Human p53 binds DNA as a protein homodimer but monomeric variants retain full transcription transactivation activity. *Oncogene* **8**: 3165–3173.
- Thiagalingam, S., K.W. Kinzler, and B. Vogelstein. 1995. PAK1, a gene that can regulate p53 activity in yeast. *Proc. Natl. Acad. Sci.* **92**: 6062–6066.
- Thompson, T.C., S.H. Park, T.L. Timme, C. Ren, J.A. Eastham, L.A. Donehower, A. Bradley, D. Kadmon, and G. Yang. 1995. Loss of p53 function leads to metastasis in *ras* + *myc*-initiated mouse prostate cancer. *Oncogene* **10**: 869–879.
- Thut, C., J.L. Chen, R. Klemm, and R. Tjian. 1995. p53 Transcriptional activation mediated by coactivators TAFII40 and TAFII60. *Science* **267**: 100–104.
- Tong, X., R. Drapkin, D. Reinberg, and E. Kieff. 1995. The 62- and 80-kDa subunits of transcription factor IIH mediate the interaction with Epstein-Barr virus nuclear protein 2. *Proc. Natl. Acad. Sci.* **92**: 3259–3263.
- Truant, R., J. Antunovic, J. Greenblatt, C. Prives, and J.A. Cromlish. 1995. Direct interaction of the hepatitis B virus HBx protein with p53 leads to inhibition by HBx of p53 response element-directed transcription. *J. Virol.* **69**: 1851–1859.
- Unger, T., M.M. Nau, S. Segal, and J.D. Minna. 1992. p53: a transdominant regulator of transcription whose function is ablated by mutations occurring in human cancer. *EMBO J.* **11**: 1383–1390.
- Unger, T., J.A. Mietz, M. Scheffner, C.L. Yee, and P.M. Howley. 1993. Functional domains of wild-type and mutant p53 proteins involved in transcriptional regulation, transdominant inhibition, and transformation suppression. *Mol. Cell. Biol.* **13**: 5186–5194.
- Van Meir, E.G., P.J. Polverini, V.R. Chazin, H.-J. Su Huang, N. de Tribolet, and W.K. Cavanee. 1994. Release of an inhibitor of angiogenesis upon induction of wild type p53 expression in glioblastoma cells. *Nature Genet.* **8**: 171–176.
- Waga, S., G.J. Hannon, D. Beach, and B. Stillman. 1994. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* **369**: 574–578.
- Wagner, A.J., J.M. Kokontis, and N. Hay. 1994. Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and ability of p53 to induce p21^{waf1/cip1}. *Genes & Dev.* **8**: 2817–2830.
- Wang, Y. and C. Prives. 1995. Increased and altered DNA binding of human p53 by S and G2/M but not G1 cyclin-dependent kinases. *Nature* **376**: 88–91.
- Wang, P., M. Reed, Y. Wang, G. Mayr, J. Stenger, and E. Anderson. 1994. p53 domains: structure, oligomerization, and transformation. *Mol. Cell. Biol.* **14**: 5182–5191.
- Wang, X.W., K. Forrester, H. Yeh, M.A. Feitelson, J.-R. Gu, and C.C. Harris. 1994. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc. Natl. Acad. Sci.* **91**: 2230–2234.

- Wang, X.W., H. Yeh, L. Schaeffer, R. Roy, V. Moncollin, J.-M. Egly, Z. Wang, E.C. Friedberg, M.K. Evans, B.G. Taffe, V.A. Bohr, G. Weeda, J.H.J. Hoeijmakers, K. Forrester, and C.C. Harris. 1995. p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nature Genet.* **10**: 188–193.
- Wang, Y., M. Reed, P. Wang, J.E. Stenger, G. Mayr, M.E. Anderson, J.F. Schwedes, and P. Tegtmeier. 1993. p53 domains: identification and characterization of two autonomous DNA-binding regions. *Genes & Dev.* **7**: 2575–2586.
- Wang, Y., J.F. Schwedes, D. Parks, K. Mann, and P. Tegtmeier. 1995. Interaction of p53 with its consensus DNA-binding site. *Mol. Cell. Biol.* **15**: 2157–2165.
- Waterman, J.L., J.L. Shenk, and T.D. Halazonetis. 1995. The dihedral symmetry of the p53 tetramerization domain mandates a conformational switch upon DNA binding. *EMBO J.* **14**: 512–519.
- White, E. 1996. Life, death, and the pursuit of apoptosis. *Genes & Dev.* **10**: 1–15.
- Williams, B.O., L. Remington, D.M. Albert, S. Mukai, R.T. Bronson, and T. Jacks. 1994. Cooperative tumorigenic effects of germline mutations in Rb and p53. *Nature Genet.* **7**: 480–484.
- Wolkowicz, R., A. Peled, N.B. Elkind, and V. Rotter. 1995. Augmented DNA-binding activity of p53 protein encoded by a carboxyl-terminal alternatively spliced mRNA is blocked by p53 protein encoded by the regularly spliced form. *Proc. Natl. Acad. Sci.* **92**: 6842–6846.
- Wu, L., J.H. Bayle, B. Elenbaas, N.P. Pavletich, and A.J. Levine. 1995. Alternatively spliced forms in the carboxy-terminal domain of the p53 protein regulate its ability to promote annealing of complementary single strands of nucleic acids. *Mol. Cell. Biol.* **15**: 497–504.
- Wu, X. and A.J. Levine. 1994. p53 and E2F-1 cooperate to mediate apoptosis. *Proc. Natl. Acad. Sci.* **91**: 3602–3606.
- Wu, X., J.H. Bayle, D. Olson, and A.J. Levine. 1993. The p53-mdm-2 autoregulatory feedback loop. *Genes & Dev.* **7**: 1126–1132.
- Wu, Y., Y. Liu, L. Lee, Z. Miner, and M. Kulesz-Martin. 1994. Wild-type alternatively spliced p53: binding to DNA and interaction with the major p53 protein in vitro and in cells. *EMBO J.* **13**: 4823–4830.
- Xiao, H., A. Pearson, B. Coulombe, R. Truant, S. Zhang, J.L. Rieger, S.J. Triezenberg, D. Reinberg, O. Flores, C.J. Ingles, and J. Greenblatt. 1994. Binding of basal transcription factor TFIIH to the acidic activation domains of VP16 and p53. *Mol. Cell. Biol.* **14**: 7013–7024.
- Xiong, Y., G.J. Hannon, H. Zhang, D. Casso, R. Kobayashi, and D. Beach. 1993a. p21 is a universal inhibitor of cyclin kinases. *Nature* **366**: 701–704.
- Xiong, Y., H. Zhang, and D. Beach. 1993b. Subunit rearrangement of the cyclin-dependent kinases is associated with cellular transformation. *Genes & Dev.* **7**: 1572–1583.
- YeARGIN, J. and M. Haas. 1995. Elevated levels of wild-type p53 induced by radiolabeling of cells leads to apoptosis or sustained growth arrest. *Curr. Biol.* **5**: 423–431.
- Yew, P.R., X. Liu, and A.J. Berk. 1994. Adenovirus E1b oncoprotein tethers a transcriptional repression domain to p53. *Genes & Dev.* **8**: 190–202.
- Yin, Y., M.A. Tainsky, F.Z. Bischoff, L.C. Strong, and G.M. Wahl. 1992. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell* **70**: 937–948.
- Yonish-Rouach, E., D. Grunwald, S. Wilder, A. Kimchi, E. May, J.-J. Lawrence, P. May, and M. Oren. 1993. p53-mediated cell death: Relationship to cell cycle control. *Mol. Cell. Biol.* **13**: 1415–1423.
- Yonish-Rouach, E., J. Borde, M. Gotteland, Z. Mishal, A. Viron, and E. May. 1994. Induction of apoptosis by transiently transfected metabolically stable wt p53 in transformed cell lines. *Cell Death & Differ.* **1**: 39–47.
- Zauberman, A., A. Lubp, and M. Oren. 1995. Identification of p53 target genes through immune selection of genomic DNA: The cyclin G gene contains two distinct p53 binding sites. *Oncogene* **10**: 2361–2366.
- Zhan, Q., F. Carrier, and A.J. Fornace Jr. 1993. Induction of cellular p53 activity by DNA-damaging agents and growth arrest. *Mol. Cell. Biol.* **13**: 4242–4250.
- Zhan, Q., S. Pan, I. Bae, C. Guillof, D.A. Lieberman, P.M. O'Connor, and A.J. Fornace Jr. 1994. Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene* **9**: 3743–3751.
- Zhang, Q., D. Gutsch, and S. Kenney. 1994. Functional and physical interaction between p53 and BZLF1: Implications for Epstein-Barr virus latency. *Mol. Cell. Biol.* **14**: 1929–1938.
- Zhang, W., W.D. Funk, W.E. Wright, J.W. Shay, and A.B. Deisseroth. 1993. Novel DNA binding of p53 mutants and their role in transcriptional activation. *Oncogene* **8**: 2555–2559.
- Zhang, W., X.-Y. Guo, G.-Y. Hu, W.-B. Liu, J.W. Shay, and A.B. Deisseroth. 1994a. A temperature-sensitive mutant of human p53. *EMBO J.* **13**: 2535–2544.
- Zhang, W., C. McClain, J.-P. Gau, X.-Y. Guo, and A.B. Deisseroth. 1994b. Hyperphosphorylation of p53 induced by okadaic acid attenuates its transcriptional activation function. *Cancer Res.* **54**: 4448–4453.
- Zhao, J., F.I. Schmieg, D.T. Simmons, and G.R. Molloy. 1994. Mouse p53 represses the rat brain creatine kinase gene but activates the rat muscle creatine kinase gene. *Mol. Cell. Biol.* **14**: 8483–8492.
- Ziegler, A., A.S. Jonason, D.J. Leffell, J.A. Simon, H.W. Sharma, J. Kimmelman, L. Remington, T. Jacks, and D.E. Brash. 1994. Sunburn and p53 in the onset of skin cancer. *Nature* **372**: 773–776.

p53: puzzle and paradigm.

L J Ko and C Prives

Genes Dev. 1996, **10**:

Access the most recent version at doi:[10.1101/gad.10.9.1054](https://doi.org/10.1101/gad.10.9.1054)

References

This article cites 243 articles, 117 of which can be accessed free at:
<http://genesdev.cshlp.org/content/10/9/1054.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.

USC
SCIENTIFIC
SIDE BY SCIENCE