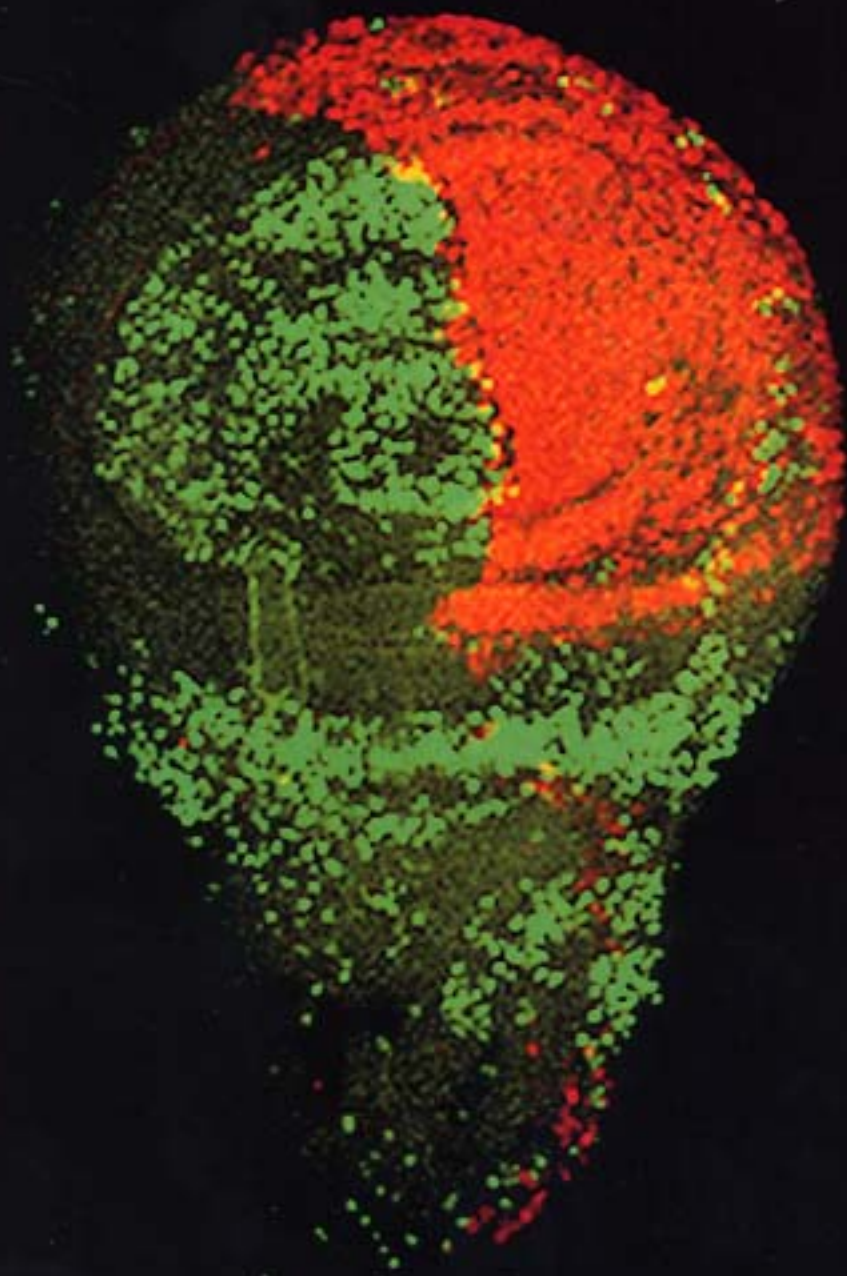


# Cell

Volume 101 Number 1

March 31, 2000



***Drosophila* p53 and Apoptosis**

**Conditional Model of Huntington's Disease**

# *Drosophila p53* Is a Structural and Functional Homolog of the Tumor Suppressor *p53*

Michael Ollmann,\*<sup>§</sup> Lynn M. Young,\*<sup>§</sup>  
Charles J. Di Como,<sup>†</sup> Felix Karim,\* Marcia Belvin,\*  
Stephanie Robertson,\* Kellie Whittaker,\*  
Madelyn Demsky,\* William W. Fisher,\*  
Andrew Buchman,\* Geoffrey Duyk,\* Lori Friedman,\*  
Carol Prives,<sup>†</sup> and Casey Kopczynski\*<sup>‡</sup>

\*Exelixis, Inc.

260 Littlefield Avenue  
South San Francisco, California 94080

<sup>†</sup>Department of Biological Sciences  
Columbia University  
New York, New York 10027

## Summary

The importance of *p53* in carcinogenesis stems from its central role in inducing cell cycle arrest or apoptosis in response to cellular stresses. We have identified a *Drosophila* homolog of *p53* ("*Dmp53*"). Like mammalian *p53*, *Dmp53* binds specifically to human *p53* binding sites, and overexpression of *Dmp53* induces apoptosis. Importantly, inhibition of *Dmp53* function renders cells resistant to X ray–induced apoptosis, suggesting that *Dmp53* is required for the apoptotic response to DNA damage. Unlike mammalian *p53*, *Dmp53* appears unable to induce a G1 cell cycle block when overexpressed, and inhibition of *Dmp53* activity does not affect X ray–induced cell cycle arrest. These data reveal an ancestral proapoptotic function for *p53* and identify *Drosophila* as an ideal model system for elucidating the *p53* apoptotic pathway(s) induced by DNA damage.

## Introduction

*p53* is a tightly regulated transcription factor that induces cell cycle arrest or apoptosis in response to cellular stresses such as DNA damage and oncogene activation (Ko and Prives, 1996; Levine, 1997; Giaccia and Kastan, 1998; Kamijo et al., 1998). Loss or inactivation of the *p53* tumor suppressor gene is the single most common mutation in human cancer (Hainaut et al., 1998). Thus, inactivation of *p53* function may provide a selective advantage to tumor cells through accrued mutations leading to deregulated cell proliferation and resistance to cell death (Graeber et al., 1996; Kinzler and Vogelstein, 1996).

Numerous studies have established that growth arrest and apoptosis are independent functions of *p53* (reviewed in Gottlieb and Oren, 1996). *p53*-dependent G1 arrest occurs largely through transcriptional induction of *p21*<sup>WAF1</sup>, which prevents entry into S phase by inhibiting G1 cyclin–dependent kinase activity (el-Deiry et al., 1993; Harper et al., 1993; Xiong et al., 1993; Deng et al., 1995). However, *p21* is not required for *p53*-dependent

apoptosis (Wagner et al., 1994). In fact, *p21* may protect against *p53*-induced apoptosis in at least some cell types (Waldman et al., 1996; Wang and Walsh, 1996; Gorospe et al., 1997; Bissonnette and Hunting, 1998; Yu et al., 1998; Asada et al., 1999).

Induction of apoptosis by *p53* is critical for the tumor suppressor function of *p53*. There appear to be multiple mechanisms through which *p53* promotes apoptosis. For example, *p53* can transcriptionally activate the proapoptotic genes *Bax* (Miyashita and Reed, 1995), *Fas* (Owen-Schaub et al., 1995), and *IGF-BP3* (Buckbinder et al., 1995), as well as a set of genes that may promote apoptosis through the formation of reactive oxygen species (Polyak et al., 1997). Furthermore, there is evidence that *p53* can induce apoptosis in the absence of its transcriptional activation function (reviewed in Ko and Prives, 1996).

The recent discovery of two *p53*-related genes, *p63* and *p73*, has revealed an additional level of complexity to studying *p53* function in vertebrates (Kaelin, 1999). Both genes encode proteins with transactivation, DNA-binding, and tetramerization domains, and some isoforms of *p63* and *p73* are capable of transactivating *p53* target genes and inducing apoptosis. It was initially thought that only *p53* was induced in response to DNA damage and other stress signals. There is now evidence, however, that *p73* is also activated by some forms of DNA damage in a manner that is dependent upon the c-Abl tyrosine kinase (Agami et al., 1999; Gong et al., 1999; Yuan et al., 1999). These data suggest that *p53*-independent apoptotic pathways may be mediated by other *p53* family members.

A better understanding of *p53*-regulated pathways could lead to improved strategies for treating *p53*-deficient tumors. However, the apparent complexity of *p53* function in vertebrates has hampered efforts to elucidate these pathways. The fruit fly *Drosophila* has proven to be a powerful tool for the genetic dissection of biochemical pathways. We report the identification of a *Drosophila* homolog of *p53* (the abbreviation "*Dmp53*" will be used here to distinguish *Drosophila p53* from other family members). *Dmp53* shares significant amino acid identity with the DNA-binding domain of *p53*, including conservation of key residues commonly mutated in human cancer. Like *p53*, overexpression of *Dmp53* induces apoptosis, and inhibition of *Dmp53* function renders cells resistant to X ray–induced apoptosis. In contrast to mammalian *p53*, *Dmp53* overexpression does not induce a G1 cell cycle block, and inhibition of *Dmp53* activity does not affect X ray–induced cell cycle arrest. These data suggest that the ancestral function of *p53* may have been restricted to eliminating damaged cells by apoptosis and identify *Drosophila* as an ideal model system for dissecting *p53*-mediated apoptotic pathways.

## Results

### Identification of *Dmp53*

We identified a partial *Drosophila* cDNA with sequence similarity to the *p53* family of genes as a result of a

<sup>‡</sup> To whom correspondence should be addressed (e-mail: caseyk@exelixis.com).

<sup>§</sup> These authors contributed equally to this work.



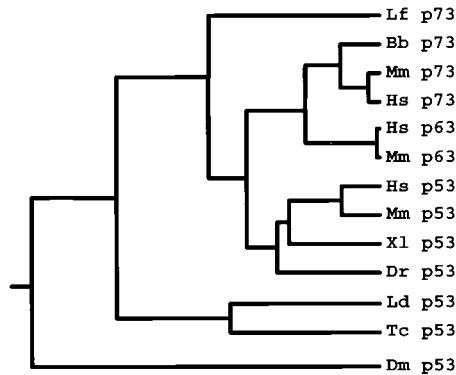


Figure 2. Phylogenetic Analysis of the *p53* Gene Family

Phylogenetic tree generated with Clustal W shows the relationship of p53-related proteins from *Loligo forbesi* (Lf), *Barbus barbuis* (Bb), *Mus musculus* (Mm), *Homo sapiens* (Hs), *Xenopus laevis* (Xl), *Danio rerio* (Dr), *Leptinotarsa decemlineata* (Ld), *Tribolium castaneum* (Tc), and *Drosophila melanogaster* (Dm).

genomic sequence downstream of *Dmp53*. The beetle p53 proteins also lack any similarity to the carboxy-terminal domains of p63 and p73. Given that the insect p53-related genes represent the most evolutionarily distant members of the p53 gene family known to date, we suggest that the p63/p73 subfamily arose from an ancestral p53-like gene after the split of the arthropod and vertebrate lineages.

#### Dmp53 Binds to Human p53 Recognition Sites in p21 and GADD45

To determine whether the sequence similarity of Dmp53 and p53 may reflect a conserved function as a DNA binding transcription factor, we tested whether Dmp53 can bind to a double-stranded DNA molecule containing a p53 recognition site using an electrophoretic mobility shift assay. Figure 3 shows that Dmp53 binds specifically to oligonucleotides containing p53 binding sites from the human p21 and GADD45 genes, demonstrating that both DNA binding and target site specificity have been conserved through more than 500 million years of evolution. This interaction is specific, as addition of unlabelled wild-type GADD45 oligoduplex DNA competes for Dmp53 binding, whereas unlabelled mutant GADD45 oligoduplex DNA does not (Figure 3A, lanes 5–8). Moreover, an anti-Dmp53 polyclonal antibody prevents DNA binding by Dmp53 (Figure 3A, lane 4), and an anti-Dmp53 monoclonal antibody supershifts the Dmp53/DNA complex (Figure 3B, lane 4). It is interesting that human p53, which was expressed and tested in an identical assay, bound p53 binding sites only in the presence of the activating antibody PAb421 (data not shown). PAb421 is thought to act by associating with a region in the carboxyl terminus of p53 that normally negatively regulates DNA binding (Hupp et al., 1992). The ability of Dmp53 to bind DNA without any activating treatments may indicate that a similar negative regulatory element does not exist in Dmp53.

We generated mutant alleles of *Dmp53* analogous to the R175H (R155H in Dmp53) and H179N (H159N in Dmp53) tumor-derived mutations in human p53. These

mutations in human p53 produce proteins with dominant-negative activity, presumably because they cannot bind DNA but retain a functional tetramerization domain (Brachmann et al., 1996; reviewed in Vogelstein and Kinzler, 1992). Thus, DNA binding by any tetramer that incorporates the mutant protein is disrupted. Both Dmp53R155H and H159N proteins inhibit binding of wild-type Dmp53 to a p53 binding site (Figure 3C, lanes 1–5), although they did not bind to DNA themselves. As described below, these mutant forms of Dmp53 are useful tools to test the function of wild-type Dmp53 in vivo.

#### Dmp53 Overexpression Induces Apoptosis but Not G1 Arrest

To determine whether Dmp53 can serve the same functions in vivo as human p53, we ectopically expressed Dmp53 in *Drosophila* larval eye discs using glass-responsive enhancer elements (Hay et al., 1994). The *glass-Dmp53* (*gl-Dmp53*) transgene expresses Dmp53 in all cells posterior to the morphogenetic furrow. The morphogenetic furrow marks the front of a wave of cellular differentiation that sweeps from the posterior to the anterior of the eye disc during larval development (Wolff and Ready, 1991). Thus, *gl-Dmp53* larvae express Dmp53 in all eye disc cells as they differentiate as well as in a subset of cells behind the furrow that undergo a final round of cell division before terminal differentiation.

Expression of Dmp53 from the *gl-Dmp53* transgene produces viable adults that have small, rough eyes with fused ommatidia (Figure 4B). TUNEL staining of *gl-Dmp53* eye discs shows that this phenotype is due, at least in part, to widespread apoptosis in cells expressing Dmp53 (Figure 4D). Similar results are seen when apoptotic cells are detected by acridine orange or Nile Blue (data not shown). TUNEL-positive cells appear within 15–30 cell diameters of the furrow. Given that the furrow is estimated to move approximately five cell diameters per hour (Wolff and Ready, 1991), this indicates that cells initiate apoptosis within 3–6 hr after Dmp53 is expressed.

The ability of p53 to induce apoptosis in some vertebrate cell types can be inhibited by overexpression of p21. The precise mechanism(s) through which p21 inhibits apoptosis is unknown, but direct inhibitory interactions with procaspase 3 (Suzuki et al., 1999a, 1999b) and apoptosis signal-regulating kinase 1 (Asada et al., 1999) have been reported. To determine if expression of human p21 can similarly suppress Dmp53-induced apoptosis, we coexpressed Dmp53 and p21 in the developing eye disc. p21 expression dramatically suppresses Dmp53-induced apoptosis in the disc (Figures 4I and 4J) as well as the adult rough-eye phenotype (data not shown). This suppression does not appear to involve reduction of p53 protein levels, since matched disc samples from larvae expressing *gl-Dmp53* (Figure 4I) or *gl-Dmp53* plus *gl-p21* (Figure 4J) show similar levels of anti-Dmp53 antibody staining. These data suggest that p53-related proteins in flies and vertebrates trigger apoptosis through similar p21-suppressible pathways. Surprisingly, we were unable to achieve similar inhibition of apoptosis by coexpression of the baculovirus p35 protein, a universal substrate inhibitor of caspases (data

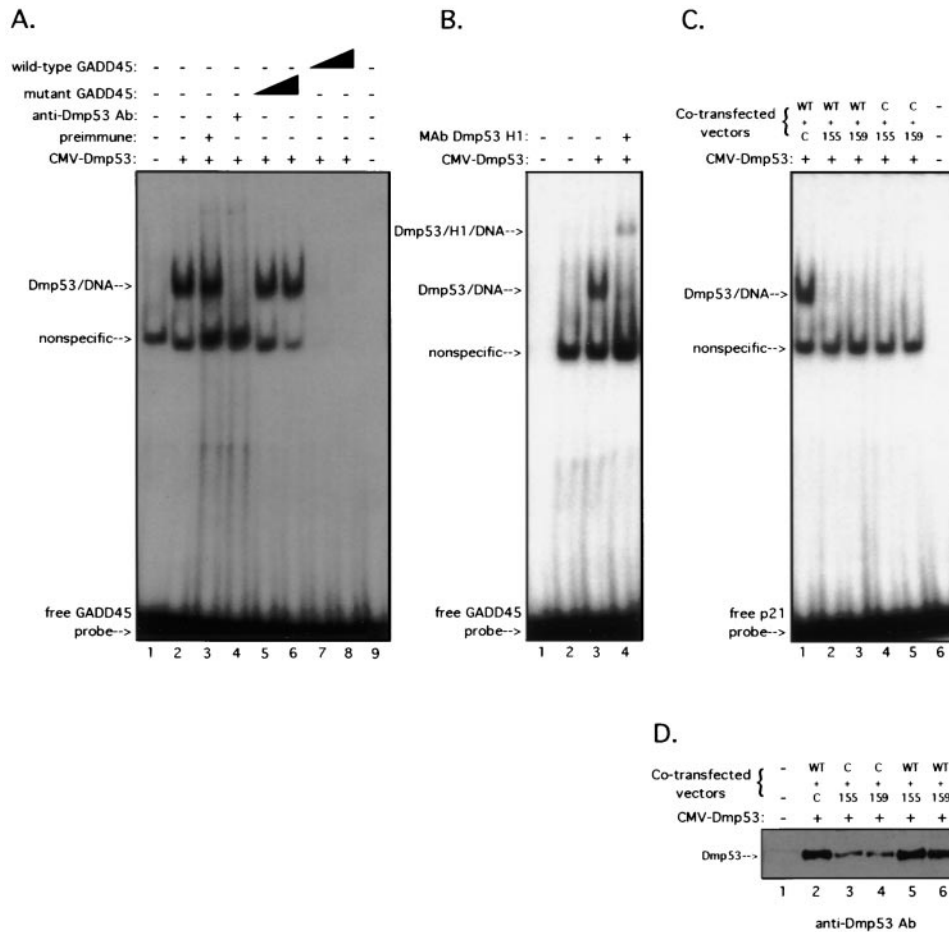


Figure 3. Wild-Type Dmp53 Binds Specifically to Human p53 Binding Sites, and This Binding Is Inhibited by Dmp53R155H and H159N Mutants H1299 cells were transiently transfected with plasmids expressing either wild-type Dmp53, Dmp53R155H (155), or Dmp53H159N (159). A mammalian whole-cell extract was prepared (see Experimental Procedures), and 20  $\mu$ g was used for electrophoretic mobility shift assays (EMSA) and Western blot analysis.

(A) EMSA showing the ability of wild-type Dmp53 to bind  $^{32}$ P-labeled oligonucleotides containing the p53-binding element from *GADD45*. Antibodies were added as indicated: lane 3, preimmune serum, and lane 4, polyclonal anti-Dmp53 serum. Wild-type or mutant *GADD45* oligonucleotides were added in 10-fold (lanes 5 and 7) or 30-fold (lanes 6 and 8) molar excess over probe. Lane 1 contains an extract from cells expressing an empty CMV vector. Lanes 2–8 contain extracts expressing wild-type CMV-Dmp53. Lane 9 contains a reaction mixture lacking extract.

(B) EMSA showing the ability of wild-type Dmp53/*GADD45* complexes to be supershifted by the monoclonal anti-Dmp53 H1 antiserum (lane 4). Lane 1 contains a reaction mixture lacking extract. Lane 2 contains an extract from cells expressing an empty CMV vector. Lanes 3 and 4 contain an extract from cells expressing wild-type CMV-Dmp53.

(C) Dmp53 mutant proteins inhibit binding by wild-type Dmp53 to *p21*-binding site oligonucleotides. For each cotransfection (lanes 1–6), a constant amount of DNA (1  $\mu$ g) was used for each expression vector ("WT," "155," and "159" refer to wild-type Dmp53, Dmp53R155H, and Dmp53H159N, respectively) or control ("C" refers to empty CMV vector) for a total of 2  $\mu$ g. Lane 6 reaction mixture lacks extract.

(D) Western blot loaded with 20  $\mu$ g of extract utilized in (C) and probed with anti-Dmp53 polyclonal antibody to detect Dmp53 in cells expressing either wild-type Dmp53 alone (WT + C), Dmp53R155H alone (C + 155), Dmp53H159N alone (C + 159), wild-type Dmp53 plus Dmp53R155H (WT + 155), or wild-type Dmp53 plus Dmp53H159N (WT + 159). Lane 1 contains 20  $\mu$ g extract expressing an empty CMV vector.

For (A), (B), and (C), "nonspecific" refers to a nonspecific protein/DNA complex present in the H1299 whole-cell extracts.

not shown). Given that p35 has previously been shown to inhibit human p53-induced apoptosis in lepidopteran (Prikhod'ko et al., 1999) and *Drosophila* cells (Yamaguchi et al., 1999), the lack of p35 suppression of apoptosis may reflect different rates and/or levels of Dmp53 and p35 protein accumulation.

In addition to its ability to affect cell death pathways, mammalian p53 can induce cell cycle arrest at the G1 and G2/M checkpoints (Levine, 1997). In the *Drosophila* eye disc, the second mitotic wave is a synchronous,

final wave of cell division posterior to the morphogenetic furrow (Tomlinson and Ready, 1987; Wolff and Ready, 1991). This unique aspect of development provides a means to assay for similar effects of Dmp53 on the cell cycle. Transition of these cells from G1 to S phase in wild-type discs can be detected by bromodeoxyuridine (BrdU) incorporation into DNA (Wolff and Ready, 1991) (Figures 4E and 4F). This transition from G1 to S phase is not blocked or delayed by Dmp53 overexpression from the *gl-Dmp53* transgene (Figure 4F). In contrast,

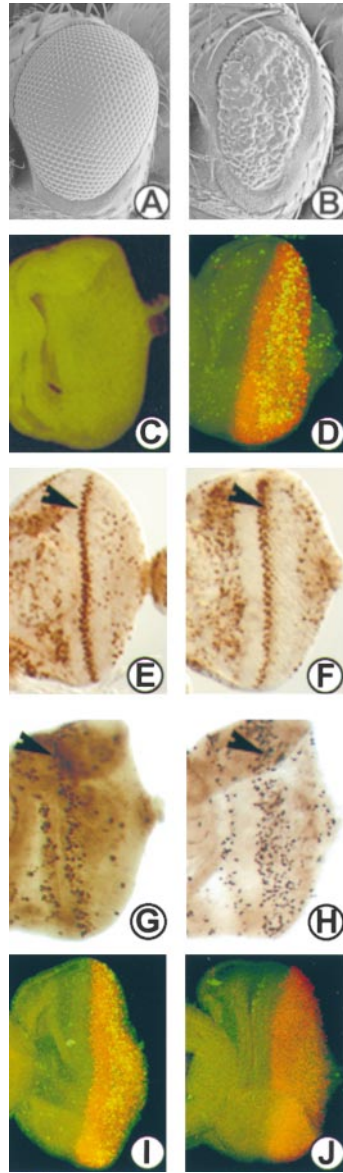


Figure 4. Effects of Dmp53 Overexpression in Eye Discs

(A and B) Scanning electron micrographs of eyes from a wild-type fly (A) or a fly carrying one copy of the *gl-Dmp53* transgene (B). (C and D) Dmp53 overexpression induces apoptosis. Eye discs were dissected from a wild-type third instar larva (C) and a larva containing two copies of the *gl-Dmp53* transgene (D). Discs are stained by TUNEL (green) to mark apoptotic cells and with anti-Dmp53 antibody to show expression of Dmp53 (red).

(E and F) Dmp53 overexpression does not cause a G1 arrest in the eye disc. A wild-type disc (E) and a disc containing one copy of the *gl-Dmp53* transgene (F) were incubated with BrdU to label cells in S phase (brown). Arrows indicate S phase cells of the second mitotic wave.

(G and H) Dmp53 overexpression alters the pattern of mitoses in cells of the second mitotic wave. A wild-type disc (G) and a disc containing one copy of the *gl-Dmp53* transgene (H) were stained with an anti-phospho-histone antibody that specifically labels mitotic cells (brown). Arrows indicate the normal position of the second mitotic wave.

(I and J) Dmp53-induced apoptosis is suppressed by coexpression of human p21. Discs containing one copy of the *gl-Dmp53* transgene (I) or one copy each of the *gl-Dmp53* and *gl-p21* transgenes (J) were stained by TUNEL (green) and anti-Dmp53 antibody staining (red).

expression of human p21 or a *Drosophila* p21 homolog, dacapo, under control of the same *glass*-responsive enhancer element completely blocks DNA replication in the second mitotic wave (de Nooij and Hariharan, 1995; de Nooij et al., 1996; and data not shown). However, overexpression of Dmp53 does affect M phase in the eye disc. In wild-type discs, an M phase-specific anti-phospho-histone antibody typically stains a distinct band of cells within the second mitotic wave (Figure 4G). In *gl-Dmp53* larval eye discs, this band of cells is present but is significantly broader and more diffuse (Figure 4H), suggesting that Dmp53 alters the entry into and/or duration of M phase.

#### Reduced Dmp53 Function Blocks X Ray-Induced Apoptosis

We next sought to determine whether loss of Dmp53 function affected apoptosis or cell cycle arrest in response to DNA damage. In order to examine the phenotype of tissues deficient in Dmp53 function, we expressed the dominant-negative *Dmp53* alleles described above as transgenes under the control of tissue-specific promoters. Coexpression of Dmp53R155H with wild-type Dmp53 suppresses the rough eye phenotype that normally results from wild-type Dmp53 overexpression (Figure 5C), confirming that this mutant protein has dominant-negative activity in vivo. The same result was obtained by expressing the Dmp53H159N protein (data not shown). Unlike wild-type *Dmp53*, overexpression of the dominant-negative alleles using the *glass* enhancer (Figure 5A) or a constitutive enhancer (*arm-GAL4*; data not shown) has no visible effect on normal development.

In mammalian systems, p53-induced apoptosis plays a crucial role in preventing the propagation of damaged DNA (reviewed in Lowe, 1995). DNA damage also leads to apoptosis in *Drosophila*. To determine if this response requires the action of Dmp53, we expressed dominant-negative *Dmp53* transgenes in the posterior compartment of the wing disc. Wild-type wing discs show widespread apoptosis detectable by TUNEL staining 4 hr after X irradiation (Figure 5E). When either dominant-negative allele of *Dmp53* is expressed in the posterior compartment of the wing disc, apoptosis is blocked in the cells expressing Dmp53, whereas the anterior compartment displays a normal amount of X ray-induced cell death (Figures 5F and 5G). Thus, induction of apoptosis following X irradiation requires the function of Dmp53. This proapoptotic role for Dmp53 appears to be limited to a specific response to cellular damage, because developmentally programmed cell death in the eye and other tissues (Abrams et al., 1993) is unaffected by expression of either dominant-negative *Dmp53* allele (data not shown).

Although our data strongly suggest that Dmp53 function is required for X ray-induced apoptosis, it does not appear to be necessary for the cell cycle arrest induced by the same dose of irradiation. In the absence of irradiation, a random pattern of mitosis is observed in third instar wing discs of *Drosophila* (Figure 5H). Upon irradiation, a cell cycle block in wild-type discs leads to a significant decrease in anti-phospho-histone staining (Figure 5I; Brodsky et al., 2000a). This cell cycle block is unaffected by expression of dominant-negative Dmp53

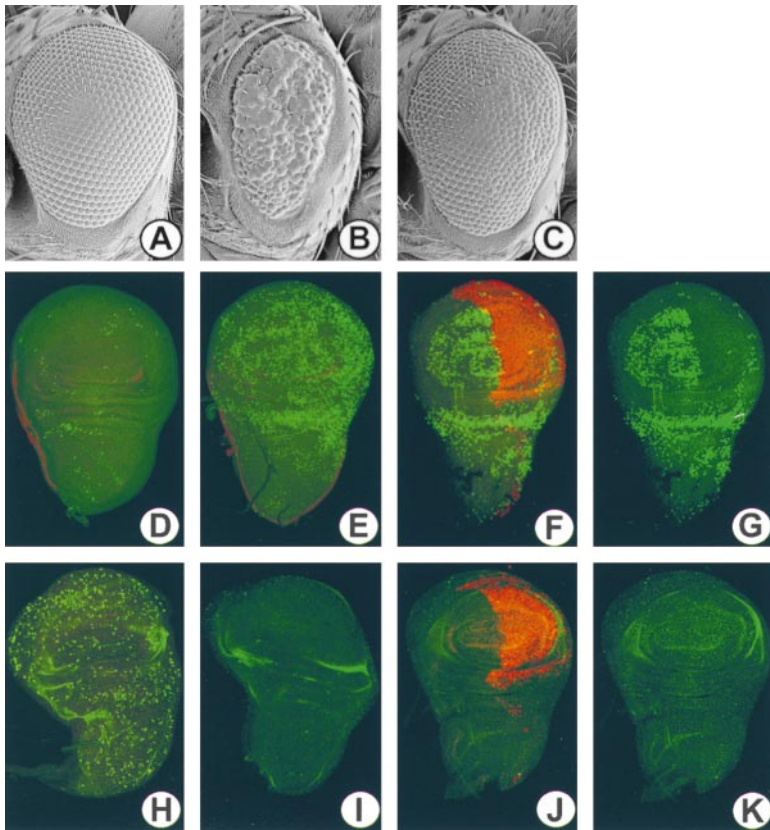


Figure 5. Dominant-Negative Dmp53 Blocks X Ray-Induced Apoptosis but Not X Ray-Induced Cell Cycle Arrest

(A–C) *Dmp53R155H* suppresses *Dmp53* activity in vivo. Scanning electron micrographs of eyes from flies carrying one copy of the *gl-Dmp53R155H* transgene (A), one copy of the wild-type *gl-Dmp53* transgene (B), and one copy each of the *gl-Dmp53* and *gl-Dmp53R155H* transgenes (C).

(D–G) *Dmp53H159N* blocks X ray-induced apoptosis. Wing discs were dissected from third instar larvae containing an *engrailed-GAL4* transgene. Apoptotic cells are stained by TUNEL (green). Panels show an unirradiated disc (D), a disc dissected 4 hr after X irradiation (E), and a disc dissected 4 hr after X irradiation carrying a *UAS-Dmp53H159N* transgene shown with (F) and without (G) anti-*Dmp53* antibody staining (red). The *UAS-Dmp53H159N* transgene is expressed specifically in the posterior compartment of the wing disc under the control of the *engrailed-GAL4* driver.

(H–K) *Dmp53H159N* does not block X ray-induced cell cycle arrest. Wing discs were dissected from third instar larvae containing an *engrailed-GAL4* transgene. Mitotic cells are stained with anti-phospho-histone antibody (green). Panels show an unirradiated disc (H), a disc dissected 4 hr after X irradiation (I), and a disc dissected 4 hr after X irradiation carrying a *UAS-Dmp53H159N* transgene shown with (J) and without (K) anti-*Dmp53* antibody staining (red).

in the posterior of the wing disc (Figures 5J and 5K). Several time points after X irradiation were examined, and all gave similar results, suggesting that both the onset and maintenance of the X ray-induced cell cycle arrest is independent of *Dmp53*.

#### Expression of *Dmp53* during Development

We characterized the expression of *Dmp53* transcripts during embryogenesis to assess potential roles for *Dmp53* during *Drosophila* development (Figure 6).

*Dmp53* RNA is maternally loaded into oocytes and is abundant until cellularization of the blastoderm. Zygotic expression of *Dmp53* begins at cellularization and is initially ubiquitous. At midembryogenesis, *Dmp53* RNA levels are highest in the mesoderm and gut, with only low levels of RNA detectable in the epidermal and neural cell layers. As development proceeds, the expression of *Dmp53* becomes progressively more restricted and falls dramatically in all tissues except for the primordial germ cells and a small patch of hindgut cells. Although

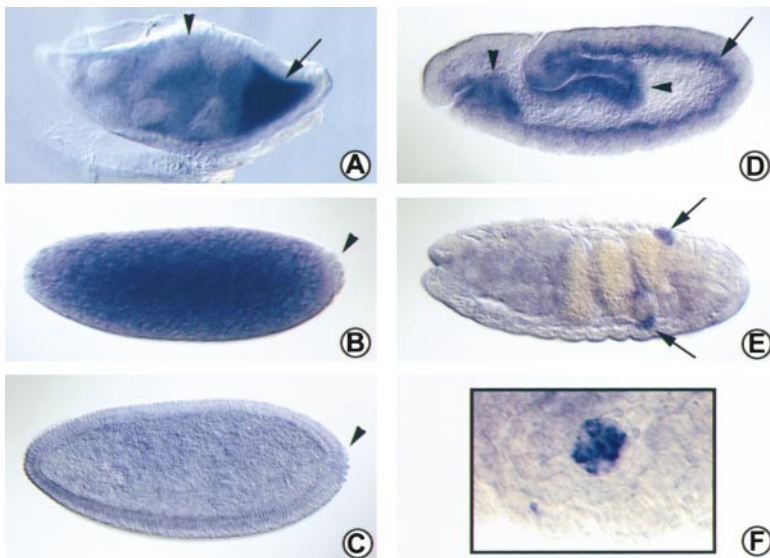


Figure 6. *Dmp53* RNA Expression during Development

(A) Stage 10 egg chamber showing high levels of *Dmp53* transcripts in the nurse cells (arrowhead) and oocyte (arrow).

(B) Stage 3 embryo, showing high levels of maternally loaded *Dmp53* RNA filling the embryo. Note that the posterior pole cells (primordial germ cells) are not stained (arrowheads in [B] and [C]). These cells are transcriptionally quiescent until midembryogenesis (Su et al., 1998).

(C) *Dmp53* maternal RNA levels are reduced by stage 5 (cellular blastoderm).

(D) At stage 10, zygotic expression of *Dmp53* RNA is highest in the mesoderm (arrow) and developing gut (arrowhead).

(E) By stage 16, *Dmp53* expression is dramatically reduced in all tissues with the exception of the primordial germ cells within the two gonads (arrows) and a small portion of the hindgut (not shown).

(F) Close up of *Dmp53* expression within the gonad of a stage 16 embryo.

one must use caution when inferring function from expression data, the high levels of *Dmp53* RNA in germ cells is likely to be significant because germline *p53* expression is a common feature in species ranging from clam (Van Beneden et al., 1997) to human (Bukovsky et al., 1995; Quenby et al., 1999). This conservation of expression suggests an important function for *p53* in germline development.

## Discussion

We have identified and characterized a *Drosophila* homolog of *p53*. Our data show that, like mammalian *p53*, *Dmp53* is a sequence-specific DNA-binding protein whose function is required to induce apoptosis in cells damaged by X irradiation. Similar results are reported in the accompanying paper by Brodsky et al. (2000b [this issue of *Cell*]). Thus, the well-established proapoptotic activity of vertebrate *p53* is an ancient, conserved function for this protein. However, there are also many intriguing differences between the mammalian and fly versions of *p53*. Perhaps the most noteworthy difference is the likelihood that the ability to serve as a G1 checkpoint regulator is a more recent evolutionary development. It is also probable that the relationship between *p53* and MDM2 is a later event in the course of evolution.

Overexpression of *Dmp53* in larval eye discs has no effect on transition of cells from G1 to S phase. While we do not know whether high levels of *Dmp53* would be capable of affecting G1 in other cell types, this result may reflect differences in the downstream targets of vertebrate and *Drosophila* *p53*. In vertebrates, *p53*-mediated G1 arrest is due primarily to transcriptional activation of the cyclin-dependent kinase (cdk) inhibitor *p21*, which can inhibit the CDKs that phosphorylate and inactivate the retinoblastoma gene product (Rb). Phosphorylated Rb family members release and activate E2F/DP transcription factor complexes that are required for cell cycle progression (reviewed in Nevins et al., 1997). The *cdk-Rb-E2F/DP* pathway is highly conserved, and homologs of these genes regulate the cell cycle in *Drosophila* (de Nooij et al., 1996; Du et al., 1996; Edgar and Lehner, 1996; Lane et al., 1996). Overexpression of *p21* or its *Drosophila* homolog, *dacapo*, in eye discs clearly blocks the cell cycle in G1 (de Nooij and Hariharan, 1995; de Nooij et al., 1996). Therefore, the lack of a G1 arrest in *gl-Dmp53* discs suggests that expression of *dacapo* is not induced by *Dmp53*. This hypothesis is consistent with reports indicating that *dacapo* is not induced by X irradiation (de Nooij et al., 1996). We speculate that, although all the necessary components of the *p53* and *Rb* pathways appear to be present in *Drosophila*, later events in evolution made *p21* a target of *p53* and thus established a dual role for *p53* in apoptosis and G1 checkpoint control.

Although overexpression of *Dmp53* in the eye disc had no detectable effect on entry into S phase, it did appear to delay entry into M phase. Given that overexpression of human *p53* can similarly delay entry into M phase (Agarwal et al., 1995; Stewart et al., 1995), it is possible that this G2 checkpoint function is conserved in *Drosophila*. However, it is also possible that the G2/M

phase delay we observed is simply an indirect consequence of the proapoptotic activity of *Dmp53*. The dominant-negative *Dmp53* proteins had no detectable effect on cell cycle arrest in response to X irradiation. However, this negative result does not rule out a function for *Dmp53* in G2 arrest because the *ATM/Chk1* pathway is conserved in *Drosophila* (Hari et al., 1995; Fogarty et al., 1997) and may be sufficient to induce G2 arrest in response to radiation. We also cannot rule out the possibility that our dominant-negative mutants might somehow block the apoptotic function of *Dmp53* without eliminating a G2 checkpoint function. Thus, it remains an open question whether *Dmp53* might play a role in radiation-induced G2 arrest.

As the most evolutionarily distant member of the *p53* gene family to be identified, *Dmp53* may shed light on the selective pressures that have maintained *p53*-like molecules throughout evolution. The absence of *p53* function in mice increases the prevalence of defective embryos and fetuses, particularly after exposure to drugs or radiation (Nicol et al., 1995; Norimura et al., 1996). This embryo-protective role for *p53* may have been a selective force for *p53* evolution, since it would have a direct impact on reproductive success (Hall and Lane, 1997). Similarly, the expression of *p53* in the germ cells of flies, clams, and mammals strongly suggests selection for germline *p53* function during evolution. The absence of *p53* in mouse testes results in reduced spontaneous germ cell apoptosis and an increased frequency of abnormal sperm (Beumer et al., 1998; Yin et al., 1998). Thus, *p53* may also have an important and conserved role in maintaining the fidelity of the germline through the elimination of defective cells.

X irradiation of imaginal wing discs induces apoptosis that requires functional *Dmp53*. This poses the question of how *Dmp53* activity is regulated in response to radiation. It is well established that mammalian *p53* receives signals from a variety of cellular stresses such as various forms of DNA damage, nucleotide deprivation, incomplete DNA synthesis, and hypoxia. These signals are likely to work through a set of signaling pathways that activate and stabilize the *p53* protein (reviewed in Giaccia and Kastan, 1998; Prives and Hall, 1999). Although understanding of the different gene products responsible for these various signaling pathways is still in its infancy, there is strong evidence that one pathway to *p53*, that induced by irradiation, requires functional ATM (reviewed in Morgan and Kastan, 1997). The ATM protein kinase shares homology with other members of the PI3 kinase family, including the *S. pombe* DNA damage mediator kinase Rad3 (Elledge, 1996). Recently, two kinases that are downstream of ATM, CHK1, and CDS1/CHK2 have been shown to phosphorylate and regulate human *p53* (Chehab et al., 2000; Shieh et al., 2000). Given that many aspects of the DNA damage checkpoint response are conserved between yeast and mammals, it is possible that *Dmp53* might be similarly regulated. Indeed, *Drosophila* homologs of ATM (*mei41*) (Hari et al., 1995) and *Chk1* (*grapes*) (Fogarty et al., 1997) have been identified, and it will be interesting to determine their relationship to *Dmp53*. It should be mentioned here that while ATM has been clearly shown to regulate *p53*-mediated cell cycle arrest, there is evidence that apoptosis induced by *p53* is independent of the function of

ATM (Barlow et al., 1997; Liao et al., 1999). Thus, the study of Dmp53-induced apoptosis in *Drosophila* may uncover new upstream regulators of p53 activity.

The ability of mammalian p53 to induce apoptosis has been studied in considerable detail. As mentioned in the introduction, a number of genes (e.g., *Bax*, *IGF-BP3*, *PIG3*, etc.) have been identified that are induced in response to p53 and can cause apoptosis. Genes such as *hid*, *grim*, and *reaper*, known to be involved in apoptosis in *Drosophila* (McCall and Steller, 1997; Abrams, 1999), are being tested for their relationship to Dmp53. Brodsky et al. (2000b) report that *reaper* is, in fact, a downstream target of Dmp53 transcriptional activation. There are reports as well that transcriptional repression may be a necessary component of some aspects of the p53 apoptotic program (Shen and Shenk, 1994; Sabbatini et al., 1995). DNA site selection (SELEX) or DNA arrays will be useful in identifying additional *Drosophila* genes that are targets of Dmp53.

Our discovery of Dmp53 and its conserved role in radiation-induced apoptosis provides a new model system to study the function of a key tumor suppressor gene. Many important cellular pathways and functions have been elucidated through the elegant genetics that *Drosophila* affords. The study of Dmp53 is likely to provide new insights into pathways that regulate mammalian p53-induced apoptosis and may suggest new therapeutic approaches to cancer.

## Experimental Procedures

### Cloning of Dmp53

A partial *Dmp53* cDNA was found by a BLAST version 2.0 (Altschul et al., 1990) search of an EST database representing embryo, disc, and adult head mRNA (C. K. et al., unpublished data). The full sequence of *Dmp53* was determined from three sources: sequencing a commercially available cDNA clone (Research Genetics, ID #31553); sequencing RACE products from embryo and adult Marathon-ready *Drosophila* cDNA libraries (Clontech); and sequencing genomic DNA from P1 clones *DS01201*, *DS02942*, *DS05102*, and *DS06254* (Berkeley *Drosophila* Genome Project). Dominant-negative versions of *Dmp53* containing the mutations R155H and H159N were generated by PCR-mediated mutagenesis. The *p53*-related genes from *Tribolium castaneum* and *Leptinotarsa decemlineata* were identified by BLAST searches of internally generated EST databases.

### Sequence Analysis and Molecular Modeling

The multiple sequence alignment and phylogenetic tree were created using the program ClustalW 1.7 (Thompson et al., 1994; freely available at [ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalW](http://ftp-igbmc.u-strasbg.fr/pub/ClustalW)). The Genbank identifier numbers for the sequences used in the alignment and/or tree are as follows: *Loligo forbesi p53/73*, gi|1244762; *Barbus barbus p73*, gi|4689086; *Mus musculus p73*, gi|4887145, *p63*, gi|3695094, and *p53*, gi|53571; *Homo sapiens p73*, gi|35214, *p63(KET)*, gi|3970717, and *p53*, gi|35214; *Xenopus laevis p53*, gi|545102; *Danio rerio p53*, 1778019.

Secondary structure predictions were made using the software Simpa (Levin, 1997; <http://www.embl-heidelberg.de/argos/simpa/>). A low-resolution three-dimensional model for Dmp53 residues 90–275 was built using comparative structural modeling with the program Modeler/InsightII 98.0 (Molecular Simulations, Inc.) (Sali and Blundell, 1993; Sanchez and Sali, 1997). The coordinates for residues 108–298 in the crystal structure of the human p53 DNA-binding domain (PDB code 1TUP) (Cho et al., 1994) were used as the template. Figure 1B shows the alignment used in the calculations.

### Electrophoretic Mobility Shift Assays

H1299 cells (American Type Culture Collection) were maintained in RPMI1640 supplemented with 10% fetal bovine serum (FBS) in 5%

CO<sub>2</sub> at 37°C. Cells were transfected by a lipopolyamine-based (Transfectam) protocol as described previously (Di Como et al., 1999). Briefly, cells were grown in RPMI1640/10% FBS and transfected with various amounts of DNA. The precipitate was left on the cells for 12 hr, after which fresh media was added for the periods indicated. *CMV-Dmp53*, *CMV-Dmp53R155H*, and *CMV-Dmp53H159N* express full-length *Dmp53*, *Dmp53R155H*, and *Dmp53H159N* cDNAs from the cytomegalovirus (CMV) promoter in *pRc/CMV* (Invitrogen).

H1299 cells were transfected in six-well, 9.6 cm<sup>2</sup> plates with 2 µg of DNA and harvested at 48 hr after transfection. Cells were lysed in 200 µl of lysis buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 0.5% NP40, 150 mM NaCl, 1 mM DTT, 10% glycerol, 0.5 mM PMSF, and a mixture of protease inhibitors), and the extracts were centrifuged at 16,000 × g for 20 min to remove cell debris. Protein concentrations were determined using the Bio-Rad assay using bovine globulin as a standard (Bio-Rad Laboratories, CA). When necessary, total cell extracts were frozen in a dry ice/alcohol bath and stored at –80°C. For Western blot analysis, samples were electrophoresed through an SDS-10% polyacrylamide gel. Protein gels were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). For Dmp53 detection, an anti-Dmp53 polyclonal antibody was used at a 1/3000 dilution. Proteins were visualized with an enhanced chemiluminescence detection system (Amersham).

Probes were made as follows: synthesized oligonucleotides (Operon Technologies, Inc.) were self-annealed and end-labeled with T4 polynucleotide kinase (PNK) in the presence of [ $\gamma$ -<sup>32</sup>P]ATP. The oligonucleotide probe containing the *p21 cis*-acting p53-responsive element (el-Deiry et al., 1993) is as follows: 5'-TAGAGCGAACATGTCCTCAACATGTTGGCGTCG-3'. The oligonucleotide probe containing the wild-type *GADD45 cis*-acting p53-responsive element (Kastan et al., 1992) is as follows: 5'-TAGAGCGAACATGTCCTAAGCATGCTGGCGTCG-3'. The oligonucleotide probe containing the mutant *GADD45 cis*-acting element is as follows: 5'-TAGAGCGAAaATITCTAAGaATICTGGCGTCG-3'. The p53 binding site is italicized, and the mutated nucleotides are in lowercase lettering. The oligonucleotides were purified over a denaturing 16% acrylamide gel, and both complementary strands were annealed and purified over a native 10% acrylamide gel.

Electrophoretic mobility shift assays were performed as described (Di Como and Prives, 1998), except for minor changes. Binding reaction mixtures contained 20 µg mammalian whole-cell extract in gel shift buffer (20 mM HEPES [pH 7.9], 25 mM KCl, 0.1 mM EDTA, 2 mM MgCl<sub>2</sub>, 0.5 mM DTT, 0.025% Nonidet P-40, 2 mM spermidine [Sigma], 10% glycerol, 0.1 µg/µl acetylated bovine serum albumin [NEB]) and 120 ng double-stranded poly(dI-C)] (Boehringer Mannheim) in a final volume of 30 µl. In all cases, volumes were equalized with lysis buffer. Reactions were incubated for 10 min at 24°C, after which 5 ng of <sup>32</sup>P-labeled oligonucleotide was added, and the incubation was continued at 24°C for 15 min. Samples were run on 4.5% native polyacrylamide gels at 180–200V (not exceeding 40 mA current) in 0.5× TBE buffer at 24°C for 1.5 hr. Gels were dried under vacuum at 80°C and exposed to Kodak XAR film at 24°C for 30–60 min.

### *Drosophila* Genetics

Fly culture and crosses were performed according to standard procedures at 22°C–25°C. *Dmp53*, *Dmp53R155H*, and *Dmp53H159N* transgenes were made by cloning a BclI/HincII fragment spanning the *Dmp53* open reading frame into *pExpress-glass* and *pExpress-UAS* transformation vectors. The *pExpress* vector is an adapted version of the *pGMR* vector (Hay et al., 1994), which contains an  $\alpha$ -*tubulin* 3' UTR for increased mRNA stabilization and a modified multiple cloning site. The *pExpress-UAS* vector is similar to *pExpress-glass* except that it contains multiple *UAS-GAL4* binding sites in place of the *glass* binding sites. Standard P element-mediated germline transformation was used to generate transgenic lines containing these constructs. For X irradiation experiments, third instar larvae in vials were exposed to 4000 Rads of X rays using a Faxitron X-ray cabinet system.

### Staining of Embryos and Discs

For immunocytochemistry, third instar larval eye and wing discs were dissected in PBS, fixed in formaldehyde at room temperature

(4% for 5 min for anti-phospho-histone or 2% for 30 min for anti-Dmp53 plus TUNEL), permeabilized in PBS + 0.5% Triton (15 min at room temperature), blocked in PBS + 5% goat serum, and incubated with primary antibody (2 hr at room temperature or overnight at 4°C). Anti-Dmp53 monoclonal antibody staining was performed using a 1:2 dilution of hybridoma supernatant. Hybridoma clone H2 was used for anti-Dmp53 staining, and a mixture of supernatant from clones H2, H4, and H5 were used to detect Dmp53R155H and Dmp53H159N. Anti-phospho-histone staining was performed using an Anti-phospho-histone H3 Mitosis Marker (Upstate Biotechnology) at a 1:500 dilution. Goat anti-mouse or anti-rabbit secondary antibodies conjugated to FITC or Texas Red (Jackson ImmunoResearch) were used at a 1:200 dilution. Antibodies were diluted in PBS + 5% goat serum. TUNEL was performed using the Apoptag Direct kit (Oncor) per manufacturer's protocol with a 0.5% Triton/PBS permeabilization step. Discs were mounted in anti-fade reagent (Molecular Probes), and images were obtained on a Leica confocal microscope. BrdU staining was performed as described (de Nooij and Hariharan, 1995). In situ hybridization was performed as described (Van Vactor and Kocczynski, 1999) using digoxigenin-labeled antisense RNA probes.

#### Generation of Anti-Dmp53 Antibodies

Anti-Dmp53 rabbit polyclonal and mouse monoclonal antibodies were generated by standard methods using as antigen a full-length Dmp53 protein fused to glutathione-S-transferase (GST-Dmp53). Inclusion bodies of GST-Dmp53 were purified by centrifugation following extraction in B-PER buffer (Pierce) and injected subcutaneously into rabbits (Josman Labs) and mice (Antibody Solutions, Inc.) for immunization. The final boost for mouse monoclonal antibody production used intravenous injection of soluble GST-Dmp53 produced by solubilization of GST-Dmp53 in 6 M Guanidine-Hydrochloride and dialysis into phosphate buffer containing 1 M NaCl. Hybridoma supernatants were screened by ELISA using soluble 6× HIS-tagged Dmp53 protein bound to Ni-NTA-coated plates (Qiagen) and an anti-mouse IgG Fc fragment-specific secondary antibody.

#### Acknowledgments

We thank Dr. Laleh Shayesteh and Kevin Keegan for sequencing *Dmp53* P1 clones, Brett Milash for sequence analysis, Sheldon Ng for assistance with in situ hybridization, and the Exelixis Sequencing Core for sequencing support. C. J. D. was supported by the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation (fellowship DRG-1427). This work was supported by NIH grants CA58316 and CA77742 to C. P.

Received January 31, 2000; revised March 1, 2000.

#### References

Abrams, J.M. (1999). An emerging blueprint for apoptosis in *Drosophila*. *Trends Cell Biol.* 9, 435–440.

Abrams, J.M., White, K., Fessler, L.I., and Steller, H. (1993). Programmed cell death during *Drosophila* embryogenesis. *Development* 117, 29–43.

Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999). Interaction of c-Abl and p73 $\alpha$  and their collaboration to induce apoptosis. *Nature* 399, 809–813.

Agarwal, M.L., Agarwal, A., Taylor, W.R., and Stark, G.R. (1995). p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proc. Natl. Acad. Sci. USA* 92, 8493–8497.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.

Asada, M., Yamada, T., Ichijo, H., Delia, D., Miyazono, K., Fukumuro, K., and Mizutani, S. (1999). Apoptosis inhibitory activity of cytoplasmic p21(Cip1/WAF1) in monocytic differentiation. *EMBO J.* 18, 1223–1234.

Barker, C.M., Calvert, R.J., Walker, C.W., and Reinisch, C.L. (1997). Detection of mutant p53 in clam leukemia cells. *Exp. Cell Res.* 232, 240–245.

Barlow, C., Brown, K.D., Deng, C.X., Tagle, D.A., and Wynshaw-Boris, A. (1997). Atm selectively regulates distinct p53-dependent cell-cycle checkpoint and apoptotic pathways. *Nat. Genet.* 17, 453–456. Erratum: *Nat. Genet.* 18(3), 1998.

Beumer, T.L., Roepers-Gajadien, H.L., Gademan, I.S., van Buul, P.P., Gil-Gomez, G., Rutgers, D.H., and de Rooij, D.G. (1998). The role of the tumor suppressor p53 in spermatogenesis. *Cell Death Differ.* 5, 669–677.

Bissonnette, N., and Hunting, D.J. (1998). p21-induced cycle arrest in G1 protects cells from apoptosis induced by UV-irradiation or RNA polymerase II blockage. *Oncogene* 16, 3461–3469.

Brachmann, R.K., Vidal, M., and Boeke, J.D. (1996). Dominant-negative p53 mutations selected in yeast hit cancer hot spots. *Proc. Natl. Acad. Sci. USA* 93, 4091–4095.

Brodsky, M.H., Sekelsky, J.J., Tsang, G., Hawley, R.S., and Rubin, G.M. (2000a). *mus304* encodes a novel DNA damage checkpoint protein required during *Drosophila* development. *Genes Dev.*, in press.

Brodsky, M.H., Nordstrom, W., Tsang, G., Kwan, E., Rubin, G.M., and Abrams, J.M. (2000b). *Drosophila* p53 binds a damage response element at the *reaper* locus. *Cell* 101, this issue, 103–113.

Buckbinder, L., Talbott, R., Velasco-Miguel, S., Takenaka, I., Faha, B., Seizinger, B.R., and Kley, N. (1995). Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature* 377, 646–649.

Bukovsky, A., Caudle, M.R., Keenan, J.A., Wimalasena, J., Foster, J.S., Upadhyaya, N.B., and van Meter, S.E. (1995). Expression of cell cycle regulatory proteins (p53, pRb) in the human female genital tract. *J. Assist. Reprod. Genet.* 12, 123–131.

Chehab, N.H., Malikzay, A., Appel, M., and Halazonetis, T.D. (2000). Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53. *Genes Dev.* 14, 278–288.

Cho, Y., Gorina, S., Jeffrey, P.D., and Pavletich, N.P. (1994). Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science* 265, 346–355.

Clore, G.M., Omichinski, J.G., Sakaguchi, K., Zambrano, N., Sakamoto, H., Appella, E., and Gronenborn, A.M. (1994). High-resolution structure of the oligomerization domain of p53 by multidimensional NMR. *Science* 265, 386–391. Erratum: *Science* 267(5203), 1995.

de Nooij, J.C., and Hariharan, I.K. (1995). Uncoupling cell fate determination from patterned cell division in the *Drosophila* eye. *Science* 270, 983–985.

de Nooij, J.C., Letendre, M.A., and Hariharan, I.K. (1996). A cyclin-dependent kinase inhibitor, Dacapo, is necessary for timely exit from the cell cycle during *Drosophila* embryogenesis. *Cell* 87, 1237–1247.

Deng, C., Zhang, P., Harper, J.W., Elledge, S.J., and Leder, P. (1995). Mice lacking p21<sup>Cip1/WAF1</sup> undergo normal development, but are defective in G1 checkpoint control. *Cell* 82, 675–684.

Di Como, C.J., and Prives, C. (1998). Human tumor-derived p53 proteins exhibit binding site selectivity and temperature sensitivity for transactivation in a yeast-based assay. *Oncogene* 16, 2527–2539.

Di Como, C.J., Gaiddon, C., and Prives, C. (1999). p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. *Mol. Cell. Biol.* 19, 1438–1449.

Du, W., Vidal, M., Xie, J.E., and Dyson, N. (1996). RBF, a novel RB-related gene that regulates E2F activity and interacts with cyclin E in *Drosophila*. *Genes Dev.* 10, 1206–1218.

Edgar, B.A., and Lehner, C.F. (1996). Developmental control of cell cycle regulators: a fly's perspective. *Science* 274, 1646–1652.

el-Deiry, W.S., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W., and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* 75, 817–825.

Elledge, S.J. (1996). Cell cycle checkpoints: preventing an identity crisis. *Science* 274, 1664–1672.

Fogarty, P., Campbell, S.D., Abu-Shumays, R., Phalle, B.S., Yu, K.R., Uy, G.L., Goldberg, M.L., and Sullivan, W. (1997). The *Drosophila* grapes gene is related to checkpoint gene chk1/rad27 and is required for late syncytial division fidelity. *Curr. Biol.* 7, 418–426.

- Giaccia, A.J., and Kastan, M.B. (1998). The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.* **12**, 2973–2983.
- Gong, J.G., Costanzo, A., Yang, H.Q., Melino, G., Kaelin, W.G., Jr., Leviero, M., and Wang, J.Y. (1999). The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* **399**, 806–809.
- Gorospe, M., Cirielli, C., Wang, X., Seth, P., Capogrossi, M.C., and Holbrook, N.J. (1997). p21(Waf1/Cip1) protects against p53-mediated apoptosis of human melanoma cells. *Oncogene* **14**, 929–935.
- Gottlieb, T.M., and Oren, M. (1996). p53 in growth control and neoplasia. *Biochim. Biophys. Acta* **1287**, 77–102.
- Graeber, T.G., Osmanian, C., Jacks, T., Housman, D.E., Koch, C.J., Lowe, S.W., and Giaccia, A.J. (1996). Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* **379**, 88–91.
- Hainaut, P., Hernandez, T., Robinson, A., Rodriguez-Tome, P., Flores, T., Hollstein, M., Harris, C.C., and Montesano, R. (1998). IARC Database of p53 gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. *Nucleic Acids Res.* **26**, 205–213.
- Hall, P.A., and Lane, D.P. (1997). Tumor suppressors: a developing role for p53? *Curr. Biol.* **7**, R144–R147.
- Hari, K.L., Santerre, A., Sekelsky, J.J., McKim, K.S., Boyd, J.B., and Hawley, R.S. (1995). The mei-41 gene of *D. melanogaster* is a structural and functional homolog of the human ataxia telangiectasia gene. *Cell* **82**, 815–821.
- Harper, J.W., Adami, G.R., Wei, N., Keyomarsi, K., and Elledge, S.J. (1993). The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **75**, 805–816.
- Hay, B.A., Wolff, T., and Rubin, G.M. (1994). Expression of baculovirus P35 prevents cell death in *Drosophila*. *Development* **120**, 2121–2129.
- Hupp, T.R., Meek, D.W., Midgley, C.A., and Lane, D.P. (1992). Regulation of the specific DNA binding function of p53. *Cell* **71**, 875–886.
- Jeffrey, P.D., Gorina, S., and Pavletich, N.P. (1995). Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. *Science* **267**, 1498–1502.
- Kaelin, W.G., Jr. (1999). The p53 gene family. *Oncogene* **18**, 7701–7705.
- Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J.-C., Valent, A., Minty, A., Chalou, P., Lelias, J.-M., Dumont, X., et al. (1997). Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* **90**, 809–819.
- Kamijo, T., Weber, J.D., Zambetti, G., Zindy, F., Roussel, M.F., and Sherr, C.J. (1998). Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. *Proc. Natl. Acad. Sci. USA* **95**, 8292–8297.
- Kastan, M.B., Zhan, Q., el-Deiry, W.S., Carrier, F., Jacks, T., Walsh, W.V., Plunkett, B.S., Vogelstein, B., and Fornace, A.J., Jr. (1992). A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* **71**, 587–597.
- Kinzler, K.W., and Vogelstein, B. (1996). Life (and death) in a malignant tumour. *Nature* **379**, 19–20.
- Ko, L.J., and Prives, C. (1996). p53: puzzle and paradigm. *Genes Dev.* **10**, 1054–1072.
- Kussie, P.H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A.J., and Pavletich, N.P. (1996). Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **274**, 948–953.
- Lane, M.E., Sauer, K., Wallace, K., Jan, Y.N., Lehner, C.F., and Vaessin, H. (1996). Dacapo, a cyclin-dependent kinase inhibitor, stops cell proliferation during *Drosophila* development. *Cell* **87**, 1225–1235.
- Lee, W., Harvey, T.S., Yin, Y., Yau, P., Litchfield, D., and Arrowsmith, C.H. (1994). Solution structure of the tetrameric minimum transforming domain of p53. *Nat. Struct. Biol.* **1**, 877–890. Erratum: *Nat. Struct. Biol.* **2**(1), 1995.
- Levin, J.M. (1997). Exploring the limits of nearest neighbour secondary structure prediction. *Protein Eng.* **10**, 771–776.
- Levine, A.J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* **88**, 323–331.
- Liao, M.J., Yin, C., Barlow, C., Wynshaw-Boris, A., and van Dyke, T. (1999). Atm is dispensable for p53 apoptosis and tumor suppression triggered by cell cycle dysfunction. *Mol. Cell. Biol.* **19**, 3095–3102.
- Lin, J., Chen, J., Elenbaas, B., and Levine, A.J. (1994). Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes Dev.* **8**, 1235–1246.
- Lowe, S.W. (1995). Cancer therapy and p53. *Curr. Opin. Oncol.* **7**, 547–553.
- McCall, K., and Steller, H. (1997). Facing death in the fly: genetic analysis of apoptosis in *Drosophila*. *Trends Genet.* **13**, 222–226.
- Miyashita, T., and Reed, J.C. (1995). Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* **80**, 293–299.
- Morgan, S.E., and Kastan, M.B. (1997). p53 and ATM: cell cycle, cell death, and cancer. *Adv. Cancer Res.* **71**, 1–25.
- Nevins, J.R., Leone, G., DeGregori, J., and Jakoi, L. (1997). Role of the Rb/E2F pathway in cell growth control. *J. Cell. Physiol.* **173**, 233–236.
- Nicol, C.J., Harrison, M.L., Laposa, R.R., Gimelshtein, I.L., and Wells, P.G. (1995). A teratologic suppressor role for p53 in benzo[a]pyrene-treated transgenic p53-deficient mice. *Nat. Genet.* **10**, 181–187. Erratum: *Nat. Genet.* **11**(1), 1995.
- Norimura, T., Nomoto, S., Katsuki, M., Gondo, Y., and Kondo, S. (1996). p53-dependent apoptosis suppresses radiation-induced teratogenesis. *Nat. Med.* **2**, 577–580.
- Owen-Schaub, L.B., Zhang, W., Cusack, J.C., Angelo, L.S., Santee, S.M., Fujiwara, T., Roth, J.A., Deisseroth, A.B., Zhang, W.W., Kruzel, E., et al. (1995). Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. *Mol. Cell. Biol.* **15**, 3032–3040.
- Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W., and Vogelstein, B. (1997). A model for p53-induced apoptosis. *Nature* **389**, 300–305.
- Prihod'ko, G.G., Wang, Y., Freulich, E., Prives, C., and Miller, L.K. (1999). Baculovirus p33 binds human p53 and enhances p53-mediated apoptosis. *J. Virol.* **73**, 1227–1234.
- Prives, C., and Hall, P.A. (1999). The p53 pathway. *J. Pathol.* **187**, 112–126.
- Quenby, S.M., Gazvani, M.R., Brazeau, C., Neilson, J., Lewis-Jones, D.I., and Vince, G. (1999). Oncogenes and tumour suppressor genes in first trimester human fetal gonadal development. *Mol. Hum. Reprod.* **5**, 737–741.
- Sabbatini, P., Chiou, S.K., Rao, L., and White, E. (1995). Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. *Mol. Cell. Biol.* **15**, 1060–1070.
- Sali, A., and Blundell, T.L. (1993). Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.* **234**, 779–815.
- Sanchez, R., and Sali, A. (1997). Evaluation of comparative protein structure modeling by MODELLER-3. *Proteins Suppl.* **1**, 50–58.
- Schmale, H., and Bamberger, C. (1997). A novel protein with strong homology to the tumor suppressor p53. *Oncogene* **15**, 1363–1367.
- Shen, Y., and Shenk, T. (1994). Relief of p53-mediated transcriptional repression by the adenovirus E1B 19-kDa protein or the cellular Bcl-2 protein. *Proc. Natl. Acad. Sci. USA* **91**, 8940–8944.
- Shieh, S.-Y., Ahn, J., Tamai, K., Taya, Y., and Prives, C. (2000). The human homologues of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev.* **14**, 289–300.
- Stewart, N., Hicks, G.G., Paraskevas, F., and Mowat, M. (1995). Evidence for a second cell cycle block at G2/M by p53. *Oncogene* **10**, 109–115.
- Su, T.T., Campbell, S.D., and O'Farrell, P.H. (1998). The cell cycle program in germ cells of the *Drosophila* embryo. *Dev. Biol.* **196**, 160–170.
- Suzuki, A., Tsutomi, Y., Miura, M., and Akahane, K. (1999a). Caspase 3 inactivation to suppress Fas-mediated apoptosis: identification of

binding domain with p21 and ILP and inactivation machinery by p21. *Oncogene* 18, 1239–1244.

Suzuki, A., Tsutomi, Y., Yamamoto, N., Shibutani, T., and Akahane, K. (1999b). Mitochondrial regulation of cell death: mitochondria are essential for procaspase 3-p21 complex formation to resist Fas-mediated cell death. *Mol. Cell. Biol.* 19, 3842–3847.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673–4680.

Tomlinson, A., and Ready, D.F. (1987). Cell fate in the *Drosophila* ommatidium. *Dev. Biol.* 120, 264–275.

Van Beneden, R.J., Walker, C.W., and Laughner, E.S. (1997). Characterization of gene expression of a p53 homolog in the soft-shell clam (*Mya arenaria*). *Mol. Mar. Biol. Biotechnol.* 6, 116–122.

Van Vactor, D., and Kopczyński, C. (1999). *Anatomical Techniques for Analysis of Nervous System Development in the Drosophila Embryo*, J.D. Richter, ed. (New York: Oxford University Press).

Vogelstein, B., and Kinzler, K.W. (1992). p53 function and dysfunction. *Cell* 70, 523–526.

Wagner, A.J., Kokontis, J.M., and Hay, N. (1994). Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21<sup>waf1/cip1</sup>. *Genes Dev.* 8, 2817–2830.

Waldman, T., Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1996). Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. *Nature* 381, 713–716.

Wang, J., and Walsh, K. (1996). Resistance to apoptosis conferred by Cdk inhibitors during myocyte differentiation. *Science* 273, 359–361.

Wolff, T., and Ready, D.F. (1991). The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. *Development* 113, 841–850.

Xiong, Y., Hannon, G.J., Zhang, H., Casso, D., Kobayashi, R., and Beach, D. (1993). p21 is a universal inhibitor of cyclin kinases. *Nature* 366, 701–704.

Yamaguchi, M., Hirose, F., Inoue, Y.H., Shiraki, M., Hayashi, Y., Nishi, Y., and Matsukage, A. (1999). Ectopic expression of human p53 inhibits entry into S phase and induces apoptosis in the *Drosophila* eye imaginal disc. *Oncogene* 18, 6767–6775.

Yin, Y., Stahl, B.C., DeWolf, W.C., and Morgentaler, A. (1998). p53-mediated germ cell quality control in spermatogenesis. *Dev. Biol.* 204, 165–171.

Yu, D., Jing, T., Liu, B., Yao, J., Tan, M., McDonnell, T.J., and Hung, M.C. (1998). Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of p21<sup>Cip1</sup>, which inhibits p34<sup>Cdc2</sup> kinase. *Mol. Cell* 2, 581–591.

Yuan, Z.M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y.Y., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1999). p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. *Nature* 399, 814–817. Erratum: *Nature* 19(400), 1999.