Drosophila Models to Investigate the Role of Regulation of Cell Death in Development and Cancer

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Drosophila Models to Investigate the Role of Regulation of Cell Death in Development and Cancer

Honors Thesis
Anam Hussain
University of Dayton Biology Department
Advisor: Madhuri Kango-Singh, Ph.D.
April 2016
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Cell death is of key importance in maintaining health and normal development. In cancer, cell death is improperly controlled provoking uncontrolled proliferation of cells, which results in severe harm to the body. The Hippo pathway is an identified pathway in Drosophila melanogaster that is involved with regulating the different mechanisms of survival and proliferation within the cells. It is known to interact with a gene known as Dronc that is a key participant in the cell death pathway of apoptosis. Previous work has shown how the loss of certain caspases, which are cysteine proteases, is linked with cell survival. My project will be investigating the mechanism by which it happens, by testing the nature of the cell survival pathway. We hypothesize that the loss of function clones of drice, dronc, or dark promote cell proliferation that support tumor growth. At the conclusion of these studies, we expect to generate insights into how loss of cell death regulating genes impacts tissues, and if it promotes aggressive growth of cancer cells.

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Abstract

Normal cells become cancerous through progressive manifestation of modifications usually introduced during DNA replication which lead first to rampant proliferation, and if unchecked, invasion and metastasis to distant sites. In normal cells, specialized response elements exist which trigger apoptosis in the event of serious damage to a cell’s genome. Apoptosis is orchestrated by the action of the caspase dependent pathway. The Hippo Pathway is another major pathway in apoptosis, which regulates the proliferation and survival of cells in *Drosophila melanogaster*, also known as the fruit fly. When the Hippo pathway is active, *dronc*, a cell death-causing gene, is also active. Specifically, *dronc* acts via the assembly of an apoptosome, which is made through the binding of *dronc* to another apoptosis regulating protein, *dark*. When this apoptosome accumulates, it allows for different interactions to come together that eventually kill the cell.

The goal of this project is to understand what the loss of *dronc*, *drice*, or *dark* does to cell division genes, to essentially note how this affects growth and development. Our aims are to focus on characterizing how genes that usually work in the cell death pathway also influence proliferation, and to test specifically how *dronc* is connected to the Hippo pathway. We used different genetic approaches and biochemical/staining protocols to compile and quantify our objectives. The significance of this research lies in understanding that a seemingly small genetic mutation within an apoptosis pathway of *Drosophila* can lead to severe consequences, specifically cancer, or perhaps other birth defects. This research will bring to light the behind-the-scene mechanisms that may
indicate tumor beginnings due to the lack of expression, or overexpression, of certain mutant genes that normally regulate cell death.

Cell death is of key importance in maintaining health and normal development. In cancer, cell death is improperly controlled provoking uncontrolled proliferation of cells, which results in severe harm to the body. While currently the care for cancers involves radiation and then chemotherapy, these treatments are ways of killing the cells, not necessarily curing the person of cancer. With this in mind, it may be helpful to understand the specific genes that usually work in the cell death pathways itself, to see how they impact the overall control of growth.

Previous work has shown how the loss of certain caspases, which are cysteine proteases, is linked with cell survival. We will be investigating the mechanism by which it happens, by testing the nature of the cell survival pathway. We hypothesize that the loss of function clones of drice, dronc, or dark promote cell proliferation that support tumor growth. It is important to note that the removal of these cell death causing genes may not directly lead to proliferation, cells may just remain suspended within a phase of replication or cell cycle. We will be testing these alternate possibilities using standard genetic and antibody staining protocols to assess protein expression levels in Drosophila mutants. At the conclusion of these studies, we expect to generate insights into how loss of cell death regulating genes impacts tissues, and if it promotes aggressive growth of cancer cells.
Chapter 1

Introduction
1.1: Apoptotic and Proliferative Means through the Hippo Pathway

*Drosophila* are used primarily due to the rapid reproducibility and easy accessibility of their genome of genetic manipulations. The Hippo pathway, a signaling mechanism used for regulating organ size, was originally discovered in flies. Furthermore, the Hippo signaling pathway in flies is similar to that of humans. Furthermore, the Drosophila Hippo pathway is not as complicated, and still easy to manipulate to understand the effects of loss or over-expression of genes within the pathway. The Hippo pathway is now identified as being the main regulator with regards to cell proliferation along with cell survival (Singh and Kango-Singh, 2009; Brumby et al., 2005). Furthermore, *dronc*, an initiator caspase, is now understood to direct cell death (Figure 1.1) (Quin et al., 2000; Meier et al., 2000). Previous work from our lab has shown that *dronc* is a transcriptional target of Hippo pathway (Verghese et al., 2012). These two work together, but more specifically, the

![Figure 1.1: Comparative Cell Death Pathways of Drosophila and Humans](Image from Hay 2004)
Hippo pathway must maintain control over the expression of *dronc*, to ensure that tissue sizes remain normal, and more particularly that there is no tumor formation or abnormal organ size growth.

It has also been found that Hippo signaling restricts *dronc* activity (Verghese et al., 2012). As shown in Figure 1.2, *dronc, drice, dark*, which is a target of *dronc*, and *dark*, a protein that binds to *dronc*, all combine and make an apoptosome, which is the protein complex required to induce cell death, and in instances where either *dronc* or *dark* are down regulated it results in over-proliferation, a cancerous situation. Taken together, cell death is required for normality within development, and in preventing cell proliferation diseases such as cancer (Verghese et al., 2012).

**Figure 1.2:** Hippo Pathway signaling in *Drosophila* *dronc, dark, and drice* are all necessary steps in the pathway that are required for either cell death or cell proliferation [Courtesy: Dr. Madhuri Kango-Singh]
The Role of Caspases in Cell Death

We began our investigations based on earlier data which showed that the loss of function of caspases hinders cell functions. Mutant clones of Dark and Drice were generated by genetic crossings to induce somatic recombination (Verghese and Kango-Singh, unpublished data), and the effects of loss of induced cell death were studied. Previous data has shown that down regulating cell death inducing genes results in cell survival (Plouffe et al., 2015). In general, the cells in which cell-death inducing genes, caspases, are depleted, reveal many interesting characteristics. As shown through Figure 1.3, one of the main findings was that alternate cell death inducing pathways are not activated in cells mutant for caspase genes—the loss of these functional clones (FRT42D dArk82 and drice[17]) stops inducing cell death within the mutant cells.
Experimentally, cell death is measured through a TUNEL assay, which by labeling the terminal end of nucleic acids, can inform us of when DNA fragmentation occurs. It is also an important side note to remember that proliferating cells within mutant crosses do not grow and mature at the same rate of their wild type comparison cells. The study of H99 and Dronc mutant clones further drove in the point that there was a failure in inducing cell death (Figure 1.4). As in boxes c and d, we can see how only a few ‘white’ spots labeling the dying cells showing only the normal cells and not the mutant cells are dying.

The second point of past research regards the following finding that caspase mutant cells induce ectopic proliferation. Ectopic proliferation is when cells continue dividing or proliferating in an environment where the proliferation is not necessarily normal or necessary. To understand the physiological significance of these cells, the mitotic index (MI) was tested. MI refers to quantification of the number of cells that have finished mitosis; this is usually assessed by phosphohistones H3 staining (PH3). Interestingly enough, the loss of caspases in Dronc mutants—the suppression of apoptosis by a deficiency—uncovers increases in mitotic

![Figure 1.5: dronc and dark mutant clones versus wild type (Verghe et al., 2012)]
figures, suggesting that cell mutant for these genes actually *induce* proliferation. The question remains, how do the cells that lack caspases cause increases in cell proliferation?

There are two possibilities, and the first is that in the absence of cell death inducing genes, these cells send stress signals to neighboring cells, which in turn activate ‘compensatory proliferation’ which then causes the observed phenotype (Donnellan et al., 1999; Ryoo et al., 2004). Alternatively, the second possibility is that because these cells cannot induce any alternate cell death pathways, they remain suspended within a state of growth arrest and actually cause abnormal signals that induce proliferation. To distinguish these possibilities, the expression of cell cycle regulatory genes called cyclins was tested (Figure 1.5). The expression of cyclin A, and cyclin E was tested in cells that were lacking caspases. These cyclins play key roles in cell division, and studies have indicated that cyclin E being over expressed continuously is strongly correlated with cell proliferation, more specifically, tumorigenesis (Kango-Singh et al., 2002). The preliminary data regarding the lack of caspases and cyclin A/E shows us that the cell cycle genes *are* affected in caspase mutant cells, as we can see that the mutant clone cells show increased amounts of cyclins A & E, when compared to the wild type.

### 1.3: Protein expression within the eye discs

The usage of larval eye discs and specifically the morphogenetic furrow allows for the ability to stain cells within the eye disc and note the specific stages of differentiation and division. Anteriorly, the morphogenetic furrow develops parallel to the ommatidial columns, depicting the wave of retinal differentiation that is occurring across the eye—the size of the eye is dependent upon this wave of differentiation that the
retina (Greenwood et al. 1999). The differentiating cells coordinate and synchronize their cell cycles and undergo a G1 arrest after entering the morphogenetic furrow. Thus, the morphogenetic furrow essentially is the border that partitions the differentiating tissue from the undifferentiated tissue; this is important to note in that wild type eye discs follow this pattern, and we can use cyclin staining of mutants to discover variants from the wild type (Figure 1.6). Protein expression and changes of activity through cyclin staining can be further studied through the morphogenetic furrow and the cell differentiations (Heberlein et al., 1993). Furthermore, the pool of un-patterned precursor cells within the eye disc give insight into organogenesis, leading to organ growth, pattern formation, and differentiation. We can also make note of the cell types, as shown in Figure 1.6, as we can comment the second stripe represents the second mitotic wave, which is then followed by the G2 phase; knowing this will allow us to find ectopic cells that are undergoing cell division outside of the wild type area.

![Figure 1.6: The morphogenetic furrow and the recruitment of cell types. Image taken from: http://slideplayer.it/slide/999922/](http://slideplayer.it/slide/999922/)
Cyclins expression can be used to identify what stage of the cell cycle (G1, S, G2, M) certain cells are in (Figure 1.7). Cyclins are a family of proteins that regulate the progression of cells within the cell cycle, by using/activating CDKs, which are cyclin-dependent kinases. While cyclins work with CDKs, we can look specifically at Cyclin E, which has been thought to be involved with cancer progression; it has been shown that the deregulation of the expression of Cyclin E is connected to many cancers and is further thought to be related to the oncogenic process (Keyomarsi et al., 1997). As Figure 1.8 depicts, Cyclin E is highest in concentration during the transition between the G1 stage and the S stage. It is important to note the function of Cyclin B, which is known to work in correlation with Cyclin A. Cyclins B and A are co-expressed during development, with the function of aiding proliferation (Lehner et al., 1990). Cyclin B, which is most concentrated during the onset of the mitotic stage, can reveal proliferation through the...
Along with Cyc B, PH3 and ELAV were also used as markers within the larval eye disc. PH3, which is histone H3 phosphorylation, is known to act as a correspondent for CDK1 activity (Su et al., 1998). This tells us that PH3 is an indicator for when a cell is within the mitotic stage. When all the genes are expressed normally, we see the morphogenetic furrow, as depicted within Figure 1.9. We can note that beyond the second mitotic wave, there is an arrest of the cells in G2 cycle of the photoreceptor neurons, as shown by the yellow marker. ELAV (Embryonic Lethal, Abnormal Vision) is an RNA-binding protein that is also used when staining to study the post-mitotic cells; ELAV is expressed within embryonic glial cells as well as within neurons (Berger et al., 2007). ELAV staining provides insight into the cells of the posterior eye disc, specifically
the photoreceptor neurons that are stained in Figure 1.9—we can make note of the wild-type pattern indicating that there is a highly regulated configuration.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cyc A</th>
<th>Cyc E</th>
<th>Cyc B</th>
<th>PH3</th>
<th>ELAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
<td>G1</td>
<td>G1-S</td>
<td>G2-M</td>
<td>M phases</td>
<td>Differentiation</td>
</tr>
</tbody>
</table>

Figure 1.9: The staining results of respective markers for wild type larval eye discs.

1.3: Hypothesis

The cell death regulating genes (*drice, dronc, or dark*) also interact with growth control genes to maintain normal cell functions.

1.4: Aims

Stemming from the preliminary data, we have two specific aims within this research.

1. **To understand how cell death regulating genes induce cell death**

2. **To understand how these cell death regulating genes are connected to the hippo pathway**
In general, our aims are to focus on characterizing how genes that usually work in the cell death pathway also influence proliferation and to test specifically how *drone*, *drice*, and *dark* are connected to the Hippo pathway.
Chapter 2

Methods
2.1: Genetics and Fly Stocks:

From the organism used, the *Drosophila melanogaster* more commonly known as the fruit fly, strains that contained specific caspase mutations were used for the experiment. The specific fly stock mutants that were used for generating somatic clones were:

- FRT82B-*drice*17/ (TM 3, Sb),
- FRT80-*dronc*2/TM6B,
- FRT80 *dronc*124/TM3,Sb,  
- FRT42D *dark*82/Cyo,
- FRT42D *dark*G8/CyO

Adult male (♂) flies were collected from these stocks and outcrossed to female virgins from the following stocks:

- Yw hsFLP; FRT82B *UbiGFP* /TM6B),
- Yw hsFLP; 42D *UbiGFP* /CYO
- Yw hsFLP; FRT80 *UbiGFP*,

The specific details are shown in Table 2.1.

2.2 Generation of GFP-negative clones:

Tissues in which *drice*, *dronc* or *dark* are mutated in each cell ultimately causes lethality for the organism, rendering these crosses impossible to obtain information from. Instead, the loss of genes must be studied through a system of smaller patches within the developing tissues. We used mosaic techniques wherein the small patches of mutant Drice, Dark, or Dronc cells were generated. The F1 larvae from the crosses were
administered the heat shock, which allows for homologous recombination to occur—thereby allowing for the creation of the small patches of mutant clones. The larvae recover following the heat shock (for usually one to two days), and are then dissected in the third instar stage to study the effect on hippo pathway targets.

The F1 larvae from the above crosses were heat shocked at 37° for twenty minutes during 24h or 48h of development, and dissected for analysis of mutant clones in the third instar or 120h of development.

<table>
<thead>
<tr>
<th>Virgin Female Stock</th>
<th>Male Stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>42D <em>UbiGFP</em> CYO (♀)</td>
<td>42-<em>dark</em>82 CYO (♂)</td>
</tr>
<tr>
<td>FRT80 <em>UbiGFP</em> (♀)</td>
<td>42-<em>dark</em>G8 CYO (♂)</td>
</tr>
<tr>
<td>FRT82B <em>UbiGFP</em> (TM6B) (♀)</td>
<td>80-<em>dronc</em>124 (♂)</td>
</tr>
<tr>
<td></td>
<td>80-<em>dronc</em>TM6B (♂)</td>
</tr>
<tr>
<td></td>
<td>FRT82B-<em>drice</em>17 (♂)</td>
</tr>
</tbody>
</table>

**Figure 2.1: The crosses that were set up during the experiment.** (♀) is the sign for Virgin Females. (♂) is the sign for Males. For each cross, around 10-15 female virgins were paired with around 5-7 males.

2.3: Immunohistochemistry
Immunohistochemistry assays are extremely important in that they are normally used to help detect the expression and localization of protein, after standard protocols (Plouffe et al., 2015). The dissection of the imaginal eye and wing discs to look for the changes in expression of the following proteins using immunohistochemistry techniques. The larvae were dissected when they reached the 3rd instar stage of development. The dissected eye/wing discs of the F1 3rd instar larvae of the crosses needed specific antibody staining to assess the results. For each cross, three antibody sets of stains were used, with the primary antibody (1°) first: 1° Antibody m DIAP1 and rat ELAV, 1° Antibody m CyclinB and rb Dlg, and 1° Antibody m PH3 and rat ELAV. The secondary antibodies were m-Cy3 and rat-Cy5, m-Cy3 and rb-Cy5, and m-Cy3 and rt-Cy5, respectively (Figure 2.2). As discussed previously, the antibodies stained specific proteins/markers that indicated ectopic growth within the eye discs, shown through deviations from the wild type growth.

<table>
<thead>
<tr>
<th>Primary antibodies (1°)</th>
<th>Secondary antibodies (2°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m DIAP1 and rat ELAV</td>
<td>m-Cy3 and rat-Cy5</td>
</tr>
<tr>
<td>m CyclinB and rb Dlg</td>
<td>m-Cy3 and rb-Cy5</td>
</tr>
<tr>
<td>m PH3 and rat ELAV</td>
<td>m-Cy3 and rt-Cy5</td>
</tr>
</tbody>
</table>

Figure 2.2: The pairing of the primary antibodies with the secondary antibodies.
Specifically, Cylin B (CYC b) was studied within this experiment. From the hippo pathway, we were able to record the amounts of Diap1, while for cell death, we were able to record Casp3\(^+\) (the \(^+\) indicating activation, activated caspase 3).

2.3: Imaging and Data Analysis:

The samples were scanned using a Laser Scanning Confocal Microscope. Most of the data for the analysis was acquired through the confocal microscopy. To compare the changes in gene expression, data from different samples (progressed using standard acquisition methods) were then quantified using the histogram function of Adobe Photoshop.
Chapter 3

Investigation of loss of the upstream caspase *dronc*
on cell cycle regulation
3.1: Functionality of \textit{dronc}

The Hippo Pathway controls cell survival and cell proliferation by regulating \textit{dronc}, (\textit{Drosophila} Nedd-2-like caspase) levels. Through studies using RNA interference techniques, it has been shown that within \textit{Drosophila} embryos, the loss of \textit{dronc} functions leads to a decrease in cell death, essentially highlighting the importance of this caspase for automatic cell death throughout embryogenesis (Quinn et al., 2000). DIAP1 and \textit{dronc} work together for proper function, but it is important to note that the loss of DIAP1 leads to cell death, through caspase activity (Orme et al., 2009). On the other hand, the total loss of \textit{dronc} within \textit{Drosophila} leads to an arrest of cell death, meaning there is no apoptotic action within the cells (Hawkins et al., 2000). While it was understood that the loss of \textit{dronc} led to no apoptosis, it was recently understood that the caspase may play a continued

\textbf{Figure 3.1:} \textit{dronc} mutant clones proliferate faster than their wild type twin spots

Verghese \textit{et al.}, 2012
role in proliferation as well. Comparison of clone sizes within eye discs showed that the size of the *dronc* mutants clones was significantly larger than the respective twin spots, indicating that the loss of *dronc* actually provokes further proliferation (Figure 3.1).

### 3.2: Loss of *dronc* expression leads to ectopic cell proliferation

Although previous studies have shown the effects of *dronc*, we wanted to study what aspect of proliferation the caspase *dronc* regulated. In order to study what was going on, two genetically different strains of *dronc* mutants were studied and we identified that there seems to be a connection in that *dronc* mutant cells go through ectopic mitosis, as shown through PH3 staining.

**Figure 3.2**: PH3 is induced in *dronc* mutant cells.

When looking at the staining, we can note that there is ectopic proliferation, shown through the growth of cells beyond the morphogenetic furrow, as indicated with the PH3 staining [bottom right]. When comparing to wild-type staining, there should be no mitotic growth after the furrow, however we can see that there is PH3 stain of cells beyond that point, meaning that there are cells at the stage of mitotic growth.
Figures 3.3/3.4: *dronc* and Cyclin B: We can note the presence of mild up-regulation in mutant cells—this is shown through the slight staining again for Cyclin B beyond the morphogenetic furrow. Furthermore, looking at the clone sizes, we can see the dark spots and light spots of non-equivalent sizes; there is a disruption of the mitotic growth of the photoreceptor neurons, as shown through the GFP staining.
Chapter 4

Investigation of loss of the apoptosome component

*dark* on cell cycle regulation
4.1: Functionality of *dark*

It is important to consider that *dronc* must combine with *dark* to make an apoptosome that further correlates with cell death. Interestingly, in *dark* loss of function mutations, cell death is diminished during development, then leading to ectopic tumors (Rodriguez et al., 1999). This insight reminds us that as a caspase, *dark* plays an equally complex role regarding apoptosis, knowing that *dark* mutants further subdue cell death activators within *Drosophila* from killing ectopic cells (Rodriguez et al., 1999). Before the *dark-dronc* complex is formed, *dark* interacts with *dronc* in a domain-dependent manner, and specifically, *dronc* is activated by this action (Chai and Shi 2014). Furthermore, this *dark* apoptosome can only be formed if dATP is presence (Yu et al. 2006). Given the *Drosophila* homologue, *dark*, is required for *dronc*-mediated cell death, for preliminary autocatalytic activation of *dronc*, the complex may be essential (Quinn et al., 2000).

4.2: Loss of *dark* induces cell cycle defects and ectopic proliferation

Understanding that *dark* plays an important role as a part of the apoptosome, we investigated the effects of the loss of *dark* as shown through the two mutant strains (FRT42D *darkG8/CYO* & FRT42D *dark82/CYO*). Our results indicate that the loss of *dark* induces PH3, meaning that there was an excess of proliferation. Furthermore, there was overall disruption of the wild-type cellular patterns of organization, indicating that the *dark* complex may act as an important regulator within more general cellular processes.
**Figure 4.1:** Looking at the basal images of the *dark* mutant cells, the disruption of the morphogenetic furrow is highly visible through Cyclin B, as it is induced near the furrow; we can note ectopic growth as Cyclin B indicates the transition from G2-Mitosis.

**Wild-type morphogenetic furrow and general eye-disc**

**Progression of layers of mutant *dark* eye disc, the top three being apical images, and the bottom three being basal images.**
**Figure 4.2:** Looking at PH3 staining of the mutant strain *dark*-G8, we see that the Loss of *dark* induces PH3, indicating proliferation. The arrests of cells within the mitotic stage that are in the photoreceptor area of the eye disc are the effects of the loss of the dark apoptosome.
Chapter 5
Investigation of loss of downstream caspase

*drice* on cell cycle regulation
5.1: Functionality of *drice*

Within the apoptotic cascade, the downstream caspase *drice* plays a significant role in regulation. As noted previously, DIAP1, an inhibitor of apoptosis, depends on the caspase *drice*, along with *dronc* to preform its task. Specifically, *drice* cleaves a specific site on Armadillo/beta-catenin (ARM), which is extremely important for disassembling epithelial cells throughout apoptosis (Kessler et al., 2009). To activate *drice*, the activated caspase *dronc* is needed—*drice* then cleaves *dronc*, allowing for protection against DIAP1 inhibition (Yan et al., 2004). It is important to note that the cell death that is usually induced by *drice*, is suppressed by DIAP1 (Kaiser 1998). However, although they play competing roles, *drice* and DIAP1 have overlapping functions within the *drosophila* apoptotic pathway (Xu et al., 2006).

5.2: Expression of *drice*

In studying the effects of mutant *drice* clones, we found that Cyclin E is up regulated within the clones. The downstream caspase, when rendered inactive, was further found to display ectopic cellular division shown through PH3 staining, as depicted within Figure 5.3. The fourth row of Figure 5.2 depicts the highly concentrated growth that occurred within the *drice* mutant clones.

![Figure 5.1](image_url): Wild-type morphogenetic furrow and general eye-disc with Cyc E. (Verghese et al., 2012)
Figure 5.2: Cyclin E gets up-regulated in *drice* mutant clones (4th row). This is shown through the highly concentrated staining/growth of cells within the dark spots, indicated by the yellow arrows. (Verghese et al., 2012).
Figure 5.3: *drice* mutant clones induce ectopic cell division (PH3 staining, second image).
Chapter 6

Conclusion
We were able to confirm our hypothesis, in that we verified that the cell death regulating genes (*drice, dronc, or dark*) also interact with growth control genes to maintain normal cell functions. In addressing our aims, we have noted that cell death regulating genes induce cell death through different means, being up stream regulation, downstream regulation, or the usage of an apoptosome complex. These cell death regulating genes essentially interact with and are transcriptionally regulated by the Hippo pathway.

Our studies show that loss of cell death inducing genes, specifically the caspases Dronc and Drice, and Dark—the apoptosome component that binds Dronc—all lead to defects in cell cycle regulation. In some instances we observed that cells underwent ectopic and likely accelerated proliferation, suggesting that although these genes are normally thought to play a very important role in the regulation of cell death; under conditions of stress or loss of function of these genes cells activate a cell cycle mediated response. The implication of this response on balancing tissue homeostasis is expected, as well as this response informs our knowledge on how cells that are no longer amenable to cell death (due to loss of cell death inducing genes) may be very hard to eliminate from tissues. Furthermore, it is interesting to note that lack or activation of caspase activity both affect cell proliferation, albeit in slightly different way.

In conclusion, these studies have paved the way for further analysis of how exactly different caspase or dark mutant cells activate different cyclins within the cell cycle. This would imply that loss of cell death has specific effects on different parts of the cell cycle, and also has implications for devising strategies to identify the underlying causes of cell cycle defects in different contexts. In the future, we will explore if caspase
mutant cells are retained in tissues (as they cannot be killed), and if these cells induce
abnormal signals to the neighboring normal or heterozygous cells to cause non-cell
autonomous effects on tissue homeostasis.
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