Drosophila p53 preserves genomic stability by regulating cell death

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When animal cells are exposed to stressful conditions, the tumor suppressor protein p53 restrains growth by promoting an arrested cell cycle or initiating a cell death program. How these distinct fates are specified through the action of a single protein is not known. To study its functions *in vivo* we produced a targeted mutation at the *Drosophila p53* (*Dmp53*) locus. We show that Dmp53 is required for damage-induced apoptosis but not for cell-cycle arrest. Dmp53 function is also required for damage-induced transcription of two tightly linked cell death activators, *reaper* and *sickle*. When challenged by ionizing radiation, *Dmp53* mutants exhibit radiosensitivity and genomic instability. Hence, elevated mutant loads were not caused by defective checkpoint functions but instead correlated with failures in p53-associated cell death. Our studies support the notion that core ancestral functions of the p53 gene family are intimately coupled to cell death as an adaptive response to maintain genomic stability.

The p53 tumor suppressor limits oncogenesis through activities that govern adaptive responses to stress. As such, p53 is thought to function as a "guardian of the genome" that becomes mutated or altered in most human cancers (1, 2). When cells are stressed by exposure to genotoxic agents, radiation, hypoxia, or inappropriate oncogene activation (1–3), p53 restrains growth through activities that reversibly arrest the cell cycle or promote apoptosis (4, 5), but how these alternative fates become specified through the action of this tumor suppressor is not understood. The primary mechanism of p53 action clearly involves transcriptional regulation (6–8), although other activities might contribute to accessory functions (9). The protein requires at least three functional domains to regulate downstream target genes but many mutations commonly found in human cancers map to the DNA-binding domain (10–13). A major effort in cancer biology is focused toward understanding downstream effectors of p53 function. The p53 target gene, p21, acts directly as a cyclin-dependent kinase inhibitor (14–16) and appears largely responsible for p53-dependent G_1/S arrest. The *in vivo* downstream targets responsible for p53-mediated apoptosis, however, are less well defined. Important candidates for *in vivo* death effectors include members of the bcl2 family, *bax* (17), *noxa* (18), *puma* (19, 20), and the death receptor *fas* (21, 22). In many cases, regulated expression of these genes is context-specific and whether there exists a generic p53 apoptosis program for all cells is not known. Genome-wide searches for p53-responsive genes have also been reported, but it is not yet clear whether these loci represent direct or indirect targets (23, 24).

A *Drosophila* homolog of p53, Dmp53, was recently identified (25–27). Like mammalian counterparts (12, 13, 28–31), Dmp53 has a well conserved DNA-binding domain with a transcriptional activation domain at its N terminus, and an oligomerization domain at its C terminus. In previous studies, forced expression of wild-type and variant *Dmp53* transgenes was used to establish that ectopic Dmp53 expression triggers apoptotic cell death and possibly affects the duration of M phase (27). Additional experiments with Dmp53 variants showed that radiation-induced apoptosis in the wing disc was suppressed by dominant negative transgenes. However, expression of these same transgenes had no effect on the damage induced cell-cycle-arrest response, even

though many cell-cycle regulators, including *p21*, are conserved in the fly genome and expression of fly p21 alone can trigger G_1 arrest (32). These observations supported a role for Dmp53 in apoptosis and, at the same time, suggest that Dmp53 may not engage checkpoint controls through the cell cycle like its mammalian counterparts.

In *Drosophila*, programmed cell death is governed by apoptosis activators mapping to a genomic interval referred to as the *Reaper* region (33). Several lines of evidence implicate one gene in this region, *reaper* (*rpr*), as a direct target of, and effector for, Dmp53 action *in vivo*. First, *rpr* transcription is acutely sensitive to damage signals, becoming induced within 90 min of γ-irradiation treatment (34). Second, this inductive response maps to a 20-bp radiation-responsive element that binds Dmp53 and resembles the consensus developed for mammalian counterparts (25). Third, recent loss-of-function analyses uncovered a partial requirement for *rpr* in a model of x-ray-induced cell death (35).

To directly examine the functions of Dmp53 and aid in the identification of direct *in vivo* targets, we isolated a mutation at the *Dmp53* locus. Because there are no available *P* elements that map near the *Dmp53* region, we adopted a gene-targeting method recently developed by Rong and Golic (36, 37). This method directs mutations at the desired locus through homologous recombination events that produce aberrant duplicates of the targeted gene. Although viable, *Dmp53* mutants exhibit pronounced defects in damage-induced apoptosis. Radiation-induced expression of two proapoptotic genes, *rpr* and *sickle* (*skl*), and a p53-responsive reporter transgene failed to regulate normally in these mutants. However, under these same irradiation conditions, the damage-induced checkpoint response occurred normally. *Dmp53* mutants are radiosensitive and also exhibit a pronounced mutator phenotype, manifested as high mutagenic loads in loss-of-heterozygosity (LOH) assays. Together, these results uncover important genomic consequences resulting from failures in p53-mediated cell death and establish the principle that preservation of genomic stability by p53 proteins can derive entirely from apoptotic determinants rather than cell-cycle checkpoint functions.

Materials and Methods

Materials and methods used for plasmid constructions, molecular verification of targeting, crosses and screen for targeting, oligonucleotide array analysis, and radiation sensitivity assay can be found in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site, www.pnas.org.

Crosses and Screens for Targeting. Flies carrying the *Dmp53* donor element on the second chromosome were crossed to flies carrying both *70FLP* and *70I-Sce*I transgenes on the second chromosome to yield *Dmp53* donor/*70FLP*, *70I-Sce*I;+/+ progeny. Induction of recombinations in the germ line as well as

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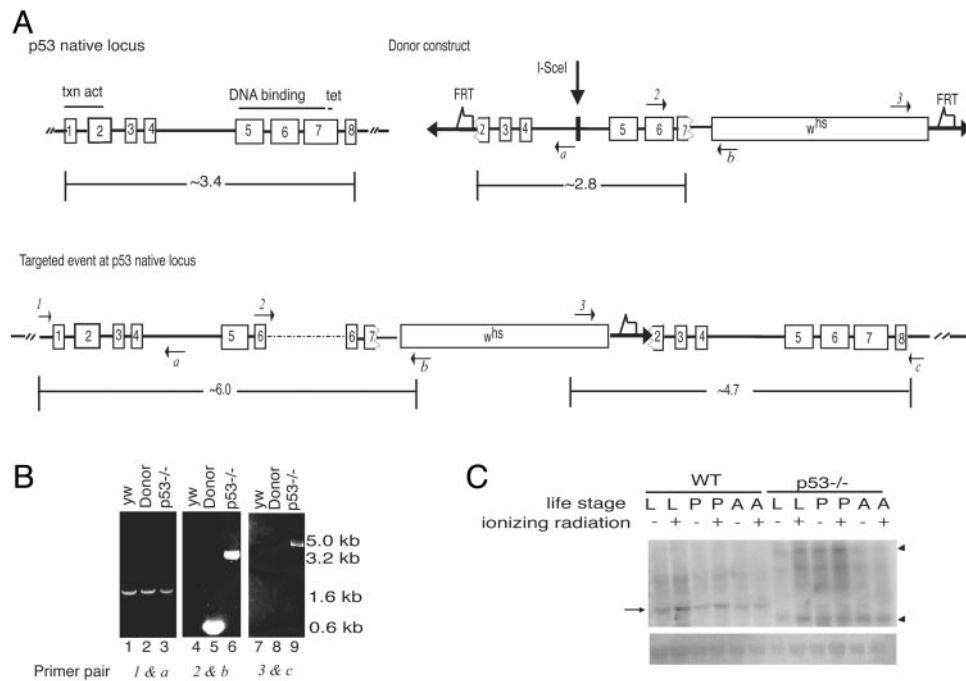
Fig. 1. Generation and verification of a targeted *Dmp53* mutation. (*A*) Targeting scheme for the *Dmp53* gene. The donor construct illustrated next to a schematic of the native *Dmp53* locus was generated by the insertion of a 2.8-kb internal fragment of the *Dmp53* gene into the targeting vector pTV2. The 2.8-kb fragment lacks part of an N-terminal transactivation domain (because of the elimination of the entire exon 1 and part of exon 2), and part of a central DNA-binding domain and an entire tetramerization domain (because of the elimination of part of exon 7 and the entire exon 8). A germ-line transformant donor strain bearing this construct on the second chromosome was used for targeted mutagenesis as described (36, 37). FRT, FLP recombination target. On heat-shock-mediated induction of FLP site-specific recombinase and I-*Sce*I endonuclease, the donor construct is excised as an extrachromosomal molecule and may create a lesion at the native *Dmp53* locus by homologous recombination. The targeted mutation at the native *Dmp53* locus, referred to as *Dmp53^{-ns}*, produced a tandem duplication of *Dmp53* variants that sandwich the *w^{hs}* gene. The dotted line in exon 6 shown for the targeted event at the *Dmp53* native locus indicates an unexpected insertion of partial pTV2 vector sequences (confirmed by PCR and sequencing). (*B*) PCR analysis of a *Dmp53*-targeting event. Genomic DNA from flies was used as template DNA for PCR to verify the targeting event at the Dmp53 locus. Lanes 1–3 use a primer pair, *1* and *a*. Lanes 4–6 use a primer pair, *2* and *b*, to verify the disruption of the N-terminal *Dmp53* template. Lanes 7–9 use a primer pair, *3* and *c*, to assess the disruption at the C-terminal *Dmp53* template. All PCR fragments were partially or fully sequenced to determine the variations introduced at the native *Dmp53* locus. Locations of each primer are listed in *A* in italics. Genotype of flies is as follows: yw refers to the parental wild-type strain at the native *Dmp53* site on the third chromosome; Donor refers to the *Dmp53*-donor construct on the second chromosome, wild-type at the native *Dmp53* site on the third chromosome; and p53−/− refers to the disrupted native *Dmp53* site on the third chromosome. (*C*) Northern blot analysis of a *Dmp53*-targeting event. Total RNA from wild-type (*yw*) and *Dmp53^{-ns}* strains was isolated, blotted to a membrane, and hybridized with a *Dmp53* probe. The arrow indicates the expected 1.6-kb *Dmp53* transcript. In wild-type larval and pupae individuals, this transcript is moderately responsive to ionizing radiation. *Dmp53* mutants lack the1.6-kb *Dmp53* transcript, and instead express aberrantly sized transcripts, which are indicated by arrowheads. (*Lower*) The membrane stained with methylene blue to show ribosomal RNA as a loading control. RNA from untreated (−) or γ-irradiated (+) individuals in the wandering third-instar larvae (L), pupae (P), and adult (A) stages were analyzed.

screens for targeting events were carried as described (36) and also can be found in *Supporting Materials and Methods*.

Histochemical Staining of *rpr-lacZ* Transgenic Embryos. Transgenic flies containing the150-bp *lacZ* reporter construct (25) were crossed to *Dmp53^{-ns}* mutant flies to generate 150-bp *lacZ;p53$^{-/-}$* flies. Collection of 2- to 5-h embryos of the appropriate genotype and histochemical staining of those were carried out as described (25).

Cell Death Assay and Checkpoint Function Assay. Cell death assay was carried out as described (25). Checkpoint function assays were done 90 min after γ-irradiation as described, with minor modifications described in *Supporting Materials and Methods* (27).

LOH Assay. Three independent recombinant lines homozygous for *Dmp53^{-ns}* and heterozygous for *multiple wing hair* (*mwh*) were generated. Each recombinant line was mock-treated or γ-irradiated with 250 rads during a third-instar larval stage. Wings were dissected and mounted in 1:1 methyl salicylate/Canada balsam (Sigma). Cells only with three or more hairs were scored as a *mwh$^{-/-}$* phenotype.

Results

Targeted Mutation at the *Dmp53* Locus. To obtain a *Dmp53* mutant, we used a gene-targeting method developed by Rong and Golic for "ends-in" homologous recombination (36, 37). Like its mammalian counterpart, Dmp53 protein contains a putative transcriptional activation domain at the N terminus, a DNA-binding domain, and an oligomerization domain at the C terminus. A donor construct using an internal 2.8-kb *Dmp53* fragment as a donor template from the target-gene sequence was prepared (Fig. 1*A*). This internal 2.8-kb fragment in the donor construct lacks two-thirds of the transcriptional activation domain, the entire oligomerization domain, and part of the DNA-binding domain (25–27). Note also that five of eight residues predicted to contact DNA (25–27) are absent in this donor template. Hence, an insertion of the 2.8-kb internal fragment at the *Dmp53* native locus is predicted to generate loss-of-function mutations at this locus.

We obtained seven individuals who had lost *white$^+$* mosaicism from the secondary screen (see *Supporting Materials and Methods*). Of these, we validated one line, *Dmp53^{-ns}*, as a targeted mutation. PCR-based assays (Fig. 1*B*), with several primers specific to the *Dmp53* genomic region as well as primers specific
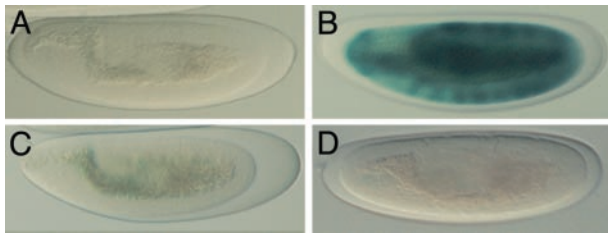
Fig. 2. The radiation-responsive enhancer at the *rpr* locus is no longer radiation responsive in *Dmp53−ns* embryos. Transgenic lines carrying a 150-bp radiation-responsive enhancer from the *rpr* locus was tested in wild-type (*A* and *B*) or *Dmp53−ns* mutant embryos (*C* and *D*). Mock-treated controls (*A* and *C*) or γ-irradiated (*B* and *D*) samples were stained for β-galactosidase activity (25).

to the marker gene *w^hs*, confirm this interpretation. For example, the primer pair 3 and *c* gives a product only when there is a targeting event at the *Dmp53* locus (Fig. 1 *A* and *B*).

Flies disrupted at the *Dmp53* locus are viable, fertile, and exhibit no overt visible defects. We used Northern blot analyses to confirm that expression of native *Dmp53* transcripts is disrupted in the *Dmp53−ns* strain. Hybridization with a *Dmp53*-specific probe to RNAs from three different stages showed a mildly radiation-responsive 1.6-kb *Dmp53* transcript in wild-type flies that was absent from *Dmp53−ns* homozygotes (Fig. 1*C*). Instead, two aberrantly sized transcripts are evident in the mutant strain, which are probably derived from one or both of the targeted variants.

Dmp53 Is Necessary for Radiation-Induced Transcription of the *Reaper* Region. Programmed cell death in *Drosophila* requires three apoptosis activator genes; *rpr*, *grim*, and *hid*, which map to a 300-kb genomic interval (38–40). Of these, *rpr* is acutely sensitive to damage signals, becoming transcriptionally activated within a short period after exposure to ionizing radiation (34). The minimal radiation-responsive cis-element lies in a 150-bp fragment, 5 kb upstream of the *rpr* start codon, and within this radiation-responsive enhancer a Dmp53 binding site was identified (25). To determine whether Dmp53 is required for activation of this radiation-responsive enhancer *in vivo*, we used a *lacZ* reporter transgene referred to as *rpr* 150-bp-*lacZ*, which contains the minimal 150-bp radiation-responsive fragment. We examined β-galactosidase expression of the 150-bp *lacZ* reporter gene in wild-type and *Dmp53−ns* mutant embryos with or without irradiation treatment. When mock-treated, neither wild-type nor *Dmp53−ns* mutant embryos showed β-galactosidase activity (Fig. 2 *A* and *C*). After irradiation treatment, wild-type embryos showed robust β-galactosidase activity (Fig. 2*B*). In contrast, *Dmp53−ns* mutant embryos showed no induction of β-galactosidase (Fig. 2*D*). These data show that Dmp53 is necessary for activation of the radiation-responsive enhancer upstream of the *rpr* locus.

Like other genes in the *Reaper* region, *skl* encodes an apparent inhibitor of apoptosis protein (IAP) antagonist. This gene maps ≈41 kb proximal to *rpr*, and, like *rpr*, is acutely radiation responsive (41). We therefore compared radiation-responsive expression of *rpr* and *skl* in embryos from two parental wild-type strains and the *Dmp53−ns* mutants (Table 1). Microarray profiles of radiation-responsive genes confirmed that both *rpr* and *skl* are strongly induced after γ-irradiation (41). However, in the *Dmp53−ns* mutant strain, neither *rpr* nor *skl* was induced after γ-irradiation treatment. These results confirm that *rpr* is a transcriptional target of Dmp53 and also indicate that *skl* could be a Dmp53 target gene as well.

Table 1. Radiation response: Fold-induction values

| Transcript	WT^yw	WT^w	*Dmp53−ns*
Reaper	6.0 (↑)	5.4 (↑)	No change
Sickle	10.6 (↑)	6.0 (↑)	No change

Expression of the indicated transcripts was examined in irradiated and control samples from two wild-type strains (WT^yw and WT^w) and the *Dmp53−ns* mutant strain. Total RNAs isolated from staged embryos were analyzed by using the Affymetrix *Drosophila* GeneChip Array. Fold-induction values relative to basal levels are reported. In agreement with previous studies (41), *reaper* and *sickle* are induced from ≈6- to 10-fold, respectively, within 90 min of irradiation exposure. In *Dmp53−ns*, levels of *reaper* and *sickle* transcripts are unchanged.

Dmp53−ns Mutant Blocks Damage-Induced Apoptosis but Not Cell-Cycle Arrest. We examined requirements for Dmp53 *in vivo* in the context of damage-induced signaling. In the wing discs of wandering third instar larvae, apoptotic cells are rarely observed in wild-type flies (Fig. 3*A*; refs. 25 and 27) or in *Dmp53−ns* mutants (Fig. 3*C*). However, after irradiation, wild-type wing discs (and wing discs from *Dmp53−ns* heterozygotes) show notably increased levels of apoptotic cell death (Fig. 3*B*). This response was clearly absent from the *Dmp53−ns* strain because we observed no acridine orange-positive cells in irradiated wing discs from this strain (Fig. 3*D*). Under the same conditions and in the same tissue, we next tested the requirement for Dmp53 on damage-induced cell-cycle arrest. We used a phosphohistone H3 antibody to examine the presence of cells in mitosis as in previous studies (42, 43). After irradiation, both wild-type and *Dmp53−ns* mutant discs showed a complete absence of mitotic cells (Fig. 4 *B* and *D*) and, hence, without functional Dmp53, normal checkpoint functions were completely unaffected. Therefore, whereas Dmp53 status is an essential determinant of radiation-induced apoptosis, the gene plays no role in governing the cell-cycle-arrest response, at least in the developing wing discs. Together, these results demonstrate a requirement for Dmp53 in damage-induced apoptosis but not in cell-cycle arrest.

Dmp53−ns Mutant Is Sensitive to Ionizing Radiation and Shows a Higher Rate of Genomic Instability. We assessed the *Dmp53−ns* line for potential mutator phenotypes by using a LOH assay (43, 44). Wandering third-instar larvae heterozygous for the *mwh* mutation and either wild-type or homozygous for the *Dmp53−ns* allele
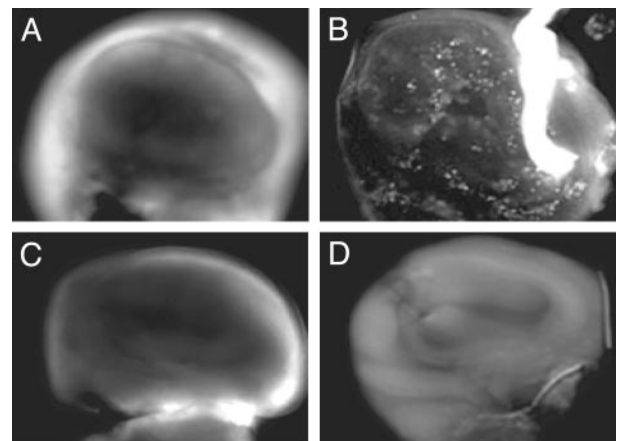

Fig. 3. *Dmp53−ns* mutants are defective in damage-induced apoptosis. A vital dye, acridine orange, specifically recognizes apoptotic cells. Wing discs from wild-type (*A* and *B*) or *Dmp53−ns* (*C* and *D*) were dissected from mock-treated (*A* and *C*) or γ-irradiation-treated (*B* and *D*) larvae, and apoptotic cells were visualized by acridine orange. For each genotype, at least five wing discs were dissected and stained. The *Dmp53−ns* phenotype was fully penetrant.
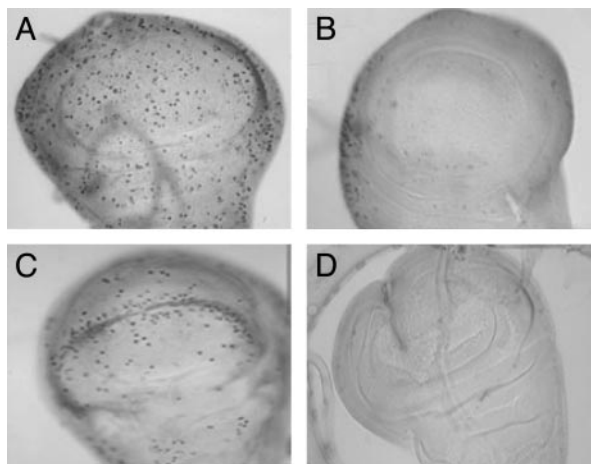
Fig. 4. $Dmp53^{-ns}$ mutants exhibit a normal cell-cycle arrest. Wing discs from wild-type (*A* and *B*) or $Dmp53^{-ns}$ (*C* and *D*) were dissected from mock (*A* and *C*) or γ-irradiated (*B* and *D*) third-instar wandering larvae. Histochemical staining with phosphohistone H3 antibody detects cells undergoing mitosis. For each genotype, approximately five wing discs were dissected and stained. Discs from wild-type or $Dmp53^{-ns}$ mutant were indistinguishable with respect to the incidence of immunoreactive cells, and staining seen for the $Dmp53^{-ns}$ control wing disc shown is within the normal range. The $Dmp53^{-ns}$ phenotype was fully penetrant.

were collected and irradiated. LOH for the wild-type *mwh* allele (arising from chromosomal aberrations or point mutations) is readily assessed by scoring the recessive *mwh* phenotype in wing
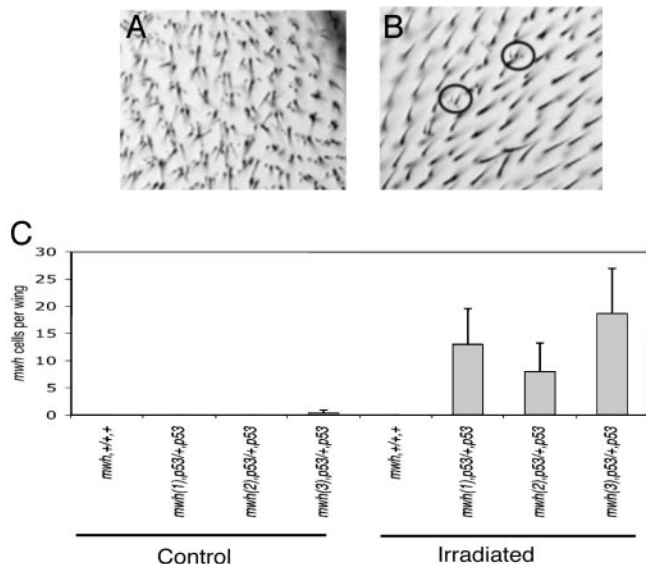
Fig. 5. $Dmp53^{-ns}$ adults carry high mutation loads after exposure to ionizing radiation. LOH is one measure of genomic instability. Here, the recessive *mwh* phenotype (*A*) was scored in flies heterozygous for *mwh* and either wild-type or homozygous for $Dmp53^{-ns}$. (*A*) A wing from homozygous *mwh* fly. Each cell contains multiple hairs. (*B*) A wing from a fly heterozygous for *mwh* and homozygous for $Dmp53^{-ns}$ that has been treated with γ-irradiation. Cells within the circles have lost the wild-type copy of *mwh*, uncovering the recessive *mwh* phenotype. (*C*) Loss of Dmp53 leads to genomic instability after irradiation. Both *mwh* and *Dmp53* genes are on the third chromosome, and three independent recombinant lines (*mwh(1)*, *p53*; *mwh(2)*, *p53*; and *mwh(3)*, *p53*) were generated and tested. For each genotype, three wings were examined. The average number of *mwh* cells per wing in wild-type or $Dmp53^{-ns}$ mutant flies is indicated in flies that had or had not been exposed to moderate levels of ionizing radiation.

Table 2. Radiation sensitivity: Percent survival to adult after exposure at 4,000 rads

	Survival, %		
Strain	Trial 1	Trial 2	Trial 3
WT	35	67	78
$Dmp53^{-ns}$	2	17	6

Wandering third-instar larvae (≈50 of each genotype per trial) were irradiated and eclosion was determined 5–6 days later. When tested at 1,000–3,000 rads, $Dmp53^{-ns}$ mutants exhibited moderate radiosensitivity. At doses of 500 rads or lower, eclosion rates for WT and $Dmp53^{-ns}$ mutants were between 80% and 90%.

cells of adult flies (Fig. 5 *A* and *B*). The absence of Dmp53 alone did not significantly affect the basal incidence of *mwh* cells (Fig. 5*C*). However, if challenged by low doses of ionizing radiation (250 rads), each independent (*mwh*, *Dmp53/+*, and *Dmp53*) line showed substantially elevated levels of *mwh* cells under conditions that had no effect in wild-type flies for Dmp53. We also note that $Dmp53^{-ns}$ mutant larvae exhibited a radiosensitive phenotype, manifested as extensive lethality rates that were particularly noticeable at doses of 4,000 rads (Table 2). These results indicate that, in the context of genotoxic damage, Dmp53 functions to preserve organismal viability and genomic stability.

Discussion

To infer *in vivo* functions for Dmp53, previous studies relied on forced expression of either wild-type or presumptive dominant-negative transgenes. Although these types of studies are informative, conclusions from them are confounded by considerations relating to highly expressed transgenes. Here we sought to determine the precise role or roles of Dmp53 through loss-of-function genetic analyses. Using the homologous recombination procedure devised by Golic and colleagues (36, 37), we isolated a targeted mutation at the native *Dmp53* locus. As the product of ends-in recombination this allele, designated $Dmp53^{-ns}$, generates two variant copies of *Dmp53*, both of which, if translated, would produce nonfunctional products. For example, the disrupted template mapping to the left of the w^{hs} gene lacks most of the oligomerization and DNA-binding domains, including five of eight residues thought to contact DNA (25–27). Likewise, the template mapping to the right of the w^{hs} gene lacks all contiguity with its native promoter and virtually all of the putative transactivation domain, including the initiating methionine and a critical serine residue (Ser-4) thought to be critical for regulation by fly Chk2 (45). Given the indispensable nature of these domains, $Dmp53^{-ns}$ can be considered a null allele with respect to transcriptional activity. It is formally possible that aberrant $Dmp53^{-ns}$-derived activity could, in principle, engender neomorphic effects (e.g., if Dmp53 encodes significant functions beyond transcription) but, given that $Dmp53^{-ns}$ phenotypes are recessive and consistent with those previously documented for the transgenic dominant-negative alleles, we believe this is a remote possibility.

In agreement with Golic and colleagues (46), who briefly reported on a *Dmp53* allele, flies homozygous for $Dmp53^{-ns}$ are viable and fertile and exhibit no overt visible defects as embryos, larvae, or adults. Knockout p53 mutations in mice are similarly viable and fertile and, hence, like its mammalian counterpart, Dmp53 function is not required for normal development. However, mice mutated at p53 also exhibit a strong predilection toward late-onset cancers and, although more subtle age-dependent effects might be uncovered in longevity studies not undertaken here, we observed no clear indications of late-onset overgrowth phenotypes in aged $Dmp53^{-ns}$ flies.

Studies on dominant-negative transgenes indicated important roles for Dmp53 in damage-responsive induction of *rpr* and the

ensuing apoptotic reaction. Three compelling results with the $Dmp53^{-ns}$ strain validate these predictions for a loss-of-function $Dmp53$ allele, permitting us to account for cell killing by an external insult in this fly. First, whereas other aspects of *rpr* regulation appear normal, an upstream enhancer governing the acute radiation response at this locus is entirely unresponsive in a $Dmp53^{-ns}$ background (Fig. 2D). Second, direct examination of irradiated $Dmp53^{-ns}$ flies corroborates this defect, extending requirements for Dmp53 to regulation at the native *rpr* locus (Table 1). Third, individuals homozygous for $Dmp53^{-ns}$ exhibit profound failures in damage-induced apoptosis when directly tested under conditions of genotoxic stress. If placed under the same conditions, it is worth noting that discs from $Dmp53^{-ns}/+$ heterozygous flies were indistinguishable from wild-type flies. Hence, in this context, we uncovered no evidence for dominant-negative effects exerted through the $Dmp53^{-ns}$ allele. Together, these data establish an absolute requirement for Dmp53 in radiation-induced apoptosis and also validate *rpr* as an authentic *in vivo* target of Dmp53.

Recently Peterson *et al.* (35) studied a synthetic deletion uncovering *rpr* and found a requirement for this gene in x-ray-induced cell death. Hence, pathways linking damage signals to *rpr* through Dmp53 are essential for optimal apoptotic cell death in this genetic model. At the same time, however, it should also be emphasized that evidence favoring the existence of additional, proapoptotic Dmp53 targets is strong. For example, flies that are singly mutated for *rpr* were only partially compromised for radiation-induced apoptotic cell death, and overexpression of Dmp53 in the eye was not significantly affected by the absence of *rpr*. Also, as seen here in the wing disc, removal of Dmp53 function completely abrogates radiation-induced apoptosis but removal of *rpr* only partially effects this same damage response. Together, these data argue that additional, proapoptotic targets of p53 exist in the *Drosophila* genome. One particularly attractive candidate in this regard is the inhibitor of apoptosis protein (IAP)-antagonist *sickle* (41, 47, 48), which, like *rpr*, also shows an acute, Dmp53-dependent induction provoked by damage signals (Table 1). This gene maps ≈40 kb to the 5′ side of *rpr* and so it is conceivable that the Dmp53-binding site regulating *rpr* might similarly govern the radiation-responsive induction of *skl* as well. Products encoded at *rpr* and *skl* provoke cell death, at least in part, by antagonizing native caspase inhibitors referred to as IAP proteins (41, 47, 48). IAP antagonists with orthologous activity (e.g., Smac/Diablo and Omi/Htra2) have also been reported in mammalian systems and it will therefore be of interest to determine whether any of these genes might represent p53 targets in mammals.

As in mammalian systems, *Drosophila* cells abruptly halt progression through the cell cycle in response to genotoxic stress. In vertebrate, and possibly invertebrate, models, the cell-cycle regulator p21 is essential for this response and, in stressed mammalian cells, p53 is the major regulator of p21 induction. Studies with dominant-negative transgenes suggested that Dmp53 may not similarly govern cell-cycle progression in flies, but, given the usual limitations on interpreting these types of studies, alternative technical explanations were also possible. Therefore, we directly examined the issue of damage-induced cell-cycle control in the $Dmp53^{-ns}$ strain. Using a mitosis marker to monitor this response, we found that irradiated wing discs from $Dmp53^{-ns}$ homozygotes were indistinguishable from wild-type samples. Hence, at least in the wing disc, Dmp53 is entirely dispensable for an arrested cell cycle and, together with earlier studies, the analyses uncover an important

distinction between flies and mammals. Although p21 is possibly the arresting agent in both systems, our data suggest alternative p53-independent mechanisms for regulating cell-cycle arrest in the context of genotoxic damage.

Important implications arise from the fact that p53 functions to govern apoptosis, but not cell-cycle arrest, in *Drosophila*. From an evolutionary perspective, it can be inferred that ancestral functions of p53 were intimately coupled to the regulation of cell death in the face of genotoxic challenge. Hence, the core means for exerting negative growth control by this family relates to the effects of these proteins on cell death. An obvious corollary here is that checkpoint arrest provoked by p53 may reflect a more recently invented function, specific perhaps to the vertebrate or mammalian lineage. In agreement with this deduction, a recent study in the nematode similarly reported a role for *Caenorhabditis elegans* p53 in damage-induced cell death but found no role for the gene in the cell-cycle-arrest response (49). A second implication from these studies argues against the hypothesis that p53 launches a sequential damage response program. According to this widely held view, genotoxic stress first provokes an attempted repair program (manifested as cell-cycle arrest), which is then followed by apoptosis if repair efforts are unsuccessful. Inherent to this scenario is the assumption that p53 forces cells toward an arrested cell cycle before engaging a cell-death program. Because Dmp53 clearly elicits apoptosis without arresting the cell cycle, our findings strongly refute this notion. If there are universal effectors of this tumor suppressor, our data argue instead for a proximate connection between p53 signaling and cell death without the need for intervening cell-cycle signaling.

Perhaps the most substantive advance from these studies relates to the effect of Dmp53 on genomic stability. If exposed to moderate doses of radiation, $Dmp53^{-ns}$ homozygotes harbored significantly elevated mutagenic loads that were not detectable in their wild-type siblings. Interestingly, we uncovered no evidence that $Dmp53^{-ns}$ promotes a mutator effect in the absence of radiation and, hence, the effect of $Dmp53^{-ns}$ is clearly limited to conditions associated with genotoxic stress. These observations are clearly distinct from checkpoint/DNA replication/repair mutants (43, 50) where high mutagenic loads are detectable even in the absence of genotoxic stress. In mammalian systems, p53 is essential for the maintenance of genomic stability (51, 52), but the precise derivation of this activity has been difficult to resolve, given the multiple functions of this protein. For example, it is not clear whether failures in cell-cycle arrest or failures in cell death, or possibly a combination of both defective activities, is responsible for maintaining genomic stability. Because $Dmp53^{-ns}$ homozygotes exhibit a mutator phenotype (and yet are normal for cell-cycle arrest) we conclude that a failure in p53-associated cell death alone can account for genomic instability. Although defective cell-cycle control could contribute to many p53 phenotypes seen in mammalian settings, our data are consistent with Schmitt *et al.* (53), who argue from study of mouse models that mutator defects leading to robust transformation can be traced to p53-associated apoptosis rather than defective checkpoint functions.

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