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Accessibility
E2F and p53 Induce Apoptosis Independently during Drosophila Development but Intersect in the Context of DNA Damage

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Abstract

In mammalian cells, RB/E2F and p53 are intimately connected, and crosstalk between these pathways is critical for the induction of cell cycle arrest or cell death in response to cellular stresses. Here we have investigated the genetic interactions between RBF/E2F and p53 pathways during Drosophila development. Unexpectedly, we find that the pro-apoptotic activities of E2F and p53 are independent of one another when examined in the context of Drosophila development: apoptosis induced by the deregulation of de2F1, or by the overexpression of de2F1, is unaffected by the elimination of dp53; conversely, dp53-induced phenotypes are unaffected by the elimination of de2F activity. However, de2F and dp53 converge in the context of a DNA damage response. Both de2F1/dDP and dp53 are required for DNA damage-induced cell death, and the analysis of rbf1 mutant eye discs indicates that de2F1/dDP and dp53 cooperatively promote cell death in irradiated discs. In this context, the further deregulation in the expression of pro-apoptotic genes generates an additional sensitivity to apoptosis that requires both de2F/dDP and dp53 activity. This sensitivity differs from DNA damage-induced apoptosis in wild-type discs (and from de2F1/dDP-induced apoptosis in un-irradiated rbf1 mutant eye discs) by being dependent on both hid and reaper. These results show that pro-apoptotic activities of de2F1 and dp53 are surprisingly separable: dp53 is required for de2F-dependent apoptosis in the response to DNA damage, but it is not required for de2F-dependent apoptosis caused simply by the inactivation of rbf1.

Introduction

Research in mammalian cells has demonstrated that the cellular effects of deregulated E2F activity are intimately connected with the p53 pathway. The p53 pathway provides a surveillance mechanism that is activated in response to a variety of cellular stresses, including DNA damage or oncogene stress. Multiple connections between p53 and RB/E2F are thought to explain why mutations in the p53 pathway (which deregulate E2F and facilitate cell proliferation) and mutations in the p53 pathway (which suppress E2F1-induced apoptosis) strongly synergize in tumorigenesis [1,2].

Experiments using immortalized cell lines first revealed that elevated levels of E2F1 are sufficient to drive cells from quiescence into S-phase [3]. Cells that are forced to cycle in this manner often undergo apoptosis and, in many cell types, this E2F1-induced apoptosis is p53 dependent [4,5]. Studies with primary cell cultures revealed that the mutation of p53 not only affects E2F1-induced apoptosis but also facilitates E2F1-driven cell cycle entry by lowering the levels of the Cdk inhibitor p21 [6]. E2F1 can also trigger p53-independent apoptosis and the pro-apoptotic activity of E2F1 has been found to be suppressed by mutation of the p53 related gene p73, or Apaf1, or by inhibition of certain BH3 only proteins [7,8,9,10,11,12,13].

E2F1 and p53 are both activated in response to DNA damage and promote the expression of pro-apoptotic genes [14,15,16,17,18]. Activation of E2F1, either through the deregulation of pRB or following DNA damage, stabilizes p53 and enhances p53-mediated transcription. E2F1 stabilizes p53 by inducing the expression of p19(p14)/ARF, an inhibitor of the mdm2 ubiquitin ligase that targets p53 for proteolysis [19]. In addition, E2F1 also stabilizes p53 via p19(p14)/ARF-independent mechanisms that may include direct binding to p53 and/or changes that promote the phosphorylation of p53 [20,21,22]. This activity of E2F, which acts upstream of p53, is thought to be a critical tumor suppressor pathway [23].

The Drosophila homologs of p53 and E2F1 have activities that are reminiscent of their mammalian counterparts. dp53 and de2F1 induce apoptosis when overexpressed and studies of mutant animals show that dp53 and de2F1 contribute to DNA damage-induced apoptosis [24,25,26]. dp53 induces the expression of several pro-apoptotic genes including reaper, an inhibitor of DIAP1.
**Materials and Methods**

**Drosophila Strains**

Unless otherwise noted, all fly crosses were done at 25°C and phenotypes are depicted from female progeny. The dp53 allele $\text{dp}53^{\text{R259H}}$ was a generous gift from Dr. Michael Brodsky [31]. The GUS-\text{dp}53 dominant-negative allele (R259H) was obtained from the Bloomington Stock Center. The following stocks were previously published in Morris et al., 2006: GMR-GAL4, UAS\text{-dp}53/Cyo, lacZ; hid05014/Df(3L)X14, rbf1120a [32].

**Irradiation of Larvae**

Third instar larvae were exposed to 40 Gy of gamma-ray using a cesium irradiator. For each experiment, minimum of ten larvae were used for analysis. Representative images are shown in the Figures.

**Immunocytochemistry and in situ Hybridization**

To visualize apoptotic cells rabbit polyclonal antibodies raised against the cleaved form of humans Caspase 3, anti-C3 antibodies (Cell Signaling), were used with 1:100 dilution. For immunostaining, discs were fixed with 4% formaldehyde for 30 minutes at 25°C, washed twice with 0.3% PBST (0.3% Triton X-100 in PBS) for 5 minutes at room temperature, then incubated with desired primary antibody in 0.1% PBST (0.1% Triton X-100 in PBS) with 5% normal goat serum at 4°C for 16 hours. After washing five times with 0.1% PBST for 10 minutes, at room temperature, discs were incubated with secondary antibody in 0.3% PBST (0.3% triton in PBS) with 5% normal goat serum at 4°C for 16 hours. After washing five times with 0.1% PBST for 10 minutes, at room temperature, wing discs were mounted for confocal microscopic imaging.

**Real Time qPCR**

Total RNA was prepared from eye-antenna discs with Trizol (Invitrogen) reagent and reverse transcription PCR (RT-PCR) was performed using Taq Man Reverse Transcription (PE Applied Biosystems) according to manufacturer specification. Real time PCR was performed using an ABI prism 7700 Sequence Detection system. Relative levels of specific mRNAs were determined using the SYBR Green I detection chemistry system (Applied Biosystems Foster City, CA). Quantification was performed using the comparative C_{\text{t}} method as described in the manufacturer’s procedures manual. Rp49 was used as normalization control. All primers were designed with Primer Express 1.0 software (Applied Biosystems Foster City, CA) following the manufacturer’s suggested conditions. The primer pairs used were:

- RP49-58F TACAGGCAGCCCAAGTGTTGTAAG
- RP49-175R GACCGAATCTCGTGGTCGATACCC
- Hid-1095F CATGAGTGCAGCAGCAGCAG
- Hid-1196R ACGAAAACGGTCAACACAGTGG
- RN21180F CATCCTGCCGAGTCTGGTGTTAC
- RN21292R GAAGTCCTGAACCGCCCTATCC
- Reaper-128F CCAGTTGTGTTAATTTCGGACG
- Reaper-241R GGATCTGGCTGCTTCTGTG

**Results**

In previous studies we exploited the GAL4/UAS-system to examine the effects of ectopic dE2F1 expression during Drosophila development [29]. Elevated dE2F1 activity causes inappropriate cell proliferation and/or apoptosis and the balance between these outcomes varies in different developmental contexts. Using tissue specific drivers we have generated stable stocks with visible phenotypes that are caused by dE2F1-induced apoptosis. One such stock, Act88F-GAL4, UASdE2f1, has multiple wing defects that can be fully suppressed by the co-expression of RBF1 or by the expression of the caspase inhibitor p35. Previously we used Act88F-GAL4, UASdE2f1 to find novel modifiers of dE2F1-induced apoptosis that are conserved in mammalian cells [29].

In mammalian cells, E2F-induced apoptosis can occur via p33-dependent and p53-independent pathways. We used genetic tools described above to investigate the role of p53 in E2F-induced apoptosis in Drosophila. Strikingly, we found that the Act88F-GAL4, UASdE2f1 wing phenotype was completely unaffected by mutant alleles of dp53 or by co-expression of a dominant negative form of dp53 [29] and Figure 1A. To exclude the possibility that this unexpected result was an unusual feature of this particular study, we looked closely at the p33dependent pathway in Drosophila and mammalian cells.
phenotype we performed similar experiments using phenotypes induced by ectopic dE2F1 in either the developing eye or bristles. These phenotypes were also unaffected by mutant alleles of dp53 or by expression of dp53DN (Figure 1B and data not shown), indicating that the lack of an interaction between dp53 and dE2F1 is a reproducible result that is true in several different genetic assays.

The adult phenotypes of these dE2F1-expressing stocks provide only an indirect measure of dE2F1-induced apoptosis. It was conceivable that the inactivation of dp53 might alter the level or distribution of dE2F1-induced apoptosis without changing the eventual visible phenotype of the stocks. To test this, we used C3, an antibody that recognizes activated caspase, to directly monitor the appearance of apoptotic cells in eye imaginal discs expressing dE2F1/dDP, in either the presence or absence of endogenous dp53. GMR-GAL4 driven expression of UAS-dE2F1 and UAS-dDP strongly induced apoptosis in eye discs that are wild-type for dp53. However, no appreciable difference in the level or pattern of C3 staining was observed following dE2F1/dDP expression in wild-type or dp53 mutant eye discs (Figure 1C).

To test the possibility that this lack of interaction was an artifact of dE2F1 overexpression, we examined a wave of apoptosis that

Figure 1. dp53 is not required for dE2F1-induced cell death. (A) The wing phenotype of Act88F-Gal4,UAS-dE2F1 was suppressed by co-expression of RBF1 but was unaffected by co-expression of dp53DN. GUS is a GMR vector derivative that contains binding sites for GAL4 proteins [34]. (B) The rough eye phenotype of GMR-Gal4; UAS-dE2F1,UAS-dDP flies was unaffected by co-expression of dominant negative p53 (dp53DN) or by a mutant allele of p53. The Drosophila wing and eye phenotypes shown in this figure are representative figures of at least 50 flies per genotype scored. No significant variation in the phenotypes was observed. (C) Apoptotic cells were visualized by C3 (an antibody that recognizes the active form of Caspase 3) staining of third instar eye discs of the genotypes shown. GMR-Gal4 drives the expression dE2F1 and dDP in the posterior region of the eye disc inducing a pattern of apoptosis that was unaffected by the homozygous mutation of dp53. (Hereon, the white arrow indicates the position of the morphogenetic furrow)
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occurs in eye discs that are hypomorphic for \( \text{rbf1} \). RBF1 is a key regulator of dE2F and \( \text{rbf1}^{120a} \) mutant flies have elevated dE2F1 activity. Previous studies have shown this wave of apoptosis, that accompanies the morphogenetic furrow as it crosses the eye discs during the third larval instar, is caused by deregulated E2F activity and can be eliminated by mutation of either \( \text{de2f1} \) or \( \text{dDP} \) [28].

\( \text{dDP} \) is an essential heterodimeric partner for both \( \text{Drosophila} \) dE2F proteins but unlike \( \text{de2f1} \) mutants, that are not embryonic lethal, \( \text{dDP} \) mutants develop to pupal stages. \( \text{dDP} \) mutants thus provide a simple way to examine imaginal discs that lack any E2F/DP complexes. Whereas the wave of apoptosis in \( \text{rbf1} \) mutant eye discs was completely suppressed by mutation of \( \text{dDP} \), it was unaffected by mutation of \( \text{dp53} \) (Figure 2). Hence, this wave of apoptosis that is triggered by the release of endogenous dE2F1/dDP from RBF1 is independent of \( \text{dp53} \).

To be thorough, we also examined the converse possibility that \( \text{dp53} \)-induced apoptosis might require E2F activity. \( \text{GMR-GAL4} \) driven expression of \( \text{dp53} \) induces apoptosis in the developing eye disc, giving a rough eye phenotype that can be suppressed by co-expression of dominant negative form of \( \text{dp53} \). This phenotype was unaffected by the co-expression of RBF1 (Figure 3A), which suppresses dE2F1 activity. Moreover, when we compared the patterns of \( \text{dp53} \)-induced apoptosis in wild-type eye discs and in eye discs mutant for \( \text{dDP} \), we found that \( \text{GMR-GAL4} \) driven expression of \( \text{dp53} \) efficiently induced apoptosis in eye discs of control larvae and showed no detectable change in \( \text{dDP} \) mutant discs. Hence, the pro-apoptotic activity of \( \text{dp53} \) does not require functional E2F/DP complexes (Figure 3B).

Together these results show that, while elevated levels of \( \text{dp53} \) and dE2F1 can both induce apoptosis, these activities cannot be

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**Figure 2.** Mutation of \( \text{dp53} \) has no effect on dE2F1/dDP-dependent cell death in \( \text{rbf1} \) mutant eye discs. Third instar eye discs of the indicated genotypes were stained with C3 to visualize apoptotic cells. A wave of cell death in \( \text{rbf1}^{120a} \) mutant eye discs was eliminated by the homozygous mutation of \( \text{dDP} \) but not by the homozygous mutation of \( \text{dp53} \). doi:10.1371/journal.pgen.1000153.g002

**Figure 3.** dDP is dispensable for \( \text{dp53} \)-induced cell death. (A) A rough eye phenotype was generated using \( \text{GMR-Gal4} \) to drive expression of \( \text{dp53} \). The \( \text{dp53} \)-dependent eye phenotype was suppressed by co-expression of dominant negative \( \text{dp53} \) but was unaffected by co-expression of RBF1. The eye phenotypes shown in this figure are representative figures of at least 50 flies per genotype scored. (B) \( \text{dp53} \)-induced apoptosis was visualized in third instar eye discs by immunostaining with C3. The \( \text{GUS-dp53} \) transgene did not induce cell death by itself, but \( \text{GMR-Gal4} \) driven expression of \( \text{dp53} \) caused widespread apoptosis at the posterior part of the eye discs. \( \text{dp53} \)-induced cell death was unaffected by homozygous mutation of \( \text{dDP} \). doi:10.1371/journal.pgen.1000153.g003
placed in a linear genetic pathway with one either upstream or downstream of the other.

Next we considered the possibility that the dE2F1-dependent apoptosis that occurs during animal development might have different requirements to the dE2F1-dependent apoptosis that occurs in response to DNA damage. There are few apoptotic cells in wild type *Drosophila* third instar eye discs. However, a distinct pattern of cell death occurs within four hours of irradiation (Figure 4). Cells in the Morphogenetic Furrow (MF) are protected from DNA damage-induced apoptosis while cells near the MF are sensitive to DNA damage-induced cell death. The DNA damage-induced cell death seen in irradiated eye discs requires the activities of both dp53 and dE2F1 (Figure 4). Virtually no apoptotic cells were observed in dp53 mutant eye discs after treatment with ionizing irradiation. A similar result was observed in dDP mutant eye discs that lack functional de2f1.

The fact that dE2F/dDP and dp53 are both required for DNA damage-induced cell death shows that, in this context, neither transcription factor is sufficient to induce apoptosis in the absence of the other. Because of this, we used irradiated eye discs as a setting to look for a functional interaction between dp53 and the dE2F1/dDP activity that is deregulated in rbf1 mutant cells.

As a first step, we examined the pattern of DNA-damage-induced apoptosis in rbf1 mutant cells. Using ey-FLP and FRT- rbf114 we generated mosaic eye discs carrying somatic mutant clones that were null for rbf1. This allowed a side-by-side comparison between wild-type cells and mutant cells with deregulated dE2F1. As described earlier, rbf1 mutant cells are prone to apoptosis in the MF. When the mosaic discs were irradiated, rbf114 mutant cells show a heightened sensitivity to apoptosis (Figure 5A). More C3 staining was detected in rbf1 mutant clones after irradiation, and this staining appears more quickly in the mutant cells. A similarly elevated pattern of DNA-damage induced apoptosis was also seen in hypomorphic rbf1 mutant eye discs, rbf1120a (Figure 5B). Here the increase in apoptosis was evident in a broadening of the stripe of apoptosis at the MF, in the appearance of apoptotic cells in the anterior region of this disc containing proliferating cells, and in the appearance of apoptotic cells among the post-mitotic cells posterior to the MF. The intensity of the C3 staining was measured using image software. This quantification shows a 2.1 fold increase in staining at the posterior regions of rbf1120a mutant eye discs, and a 2.6 fold increase in the anterior regions of the discs.

Next we asked whether this heightened sensitivity of rbf1 mutant cells to apoptosis requires p53 or E2F (Figure 5B). The elevated level of DNA-damage-induced apoptosis in rbf1120a mutant eye discs was suppressed by mutation of dDP, consistent with the idea that this change is caused by deregulated dE2F1. In addition, this elevated level of apoptosis was also eliminated by the mutation of dp53, indicating that this dE2F1-dependent apoptosis is also dependent on dp53. Remarkably, within the same disc, the wave of dE2F1/dDP-dependent apoptosis in the MF, that appears in the absence of irradiation (Figure 2), was unaffected by the mutation of dp53 (Figure 5B). These results show that irradiated rbf1 mutant eye discs contain two distinct patterns of apoptosis: a developmentally induced wave of apoptosis that is dE2F1/dDP dependent but independent of dp53, and a hypersensitivity to DNA-damage induced apoptosis that depends on both dE2F1/ dDP and dp53. Hence, dp53 is not required for E2F-dependent
apoptosis that occurs during animal development following the mutation of *rbf1*, but it is required for dE2F-dependent apoptosis in the context of a DNA damage response. This includes the broadening of the stripe of apoptosis at the MF (Figure 5C).

How do deregulated dE2F1 and dp53 cooperate to sensitize cells to apoptosis following DNA damage? Potentially, dE2F1 and dp53 might regulate different pro-apoptotic genes and these targets could collectively promote cell death. Alternatively, dE2F1
and dp53 might converge on a common target that is critical for DNA damage-induced cell death. Previous studies have suggested that the pro-apoptotic genes hid and reaper are likely to be points of convergence between dp53 and dE2F1. Both genes are up-regulated following DNA damage in a dp53-dependent manner and both have also been identified as dE2F1-regulated genes [24,25,27,35].

Using quantitative PCR, we measured the level of hid and reaper mRNA, both before and after DNA damage, in control, rbf1, dp53, and rbf1dp53 mutant eye discs (Figure 6A). In control discs, DNA damage increased the levels of hid and reaper mRNAs 4.0 and 3.2 fold respectively. Both genes were also expressed at an elevated level in rbf1 mutant eye discs prior to DNA damage, again with a similar increase (2.0 fold for hid, 1.9 fold for reaper). Interestingly, this increase in rbf1 mutant eye discs is dp53 independent—since mutation of dp53 mutation had no effect on hid and reaper expression in (unirradiated) rbf1 mutant animals. Irradiation of rbf1 mutant eye discs further increased the expression of reaper (5.8 fold) compared to the irradiated control eye discs but, interestingly, gave only a very slight increase in the level of hid mRNA (5.4 fold) compared to the control. These changes were specific to these pro-apoptotic targets, since expression of rrm2, a cell cycle-regulated target of dE2F1, did not show the same increase (data not shown). These results demonstrate that elevated dE2F1 activity (resulting from the inactivation of rbf1) and DNA damage-induced activation of dp53 have co-operative effects on the expression of some pro-apoptotic genes.

We measured the level of hid and reaper mRNA, both before and after DNA damage, in control, dDP, and dDDPdp53 mutant eye discs. The levels of hid and reaper expression were similar in control and dDP mutant eye discs, both before and after irradiation. This indicates that the ability of dp53 to activate hid and reaper after irradiation does not require dE2F1/dDP activity. In summary, and in agreement with the genetic experiments presented in Figure 1 and 3, dE2F1 and dp53 cooperate after DNA damage but their abilities to activate transcription of pro-apoptotic genes do not depend on one another.

To test the functional significance of these gene expression changes we examined the effects of removing either hid or reaper on the level of DNA damage-induced apoptosis in wild-type or rbf1 mutant eye discs. Previous studies have shown that the gene dosage of hid affects the levels of DNA damage-induced cell death [35] but it has not been possible to compare the effects of homozygous mutation of reaper and hid, in large part because of the lack of specific mutant alleles of reaper. For these experiments we used a recently generated, specific allele of reaper, rpe77, and a combination of viable hid mutant alleles (see materials and methods).

Strikingly, mutation of hid completely abolished DNA damage-induced apoptosis in control and rbf1 mutant discs (Figure 6B). In contrast, mutation of reaper had different consequences in control and rbf1 mutant discs. Mutation of reaper caused no appreciable change in the level of DNA damage-induced cell death in the presence of RBF1, however, the level of cell death seen in rbf1<sup>1206a</sup> discs was clearly reduced when reaper was mutated (Figure 6B). Interestingly, measurement of C3 staining intensity showed that the reaper mutation had greater effects at the posterior region of the MF while only a slight change was evident in the vicinity of the MF (Figure 6C). It is important to point out that reaper mutations did not affect the level or pattern of cell death in rbf1<sup>1206a</sup> eye discs before irradiation treatment (data not shown). In summary, we conclude that the expression of hid is very important for the apoptosis induced by DNA-damage in wild-type discs. Similarly, the wave of apoptosis that occurs in rbf1 mutant discs requires hid but not reaper. However, irradiation of rbf1 mutant discs causes a further increase in reaper expression and a more extensive pattern of apoptosis that is dependent on both hid and hid.

Discussion

In both mammalian cells and in Drosophila, an elevated level of E2F1/dE2F1 activity delays cell cycle exit and sensitizes cells to apoptosis. Studies in mammalian cells have led to the conclusion that, in many cellular contexts, E2F1 acts upstream of p53. In the experiments described above we show that while homologs of p53 and E2F1 exist in Drosophila, the functional relationship between dp53 and dE2F1 is different from that seen in mammalian cells. Epistasis experiments demonstrate that dE2F1-induced apoptosis does not require dp53, and that dp53-induced apoptosis does not require dE2F1 activity. Consequently, animal phenotypes caused by the over-expression of de2f1 and attributable to de2f1-induced apoptosis are unaffected by mutation of dp53. However, we do find that, like in mammals, de2f1 can cooperate with dp53 to promote DNA damage-induced cell death.

It is satisfying when homologous genes have conserved functions, but it is perhaps more intriguing when they do not. Why are the genetic interactions between E2F1/dE2F1 and p53/ dp53 so different between flies and humans? One general explanation is that de2f1 and dp53 are unique genes in Drosophila, whereas E2f1 and p53 are members of families of related mammalian genes. p53, p63 and p73 have distinct functions, as do E2F1, E2F2 and E2F3, and the roles of dp53 and dE2F1 may resemble the function of an ancestral protein, rather than the more specialized roles of the individual mammalian family members. A second, and more detailed explanation, is that a key connection between E2F1 and p53 is provided in mammalian cells by p19 (p14)/ARF and Mdm2. The Drosophila genome lacks clear homologs of either of these genes. Hence, a major pathway by which E2F1 induces p53 in mammalian cells is likely to be absent in Drosophila (Figure 7). Although it is possible that functional homologues might exist in Drosophila, the absence of genetic interaction presented in this study argues otherwise. Interestingly, dp53 also fails to induce expression of dacapo (the Drosophila ortholog of p21<sup>CIP1</sup>) and also appears to be defective for G1/S checkpoint functions [26,35]. Drosophila cells arrest primarily at G2/M in response to DNA damage, and there is scant evidence for G1/S damage checkpoint in irradiated discs. Thus, while dp53 is important for DNA-damage induced apoptosis, its activity appears to be largely uncoupled from some of the key sensors and effectors of mammalian p53 checkpoint pathways.

As seen in mammals, the expression of critical pro-apoptotic genes is regulated by both E2F and p53 in Drosophila. The convergence of dE2F1 and dp53 on common target genes provides a simple molecular explanation for how dE2F1 and dp53 cooperate to promote DNA damage-induced cell death (Figure 7). Our result indicates that elevated dE2F1 activity can influence the basal level of hid and reaper expression while dp53 is essential for the induction of gene expression after DNA damage (Figure 6A). This difference in the physiological context in which they function may explain why dE2F1 and dp53 proteins act independently of one another and appear to function in parallel pathways. Interestingly, the levels of hid and reaper expression before irradiation were unaffected by mutation of dDP. This indicates that dE2F1 does not contribute to the basal expression of these pro-apoptotic genes. This is somewhat expected since hid and reaper are not expressed in a cell cycle-dependent manner and are likely to be expressed at a low level in the absence of stress. Moreover, since hid and reaper are induced at a similar level
Figure 6. *hid* and *reaper* are deregulated, and functionally important, for DNA damage-induced apoptosis in *rbf1* mutant eye discs.

(A) Eye discs from control and *rbf1* mutant third instar larvae were dissected before and three hours after ionizing irradiation. RNA samples were isolated from each genotype and the expression levels of indicated genes were measured by quantitative real-time PCR. Relative expression levels are shown from three independent experiments, each with triplicate samples. A similar analysis was performed using *dDP* and *dDP;dp53* mutant larvae.

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<td><em>rbf1</em>&lt;sup&gt;120a&lt;/sup&gt;</td>
<td>13.0 ± 2.2</td>
<td>37.6 ± 4.3</td>
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<tr>
<td><em>rbf1</em>&lt;sup&gt;120a&lt;/sup&gt;;<em>rpr</em>&lt;sup&gt;87&lt;/sup&gt;</td>
<td>8.5 ± 0.8</td>
<td>33.1 ± 4.4</td>
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<td>Fold change</td>
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*E2F and p53 Pathways in Mammals and Flies*
The intensity of C3 staining signals in Drosophila operate to promote cell death. While DNA damage is shown to activate a common set of pro-apoptotic target genes, dE2F1 and dp53 co-p53. In the context of DNA damage, where dE2F1 and dp53 converge and lack of this connection may limit the crosstalk between E2F1 and p53, the hypersensitivity of rbf1 mutant cells to DNA damage induced apoptosis requires both dE2F1 and dp53. Given the recent studies showing that an activated DNA damage response is a hallmark of pre-neoplastic lesions [40], it is particularly interesting that DNA damage-induced apoptosis is one setting where the strong synergy between E2F1 and p53 is conserved between flies and humans.

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Author Contributions

Conceived and designed the experiments: NSM LDS EJM KW NJD. Performed the experiments: NSM LDS EJM RP. Analyzed the data: NSM LDS EJM KW NJD. Contributed reagents/materials/analysis tools: NSM LDS EJM RP KW. Wrote the paper: NSM LDS EJM KW NJD.

Reference


Figure 7. E2F and p53 network between mammals and flies. In mammalian cells, the activation of E2F1 is sufficient to activate p53. One of the key links between E2F2 and p53, is provided by p19/p14ARF and mdm2. Drosophila lack any clear orthology of p19/p14ARF and mdm2 and lack of this connection may limit the crosstalk between E2F1 and p53. In the context of DNA damage, where de2F1 and dp53 converge on a common set of pro-apoptotic target genes, de2F1 and dp53 cooperate to promote cell death. While DNA damage is shown to activate E2F1 and p53 pathways in mammals, it remains uncertain whether de2F1 is similarly activated by DNA damage.

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