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p53-Independent Apoptosis and Autophagy as a result of RNAi Knockdown of Nopp140 in Drosophila melanogaster

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p53-Independent Apoptosis and Autophagy as a result of RNAi Knockdown of Nopp140 in *Drosophila melanogaster*

By

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Abstract

This thesis describes cell stress responses (apoptosis and autophagy) in *Drosophila* tissues that are depleted for the nucleolar protein, Nopp140, a ribosome assembly factor. Depletion of Nopp140 likely results in the loss of functional ribosomes and thus protein synthesis. Cells incapable of adequate protein synthesis thus respond to this stress by inducing either apoptosis or autophagy. Apoptosis is a method of programmed cell death that allows the organism to get rid of damaged cells as a result of normal developmental programs, excess cell stress, or excess DNA damage. p53 is a tumor suppressor that is known to play a vital role in apoptosis. Apoptosis however can be p53-dependent or p53-independent. Both types involve activation of caspases in the apoptotic pathway. This work focuses on using immunofluoresence to detect for activated caspases in p53-independent apoptosis. Autophagy is also a method of programmed cell death that involves autophagosomes and lysosomes self-digesting organelles. Part two of this work focuses on autophagy that results from depletion of Nopp140.

Introduction

Apoptosis is a form of programmed cell death in response to adverse external and internal insults leading to stress. It involves signaling cascades that result in nuclear fragmentation and chromatin condensation (Maiuri et al, 2007). Apoptosis can be caspase-dependent or caspase-independent. Both pathways can be caused by a form of cellular stress, such as ionizing radiation, by death receptor stimulation, or by DNA damage (Maiuri et al, 2007). Apoptosis is important because it allows organisms to get rid of damaged cells that could otherwise be harmful; these damaged cells could lead to tumor growth if not destroyed. The transcription factor p53 plays a large role in inducing genes necessary for apoptosis in cells with DNA damage (Wichmann et al, 2006).

It is known that the p53 tumor suppressor protein plays a role in apoptosis. p53 induces apoptosis through intrinsic and extrinsic pathways. The intrinsic pathway involves mitochondria and the extrinsic one involves death-receptor genes (Sidi et al, 2008). p53 activates the transcription of BH3 proteins, which in turn activate mitochondrial outer membrane permeabilization (MOMP). MOMP causes the release of cytochrome c, which signals assembly of the apoptosome composed of the initiator caspase-9 and APAF1 (Maiuri et al, 2007). Caspases are from a family of cysteine proteases that are activated in response to a specific event (Maiuri et al, 2007). Caspase 9 then activates down stream caspases (e.g. Caspase 3) that destroy cellular proteins effectively killing the cell. This is one known pathway of caspase-dependent death involving p53.

Most cells already house the equipment needed for apoptotic programmed cell death (Lockshin and Zakeri, 2004). This equipment is usually found in the form of "pro-

caspases", which are waiting to become activated in order to induce apoptosis (Lockshin and Zakeri, 2004). Often times, this machinery is used for partial apoptosis in which major organelles are discarded because they are no longer needed (Lockshin and Zakeri, 2004). An example of this would be in mammalian red blood cells that do not contain nuclei. This means that apoptosis can be a selective process where the entire cell is not destroyed, just certain parts of it (Lockshin and Zakeri, 2004).

p53 is a transcription factor that is known to arrest the cell cycle and induce apoptosis. It is known to play a role in tumor suppression and is frequently mutated or inactivated in cancer (Maiuri et al, 2007). Research suggests that p53 also plays a role in cell survival in response to stress through either antioxidant pathways or autophagy (Maiuri et al, 2007). p53 is also known to be an inducer of apoptosis, mostly by activating transcription of redox-related genes (Polyak et al, 1997). Mutations involving p53 have been found in human diseases including cancers. Inherited p53 mutations have been found in individuals with Li-Fraumeni syndrome, demonstrating the importance of p53 in maintaining genome consistency (Wichmann et al, 2006).

Apoptosis in *Drosophila melanogaster* occurs through the induction of *reaper*, *hid*, and *grim* three closely linked genes located on the third chromosome (Goyal et al, 2000). These genes are activated transcriptionally by "death-inducing signals" (Goyal et al, 2000). When cell survival signals are present, *hid* is phosphorylated by RAS-MAP kinase to inhibit apoptosis. *Reaper, hid,* and *grim* induce apoptosis by activating caspases. *Hid* stands for head involution defect (Grether et al, 1995). *Drosophila* lacking the *hid* gene have reduced levels of apoptosis, indicating that *hid* plays an important role in inducing apoptosis (Grether et al, 1995). However, baculovirus p35 inhibits caspase

activation (Goyal et al, 2000). Another molecule known as IAP plays an important role in p53 induced apoptosis. IAPs are inhibitor of apoptosis proteins. In *Drosophila*, they are known as *diap1* and *diap2* (Goyal et al, 2000). When *diap1* is not functional, *reaper, hid,* and *grim* are enhanced, leading to apoptosis (Goyal et al, 2000). *Diap1* functions by inhibiting caspase function. *Reaper, hid,* and *grim* are able to functionally inhibit *diap1* by binding directly to it, allowing caspases to become activated (Goyal et al, 2000).

Caspases play an important role in apoptosis. In testing for apoptosis, an anti-caspase antibody is used.

An antibody directed against human Caspase-3 has been used in *Drosophila* apoptosis research. It is obtained from rabbits immunized by the activated (cleaved) human Caspase-3 (Fan and Bergmann, 2009). *Reaper, hid,* and *grim* are pro-apoptotic genes in *Drosophila*, which induce proteolytic degradation of *Diap1* (Fan and Bergmann, 2009). *Diap1* degradation activates the caspases. The caspases involved in *Drosophila* apoptosis are DRONC, which is similar to mammalian Caspase-9, and DRICE and DCP-1, which are Caspase-3 like (Fan and Bergmann, 2009). DRONC eventually becomes part of the apoptosome, which cleaves and activates the Caspase-3 like DRICE and DCP-1 (Fan and Bergmann, 2009). The antibody likely recognizes activated DRICE or DCP-1.

Apoptosis due to ionizing radiation in *Drosophila* usually requires activation of the ATM and Chk2 kinases, as well as p53 (McNamee and Brodsky, 2000). p53 activates the transcription of *reaper*, *hid*, and *grim*. It is thought that *hid* is essential, while *reaper* and *grim* play lesser roles (McNamee and Brodsky, 2009). *Reaper, hid,* and

grim bind to and inhibit *Diap1*, activating DRONC and other caspases (McNamee and Brodsky, 2009).

p53 independent apoptosis had been seen as a result of chromosomal damage (McNamee and Brodsky, 2009). In *Drosophila*, the damage-signaling pathway causes unprotected telomeres and as a result, high levels of apoptosis (McNamee and Brodsky, 2009). When p53 is lost, it partly suppresses apoptosis in this instance and apoptosis is slightly delayed. However, *Drosophila* has only one homolog of p53, so there are no other p53 family members contributing to the response (McNamee and Brodsky, 2009). According to McNamee and Brodsky, double mutant *tefu*, *mus304*, and *nbs* flies showed apoptosis, indicating that apoptosis due to chromosomal damage is independent of the DNA damage pathway components (McNamee and Brodsky, 2009).

McNamee and Brodsky were able to demonstrate p53 independent apoptosis as a result of ionizing radiation. They found that p53 independent apoptosis requires *hid* and DRONC. *Hid* is induced through regulation of the JNK phosphatase *puc* (McNamee and Brodsky, 2009).

E2F1 can also play a role in p53-independent apoptosis. E2F1 is a transcription factor that induces *cyclin E* and *DNA polymerase-α* (Wichmann et al, 2010). In *Drosophila*, it is known as dE2F1. When dE2F1 is over-expressed, it induces the expression of *reaper* (Wichmann et al, 2010). In certain *Drosophila* mutations, dE2F1 can activate *reaper, hid* and *grim* (Wichmann et al, 2010). Because *Drosophila* has only one p53, it is thought that p53 deficient flies lack all p53 activity (Wichmann et al, 2010). Further research using p53-deficient flies has found that p53-independent apoptosis is still dependent on caspase activity, but the caspases are activated without p53 being

present (Wichmann et al, 2010). *Hid* is thought to play a major role because flies with *hid* mutations have highly reduced levels of p53-independent apoptosis (Wichmann et al, 2010).

The goal of Part One of this thesis is to test for the presence of p53-independent apoptosis, not the mechanisms behind it. To do this, p53 deficient flies were mated and the larvae was dissected for immunofluorescence microscopy using anti-Caspase 3 antibody.

Part Two of this thesis describes a second stress response pathway known as autophagy. Autophagy is a process of "self-eating" where the cell's own lysosomes digest organelles, parts of organelles, or the entire cell. Autophagosomes engulf the cytoplasm and organelles and deliver them to the lysosomes to be digested (Maiuri et al, 2007). Autophagy can also be used as a survival mechanism during cellular starvation in which it can generate necessary nutrients for the cell through the digestion of macromolecules (Maiuri et al, 2007). This mechanism can be used to inhibit apoptosis. Autophagy is useful in that it helps ward of infection and tumor development (Thorburn, 2007). Autophagy is caspase-independent and relies on a large buildup of autophagosomes that allow organelles to be digested from within while the cytoskeleton remains intact until late in the process (Thorburn, 2007). Autophagy is regulated by the *ATG* genes that control the formation of the autophagosomes and their fusion with lysosomes (Thorburn, 2007).

Nopp140 stands for nucleolar phosphoprotein of 140 kilodaltons (Cui and DiMario, 2007). Nopp140 is found in the nucleoli and in extra-nucleolar Cajal bodies (Cui and DiMario, 2007). It is thought that Nopp140 facilitates the assembly of

ribosomes because it shuttles between the nucleus and cytoplasm and between the nucleolus and Cajal bodies delivering small nucleolar ribonucleoprotein particles necessary for proper ribosome assembly (Cui and DiMario, 2007). In mammals, Nopp140 may also regulate the transcription of rRNA (Cui and DiMario, 2007). In *Drosophila*, two forms of the protein exist: Nopp140-true, which has 686 amino acid residues, and Nopp140-RGG, which has 720 amino acid residues (Cui and DiMario, 2007). Nopp140-true is considered the true ortholog of vertebrate Nopp140 (Cui and DiMario, 2007). Nopp140 interacts with small nucleolar ribonucleoproteins (snoRNPs), particularly the H/ACA snoRNPs that function in pre-rRNA processing and telomere maintenance (He and DiMario, 2011). Nopp140 perhaps functions in bringing snoRNPs from the Cajal bodies to the nucleolus, where spliceosomal assembly and processing of snoRNAs takes place to the nucleolus (He and DiMario, 2011). This shuttling might aid in the import of ribosome assembly factors (Cui and DiMario, 2007). When Nopp140 is lost, ribosome assembly is likely to be impaired resulting in the loss of functional ribosomes, and thus protein synthesis.

The goal of part two of this thesis is to demonstrate autophagy due to loss of Nopp140 in larval midgut cells. This was done using mCherry-ATG8a transgene in *Drosophila* as a marker for autophagy and daGAL4 as the driver to induce expression of the ATG8a transgene.

Autophagy and apoptosis are two methods of programmed cell death that are very different from one another, but can share some of the same characteristics. Research has found that autophagy can block apoptosis and that p53, an inducer of apoptosis, can also induce autophagy (Thorburn, 2008). *ATG* genes are largely regulators of autophagy, but

they may also play a role in regulating apoptosis (Thorburn, 2008). What determines whether cells use apoptosis or autophagy as the method of cell death may not be as obvious as once hypothesized. When Nopp140 is lost, it is thought to induce autophagy in larval midgut cells but apoptosis in imaginal discs (He and DiMario, 2011).

Materials and Methods

Part 1

Fly Genetics:

To test for apoptosis in the absence of p53, the progeny from the following cross was used:

A9/Y; +//+; Δp53//Δp53 (males) X w^-//w^-; TdsCom.C4//TdsCom.C4; Δp53//Δp53.

A9 is a transgene on the X chromosome that encodes the yeast transcription factor GAL4, but only in the larval imaginal wing disc (progenitor tissue for the adult wing). Δp53 is a deletion of the p53 gene on the third chromosome. TdsCom.C4 is a transgene on the second chromosome that is induced by GAL4 expressed from the A9 "driver" gene. TdsCom.C4 expresses RNAi that depletes Nopp140 mRNAs. Thus, Nopp140 is only depleted in the wing discs.

Immunofluorescence:

To detect for apoptosis, immunofluorescence microscopy was used. For the first step, the larvae were dissected into Spradling's B Buffer at room temperature. To dissect larvae, you simply use two sets of tweezers and pull the larvae apart, making sure to grab the larvae around the mouth hooks. Spradling's B Buffer consists of 0.852 ml of 200 mM KH_2PO_4, 0.818 ml of 200 mM K_2HPO_4, 1.5 ml of 1 M KCl, 0.5 ml of 1 M NaCl, 0.066 ml of 1 M $MgCl_2$, 8.00 ml of 5% Paraformaldehyde, and 8.264 ml of MilliQ water for a total volume of 20 ml. The larvae were dissected in the B Buffer in a three well dish that holds an approximate volume of 0.5-0.75 ml in each well. Tissues were teased apart but not separated from the larvae.

After dissection, the larvae were fixed in the same Spradling's B Buffer at room temperature for approximately 30-60 minutes. They were then washed for about 30 minutes at room temperature. The solution used for the washing was PBS with 0.1% Triton X-100. In order to wash the larvae, the solution must be changed multiple times within the suggested period, averaging about one change every five minutes during the allotted time. The larvae remain in the same well during each step of the process, but the solutions are pulled off using a glass pipette that has been heated and stretched to create a thinner tip.

After the wash, the dissected larvae were incubated at room temperature for 30 minutes in PBS with 0.1% Triton X-100 with 3% BSA. BSA stands for Bovine Serum Albumin. To make the BSA with PBS and Tritonx-100, 10 ml of PBS with 0.1% Triton X-100 is mixed with 0.3 g of BSA.

The larvae were then incubated overnight in the refrigerator at 4°C in PBS with 0.1% Triton X-100, 3% BSA and the diluted primary antibody. The antibody against cleaved Caspase 3 was purchased from Cell Signaling Technology. The antibody was used at a dilution of 1/200. To make the antibody dilution, 1.5 ml of PBS with 0.1% Triton X-100 and 3% BSA were mixed with 7.5 µl of antibody.

The next day, the larvae were washed for two hours at room temperature in PBS with 0.1% Triton X-100 and 0.2% BSA. They were then incubated for 30 minutes at room temperature in PBS with 0.1% Triton X-100 and 3% BSA. The next step was to incubate the larvae at 4°C in PBS with 0.1% Triton X-100 and 3% BSA along with the secondary antibody. The incubation period should last for 4 hours. The secondary antibody was Alexa Fluor 488 Goat α Rabbit (Molecular Probes) used at a dilution of

1/250. To make the antibody dilution, 1.5 ml of PBS with 0.1% Tritonx-100 and 3% BSA are combined with 6 µl of antibody.

After the 4-hour incubation, the larvae were washed in PBS with 0.1% TritonX-100. In the last wash, 4',6-diamidino-2-phenylindole (DAPI) was added to stain the DNA. 1.00 µl of DAPI is added to every 1 ml of solution used.

The wing discs were then put on slides to view using phase-contrast and fluorescence microscopy. In order to make the slides, one drop of PBS with 0.1% TritonX-100 was placed on the slide to keep the tissue moist. The wing disc was then added, along with a cover slip.

Part 2

Fly Genetics:

To test for autophagy, the following cross was used:

w^{1118}//Y; TdsCom.C4//TdsCom.C4; mCherry-ATG8a//mCherry-ATG8a (males) X

+//+; +//+; da-GAL4//da-GAL4 (females)

The mCherry-ATG8a transgenic fly line was a gift from Dr. Tom Neufeld at the University of Minnesota.

Dissection:

The larvae were dissected in a three well dish that can hold approximately 0.5-0.75 ml of fluid. To dissect the larvae, two sets of tweezers were used, along with a microscope. While viewing the larvae under the microscope, the larvae are grabbed around the middle with one set of tweezers, while the other set of tweezers is used to pull

the larvae apart. One must make sure to pull all of the tissue out from the cuticle completely in order for each tissue to be exposed to the DAPI for DNA staining.

The larvae were dissected in Brower's solution. Brower's solution consists of 3.00 ml of Brower's mix, 0.4 ml of 37% formaldehyde, and 0.6 ml of MilliQ water. To that mixture of 4 ml, 4 µl of DAPI is added for DNA staining. The larvae were fixed for at least 10 minutes.

After fixation, the larval tissues were put onto microscope slides in order to view using phase contrast and fluorescence microscopy. One drop of Brower's solution was added to the slide to keep the tissue moist. For the purpose of this thesis, the gut tissue was observed primarily.

Results

Part 1: Apoptosis

The A9//A9; +//+; Δp53//Δp53 newly-eclosed female flies were crossed with the w^-//Y; TdsCom.C4//TdsCom.C4; Δp53//Δp53 males. This cross was made three times and kept in the 27°C incubator. Each time, no larvae were present and it appeared that the females had died. More newly-eclosed females were added, but only to yield the same results. The cross was made again for the fourth time but kept at 22°C on the lab bench but there were still no larvae. It was concluded that this cross is a sterile cross, producing no offspring.

The cross was made again, only this time using A9//Y; +//+; Δp53//Δp53 males and w^-//w^-; TdsCom.C4//TdsCom.C4; Δp53//Δp53 newly-eclosed females. This cross was kept in the 27° incubator. A9 is a transgene that expresses the yeast GAL4 transcription factor only in wing discs. GAL4 induces the TdsCom.C4 gene to produce interfering RNA that depletes Nopp140 mRNAs and thus Nopp140. The cross was kept in the incubator for about a week to give the flies time to reproduce and allow the larvae to develop. Immunofluorescence microscopy was used to detect for Caspase-3, a marker of apoptosis. Observation of the wing discs on the microscope slides demonstrated that apoptosis was indeed occurring in the third instar larval wing discs (Figures 1 and 2).

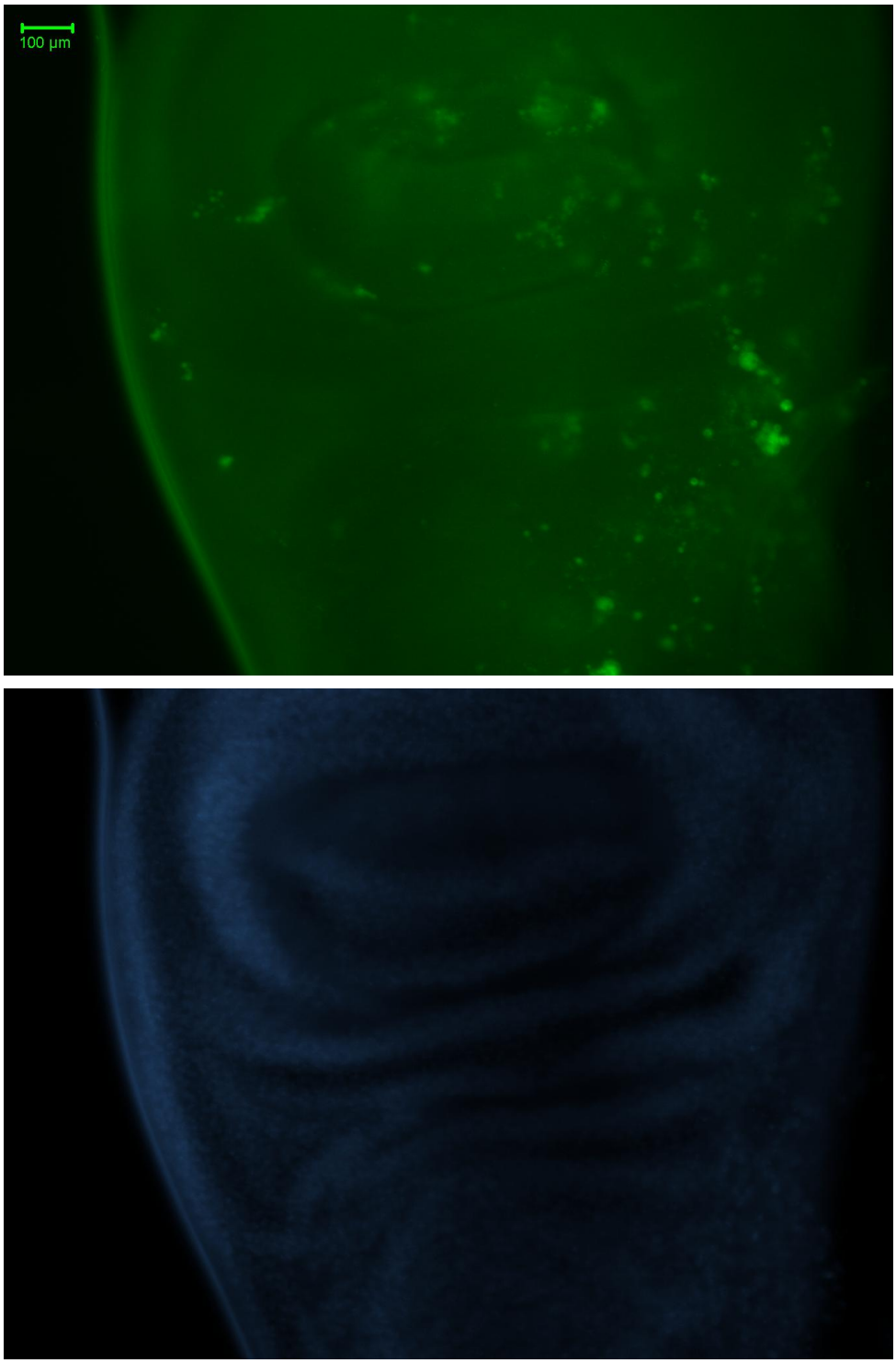
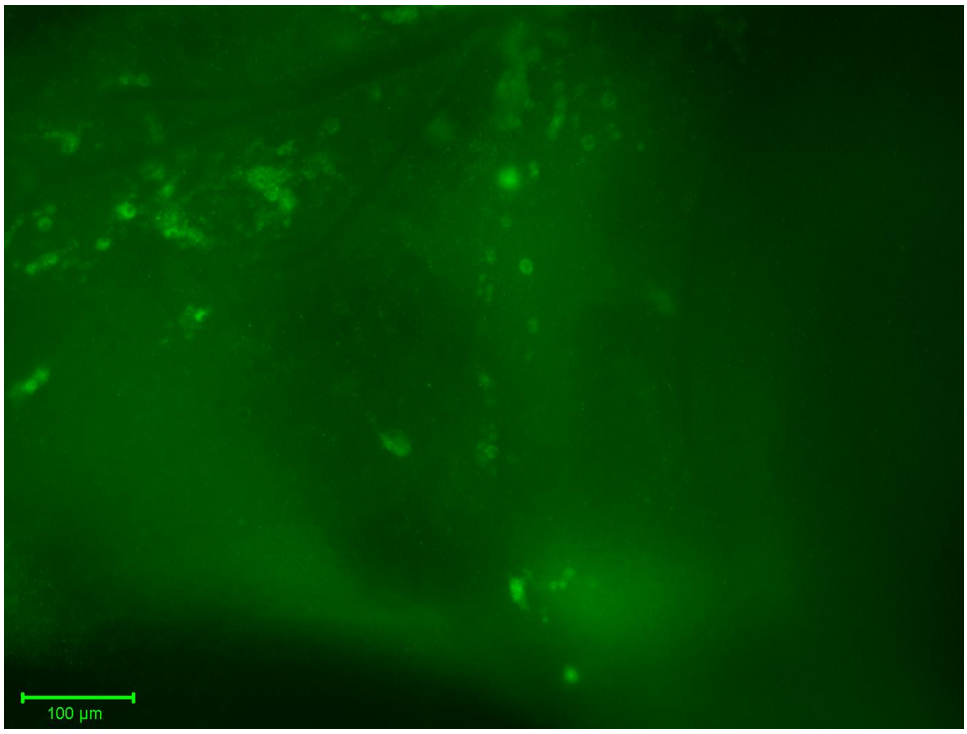


Figure 1a. Caspase 3 antibody staining of a third instar wing disc from Δp53 larvae. This picture was taken at 20x magnification. Apoptotic cells are shown as bright green.

Figure 1b. DAPI staining of a third instar wing disc from Δp53 larvae. This picture was taken at 20x magnification. DNA is stained in blue.



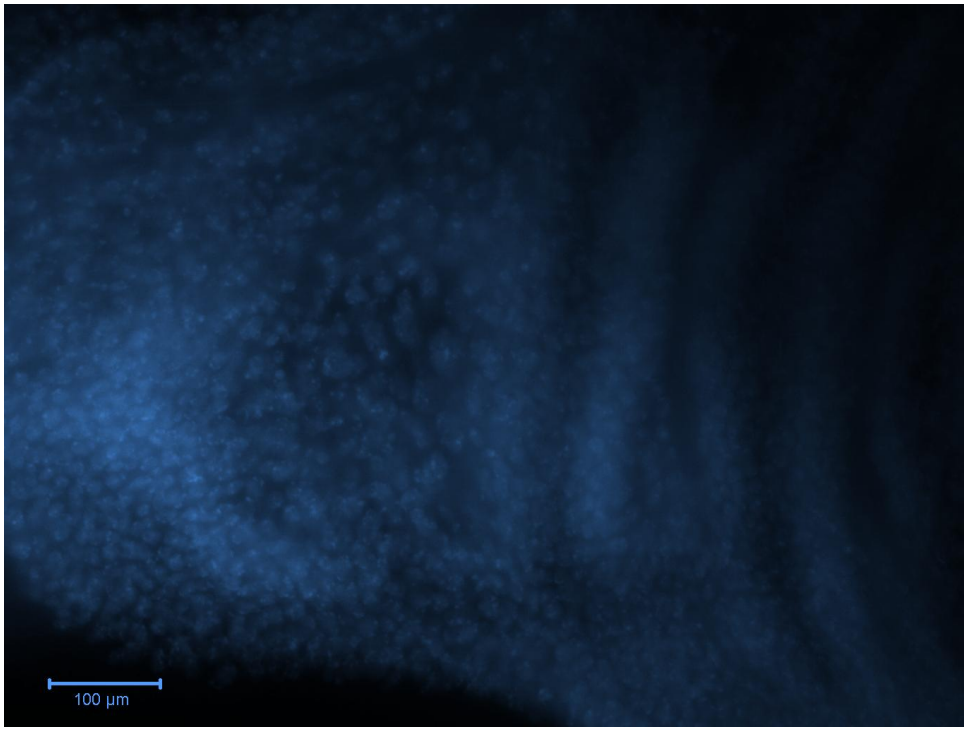


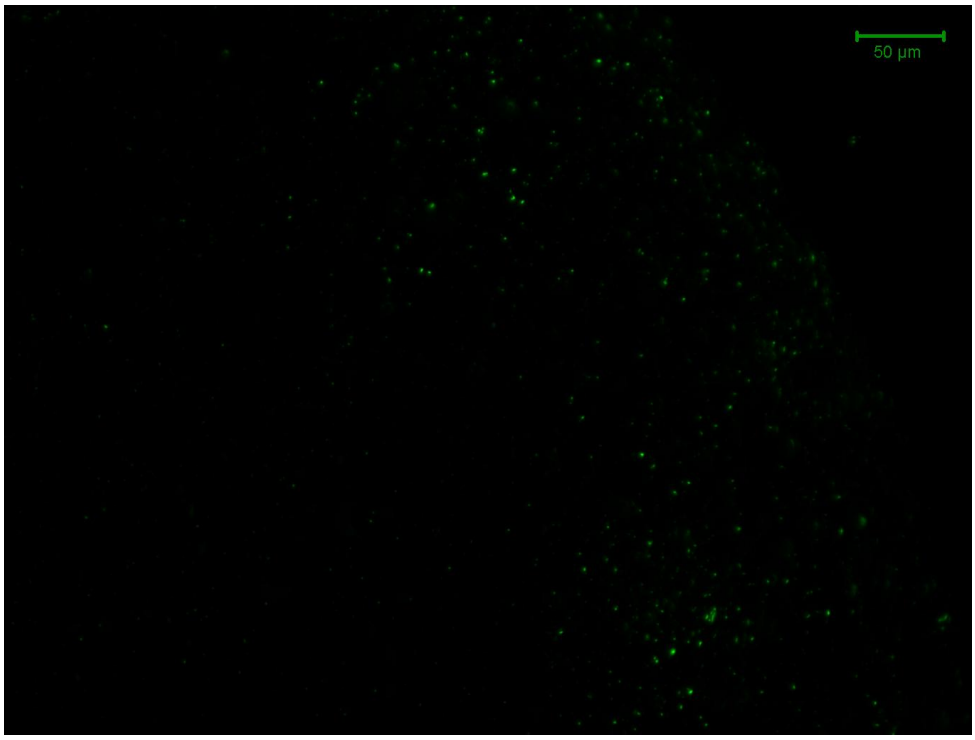
Figure 2a. Caspase 3 antibody staining of a third instar wing disc from Δp53 larvae. This picture was taken at 20x magnification. Apoptotic cells are shown as bright green.

Figure 2b. DAPI staining of a third instar wing disc from Δp53 larvae. This picture was taken at 20x magnification. DNA is stained in blue.

The genotypes of the progeny are A9//w^-; TdsComC4//+; Δp53//Δp53 females and w^-//Y; TdsComC4//+; Δp53//Δp53 males. The A9 gene is located on the X chromosome, so the male progeny do not have that gene. Since A9 is expressed in the wing discs, the phenotype of the progeny had curled wings. Only the females had an upward curl of the wings. The males had normal wings.

For a negative control, A9//Y; +//+; +//+ males were crossed with w^-//w^-; TdsCom.C4//TdsCom.C4; +//+ females. No apoptosis was seen and all the progeny had normal wings.

For a positive control, A9//Y; +//+; +p53//+p53 males were crossed with w^-//w^-; TdsCom.C4//TdsCom.C4; +p53//+p53 females. Some very fine staining was seen. However, it is hypothesized that the mitochondria are being stained (Figure 3). The caspase-3 antibody is made against the human caspase-3. It is supposed to detect activated caspases during apoptosis, but since it is made against human caspase-3, it is possible that it is detecting non-activated caspase-3 associated with mitochondria in healthy non-apoptotic cells.



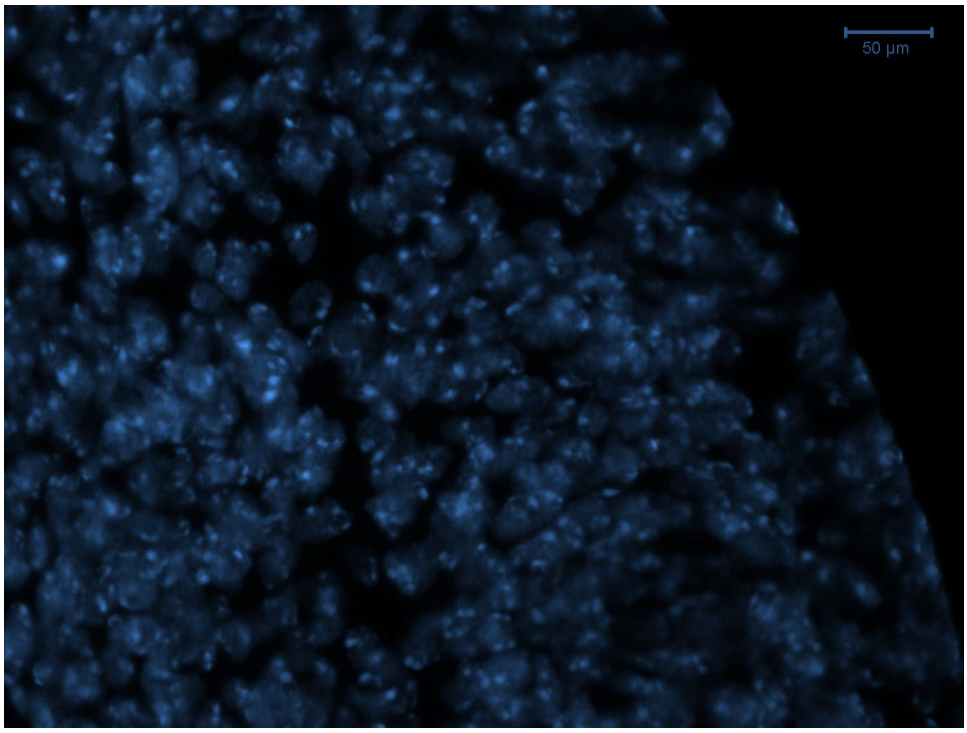


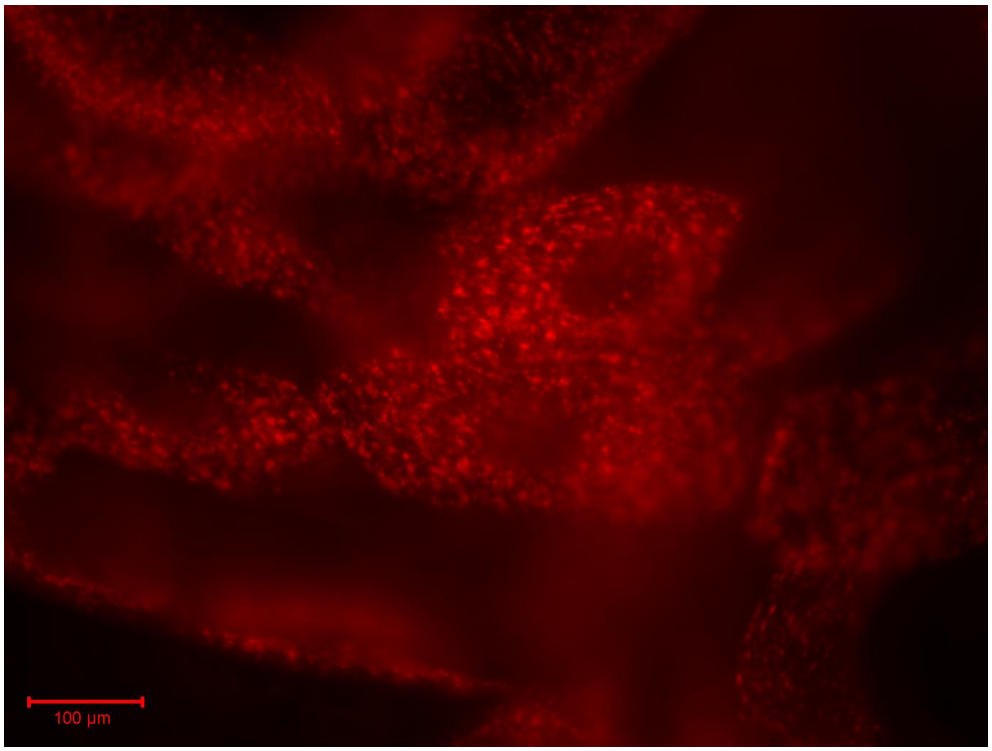
Figure 3a. Caspase 3 antibody staining of +p53 third instar larvae wing disc. Picture taken by Dr. DiMario. It is hypothesized that the small bright green specks are actually mitochondria and not apoptotic cells.
Figure 3b. DAPI staining of +p53 third instar larvae wing disc. Picture taken by Dr. DiMario.

Part 2: Autophagy

The TdsCom.C4//TdsCom.C4; mCherry-ATG8a//mCherry-ATG8a male flies were crossed with +//+; da-GAL4//da-GAL4 newly eclosed females. This cross was kept in the 27° incubator. ATG8a is an autophagy specific gene that is tagged to mCherry. TdsCom.C4 is a transgene prepared by Cui and DiMario (2007) which expresses RNAi to deplete Nopp140 and is located on the second chromosome. RNAi expression was induced by crossing the TdsCom.C4//TdsCom.C4; ATG8a//ATG8a male flies to the *daughterless*-GAL4 (da-GAL4) (Cui and DiMario, 2007). Even though the progeny will be heterozygous for da-GAL4, the *daughterless* promoter induces expression of GAL4 in nearly all larval and adult tissues (Cui and DiMario, 2007).

This cross proved to be lethal, as no progeny lived to adulthood. Cui and DiMario state that lethality occurs during the late third instar period, which potentially occurs due to RNAi expression in larval polyploid tissues (2007).

Fluorescence microscopy was used to detect autophagy in these polyploidy tissues as a result of RNAi knockdown of Nopp140. The ATG8a gene is tagged to mCherry, which appears as a bright red color under fluorescence microscopy. Figures 4 and 5 demonstrate that autophagy does in fact occur in these tissues. Third instar larvae lacking spherical eversions were used for this experiment. The non-everted sphericals indicate that the larvae are in the early to mid third instar stage. These larvae were used to make sure that the autophagy seen was not due to normal programmed cell death as the larvae begin to enter the pupal stage.



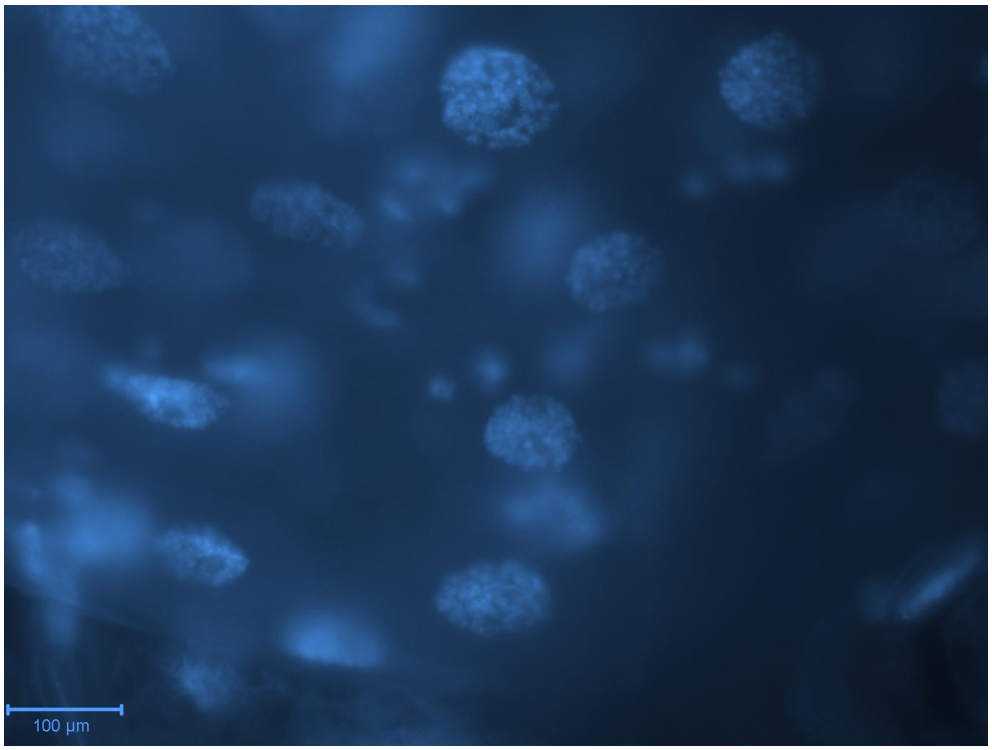
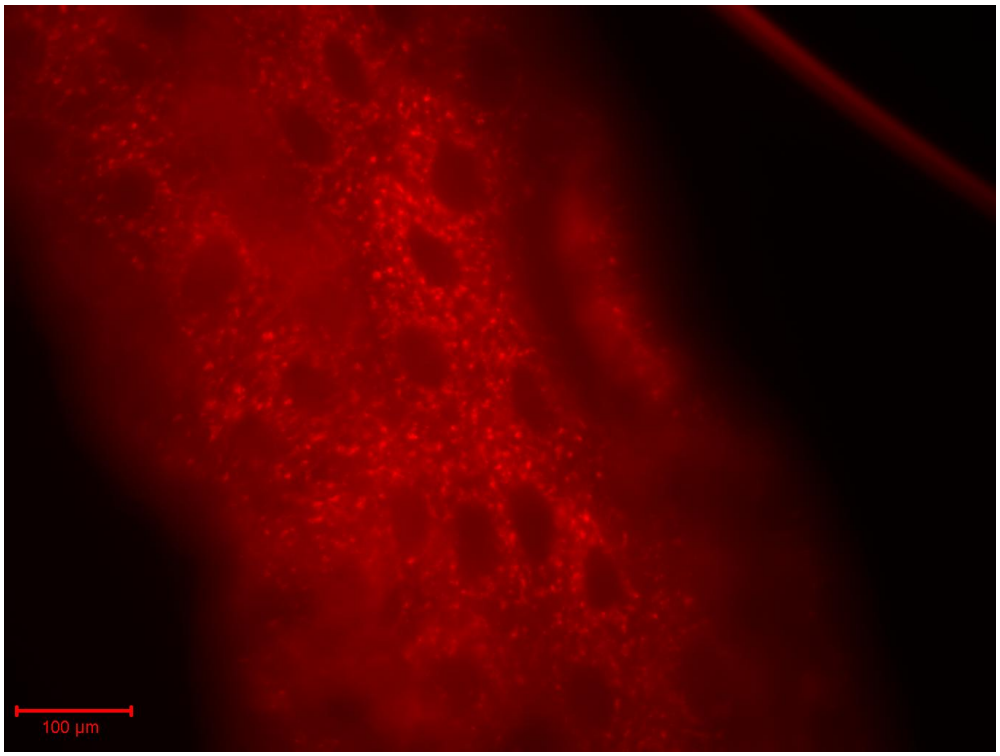


Figure 4a. mCherry-tagged ATG8a demonstrating autophagy. Autophagic vesicles are labeled as the bright red spots. Picture taken at 40x magnification.
Figure 4b. DAPI staining of the ATG8a flies. DNA is labeled as the bright blue areas. Picture taken at 40x magnification.



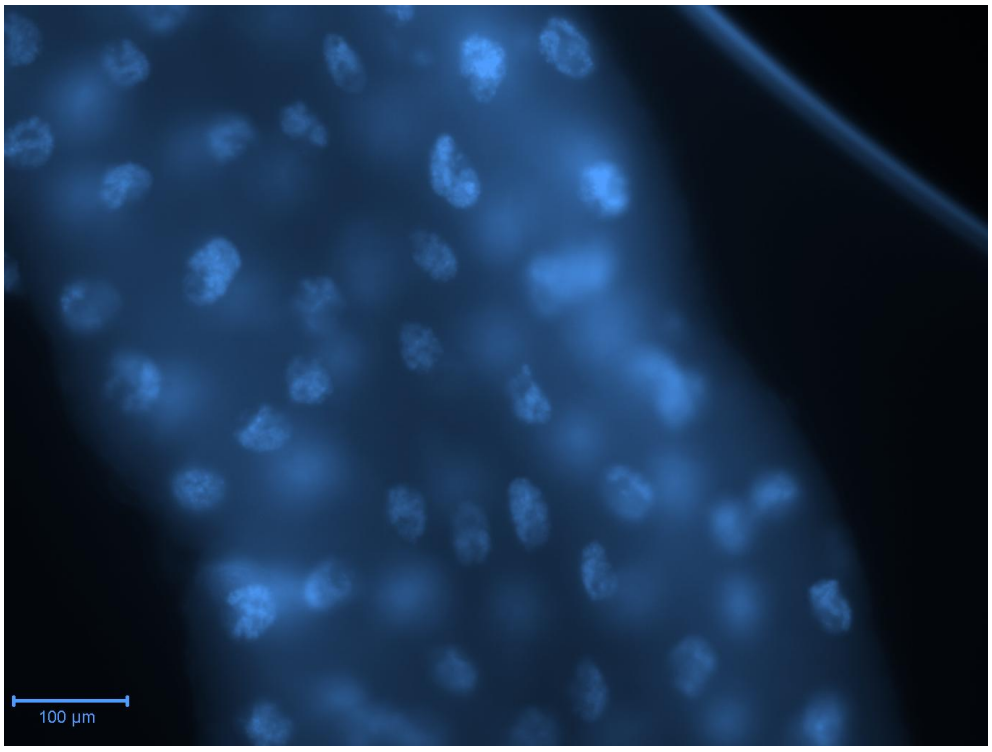


Figure 5a. mCherry-tagged ATG8a demonstrating autophagy. Autophagic vesicles are labeled as the bright red spots. Picture taken at 40x magnification.
Figure 5b. DAPI staining of the ATG8a flies. DNA is labeled as the bright blue areas. Picture taken at 40x magnification.

The control used in this experiment was deficient in TdsCom.C4, the RNAi transgene. Therefore, there was no RNAi knockdown of Nopp140 and very limited autophagy (Figure 6). Figure 6 does not show autophagic vesicles clearly labeled, but it does show mCherry labeling much more diffusely. In other words, mCherry-ATG8a was present but not found in autophagic vesicles. The lack of autophagic vesicles demonstrates the lack of autophagy.

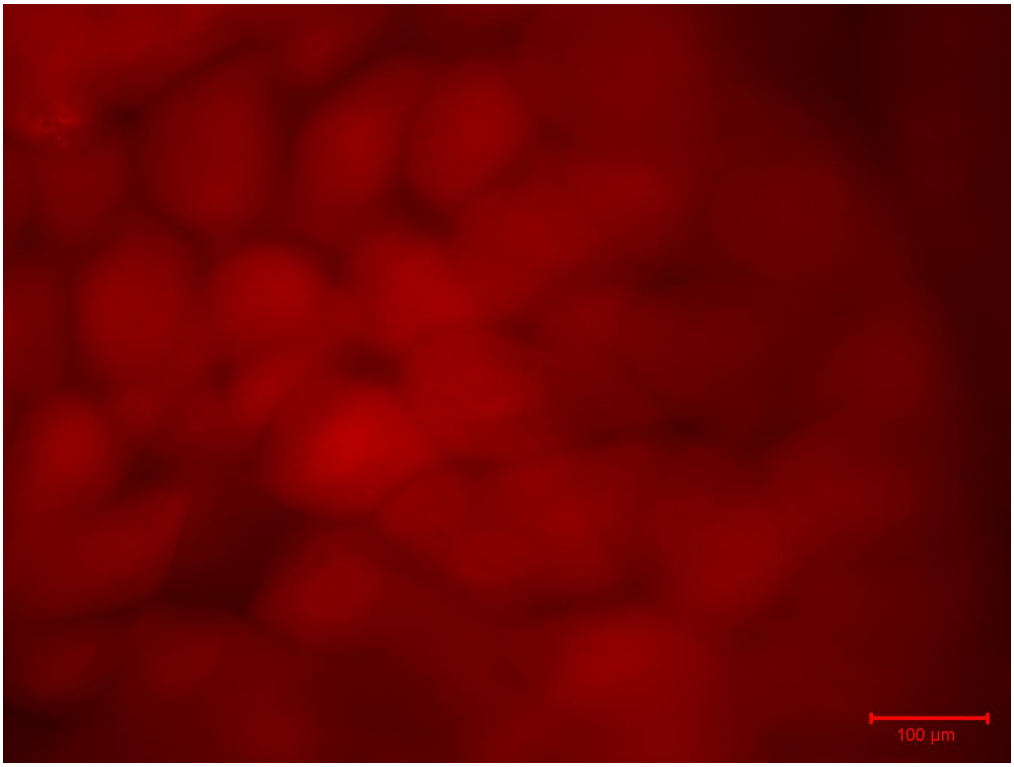


Figure 6a. Control lacking the TdsCom. C4 gene. No Autophagosomes labeled. Picture taken at 40x magnification.
Figure 6b. DAPI labeling DNA in the control flies. Picture taken at 40x magnification.

Discussion

The results show that apoptosis occurred in the absence of the p53 gene but in the presence of activated caspases, as detected by the Caspase-3 antibody. Other evidence suggests that although this is p53-independent apoptosis, it still uses many of the same downstream p53-dependent components, such as *reaper, hid,* and *grim* (Wichmann et al, 2006). p53 is an important gene because it is the one most commonly mutated in human cancers (Wichmann et al, 2006). When p53 is mutated, it can no longer stimulate p53-dependent apoptosis in tumor cells. Therefore, finding the p53-independent pathway for apoptosis could lead to future drug therapy in humans (Wichmann et al, 2006). This thesis only proved that p53-independent apoptosis could occur. Investigating the pathways of this p53-independent apoptosis is ongoing research in the DiMario lab.

Apoptosis was only one aspect of this thesis project. Autophagy as a result of RNAi knockdown of Nopp140 was the other. To test for autophagy, the *daughterless*-GAL4 transgene was used to drive the RNAi-expressing transgene, TdsCom.C4. All of the progeny from this experiment experienced lethality, signaling an effective knockdown of Nopp140.

Nopp140 is important because it is the *Drosophila* homologue to the human treacle (Cui and DiMario, 2007). Mutations in the human treacle can lead to Treacher Collins Syndrome (TCS) (Cui and DiMario, 2007). TCS is characterized by craniofacial deformities including underdevelopment of the facial bones and outer ear (Cui and DiMario, 2007). Cui and DiMario (2007) demonstrated that some flies with RNAi knockdown of Nopp140 have *Minute* phenotypes that are similar to human TCS. The cause of TCS is the loss of neural crest cells in the early embryonic development by

apoptosis. The cells normally give rise to the craniofacial structures that are deformed in TCS.

Autophagy and apoptosis are two methods of programmed cell death that have always been thought to be two non-related processes. However, recent discoveries may change this perspective. Autophagy and apoptosis may both involve some common upstream triggers (Maiuri et al, 2007). p53 has been proven to be an inducer of apoptosis, but it may also play a role in mTOR inhibition leading to autophagy (Maiuri et al, 2007). It is unknown how a cell decides between apoptosis and autophagy, but one hypothesis is that the initiating stimulus might play a role (Mairui et al, 2007). Discovering the interactions between apoptosis and autophagy can lead to better understanding of the mechanisms involved. The working hypothesis that this Honors Thesis supports is that imaginal disc cells are capable of apoptosis but not autophagy, while polyploidy gut cells opt for autophagy.

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