REVIEW

p53: puzzle and paradigm

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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 products 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

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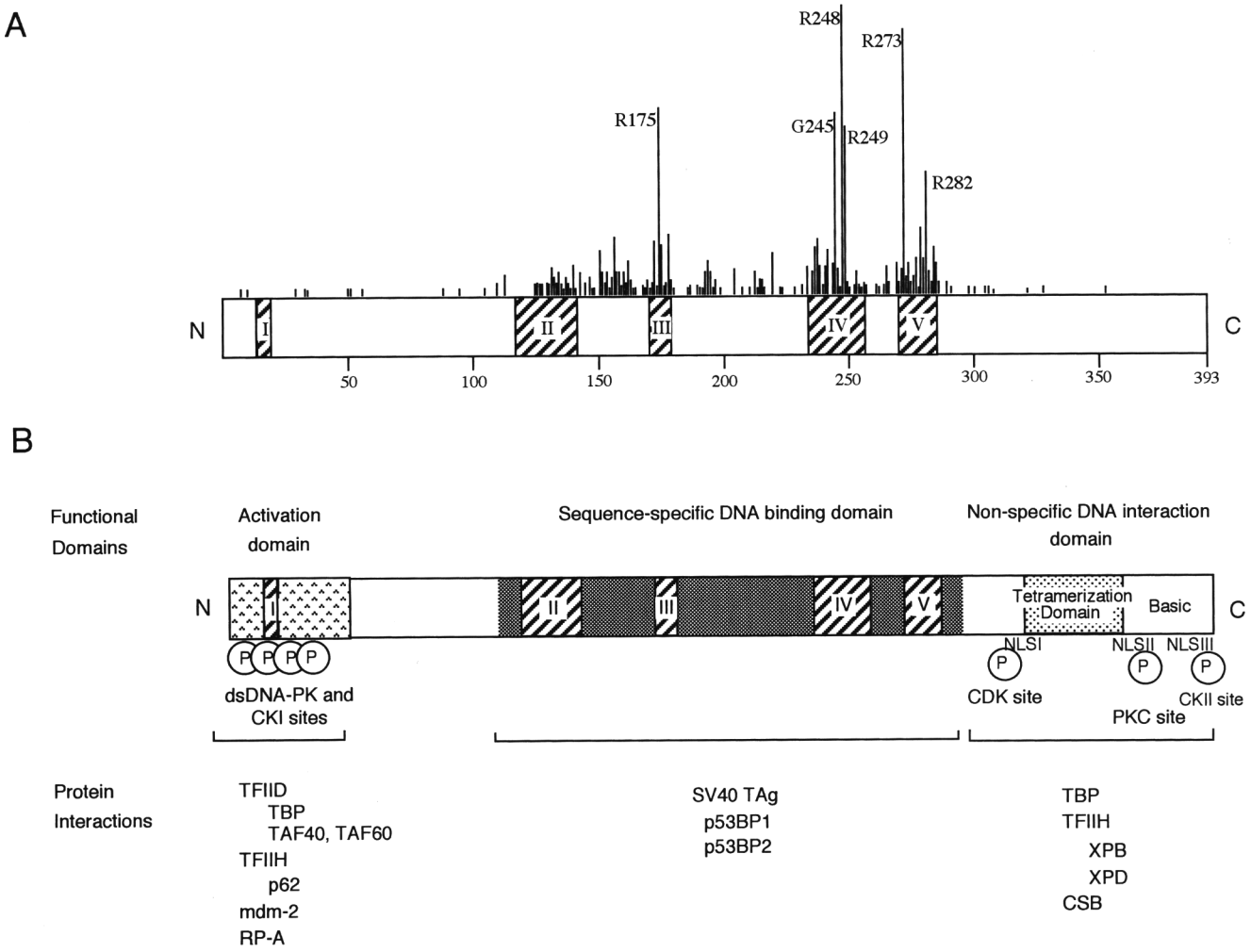

Figure 1. Landmarks of the human p53 protein. (*A*) p53 mutations found in human cancer. Hatched boxes represent evolutionarily conserved regions. Vertical lines above represent the frequency at which mutations are found at each particular residue and are clustered in conserved regions II–V. Several 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 important 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
General transcription factors	*Transcriptional activators*	SV40 TAg
TFIID components	WT-1	AdE1B55 kD
TBP	Sp1	HPV E6
dTAF40/hTAF31	CBF	HBV HBx
dTAF60		EBV BZLF1
TFIIH components	*Kinases*	EBV EBNA-5
hERCC2/hXPD/yRAD3	casein kinase II	
hERCC3/hXPB/ySSL2	c-abl	
hp62/yTFB1		
	Calcium-binding proteins	
	S100b	
Replication and repair proteins	*Ubiquitin-system proteins*	
RP-A (p70)	E6-AP	
TFIIH components		
XPD		
XPB	*Uncharacterized*	
p62	53BP1	
CSB	53BP2	
Oncogene products		
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-κB 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 G_2 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 ubiquitinization 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 demonstration 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 (Thiagalingam 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 G_1 phase of the cell cycle (Gu et al. 1993; Harper et al. 1993; Xiong et al. 1993a). In response to irradiation, p53-dependent G_1 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 G_1 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 G_1 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.

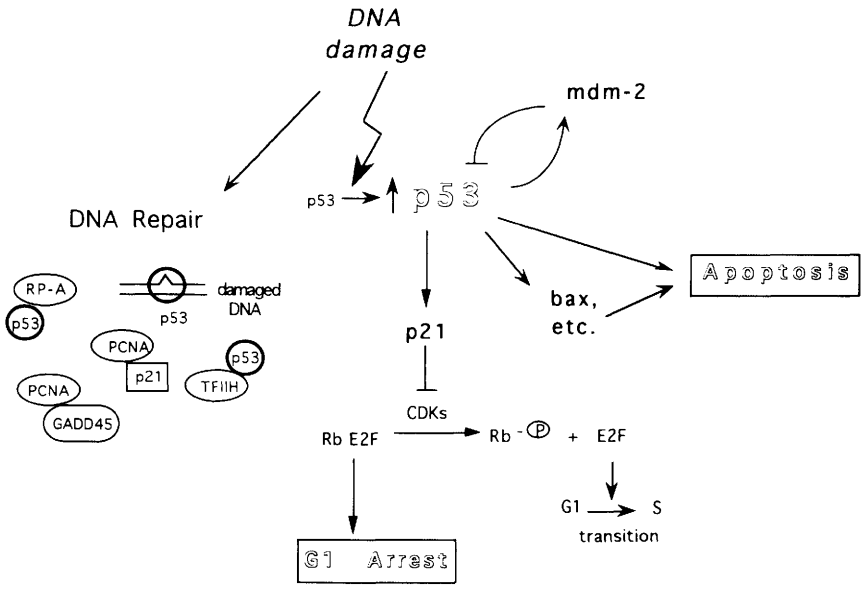

Figure 2. Pathways of the biological effects of p53.

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 G_1 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 G_1 arrest. Whether there are other p53 targets whose activation is required for G_1 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.

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