

# p53, the Cellular Gatekeeper for Growth and Division

## Review

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The *p53* gene and its protein product have become the center of intensive study ever since it became clear that slightly more than 50% of human cancers contain mutations in this gene. An extensive database (Hollstein et al., 1994) catalogs these mutations in more than 50 different cell and tissue types, although some types of cancers never appear to select for *p53* mutations (Lutzker and Levine, 1996). The nature of these genetic changes in cancer cells is most commonly a missense mutation in one allele, producing a faulty protein that is then observed at high concentrations in these cells, followed by a reduction to homozygosity. More rarely, deletions or chain-termination mutations in the *p53* gene indicate that the null phenotype predisposes to cancer, as has been observed in mice with a homozygous *p53* null mutation (Donehower et al., 1992). There have been some suggestions that the missense mutant producing a faulty *p53* protein could contribute a “gain of function” phenotype (Dittmer et al., 1993), but this remains to be substantiated by additional experimentation.

A study of the mutational spectra at the *p53* locus in different tissue types indicates a strong role for diverse environmental mutagens, with a set of tissue preferences. In addition, there is strong selection for a subset of mutations localized predominantly in the DNA-binding domain of the protein. Several codons in this domain that are never observed in the *p53* mutational spectra have been altered by site-specific mutagenesis, and in all cases these mutations have no phenotype and behave like the wild-type (Lin et al., 1995). Thus, both selection and a strong set of environmental mutagens combine to produce mutations in the *p53* gene in human cancers.

### p53 Domains: Structure–Function Relationships

The *p53* protein is a transcription factor that enhances the rate of transcription of six or seven known genes that carry out, at least in part, the *p53*-dependent functions in a cell (Table 1). The human *p53* protein contains 393 amino acids and has been divided structurally and functionally into four domains. The first 42 amino acids at the N-terminus constitute a transcriptional activation domain that interacts with the basal transcriptional machinery in positively regulating gene expression. Amino acids 13–23 in the *p53* protein are identical in a number of diverse species. The *p53* amino acids F19, L22, and W23 have been shown to be required for transcriptional activation by the protein in vivo (Lin et al., 1995). These same amino acids make contacts with and bind to (in vitro) the TATA-associated factors TAF<sub>II</sub>70 and TAF<sub>II</sub>31, both of which are subunits of TFIID (Lu and Levine, 1995; Thut et al., 1995). *p53* transcriptional activation is

negatively regulated by the adenovirus E1B-55Kd protein and the human MDM2 protein. In both cases, *p53* amino acid residues 22 and 23 play a key role in the binding of *p53* to E1B-55Kd or MDM2 (Lin et al., 1995). Thus, the negative regulators of *p53*-mediated transcription target some of the same *p53* amino acids critical to positive regulation of transcriptional activation.

These results clearly point out that *p53* uses a hydrophobic interface in its N-terminal domain to interact with the transcriptional machinery of the cell and its negative regulators. Recently, the N-terminal domain of MDM2 was cocrystallized with a peptide containing *p53* amino acid residues 13–29, covering the portion of *p53* that was shown, by mutational analysis, to interact with MDM2. The MDM2 domain forms a deep hydrophobic pocket, and the *p53* peptide forms an amphipathic helix with its hydrophobic surface, pointing into and filling the hydrophobic pocket. F19, W23, and L26 all stabilize these hydrophobic interactions in this pocket between *p53* and MDM2 (Kussie et al., 1996).

The sequence-specific DNA-binding domain of *p53* is localized between amino acid residues 102 and 292. It is a protease-resistant and independently folded domain containing a Zn<sup>2+</sup> ion that is required for its sequence-specific DNA-binding activity. This domain folds into a four-stranded and five-stranded antiparallel  $\beta$  sheet that in turn is a scaffold for two  $\alpha$ -helical loops that interact directly with the DNA (Cho et al., 1994). The tetrameric *p53* protein (which is a dimer of a dimer) binds to four repeats of a consensus DNA sequence 5'-PuPuPuC(A/T)-3', and this sequence is repeated in two pairs, each arranged as inverted repeats such as  $\rightarrow\leftarrow\leftarrow\rightarrow$ , where  $\rightarrow$  is the sequence given above. Residues K120, S241, R273, A276, and R283 make contacts with the phosphate backbone in the major groove, while K120, C277, and R280 interact via hydrogen bonds to the DNA bases. R248 then makes multiple hydrogen bond contacts in the minor groove of the DNA helix (Cho et al., 1994). More than 90% of the missense mutations in *p53* reside in this sequence-specific DNA-binding domain, and these mutations fall into two classes. Mutations in amino acid residues such as R248 and R273, the two most frequently altered residues in the protein, result in defective contacts with the DNA and loss of the ability of *p53* to act as a transcription factor. A second class of *p53* mutations disrupts the structural basis of the  $\beta$  sheet and the loop-sheet helix motif that acts as a scaffold in this domain. These structural mutations alter the conformation of the *p53* protein and produce a protein that now reacts with the monoclonal antibody PAb240, whose epitope (residues 212–217) is not accessible to the binding of this antibody in the native or wild-type structure. More than 40% of the missense mutations are localized to residues R175, G245, R248, R249, R273, and R282, which play a role in the structural integrity of this domain or the DNA contact sites directly (Cho et al., 1994; Hollstein et al., 1994).

The native *p53* protein is a tetramer in solution, and amino acid residues 324–355 are required for this oligomerization of the protein. The structure of this domain

Table 1. Products of Genes Transcriptionally Activated by p53

p21, WAF1, Cip1	Inhibits several cyclin-cyclin-dependent kinases; bind cdk's, cyclins, and PCNA; arrest the cell cycle
MDM2	Product of an oncogene; inactivates p53-mediated transcription and so forms an autoregulatory loop with p53 activity
GADD45	Induced upon DNA damage; binds to PCNA and can arrest the cell cycle; involved directly in DNA nucleotide excision repair
Cyclin G	A novel cyclin (it does not cycle with cell division) of unknown function and no known cyclin-dependent kinase
Bax	A member of the BCl2 family that promotes apoptosis; not induced by p53 in all cells
IGF-BP3	The insulin-like growth factor binding protein-3; blocks signaling of a mitogenic growth factor

While a large number of other genes have been suggested to be regulated by p53 (Ko and Prives, 1996), those listed above all have been shown to contain p53-dependent, *cis*-acting, DNA-responsive elements.

contains a dimer of a dimer with two  $\beta$  sheets and two  $\alpha$  helices. The two dimers are held together by a large hydrophobic surface of each helix pair, which then forms a four-helix bundle. This tetramerization domain is linked to the sequence-specific DNA-binding domain by a flexible linker of 37 residues (287–323) (Jeffrey et al., 1995).

The C-terminal 26 amino acids form an open (protease-sensitive) domain composed of nine basic amino acid residues that bind to DNA and RNA readily with some sequence or structural preferences (Lee et al., 1995). There is considerable evidence demonstrating that the p53 protein derived from several sources requires a structural change to activate it for sequence-specific binding to DNA. This non-DNA-binding or latent form of p53 can be regulated by this basic C-terminal domain. Deletion of this domain, phosphorylation at residue S378 by protein kinase C or residue S392 by casein kinase II, or binding of antibody PAb421 (to residues 370–378) all activate site-specific DNA binding by the central domain (residues 102–292) of this protein (Hupp and Lane, 1994). Short (20–39 nucleotides) single strands of DNA interacting with this C-terminal domain can also activate specific p53 DNA binding, but longer, double strands of DNA inhibit p53 sequence-specific binding through this region of the protein (Jayaraman and Prives, 1995). The C-terminal domain helps to catalyze the reassociation of single-stranded DNA or RNA to double strands. It also binds preferentially to DNA ends and to internal deletion loops in DNA as generated by replication errors that are then detected and fixed by mismatch-repair processes. There is some sequence or structural specificity in this binding (Lee et al., 1995). Clearly, the C-terminal domain either sterically or allosterically regulates the ability of p53 to bind to specific DNA sequences at its central or core domain.

#### The p53 Protein Functions to Integrate Cellular Responses to Stress: Upstream Events

Normally, in a cell, the p53 protein is kept at a low concentration by its relatively short half-life (about 20 min). The proteases responsible for this are not known,

but some evidence has suggested that ubiquitin-mediated proteolysis plays a role. In addition to this low protein concentration, in some cells p53 probably also exists in a latent form, inactive for transcription. Under these conditions, the p53 protein must receive a signal or alteration to activate it to function. The upstream events or signals that flow to p53 are mediated by several stressful situations. Several different types of DNA damage can activate p53, including double-strand breaks in DNA produced by  $\gamma$ -irradiation and the presence of DNA repair intermediates after ultraviolet irradiation or chemical damage to DNA. This results in a rapid increase in the level of p53 in the cell and activation of p53 as a transcription factor. The p53 level increases because the half-life of the protein is lengthened and possibly because the rate of translational initiation of p53 mRNA in the cell is enhanced. This increase in p53 levels is proportional to the extent of DNA damage, but both the extent of increase and the kinetics of p53 enhancement differ for different types of radiation damage. The cell uses different functions and proteins to recognize different classes of DNA damage (such as breaks in the DNA and excision repair of ultraviolet-irradiation dimers) and different systems of enzymes to repair them. It is an attractive idea that the cellular proteins that recognize DNA damage may communicate with p53 and activate it. For example, cells in culture defective in the *ATM* gene (ataxia-telangiectasia) have a delayed and attenuated p53 response to ionizing radiation, suggesting that the ATM protein, which may recognize damaged DNA and which contains a protein kinase domain, may signal the p53 protein in this fashion (Kastan et al., 1992). While a signal transduction pathway of this type linking p53 to DNA damage must be postulated, no clear-cut components of this pathway have been identified. Because the p53 protein itself can bind to DNA ends and excision-repair damage sites or internal deletion loops (Lee et al., 1995), it is possible that both the p53 protein and a damage-detector protein are localized at the site of DNA damage and repair, where phosphorylation or other activating signals can then be processed (Figure 1). This concept has the advantage of requiring two independent checks (the damage-detector protein and p53) for the presence of DNA damage prior to a p53 functional response to such damage.

DNA strand breaks in a cell appear to be sufficient for activating p53. The introduction of restriction enzyme nucleases into the nucleus of a cell stimulates p53 levels and activity. Transgenic mice defective in nucleotide excision repair (leaving repair intermediates) have elevated levels of p53 in several tissues. Mice defective in V-D-J receptor recombination (SCID mice) also have an activated p53 owing to the persistence of DNA recombination intermediates (Guidos et al., 1996).

In addition to DNA damage, hypoxia is able to stimulate p53 levels and activate the p53 protein (Graeber et al., 1996). It has been suggested that this process represents yet another way that p53 may act as a gatekeeper against the formation of cancers. Many tumors begin to replicate and reach a critical size when the blood supply becomes rate-limiting, requiring angiogenic factors to sustain growth. The resultant hypoxia might trigger p53 activity and kill such cells. It has also

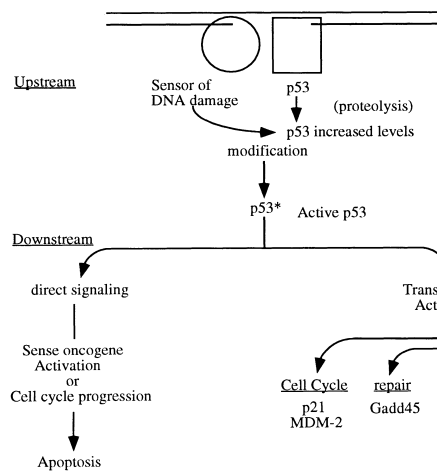


Figure 1. The Events in p53 Activation

DNA damage (indicated by the break in the double line at the top) is recognized by a “sensor” molecule that identifies a specific type of lesion and possibly by the p53 protein, using its C-terminal domain. The sensor modifies p53 (by phosphorylation) when both molecules correctly determine that there is damage. A modified p53 is more stable (enhanced half-life), and a steric or allosteric change in p53 permits DNA binding to a specific DNA sequence regulating several downstream genes (*p21*, *MDM2*, *GADD45*, *Bax*, *IGF-BP*, and *cyclin G*). Two modes of signaling for cellular apoptosis are possible: one requiring transcription and one involving direct signaling with no transcription of downstream genes required.

been reported that the thrombospondin gene is a p53-regulated gene. If that is correct, thrombospondin is an antiangiogenic factor that could further reduce the blood supply to these tumors.

Yet a third signal to activate p53 is sent when ribonucleoside triphosphate pools fall below a critical threshold. Clearly, the need to have normal nucleoside triphosphate pools to support DNA replication and progression through the cell cycle is monitored and reported to p53, but the pathway or proteins involved in this remain unclear (Linke et al., 1996).

Other signals of cellular distress that use p53 as an integrator of a response have been reported. Recognition of birth defects in response to teratogenic agents has been singled out as one possible role of p53 (Nicol et al., 1995). For example, in the absence of MDM2, the negative regulator of p53, mouse fetuses are aborted just after implantation, in a p53-dependent fashion (Montes de Oca Luna et al., 1995). Implantation may trigger stressful signals responded to by p53 and modulated appropriately by MDM2. Mice nullizygous for the *p53* gene develop giant cells (polyploid cells) in the testes, which could result from a lack of surveillance of recombination intermediates in spermatocytes. This could mean that one role of wild-type p53 is to detect abnormal intermediates in recombination and to eliminate such clones during spermiogenesis, as is more clearly the case with the T cell receptor recombination intermediates (Guidos et al., 1996). Perhaps related to this observation is the finding that the most common tumor that arises in *p53* nullizygous mice is a thymic lymphoma (Donehower et al., 1992).

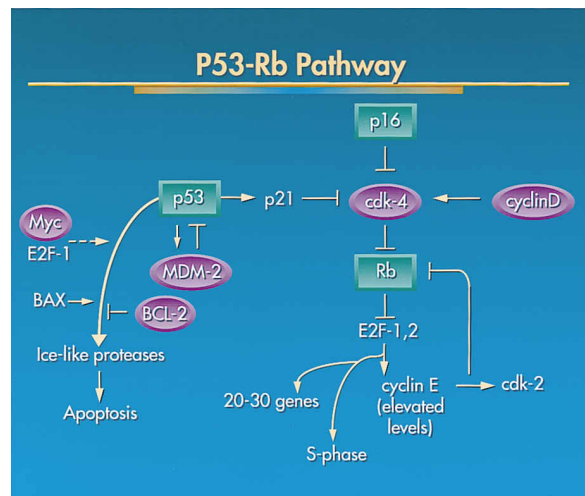


Figure 2. The p53–Rb Pathway

Experiments discussed in the text permit one to postulate the interrelationships among a number of oncogenes (purple circles) and tumor suppressor genes (green squares) that regulate the G1–S phase restriction point, its relation to a DNA damage checkpoint mediated by p53, and the choice by p53 whether to initiate a G1 arrest (via p21) or apoptosis. The available evidence suggests an important role for Rb and its two related gene products, p107 and p130 (along with E2F-4 and -5), in p53-mediated G1–S phase regulation. Shown are the p53–MDM2 autoregulatory loop that reverses this checkpoint control and the gene products that positively or negatively act on the probability of entering apoptosis.

### Responses to the Activation of p53: Downstream Events

The downstream events mediated by p53 take place by two major pathways: cell cycle arrest and apoptosis.

#### Cell Cycle Regulation

The *p16*–*cyclin D*–*cdk4*–*Rb* pathway is central to the regulation of the G1-to-S phase transition and to the understanding of human cancers (Figure 2). One of these four genes is altered or mutated in nearly every cancer examined. *p16* is a negative regulator of cyclin D<sub>1</sub>–Cdk4, and the gene is shut off (heavily methylated) in some cancer cells or mutated in other cancers. Cyclin D<sub>1</sub> is amplified and overexpressed in a number of cancers (about 16% of breast cancers), and *cdk4* mutations (no longer sensitive to p16) and *cdk4* gene amplifications have been reported in selected tumors. The retinoblastoma protein (Rb) is the major target of cyclin D<sub>1</sub>–Cdk4 for cell cycle regulation and is also present in a mutant form in a number of cancers (such as small-cell lung cancer and osteosarcomas). The Rb protein regulates E2F–DP transcription factor complexes (E2F-1, -2, and -3, and DP-1, -2, and -3), which in turn regulate a number of genes (including those encoding cyclin E, cyclin A, dihydrofolate reductase, and proliferating cell nuclear antigen [PCNA]) required to initiate or propagate the S phase of the cell cycle. Phosphorylation of Rb by cyclin D<sub>1</sub>–Cdk4 releases E2F–DP proteins from the Rb complex, relieving repression of these genes or activating their transcription. The Rb protein regulates the restriction point or start, as a “go–no go” signal for cell cycle progression that is sensitive to the impact of various exogenous growth factors (via the regulation of cyclin D<sub>1</sub>–Cdk4 and possibly p16).

In response to some forms of DNA damage, p53 is activated and turns on the transcription of one of its downstream genes, *p21* (*WAF1*, *Cip-1*) (El-Deiry et al., 1993). p21 binds to a number of cyclin and Cdk complexes: cyclin D1–Cdk4, cyclin E–Cdk2, cyclin A–Cdk2, and cyclin A–Cdc2. One molecule of p21 per complex appears to permit Cdk activity (and may even act as an assembly factor), while two moles of p21 per complex inhibit kinase activity and block cell cycle progression. p21 also binds to PCNA (at its C-terminal domain). The available evidence suggests that p21–PCNA complexes block the role of PCNA as a DNA polymerase processivity factor in DNA replication, but not its role in DNA repair. Thus, p21 can act on cyclin–Cdk complexes and PCNA to stop DNA replication. Mice deficient in the *p21* gene (null phenotype) develop normally, and mouse embryo fibroblasts derived from these mice are partially deficient in their ability to arrest cells in G1 in response to DNA damage (Deng et al., 1995). Based on these observations, there should be a p21-independent pathway that contributes to the p53-mediated G1 arrest. The overexpression of the product of the *GADD45* gene (a p53-responsive gene) in some cells in culture arrests cells in G1. Perhaps *GADD45*, also binding to PCNA, plays this backup role for p21. By contrast, the removal of both *p21* alleles from a cancerous cell line in culture that contained a wild-type *p53* allele completely eliminated the DNA damage–induced G1 arrest in these cells, indicating that p21 is sufficient to enforce a G1 arrest in this experimental situation (Polyak et al., 1996). Perhaps the particular set of mutations in this cancer cell line or the genetic background of these cells plays a role in the degree to which p21 alone can regulate the G1 checkpoint.

While this model for p53-mediated G1 arrest (Figure 2) predicts that cells with a mutant or faulty Rb protein should not be blocked in G1 after DNA damage and p53 activation, the evidence that supports this rests heavily on the use of viral oncogenes, which undoubtedly have multiple functions. In these experiments, the SV40 T antigen, E1A proteins of adenovirus, or E7 of the human papilloma viruses are used to block Rb functions and test for p53-mediated G1 arrest. In the absence of Rb regulation of E2F–DP-1, p53 fails to arrest these cells. When a simpler protocol is used, such as mouse embryo fibroblasts from *Rb*<sup>-/-</sup> (null) mice that are irradiated in cell culture, they in fact undergo G1 arrest. One distinction between these experiments and those using viral oncogenes to eliminate Rb function is that such viral oncogene products also alter the other two Rb family members, p107 and p130, which can certainly play a role in a p53-mediated G1-arrest phenotype. Thus p21 inhibition of cyclin D<sub>1</sub>–Cdk4 and cyclin E–Cdk2 can still affect the activities of p107 and p130, which regulate E2F-4 and -5. These interrelationships must be understood better if we are to know how p53 regulates G1 arrest.

Cells with wild-type p53 do not undergo gene amplification readily (Livingstone et al., 1992). The drug PALA (N-phosphonacetyl-L-aspartate), which is used to select for the amplification of the *CAD* gene, results in the depletion of pyrimidine triphosphate pools, which in turn activates p53, resulting in G1 arrest. Cells with mutant

p53 enter S phase and can also amplify segments of DNA. Gene amplification is thought to arise from nonhomologous recombination in a bridge–breakage–fusion cycle. Although the mechanism by which gene amplification is curtailed and inhibited by p53 remains unclear, one possibility is that p53 plays a role in monitoring abnormal recombination intermediates and acts to kill such cells (Guidos et al., 1996).

More recently, p53 has been implicated in a G2/M phase checkpoint. When mitotic spindle inhibitors, such as nocodazole, are added to cells with wild-type p53, the cells are blocked in G2. In the absence of wild-type p53, these cells will reinitiate DNA synthesis, increasing the ploidy of the cells (Cross et al., 1995). These data suggest that p53 may be part of a G2/M checkpoint, preventing premature entry into another S phase. In addition, p53 appears to be an integral part of the process that regulates the number of centrosomes in a cell (Fukasawa et al., 1996). Mouse embryo fibroblasts from *p53*<sup>-/-</sup> (null) mice produce abnormal numbers of centrosomes (not observed in normal cells in culture at the same passage level) after a few doublings in cell culture and initiate spindles with three or four poles (Fukasawa et al., 1996). This surely contributes to the phenotype of *p53*<sup>-/-</sup> cells in culture that rapidly become aneuploid at times when cells with wild-type p53 remain diploid during their passages. Of note, p53 has been found to copurify with the centrosomes isolated from some cells in culture (Brown et al., 1994). This p53-mediated G2/M checkpoint may account for the phenotype of genomic instability that is commonly associated with a p53 mutation.

A clear example of this phenotype was demonstrated using the transgenic mouse carrying the *MMTV-LTR-Wnt1* transgene that promotes breast cancer. Crossing this transgene with a *p53*<sup>-/-</sup> mouse resulted in a more rapid appearance of breast tumors, and these tumors were markedly more aneuploid, demonstrating the *p53* null genomic instability phenotype in vivo (Donehower et al., 1995).

Evidence for a third cell cycle checkpoint for p53 comes from an examination of the G0–G1–S phase transition. The *Gas1* gene is a membrane protein that keeps cells in G0 arrest and is expressed only at that time. *Gas1* can act to place cells into a G0 arrest only when wild-type p53 is present in such cells. In this case, however, p53 is not acting as a transcription factor regulating its downstream genes. The codon 22, 23 mutant of p53, which cannot act as a transcription factor, still functions to transmit *Gas1* signals for G0 arrest (Del Sal et al., 1995). This brings up the possibility that p53 can function not as a transcription factor but in a second way, perhaps by direct protein–protein signaling. This idea is strongly supported by additional experiments examining the role of p53 in apoptosis.

#### **Regulation of Apoptosis**

Several experimental protocols have demonstrated that p53 plays a role in triggering apoptosis under several different physiological conditions. Normal thymocytes will undergo apoptosis in response to DNA damage, whereas thymocytes from *p53*<sup>-/-</sup> mice do not undergo apoptosis in response to the same stimulus (Lowe et al., 1993b). Similarly, in mice that are irradiated, the stem

cells of the small and large intestine undergo apoptosis, which does not occur in  $p53^{-/-}$  mice. However, it is equally clear that not all apoptotic events are p53-mediated. For example, immature thymocytes from  $p53^{-/-}$  mice die of apoptosis when exposed to glucocorticoids or compounds that trigger T cell receptor pathways (Lowe et al., 1993b).

Physiological signals or activation of a specific signal transduction pathway can block p53-mediated apoptosis. Myeloid leukemic cells or murine erythroleukemic cells will undergo apoptosis when p53 is overexpressed and activated (Yonish-Rouach et al., 1991). Treatment of the myeloid cells with interleukin-6 or the erythroid cells with erythropoietin blocks the p53-mediated apoptotic pathway (Yonish-Rouach et al., 1991; Johnson et al., 1993). In this case, communication between p53 and a signal transduction pathway results in the reversal of p53-mediated apoptosis. Thus, it is possible that p53 may play a role in monitoring developmental factor dependence in the hematopoietic system.

p53 can also initiate apoptosis in response to the expression of a viral or cellular oncogene or the absence of a critical tumor suppressor gene product (Rb). The expression of the adenovirus E1A protein in rat embryo fibroblasts stabilizes and activates p53. The resultant cells die of apoptosis (Debbas and White, 1993). The adenoviruses normally express the E1B-55Kd protein, which binds to p53 and blocks its transcription factor activity, and the E1B-19Kd protein, which acts like BCl2 to block apoptosis downstream of p53 activation. The human papilloma virus E7 protein acts like E1A and induces p53-mediated apoptosis. In response, the human papilloma virus genome encodes the E6 oncogene product to bind to p53 and degrade it. Transgenic mice expressing E7 in the retina photoreceptor cells show extensive apoptosis. The expression of E7 in these same cells but in a  $p53^{-/-}$  mouse results in a reduced frequency of apoptosis and an increased frequency of development of retinal tumors (Howes et al., 1994). The E1A and E7 proteins bind to the Rb protein and inactivate its ability to regulate E2F-1 activity. Such an unregulated E2F-1 activity in concert with activated p53 results in apoptosis. Cells overexpressing E2F-1 and a temperature-sensitive p53 undergo apoptosis at 32°C but not 37–39°C, where p53 is inactive (Wu and Levine, 1994). Other oncogenes also trigger p53-mediated apoptosis. Cells overexpressing *myc* and a temperature-sensitive p53 have a temperature-sensitive apoptotic response (Wagner et al., 1994).

Thus, a number of factors affect the decision of a cell to enter a p53-mediated cell cycle arrest or apoptotic pathway. Under conditions in which the DNA is damaged, survival factors for the cells are limiting, or an activated oncogene is forcing the cell into a replicative cycle (*E1A*, *E7*, *E2F-1*, or *myc*), p53-mediated apoptosis prevails. In this way, cells with unstable genomes (due to DNA damage) or cells in an abnormal environment (i.e., located in a place with limiting survival factors) with activated oncogenes that commit them to enter the cell cycle are eliminated in a p53-dependent apoptotic event. This is most likely the reason why so many cancerous cells select against wild-type p53 function.

These observations bring up a number of questions.

Clearly, the p53-mediated cell cycle arrest requires p53-dependent transcription and one or more of its downstream genes. Does p53-mediated apoptosis require the transcriptional activity of p53? Furthermore, how does p53 sense the presence of an activated oncogene in a cell (or a pathway activated by an oncogene)? A number of experiments addressing the first question, concerning the need for p53-mediated transcription to induce apoptosis, have concluded that both a p53-mediated transcriptional activity and a p53 activity not requiring transcription can play a role in apoptosis, and the choice depends on the cell type or experimental situation. In several cell types, p53-mediated apoptosis initiated by DNA damage occurs in the presence of actinomycin D or cycloheximide, which block RNA or protein synthesis (Caelles et al., 1994). Similarly, a temperature-sensitive p53 in a cell with an activated *myc* induces apoptosis normally at 32°C in the presence of cycloheximide (Wagner et al., 1994). Clearly, transcriptional activation or the translation of p53-regulated gene products are not required in these cases for apoptosis. Similarly, the introduction of a p53 cDNA fragment that encodes amino acid residues 1–214 into HeLa cells induces apoptosis (Haupt et al., 1995). p53 (residues 1–214) fails to bind to DNA or act as a transcription factor. Similarly, p53 with mutations in the transactivating domain (residues 22 and 23) that is unable to activate transcription still induces apoptosis in HeLa cells. By contrast, the expression of E1A in baby rat kidney cells or mouse cells failed to induce apoptosis when p53 contains mutations that alter p53-specific DNA binding or transcriptional activation (Sabbatini et al., 1995). Indeed, the mutant p53 that produces amino acid residues 1–214 and induces apoptosis in HeLa cells fails to induce apoptosis in another cell type, a human lung carcinoma, cell line H1299 (Haupt et al., 1995). Thus, it appears that p53 may use transcriptional activation or direct protein signaling (protein-protein interactions or some other activity) or both to initiate apoptosis.

Two of the genes that are regulated by p53 (in at least some cell types) could influence the decision to commit to an apoptotic pathway: *bax* and *IGF-BP3* (Miyashita and Reed, 1995; Buckbinder et al., 1995). It is clear that overexpression of BCl2 (or the adenovirus E1B-19Kd protein) can block p53-mediated apoptosis. Bax binds to BCl2 and antagonizes its ability to block apoptosis, so a p53-dependent *bax* synthesis could tip the scales toward apoptosis. The mechanism of action of the BCl2-Bax family of gene products remains unclear and is central to understanding this important aspect of the development of cancer cells. A second p53-regulated gene product that could affect growth regulation is the insulin-like growth factor-binding protein-3 (IGF-BP3) (Buckbinder et al., 1995). IGF-BP3 blocks the IGF mitotic signaling pathway by binding to IGF and preventing its interaction with its receptor. Thus, the blocking of IGF activity could enhance apoptosis or lower the mitogenic response of cells. It has been reported that the *fas/apo1* gene may also be regulated by p53. p53 responsive elements have not yet been demonstrated in the *fas/apo1* gene and so proof that it is truly a p53-regulated gene remains to be provided.

Table 2. Proteins That Have Functional Interactions with p53

Viral proteins	
SV40 T antigen	Blocks p53 DNA binding domain
AdE1B 55 kDa	Blocks p53 transcriptional activation domain
Human papilloma virus E6	Promotes the degradation of p53
Oncogene products	
MDM2	Blocks p53 transcriptional activation domain
c-Abl	p53-mediated cell cycle arrest
Transcriptional factors	
TATA-binding protein	Binds amino and carboxyl termini of p53
TAF70, TAF31	Binds to amino-terminal domain of p53
TFIIH	
ERCC2, XPD	Helicase modulated by wild-type p53, not mutant p53
ERCC3, XPB	Helicase modulated by wild-type p53, not mutant p53
WT1 (Wilms tumor-1)	Alters p53 activities

A detailed list of references for these observations and additional proteins thought to interact with p53 is presented by Ko and Prives, 1996.

### p53-Protein Interactions

It has been shown that p53 directly binds to and acts on several cellular proteins (Table 2). Among these, the c-Abl protein has several noteworthy properties. First, nuclear c-Abl is activated for its kinase activity after DNA damage, and c-Abl binds to p53 and enhances its transcriptional activity. The overexpression of c-Abl in normal cells blocks cell cycle progression, and this is dependent on a wild-type p53 activity (Goga et al., 1995). Thus, there is a growing linkage between Abl and p53 in a common pathway. c-Abl, but not the product of *v-abl* activated as an oncogene, contains an SH3 (Src homology domain 3) binding domain. The human p53 protein contains five copies of the amino acid motif proline-X-X-proline (where X is any amino acid) localized between residues 61–94, and this motif has been shown to bind to SH3 domains. Deletion of this proline-rich region of the p53 protein permits normal p53-mediated transcriptional activation but significantly reduces the ability of this p53 mutant protein to mediate apoptosis or cell cycle arrest (Walker and Levine, 1996). The possibility of a regulated interaction between p53 and SH3 domain-containing proteins is one way to communicate with signal transduction pathways and sense oncogene activities. Recently, a Li-Fraumeni syndrome family, with three late-onset breast cancers, has been described with a mutation in the proline at codon 82, disrupting one of the proline-X-X-proline repeats. In two of the three tumors, there was a partial p53 reduction to homozygosity in recurrent tumors, eliminating the wild-type allele. This mutation, however, did not appear to be the major cause of the neoplastic disease, but rather conferred only a portion of the phenotype on the progression of the breast cancer (Sun et al., 1996).

The p53 protein has also been shown to bind to an RNA polymerase II basal transcription factor, TFIIH (Wang et al., 1995). TFIIH consists of two helicases, ERCC2 and ERCC3, which are the proteins encoded for

by two genes, xeroderma pigmentosum complementation groups D and B (*XP-B* and *XP-D*). Other subunits of TFIIH are the cyclin H-Cdk7-p36 proteins involved in DNA repair (the cyclin activating kinase) and transcription initiation by RNA polymerase II. p53 binds to these two helicases in vitro (*XP-B* and *XP-D* gene products) as well as to a third helicase, which is defective in Cockayne syndrome. This helicase, termed CsB, is involved in yet another DNA repair pathway. While it has been difficult to prove that those protein-protein interactions observed in vitro have a physiologically meaningful counterpart in vivo, it was recently shown that cells deficient for the XP-B or XP-D helicases fail to undergo apoptosis mediated by p53. These observations leave open the possibility that p53 itself plays a role in modulating nuclear excision repair. For example, the binding of p53 to XP-B and XP-D proteins in vitro blocks their helicase activity, while mutant p53, R273H, binds to these proteins but fails to inhibit their helicase activity in a manner comparable to the wild-type p53 protein (Wang et al., 1995).

The Wilms tumor suppressor gene product, WT1, has been shown to associate with p53 when both are overexpressed in the same cell (Maheswaran et al., 1995). Coexpression of p53 and WT1 results in higher steady-state levels of p53, an increased level of p53 binding to sequence-specific DNA targets, and an enhanced transcriptional activity. In U20S cells in culture, the expression of WT1 blocks a p53-mediated apoptosis which is triggered by ultraviolet irradiation. Thus WT1, at least at abnormally high concentrations, can have specific effects on p53 activity and physiological actions.

### A Role for p53 in Senescence

Normal mouse or human cells recently obtained from their host undergo a limited number of divisions in culture. The murine cells undergo senescence more rapidly, after only a few divisions, but it is common that some cells escape and can form permanent cell lines in culture. The human cells, on the other hand, slow their growth rate and stop dividing with little or no evidence of a cell that can form a permanent cell line. SV40 large T antigen, which inactivates p53 and Rb and alters p300 and the related CREB-binding protein activities, can extend the life span of human cells providing additional generations, but does not by itself (without additional events or mutations) produce a permanent cell line in culture. Rather, T antigen-expressing human cells in late passage enter a crises phase and fail to reproduce. Only rare cells emerge expressing T antigen and form permanent cell lines. These cells are dependent on T antigen expression for their ability to divide.

p53-deficient murine cells from a p53 nullizygous mouse readily escape senescence and produce aneuploid immortalized cell lines. This is most likely due to the loss of p53-mediated control over centrosome duplication (Fukasawa et al., 1996) and the G2/M checkpoint (Cross et al., 1995) preventing reinitiation of S-phase prior to mitosis or the next G1 phase. In addition, it has become clear that p53 responds to signals provided by normal cells undergoing progressive passages in culture. p53 activity increases in late-passage cells, and



p21 levels increase as well, slowing or stopping the division rate of the culture. The addition of a transdominant-acting p53 mutant to such cells provides a significant enhancement in the lifespan of those cells (Bond et al., 1995). These cells will still undergo senescence at a later passage level, showing that further oncogenic events are required for immortalization. For example, the addition of cDNA clones encoding the *c-myc* or *raf* oncogenes readily immortalize p53-deficient cells at a higher rate than cells containing wild-type p53.

### The Role of p53 in Tumor Formation

p53 mutations are found in 50–55% of all human cancers (Hollstein et al., 1994). These mutations strongly select for p53 proteins that fail to bind to DNA in a sequence-specific fashion. While the cell cycle arrest functions of p53 require p53 transcriptional activity, at least some of the apoptotic activities of p53 do not require p53-dependent gene products. This could mean that transcription of selected p53 target genes is critical to its tumor suppressor function (i.e., some forms of apoptosis are not critical) or that the p53 direct signaling pathway, in the absence of transcription, requires p53 to bind to DNA in a sequence-specific fashion. Clearly the enhancer elements recognized by p53 in the DNA are not all equally regulated by it. Some p53 mutant proteins can activate a p53 responsive sequence in the *p21* gene but not the *bax* gene (Friedlander et al., 1996). This could indicate that *bax* transcriptional activation (apoptosis) is selected against more frequently than is that of *p21* and *bax* together (G1 arrest). In addition to p53 mutations, some tumors inactivate p53 by the amplification of the *MDM2* gene (about one third of all sarcomas) (Oliner et al., 1992) or by the localization of p53 in the cell cytoplasm (a small number of breast tumors and neuroblastomas). In other cancers, p53 mutations are never selected (teratocarcinomas). In this case, the wild-type p53 protein in the cancerous stem cells is not a functional transcription factor, and that is presumably the reason for the absence of selection of p53 mutations (Lutzker and Levine, 1996).

Humans who are heterozygous for the wild-type allele of p53 develop cancer with a very high frequency (greater than 90–95%) and often at an early age. The cell and tissue distributions of these cancers (sarcomas, breast, and adrenal carcinomas) is not random, and it is not clear what this means either for p53 function or the additional mutations that are required to develop cancer in a p53 heterozygous cell. There are some indications that p53 heterozygous cells show dosage effects in chemically induced mouse skin tumors (Kemp et al., 1993), suggesting that the level of p53 in a cell influences the phenotype. The timing of p53 somatic mutations in human cancers contributing to malignant tumors apparently depends on the tumor type. Most colorectal tumors arise via a series of genetic changes, first in the *APC* gene and then in the *ras* gene, followed by mutations in the *DCC* gene and finally in the p53 gene. While the *APC* mutation may have to occur prior to the others, the order is not rigidly fixed, but it is the most commonly observed one. In skin cancers, by contrast, a p53 mutation appears to occur early in pre-malignant lesions (Ziegler et al., 1994). The timing of

the selection for p53 mutations surely depends on a complex set of variables. Because p53 is induced and activated by hypoxia, the need for angiogenic factors (or the loss of antiangiogenic factors) is balanced with the elimination, via mutation, of p53. A rich blood supply early in tumor development may leave p53 mutations to be selected at a later time.

Clearly, the genetic background of the host has a significant impact on the tumor type initiated by p53 mutations. In 129 mice, which develop testicular teratocarcinomas at a 1–3% frequency, a null genotype (*p53*<sup>-/-</sup>) increases this frequency to 25–40% of male mice. 129XC57Bl/6 hybrid mice rarely, if ever, develop these tumors (Donehower et al., 1992). Thus, the absence of p53 can enhance an inherited predisposition to a particular cancer. Mice that are nullizygous for p53 and heterozygous for *Rb* (*Rb*<sup>+/-</sup>) are susceptible to a much wider range of tumors, with different cell and tissue types involved, than are mice with either mutation alone (Williams et al., 1994). Therefore, these two tumor suppressor genes, p53 and *Rb*, can cooperate to produce new phenotypes. As indicated previously, Wnt1-induced breast tumors (oncogene-transgene) appear earlier and have abnormal ploidy when they arise in *p53*<sup>-/-</sup> mice (Donehower et al., 1995). In addition, mice that are heterozygous for p53 (*p53*<sup>+/-</sup>) produce a different spectrum of tumors than *p53*<sup>-/-</sup> mice (Donehower et al., 1995). Clearly, cancer is a disease in which allelic differences and the genetic background of the host affect the age of onset, incidence, and tumor type that is detected first in life.

As might be predicted from the known functions of p53, mice that are nullizygous for p53 are more susceptible to  $\gamma$ -irradiation or to carcinogens for inducing tumors in animals. However, there is no evidence for an enhanced rate of point mutations in *p53*<sup>-/-</sup> mice treated with these agents. Perhaps p53 plays a more central role in the surveillance of gene amplifications, abnormal recombination processes, and the control over ploidy. Certainly, the observations that the p53 nullizygous mice develop thymic lymphomas (Donehower et al., 1992) and that p53 plays a critical role in the surveillance of T cell recombination intermediates (Guidos et al., 1996) supports these ideas.

It is equally clear that p53 acts to reduce the incidence of cancers by mediating apoptosis in cells with activated oncogenes. Transgenic mice expressing the SV40 large T antigen develop choroid plexus papillomas during the first 3 months of life. The tumor growth is slowed considerably by p53-mediated apoptosis when a T antigen mutant is used that cannot inactivate p53 functions in these cells. When this mutant is expressed in *p53*<sup>-/-</sup> mice, the rapid tumor growth resumes with less apoptosis (Symonds et al., 1994). Clearly, then, p53-mediated apoptosis is an important part of the tumor suppressor phenotype and subsequent selection for mutant p53 genes in cancers.

### p53 and Cancer Treatment and Prognosis

The treatment of neoplasia using radiation and chemotherapy results in extensive DNA damage and signaling

to p53 in those cells. In some selected cases, it is becoming clear that p53-dependent apoptosis can modulate the toxic effects of anticancer agents. Cells in culture activated by the expression of the adenovirus oncogene *E1A* undergo a p53-dependent programmed cell death in response to ionizing radiation or treatment with 5-fluorouracil, etoposide, or adriamycin (Lowe et al., 1993a). A similar situation exists with testicular teratocarcinomas. All human and mouse testicular teratocarcinomas examined to date contain wild-type p53 genes (Lutzker and Levine, 1996). This is one of the human tumors that respond very well (90–95% cures) to chemotherapy with cisplatin. In cell culture the stem cells of this tumor undergo a p53-dependent apoptosis in response to etoposide (Lutzker and Levine, 1996). A similar situation has been noted with childhood acute lymphoblastic leukemias, which most often have wild-type p53 and respond well to chemotherapeutic intervention (Lowe et al., 1993a). When these tumors relapse, ineffectiveness of therapy correlates well with the acquisition of p53 mutations. Other tumors that frequently contain p53 mutations (melanoma, lung cancers, colorectal tumors, and bladder and prostate cancers) often respond poorly to radiation or chemotherapy. In breast cancers, p53 mutations were well correlated with de novo resistance to doxorubicin treatment (Aas et al., 1996). Similar findings have been reported for chemotherapy with small-cell lung cancers responding to cisplatin or in mice in which anthracyclines were used to treat sarcomas. It is not surprising therefore that in some cases p53 mutations have been reported to result in shorter disease-free survival and lower total survival of patients (Aas et al., 1996).

This good correlation between p53 functional status and treatment response or prognosis is not always the case. It is clear that cancer cells suffer a large number of genetic changes, some of which contribute to the overall phenotype and others that do not. The changes in the cellular environment during treatment, the possibility of an altered mutation rate, and the large number of physiological variables all confound clear-cut correlations between the status of a single gene and a response. We must begin to understand several variables at a time if we are to accurately make predictions of treatment responses and outcomes of diseases. At least we now know some of the variables.

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