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The Role of MMP2 in *Drosophila melanogaster* Fat Body Remodeling

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A Paper Presented to the Faculty of Mount Holyoke College in
Partial Fulfillment of the Requirements for the
Degree of Bachelor of Arts with Honor
Department of Biological Sciences
South Hadley, MA 01075
May, 2014

This paper was prepared
under the direction of
Professor Craig Woodard
for eight credits.

To my family

ACKNOWLEDGEMENTS

Working in Carr 116 has been the backbone of my college experience. Being offered the opportunity to be one of five Science Scholars in my class, and participate in a lab internship experience the summer before first year, was a deciding factor in my choice to matriculate to Mount Holyoke. I left Texas and essentially began my college career in the lab. My experience in the lab began as one of wonder and curiosity, and has developed into a passion for research. The routine of lab work kept me busy, but it was also a source of distraction during hectic semesters and homesickness.

I wouldn't have stuck with it if I didn't feel encouraged the entire time by Professor Craig Woodard. He not only advised my research, but also my class schedules. He was understanding and motivated me to keep working as hard as I could. I appreciated that he could see that my heart was in my lab work, even when I was not getting results. I will always be thankful for his support.

Thank you to my first lab mate Liz Perez. We spent many hour collecting virgin flies together and collecting prepupae for dissection. A big thanks to Giulia Notarangelo, who kept me company in the lab my senior year as she worked on her own project. It was great having a friend in lab who could relate to the excitement, anxiety, and frustration experienced throughout the honors thesis process. My other friends in lab, Jackie, Jasmine, and Beza also made the long hours more bearable.

Finally, I would like to thank my mom, my sister, and my best friend for constantly reminding me why I chose to go to Mount Holyoke,. It was hard to be almost 2,000 miles away from home and their company for most of the year, but they helped me keep my eye on the prize. Christian kept my spirits up when I was overwhelmed by my schedule, and when I was homesick. Our many texts, phone calls, skype calls, and face time sessions over four years were an enormous part of my college experience. When I had doubts, these three people reminded me where I came from and how far I was going.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Matrix metalloproteinases are enzymes involved in important tissue remodeling mechanisms in many animal systems, including mammalian. They are required for scar resorption during wound healing, and are believed to also influence inflammation and re-epithelialization. MMPs work by loosening ECM contacts between cells at the wound edge, allowing uninjured cells behind the edge to proliferate and cover the damaged tissue (Gill and Parks, 2008). The proteases also take part in metastatic activity of tumor cells because they degrade the ECM of tumor cells, allowing them to detach and migrate to other parts of the body (Sato et al, 2005). In *Drosophila*, Matrix metalloproteinase 2 (MMP2) plays a vital role in tissue remodeling and programmed cell-death during metamorphosis (Page-McCaw, 2008).

The larval fat body of *Drosophila* develops in the larva during the beginning stages of its life. This organ stores nutrients that power the animal through the non-feeding periods of its life, including metamorphosis. Metamorphosis is triggered by a pulse of 20-hydroxyecdysone (20E), which induces pupariation. 20E controls expression of dBlimp-1, a rapidly degrading protein that transcriptionally represses *ßftz-f1*. As the 20E titer declines, dBlimp-1 is degraded and the ßFTZ-F1 transcription factor is expressed (Agawa et al., 2007). ßFTZ-F1 functions as a nuclear receptor and confers competence upon tissues to be able to respond to a second pulse of 20E. *MMP2* is expressed during the second pulse of 20E, and cleaves proteins in the ECM, enabling fat body cells to migrate. This second pulse induces the prepupal to pupal transition (Woodard et al., 1994). It has been shown that *ßftz-f1* is necessary and sufficient to induce fat body remodeling in the presence of 20E. Without *MMP*2, the larval fat body fails to dissociate, and the transition does not occur normally (Bond, 2011).

I am examining the regulation of *MMP2* expression in larval fat body remodeling in *D. melanogaster*. I am testing the hypothesis that *ßftz-f1* is necessary for the induction of *MMP2* transcription by 20E in the late prepupa. I used transgenic flies to overexpress *dBlimp-1* in the larval fat body, and examined the expression of MMP2 in the transgenic fat body compared to controls.

CHAPTER 1: INTRODUCTION

Matrix metalloproteinases are enzymes involved in important tissue remodeling mechanisms in many animal systems, including mammalian. MMP activity is strictly regulated by transcription, interaction with certain components of the extracellular matrix (ECM), and endogenous inhibitors (Sato et al., 2005). They are required for scar resorption during wound healing, and are believed to also influence inflammation and re-epithelialization. MMPs work by loosening ECM contacts between cells at the wound edge, allowing uninjured cells behind the edge to proliferate and cover the damaged tissue (Gill and Parks, 2008). The proteases also take part in metastatic activity of tumor cells because they degrade the ECM of tumor cells, allowing them to detach and migrate to other parts of the body (Sato et al., 2005).

The MMP family is evolutionarily conserved among multicellular organisms, including plants and animals. *Drosophila melanogaster* is a main model organism in research exploring the complex physiological and developmental functions of MMPs (Page-McCaw, 2007). *Drosophila melanogaster*, the common fruit fly, has been studied extensively by biologists for more than 100 years, serving as a model organism for the analysis of genetics and development. It has remained a choice model organism due to the many similarities it shares with humans and other vertebrates. As a holometabolous insect, the fruit fly undergoes metamorphosis. This process causes the organism to undergo dramatic changes in morphology as it grows from egg to adult. The

development of *Drosophila* is regulated and mediated by numerous signaling pathways, many of which are homologous to those in vertebrate systems. Each stage in development is directed by interacting processes and molecular mechanisms.

The larval fat body of the fly is one of the larval organs that undergoes dramatic changes during metamorphosis. The larval fat body serves as a source of energy and nutrient storage, which supports the prepupa and pupa through metamorphosis. Its dissociated cells persist in the animal even after eclosion, the point at which the adult fly emerges from its pupal case. Its presence in the adult allows resistance to starvation. Understanding the mechanisms involved in the remodeling of the larval fat body organ in *Drosophila* can help expand the understanding of tissue remodeling in higher organisms.

CHAPTER 2: *DROSOPHILA* AS A MODEL ORGANISM

At the beginning of the twentieth century, *Drosophila* was identified as a valuable organism in genetics research, largely through the pioneering work of Thomas Hunt Morgan. Since then the use of *Drosophila melanogaster* as a model organism has increased and expanded outside of the field of genetics to include other areas of medical and scientific research. As in all vertebrate systems, *Drosophila* essential cell biology such as the regulation of gene expression, cell death, and membrane trafficking are conserved (Stephenson and Metcalfe, 2013).

Drosophila became a popular research organism because it is not only less expensive but also faster at breeding than other popular lab organisms, such as mice and guinea pigs (Stephenson and Metcalfe, 2013). The fruit fly develops from an egg to an adult fly in approximately 10 days at 25º Celsius. The adult female becomes sexually mature about 8 to 10 hours after eclosion. This allows for a short generation time. However, the time from egg to adult is dependent on temperature and the generation time will be faster with a higher temperature. Fast generation time is an efficient way for researchers to observe changes in organisms over their short life-span, and also observe the changes across many generations in a short period of time.

CHAPTER 3: FAT BODY AND TISSUE REMODELING

A key feature of the *Drosophila* life cycle is that there are a number of distinct developmental stages the fly goes from egg to adult. The developmental stages include the embryonic, larval, the non-feeding pupal, and the adult stages. During the larval period, the organism can be classified as one of three instars. The first two larval instars (stages) are almost solely characterized by the larva eating food and growing. Throughout the final three days of larval development, as a third instar larva, the animal increases in mass by 200-fold (Figure 1) (Aguila et al., 2007). The fat body is a single-cell-layer thick tissue that functions as the source of energy supporting the fly during the non-feeding period, before, during and after metamorphosis.

At the end of the third instar larval stage, the larva emerges from the food source and enters the wandering state during which it searches for an attachment location. About 24 hours after the animal enters the wandering state, the larva attaches and becomes a motionless prepupa. This event marks the onset of puparium formation (pupariation), and the beginning of metamorphosis. During metamorphosis, the previously single-cell-layer, sheet-like larval fat body begins to dissociate. The tissue comes apart as the polygonal cells become spherical, individual, floating cells (Bond et al., 2011). This dissociation is referred to as fat body remodeling. Fat body remodeling is regulated by hormone signals that must control the programmed cell death (PCD), which removes other larval organs

such as the salivary glands and the midgut. As the fat body is remodeled, adult tissues grow and differentiate (Liu et al., 2009).

The morphology of the larval fat body changes dramatically during early metamorphosis. This process can be categorized in three parts: retraction, disaggregation, and detachment. The retraction phase takes place at 4 to 6 hours after puparium formation(APF), during which there is a shift or retraction of fat body away from the anterior region of the prepupa. At 6 hours APF, the fat cells begin to change from their polygonal, attached, sheet-like form into individual spherical cells. They then dissociate from one another in what is referred to as the disaggregation phase. During the detachment phase, the anterior fat body cells translocate into the head capsule (Nelliot et al., 2006). This time period precedes pupal ecdysis (Figure 5) (Bond et al., 2011). The puparium is composed of the larval cuticle, which separates from a newer underlying pupal cuticle during pupal ecdysis (Rewitz et al., 2006).

As mentioned, the fat body also serves as nutrient storage reservoir, which provides energy after metamorphosis for the adult fly. The larval fat body persists in the early adult fly for up to a period of 3 days (Aguila et al., 2013). Aguila et al. (2007) found that newly eclosed flies are more resistant to starvation than older flies, which no longer have larval fat body cells (Figure 2). Only after larval fat cells undergo cell death in the adult fly do sheets of adult fat body tissue begin to form. Like the larval fat body, the adult fat cells also function in energy storage for the fly (Aguila et al., 2007). However, adult fat cells arise from different cell

lineages, which are thought to be from the cells found in the larval body wall and from adepithelial cells connected with imaginal discs (Hoshizaki et al., 1995).

Drosophila fat body is considered an organ analogous to the adipose tissue and liver in vertebrates (Liu et al., 2009). *Drosophila* larval fat body is similar to the liver because the tissue not because it stores fat but because it is detoxifying and immune responsive. This has been shown by Musselman et al. (2013), who examined the response of fat body to high caloric intake in *Drosophila* and demonstrated the necessity of lipogenesis (the metabolic formation of fat) in animals for toleration of high sugar intake.

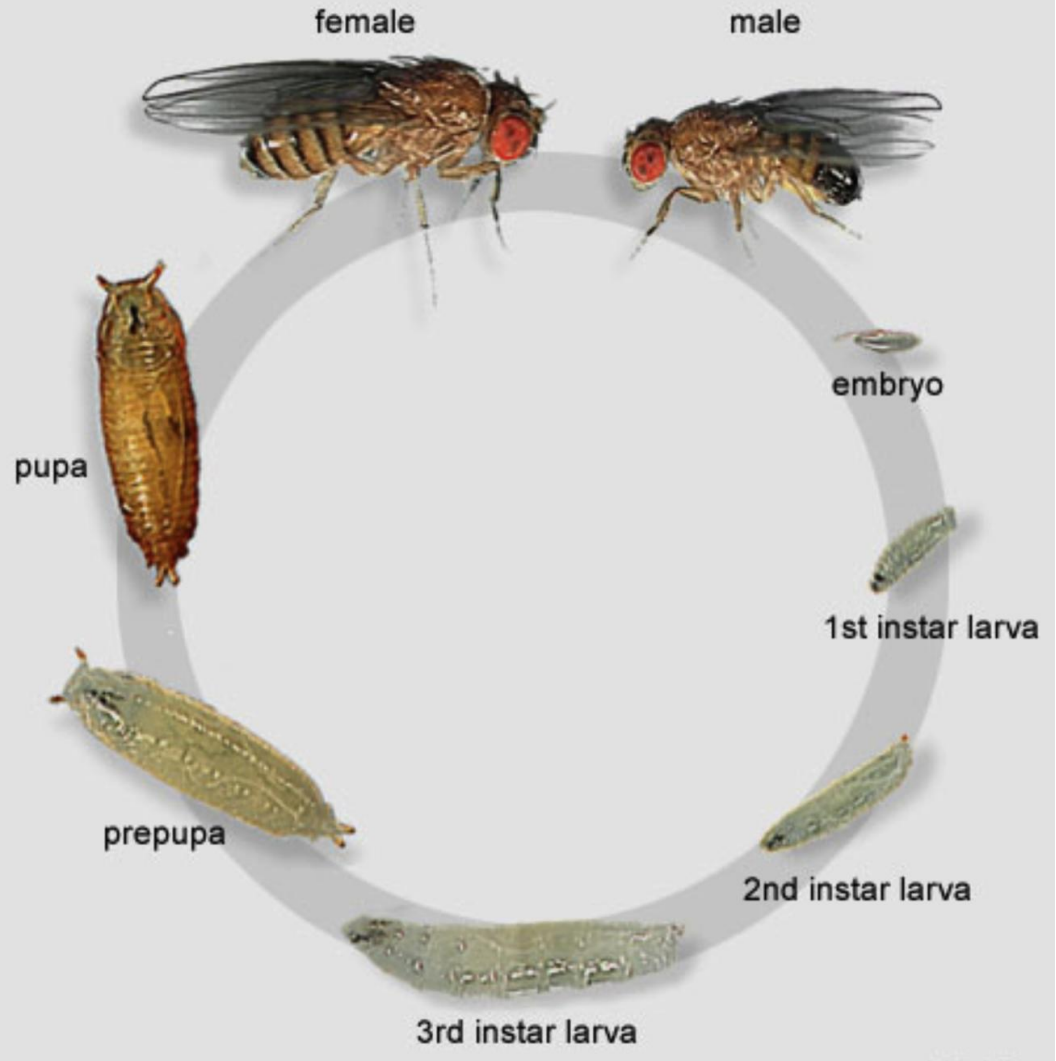

Figure 1. The life cycle of *Drosophila melanogaster*. The embryonic stage is complete after 24 hours. The first two instar larval stages are characterized by feeding and growing, followed by a molting process (all completed in one day). The third instar larval stage is completed in two days. The large larva searches for a location to pupariate. After 12 hour of pupation, the organism is a pupa. The organism becomes a full adult fly at 9 days. Taken from Weigmann et al. (2003).

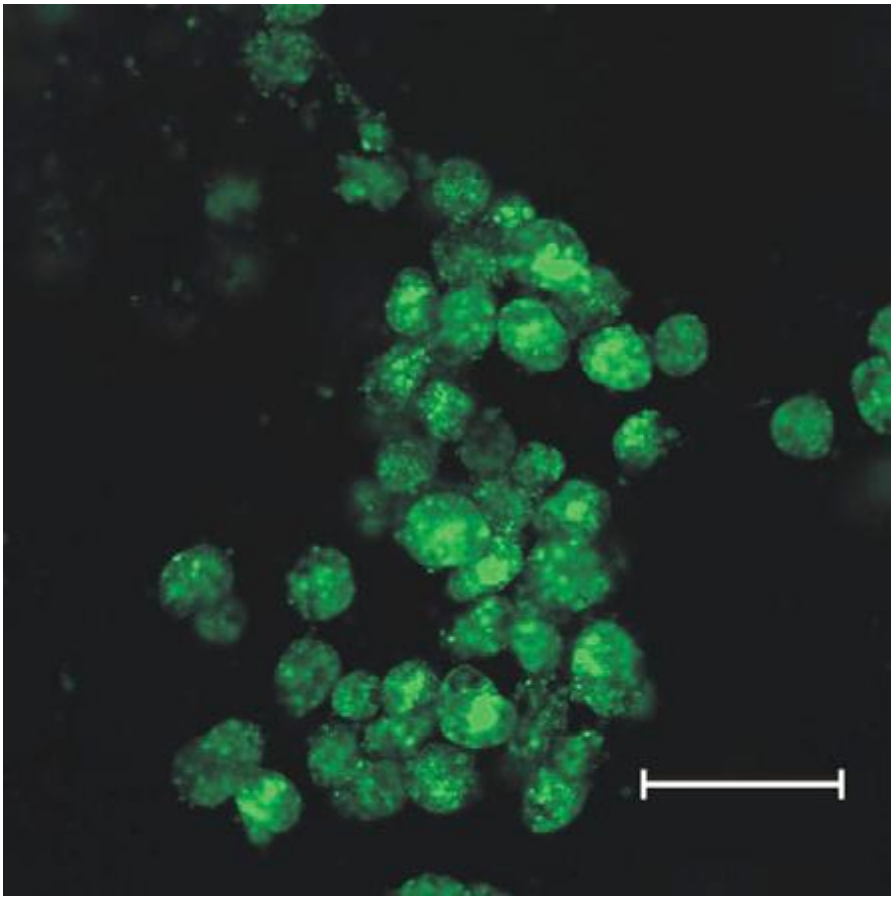

Figure 2. Free-floating larval fat body cells in adult *Drosophila*. The fat body of the larva persists in the adult for up to three days and are different than the adult fat body. The larval fat cells are labeled with a polytene chromosome GFP marker. Scale bar, 200 µm. Taken from Aguila et al. (2007).

CHAPTER 4: INSULIN SIGNALING

Drosophila development serves as model for investigating the relationships between development, division, and death. Ecdysone signaling, insulin and insulin-like growth factor signaling, and autophagy are regulators of these cellular processes (Tracy and Baehrecke, 2013). Growth is regulated by acquired nutrient levels. The larva must eat continuously in order to acquire the required nutrients to increase in size and reach the next stage in development. Growing to a certain size improves both the chances of surviving metamorphosis, and becoming a reproductively successful adult fly. The organisms must reach three checkpoints when increasing in size. The first checkpoint determines whether the larva will move forward in development and molt, and is called the threshold size (Zhou et al., 2004). Secondly, the animal acquires the body mass required for the completion of larval and pupal development during nonfeeding periods, also called minimal viable weight (Bakker, 1959). The next checkpoint, critical size, occurs at the third instar larval stage and determines whether the critical weight of the animal is sufficient for pupation. The third instar stage is prolonged if the critical size of the larva is insufficient (Nijhout, 2003). This occurs via a delay in ecdysone pulses. this checkpoint can still be reached if the nutrient supply increases. Additionally, animals can continue to develop their nutrient supply and increase body mass if nutrients are still abundant after the critical size checkpoint (Tenneson and Thummel, 2011).

The larval fat body is the organ holding most of the nutrients in the larval body (like mammalian adipose tissue), but it can also function like the mammalian liver by processing sugars and nutrients. It is a sensing organ that responds to nutritional signals, and regulates nutrients through the insulin pathway. *Drosophila* regulate their sugar levels and store glycogen and lipids for energy use. When the animal experiences starvation, the fat body can release nutrients to support the vital tissues. The insulin pathway is regulated by the insulin/insulin-like growth factor signaling pathway (IIS), the phosphatidylinositol 3-kinase signaling pathway (PI3K) and the target of rapamycin signaling pathway (TOR). *Drosophila* insulin-like peptides (dILPs) function as insulin-like growth factors (IGFs), and are major players located upstream in the insulin pathway. *Drosohila* has an insulin receptor (InR) with two subunits which bind the ligand, and two transmembrane ß-subunits, which both contain a tyrosine kinase domain (Brogiolo et al., 2001). dILPs are ligands for the InR and constitute a family of seven peptides, which are structurally similar, but are expressed in different tissues and have differential functions (Colombiani et al., 2003; Teleman, 2010). Three dILP genes (2,3, and 5) are expressed in seven neurosecretory cells in the larval brain, suggesting their role in endocrine function (Brogiolo et al., 2001). dILP2 secretion is the major target of nutrient-dependent fat body signaling (Géminard et al., 2009).

Once the InR binds to the dILPs ligand, the InR promotes the phosphorylation of the insulin receptor substrate complex (IRS). Phosphorylation

of the IRS activates additional proteins in the insulin pathway (Taguchi and White, 2008). The PI3K pathway is regulated by the InRs and are involved in cell growth management. The pathway coordinates the growth of the larva according to nutritional conditions. Increased starvation resistance occurs by repressing the PI3K pathway. Additionally, increasing PI3K activity results in enhanced cell growth and nutrient storage, even during starvation (Britton et al., 2002).

Cell growth in *Drosophila* is also mediated by TOR-signaling (target of rapamycin), which works in conjunction with autophagy. TOR is a conserved kinase, and has been known to be cell autonomously required for growth in a multicellular organism. TOR signaling interacts with PI3K signaling. The deactivation of the TOR signaling pathway inhibits cell growth and halts the cell cycle (Hennig et al., 2006). *Drosophila* with mutations in the TOR homolog (*dTOR*) exhibit cellular and physiological responses suggestive of amino acid deprivation (Colombiani et al., 2004). During periods of sufficient nutrition, autophagy is normally suppressed by TOR and the nutrients are kept stored in the fat body. If experiencing starvation, TOR is down-regulated and autophagy of the fat body occurs, allowing the release of nutrients (Rusten et al., 2004).

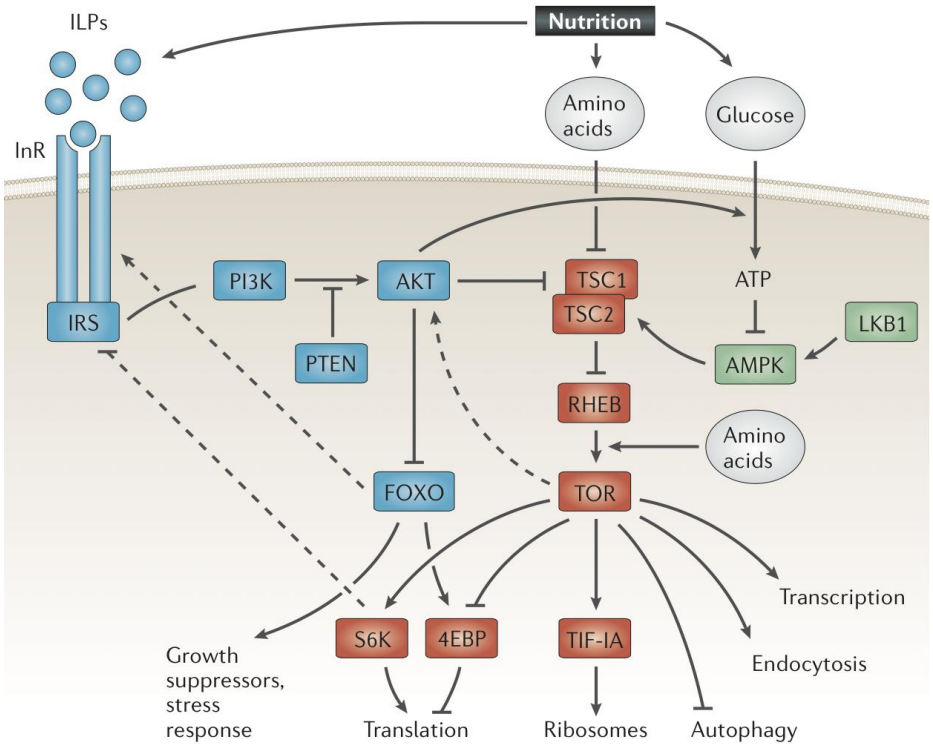

Figure 3. *Drosophila* insulin signaling pathways. *Drosophila* ILPs binds to the InR, prompting a signaling cascade. PI3K signaling is activated downstream, encouraging cell growth. The TOR signaling pathway interacts with the PI3K pathway, and regulates fat body autophagy in the presence of nutritional stimuli. Taken from Edgar (2006).

CHAPTER 5: THE ROLE OF 20E AND ßFTZ-F1 IN *DROSOPHILA* DEVELOPMENT

The distinct developmental processes oogenesis, embryogenesis, molting, and metamorphosis are temporally regulated by mechanisms initiated by both the steroid hormone ecdysone and its active form 20-hydroecdysone (20E) (from now on collectively referred to as ecdysone) (Agawa et al., 2007). There are pulses of 20E throughout the development of the fly that vary in concentration and duration (Figure 3). The amplitude and duration of the pulses depend on the rate at which the hormone ecdysone is converted to into 20E and how quickly it is degraded (Moeller et al., 2013). The hormone regulated onset of gene expression corresponds to developmental stages in insects that undergo metamorphosis. This makes holometabolous insects an invaluable model for studying hormone function in relation to the ecdysone response (Figure 2).

Ecdysone is the major steroid hormone in all holometabolus insects responsible for coordinating the juvenile-adult transition. Steroid hormones function as powerful regulators of diverse biological processes, including homeostasis, embryonic development, cellular differentiation and detoxification (King-Jones and Thummel, 2005). All steroids share a distinctive core structure of four fused rings. Steroid hormones are derived from cholesterol in the adrenal cortex and gonads. Hormones are released in the circulatory system, and travel bound to specific carrier proteins to target cells in peripheral parts of the body, driving changes in gene expression. Because of their hydrophobicity, steroid

hormones can passively diffuse through the cell membrane and bind to specific cytosolic receptors. After binding, the steroid-receptor complex becomes activated and translocates to the nucleus, where it interacts with specific chromosomal enhancers and exert positive or negative effects on the expression of target genes (Falkenstein, 2000). For ecdysone in particular, the pulse critical to *Drosophila* transition is determined by a feedback loop in the prothoracic gland (PG), the main steroid-producing tissue in insect larvae. Ecdysone has been shown to have a positive feedback effect on the PG, and amplifies its own production, which induces pupariation. A negative-feedback effect occurs during the prepupal stage, which keeps ecdysone levels lower and the fly development moving forward (Moeller et al., 2013).

There are two ecdysone pulses during the first half-day of metamorphosis. The first occurs during the late third-instar larval stage, and it initiates pupariation and the onset of metamorphosis (Agawa et al., 2007). As this pulse declines, mid-prepupal genes are activated. The transcription factor ßFTZ-F1 is encoded by one of these mid-prepupal genes, and functions as a nuclear receptor that confers competence upon target genes to be able to respond to a second ecdysone pulse. This second pulse induces the prepupal to pupal transition, marked by head eversion, roughly 10-12 hours after puparium formation (APF), (Woodard et al. 1994). The prepupal to pupal transition is accomplished by the induction of necessary transcriptional cascades by ßFTZ-F1and the second ecdysone pulse (Bond et al., 2011) (Figure 4).

ßftz-f1 transcription occurs only after the first ecdysone pulse declines. Temporally controlled expression of *ßftz-f1* is essential to its role as a competence factor, and its only appears upon a decrease in the ecdysone titer. This is partially due to *ßftz-f1* expression induction by the ecdysone-induced proteins DHR3 and DHR4 upon the initiation of *Drosophila* metamorphosis. *DHR3* is an orphan nuclear receptor gene whose protein product binds to the *ßftz-f1* promoter and activates transcription of the *ßftz-f1* gene (Lam et al., 1997). The ability of DHR3 to act on the *ßftz-f1* promoter region is repressed by the E75B early gene product when E75B binds DHR3 (White et al., 1997). The DHR4 orphan nuclear receptor must also be present to achieve maximum levels of *ßftz-f1*, because like DHR3 it activates the *ßftz-f1* gene. The disappearance of the repressor *Drosophila* homolog of mammalian B lymphocyte-induced maturation protein-1 (dBlimp-1) is also involved in the molecular mechanism controlling *ßftz-f1* expression. dBlimp-1 is a product of a gene that is induced by ecdysone, and aids in the regulation of *ßftz-f1* expression. Once expressed after the first ecdysone pulse, dBlimp-1 directly binds to the *ßftz-f1* promoter region, acting as a transcriptional repressor (Agawa et al., 2007). dBlimp-1 is a rapidly degrading protein, which helps to provide close regulation of *ßftz-f1* expression. As follows, the transcriptional program is modified by the ßFTZ-F1 protein present after the second ecdysone pulse to moderate the genetic events which occur during pupal development (Bond et al., 2011).

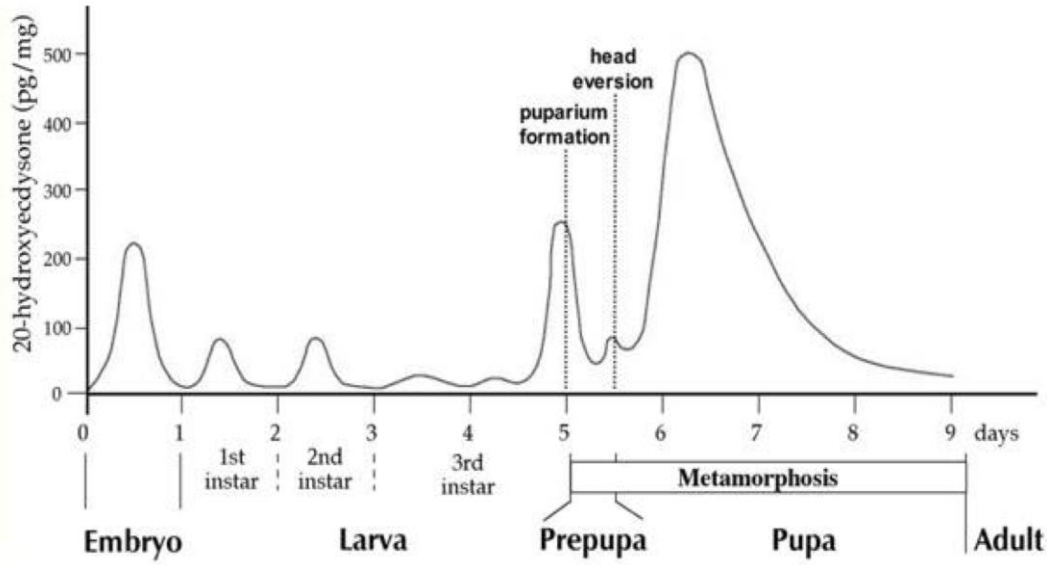

Figure 4. Ecdysone pulses throughout the *Drosophila* life cycle. Each instar in the larval stages is correlated to a rise in ecdysone titer. The rise in ecdysone after the third instar larval stage precedes puparium formation and the onset of metamorphosis. The next rise in ecdysone titer occurs at 10 hours APF and leads to head eversion. The final rise in ecdysone occurs around 30 hours APF and ends with the full development of the adult fly. Taken from Riddiford (1993).

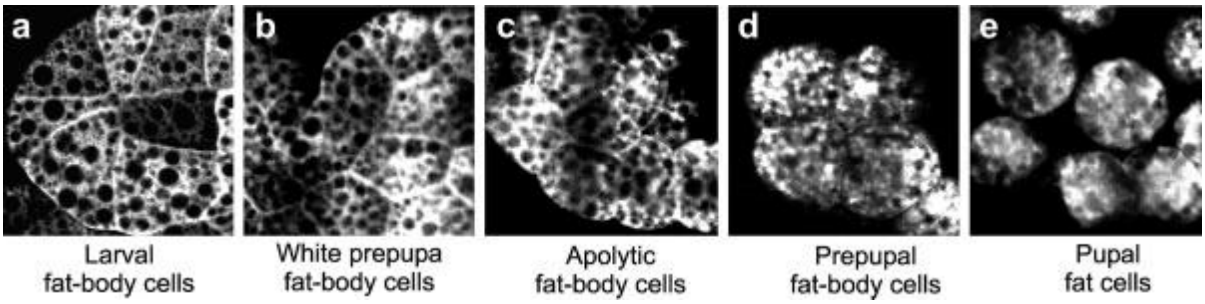

Figure 5. *Drosophila* fat-body cell changes during remodeling. As the fat body cells change shape, they dissociate from one another. They are flat and polygonal and transform into individual spherical cells as the animal goes from larva to pupa. From Nelliot et al. (2006).

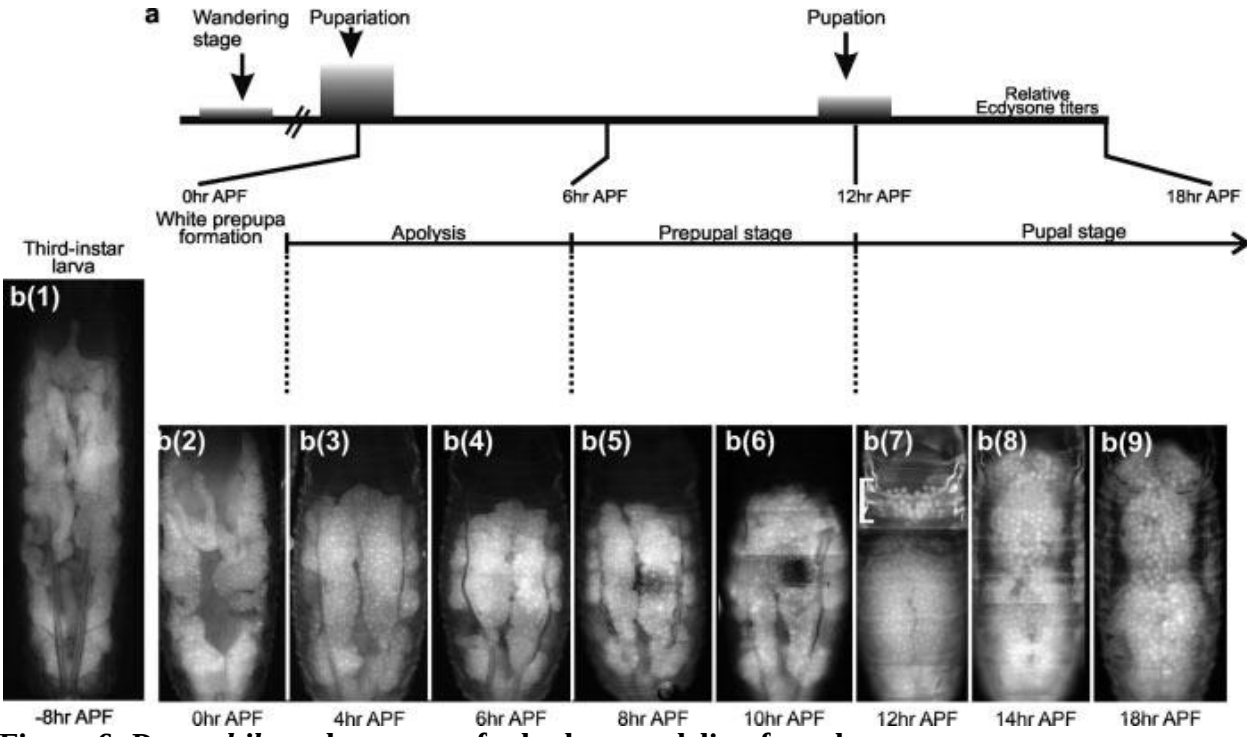

Figure 6. *Drosophila* melanogaster fat body remodeling from larva to pupa. The retraction phase occurs at 4 to 6 hours APF, and is characterized by the shift of fat body away from the anterior region of the prepupa. Afterwards, the fat body cells begin to change into individual spherical cells in what is referred to as the disaggregation phase (6 hours APF). The detachment phase occurs once the animal reaches prepupal development the fat body cells migrate into the head capsule. Taken from Nelliot et al. (2006).

CHAPTER 6: THE ROLE OF MMP2 IN TISSUE REMODELING

Larval fat body remodeling involves the breakdown of the extracellular matrix (ECM) which regularly maintains the integrity of tissue. This allows the remodeled cells to detach and migrate. The degradation of the ECM is catalyzed by matrix metalloproteinases (MMPs), a specialized class of ZN^{2+}- and Ca^{2+}-dependent endopeptidases, involved in the remodeling of tissues in both mammals and *Drosophila* (Bond et al., 2011; Page-McCaw et al., 2003). MMPs cleave substrates in the ECM allowing cells to detach and mobilize. *D. melanogaster* has two MMP homologs, *MMP1* and *MMP2*. Both are required for different components of tissue remodeling and programmed cell-death during metamorphosis. Both MMPs have the canonical MMP domain structure, which includes a signal sequence, prodomain, catalytic domain, hinge, and hemopexin domain (Figure 6). Despite this structure, they are not orthologous to the MMPs with the same name in mammals. There are actually no MMP orthologs found between vertebrates and *Drosophila* (Page-McCaw, 2008).

MMP1 is a secreted protein while MMP2 is membrane bound and has a GPI anchor. *MMP1* expression coincides with the first ecdysone pulse, the pulse for pupariation, in the salivary glands. This had lead researchers to suggest MMP1s role in the destruction of the salivary glands during metamorphosis (Bond et al., 2011). MMP1 and MMP2 expression coincides with the larval fat body detachment phase in metamorphosis. Both MMPs have roles in tissue remodeling and PCD in the larval tissue, with MMP1 closely associated with

tracheal remodeling in the larva, and MMP2 with PCD of the midgut (Page-McCaw et al., 2003).

TIMPs (tissue inhibitor of metalloproteases) are a family of proteins that inhibit MMP catalytic activity by occupying the active site of the MMP proteinase. Mutants over-expressing *TIMP* (and therefore inhibiting MMP1 and MMP2) have been shown to have defects in fat body remodeling and pupal ecdysis, with arrested head eversion (Bond et al., 2011). Animals with a misexpression of *TIMP* failed to undergo the disaggregation stage of larval fat body remodeling, and resulted in death. This suggests the importance of MMPs in fat body remodeling (Figure 8). Further experimentation by Bond et al. (2011) shows that *MMP1* mutants have normal disaggregation of fat, but the fat body of *MMP2* mutant animals shows a failure to disaggregate (Figure 7). The data suggested that MMP2, specifically, is required in fat body remodeling. Inspired by the results of Bond et al. (2011), I hypothesize that *ßftz-f1* is necessary for the induction of *MMP2* expression by the steroid hormone, 20-hydroxyecdysone (20E). To test my hypothesis, I used the transgenic genotype *cg-Gal4;UAS-dBlimp-1* to over-express d-Blimp-1 in the larval fat body. d-Blimp-1 transcriptionally represses *ßftz-f1* expression at 6 hours APF. I expect to see reduced *MMP2* expression at 10 and 12 hours APF in the transgenic prepupa.

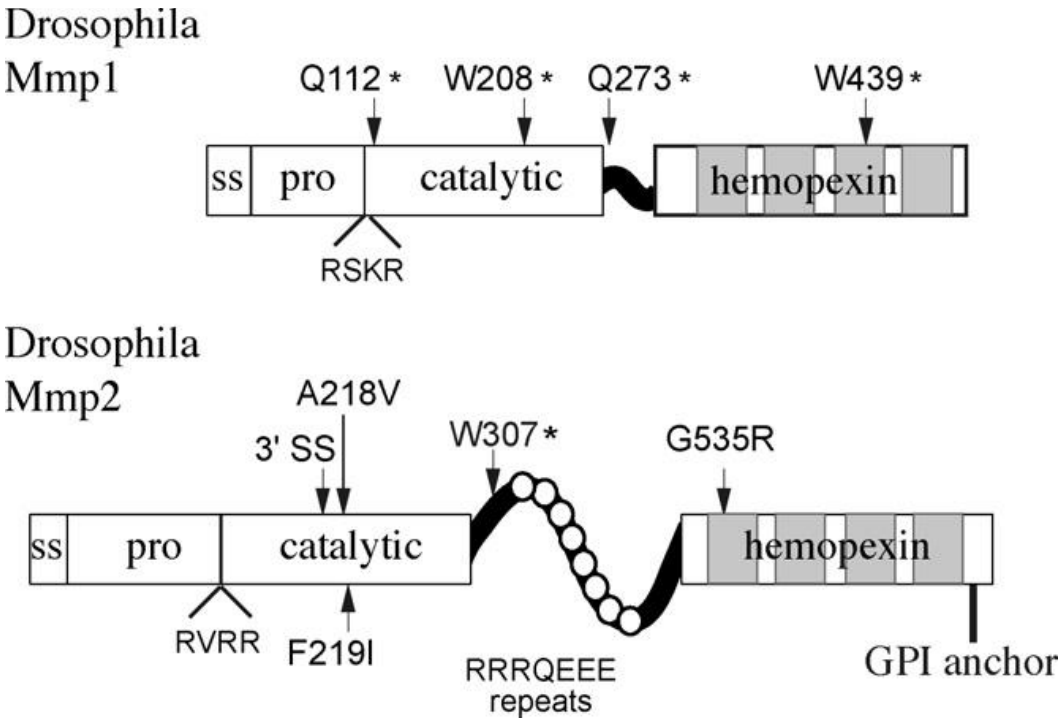

Figure 7. Matrix metalloproteinase structure in *Drosophila melanogaster*. Domain organization for MMP1 and MMP2 can be seen, each containing a signal sequence (SS), pro domain (pro), catalytic domain, and hemopexin domain. The MMP2 is shown with unusual repeats in its hinge domain (RRRQEEE). Taken from Page-McCaw (2008).

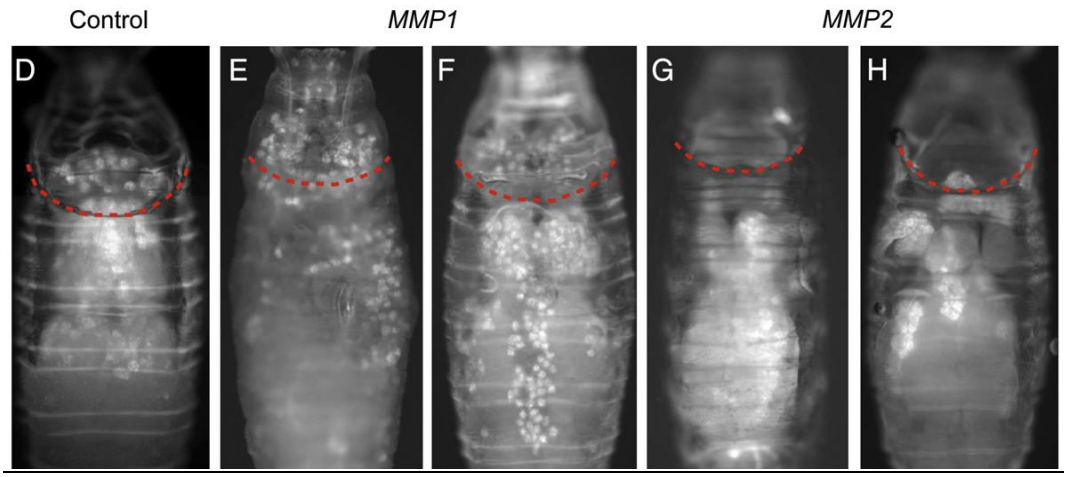

Figure 8. MMP2 is necessary and sufficient in induction of larval fat body remodeling of *Drosophila melanogaster* metamorphosis. Fat body remodeling occurred normally in the control animal (D). Fat body remodeling occurs in the *MMP1* mutants, and fat cells are translocated to the head capsule, and disaggregation of the fat cells is detected (E-F). *MMP2* mutants result in failure to disassociate, and there is no translocation of fat cells to the head capsule (G-H). Taken from Bond et al. (2011).

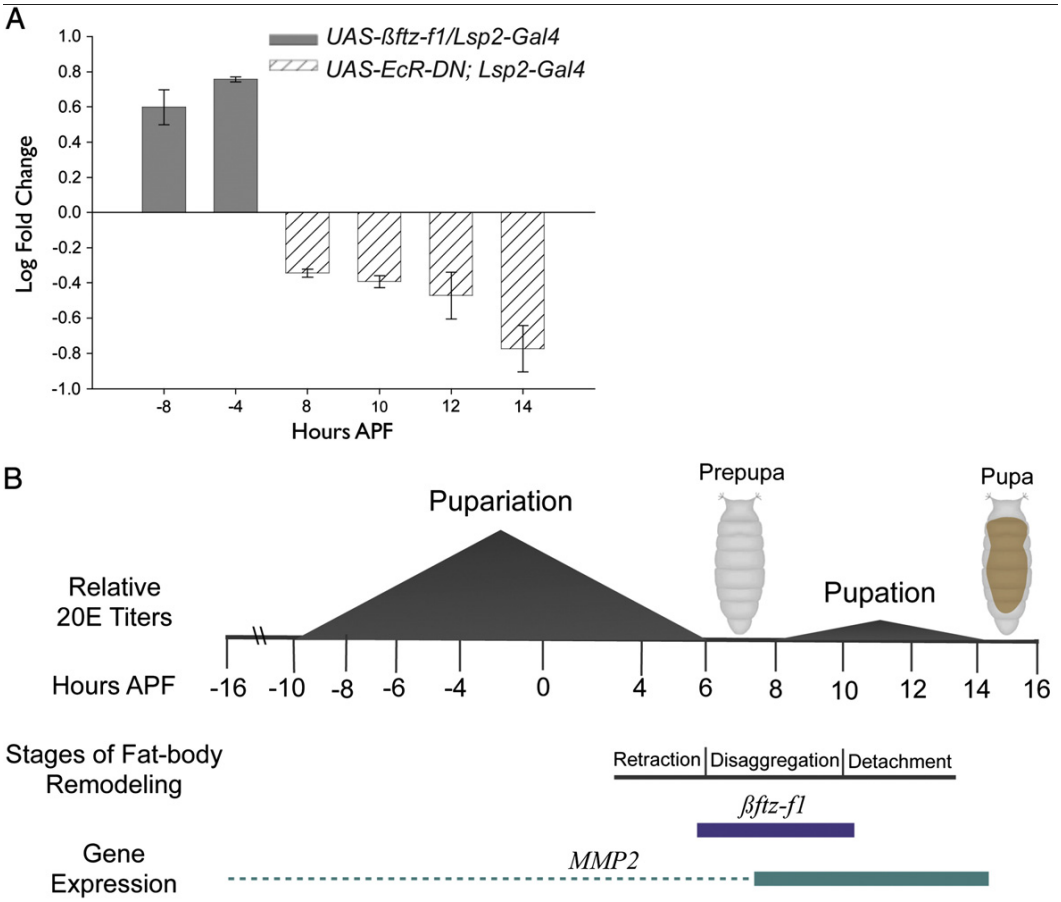

Figure 9. MMP2 expression in the fat body coincides with ecdysone signaling and ßftz-f1. (A) Log fold change in *MMP2* expression in fat body. Relative expression of *MMP2* in fat body expressing *UAS-EcR-DN*, *UAS-GFP; Lsp-Gal4* or *UAS-GFP; Lsp2-Gal4/UAS-ßftz-f1* was compared to *w1118* expression levels at several time points. MMP2 expression was down-regulated from 8 hours APF through 14 hours APF in fat bodies expressing EcR-DN, supporting the hypothesis that *MMP2* expression in the fat body is regulated via ecdysone signaling. (B) This is a model for the signaling cascade in fat-body remodeling. The figure shows ecdysone titers above hours APF, and corresponding stages of fat body remodeling. *ßftz-f1* is expressed from 6 hours to 10 hours APF. The model shows that expression of *ßftz-f1* and ecdysone signaling is required for fat-body remodeling. It also shows *MMP2* expression coinciding with ecdysone signaling in combination with prior expression of *ßftz-f1*. Taken from Bond et al. (2011).

MATERIALS AND METHODS

Drosophila Husbandry

The wild type (w^{1118}), *cg-Gal4*, and *UAS-dBlimp-1* stocks were kept at 25ºC during the collection stages. They were maintained in bottles with standard yeast medium with additional dry yeast. In this study, w^{1118} flies served as a wild-type control. For virgin collection of the *cg-Gal4* and *UAS-dBlimp-1* animals, the bottle was cleared and stored at 25ºC for up to 8 hours before collection, or stored at 18ºC for up to 18 hours until flies were sexed.

UAS-dBlimp-1 and *cg-Gal4* virgins were used to create the transgenic genotype *cg-Gal4; UAS-dBlimp-1*. About 20 virgin females of one genotype and 20 males of the other genotype were added to a new bottle to produce the transgenic progeny.

The following cross was performed:

UAS- dBlimp-1 x cg-Gal-4 → *cg-Gal4; UAS-dBlimp-1*

The *UAS/Gal4* system

The transgenic genotype for this experiment was derived using the *UAS-Gal4* system. Gal4 is a transcription factor from *Saccharomyces cerevisiae* (Duffy 2002). The Gal4 protein acts as a promoter inducer of the two sequences that constitute the Upstream Activation Sequences (UAS), *GAL1* and *GAL10*. Through binding, Gal4 activates transcription of the gene of interest adjacent to the UAS (Duffy 2002). Without the expression of Gal4 protein, the transcription of the adjacent gene will not be activated despite the presence of UAS (Duffy et

al., 2002). The activation of the UAS allowed the acquisition of the desired genotypes for this experiment. Hence, the *UAS* here regulates the ectopic expression of *dBlimp-1*.

Dissection of prepupae

The wild-type and transgenic prepupae were collected from their bottles, mounted on damp filter paper in a petri dish, and aged to either 10 or 12 hours APF (after puparium formation) at 25ºC. The time frame was chosen because it has been shown that *MMP2* expression peaks during this time in the pupation phase of *Drosophila* (Bond et al., 2011). For each time point, 5-8 pupae were dissected under a microscope using 1x phosphate buffered saline (PBS) and fat body was collected into a tube containing 30 µl of 1x PBS. 300 µl of Invitrogen TRIzol reagent (from Life Technologies), were added immediately after dissection was completed, and the fat body was ground with a homogenizer. Once the tissue was thoroughly homogenized, the samples were stored at -80ºC until RNA isolation was performed.

RNA Isolation

To begin total RNA isolation, the frozen TRIzol/ tissue samples were thawed and incubated at room temperature for a few minutes. Each sample was then transferred to a pre-spun Phase Lock Gel-Heavy 2ml microfuge tube. 60µl of chloroform was added to each tube before the tube was shaken vigorously by hand for 15 seconds. The samples were then centrifuged at 12,000 rpm for 10 minutes at 4ºC. The aqueous phase was then transferred by pipette into a clean

RNase-free tube, and 0.8 volumes (about 160µl for about 200µl of RNA product) of isopropanol added. The mixture was vortexed, and stored overnight for precipitation at -80ºC.

Once precipitated the samples were thawed and centrifuged at 13,400rpm for 30 minutes at 4ºC. The supernatant was removed before 500µl of RNase-free 75% ethanol was added to wash the pellet. The sample was then re-centrifuged at 13,400 rpm for 10 minutes, and the supernatant removed by pipette. The pellet was air dried for a minute, and 5µl of RNase-free water was added to resuspend and redissolve the pellet. Each tube was incubated at 55-60ºC for 10 minutes. The tubes were then flicked, vortexed, and incubated at room temperature for 5 minutes. At this point, the samples were quantified via a ThermoScientific NanoDrop 2000c spectrophotometer using 1µl of isolated RNA. The values evaluated were the absorbance curve, nucleic acid concentration, and OD_{260}/OD_{280} values. OD (optical density) values are used to measure the ratio of absorbance at 260 and 280 nm, in order to assess the purity of nucleic acids in the samples. Pure RNA yields OD_{260}/OD_{280} of ~2. The samples were then frozen and stored at -80ºC.

DNase treatment to remove contaminating genomic DNA

The Ambion DNA-free DNase treatment kit was used to remove any genomic DNA from the total isolated RNA sample. First, the frozen RNA-isolated sample was thawed, then 1µl of 10X DNase buffer and 1µl of rDNase-I were added for each 10µl of RNA sample. Then the sample was mixed by flicking

gently, and spun down. The samples were then incubated for 30 minutes at 37ºC. Next, DNase Inactivation Reagent was vortexed and 2µl was added to each sample. The tubes were mixed a few times as the sample incubated at room temperature for two minutes. After the incubation period, the samples were centrifuged at 10,000rpm for 90 seconds. The supernatant was then transferred to a fresh RNase-free tube.

The DNA-free procedure described above was repeated almost identically, but 1.5µl of 10X DNase buffer was added in the first step instead of 1µl. The samples were examined again using spectrophotometry. The values for absorbance curve, nucleic acid concentration, and OD_{260}/OD_{280} values were recorded.

First strand cDNA synthesis

cDNA polymerization was then be performed using the Invitrogen First-Strand Synthesis Kit for RT-PCR. For each vial of isolated RNA, both RT and No RT cDNA products were produced. No RT is a control set prepared without reverse transcriptase. This is another way to control for genomic DNA remaining in the sample. The No RT samples are used as the negative control in electrophoresis. The first master mix was made using 10 mM dNTP mix and oligo$(dT)_{12-18}$ primer with the ratio of 1µl of the component for 1 reaction, and 7µl of DEPC-treated water for 1 reaction. 9µl of the master mix was then put into RNA-free tubes with 1µl of the RNA sample. This mixture was incubated at 65ºC for 5 minutes, then moved to ice for 1 minute.

A second master mix was prepared for n+1 reactions, using 2µl of 10X RT buffer, 4µl of 25mM $MgCl_2$, 2µl of 0.1 M DTT, and 1µl of RNaseOUT for each reaction. 9µl of this master mix was added to each of the tubes containing RNA/ primer mixture. They were mixed gently, spun down, and incubated at 42ºC for 2 minutes. 1µl of SuperScript™ II RT was added to each tube (except the minus RT control, to which RNA-free water was added). The tubes were incubated at 42ºC for 50 minutes, followed by incubation was 70ºC for 15 minutes. The RNA/primer mixtures were chilled on ice and spun down briefly. Then 1µl of RNase H was added to each tube and they were incubated once more at 37ºC for 20 minutes. With the completion of this process, the reaction was ready for PCR.

Primers

Primers specific to *MMP2* are the same as used by Bond (2010). They were synthesized by Intergrated DNA Technologies (IDT), and designed from sequences from Flybase by using an IDT program, which can be found at www.idtdna.com.

Table 1. Sequences of the primers used in this experiment.

Gene	Primer ID	Sequence
MMP2	Forward	5'-AGCAATCCGGAGTCTCCAGTCTTT-3'
	Reverse	5'-TGGAGCCGATTTCGTGATACAGGT-3'
Actin 5C	Forward	5′-TCTACGAGGGTTATGCCCTT-3′
	Reverse	5′-GCACAGCTTCTCCTTGATGT-3′

The second primer is for *Actin 5C*, the endogenous control gene in this experiment.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Polymerase chain reactions were used to amplify a target gene sequence. A pipetting scheme for RT-PCR, shown in Table 2, was used to make a master mix for the cDNA products. The only product added to the individual mixtures instead of the master mix was *Taq* Polymerase.

Table 2. RT-PCR Reaction Mix. (Gendzhova, 2011)

Reagent	**Amount per reaction**	**Final concentration**
10X PCR Buffer-$MgCl_2$	5µl	1X
50mM $MgCl_2$	3µl	3mM
10mM dNTPs	1µl	200µM
10µM forward primer	2µl	400µM
10mµM reverse primer	2µl	400µM
cDNA	2µl	
Nuclease-free water	34.6µl	
Taq Polymerase	0.4µl	2 units
Final volume	50µl	

Once all of the reagents were added to the individual cDNA product tubes, they were placed in the thermocycler. The thermocycler was used to heat the reactions as described in the temperature profile (Table 3). After completion of cycling, the PCR products were stored at -20°C.

Table 3. Thermocycler profile for RT-PCR specific to *MMP2* and *Actin5c* primers.

Stage	**Temperature (**°C)	**Time**	**Cycle count**
Denaturation	94	30 seconds	35 cycles
Annealing	55	30 seconds	
Extension	72	30 seconds	
Final Extension	72	5 minutes	1 cycle
Final Hold	4	-	-

Gel Electrophoresis for visualizing RT-PCR

An agarose gel is used to visualize the PCR products. This was done using 1.6% agarose gels, consisting of 1x TAE and ethidium bromide (used as a fluorescent tag for viewing DNA under UV light). The eletrophoretic gels were run in 1x TAE at 150V, and the movement of the electric current from negative to positive allowed the DNA to from bands as they moved. This visualization is telling because the distance the product moves depends on the physical size of the DNA fragments. The bands were compared to the DNA ladder, or molecular weight marker, in order to estimate the size of the bands. After electrophoresis, the gels were imaged using the Fujifilm LAS-3000 Luminescent Image Analyzer.

RESULTS

Dissection of larval fat body

The dissection of larval fat body collection from w^{1118} at 10 and 12 hour APF was difficult due to the dissociation of the cells during this stage of metamorphosis. Although the fat body was not completely intact in the transgenic fly, it was noticeably easier to collect. Importantly, the fat body at 12 hours APF was more dissociated than at 10 hours APF. After the fat body was dissected, it was quantitated for nucleic acid concentration (Table 4). The quantitation describes the quality of the sample collected from dissection. This helps estimate the success of the steps following up to PCR.

Table 4. ***w*^{1118} and *Cg-Gal4/UAS-dBlimp-1* RNA isolated samples**

Genotype	Hour (APF)	Set	OD_{260}/OD_{280}	OD_{260}/OD_{280} (DNase Treated)	Nucleic Acid Concentration (ng/µl)	Nucleic Acid Concentration (DNase Treated)
Transgenic	8	1	2.07	1.96	2669.8	586.7
	8	2	1.82	1.92	213.9	230.6
	10	1	2.11	2.00	2798.6	740.3
	10	2	2.04	1.94	2559.3	656.1
	10	3	2.14	1.97	3835.2	1007.4
	10	4	2.01	1.97	1458.0	1111.1
	10	5	2.12	2.02	1983.3	1277.6
	10	6	2.02	1.93	903.6	672.4
	12	1	2.12	1.98	2946.7	687.9
	12	2	2.04	2.01	1434.3	447.1
	12	3	2.12	1.99	1978.7	576.1
	12	4	2.08	2.01	1649.3	510.5
	12	5	2.09	1.97	2184.7	686.0
	12	6	2.09	1.97	1908.6	529.4
	12	7	2.07	1.98	1372.4	418.5
	12	8	2.09	1.96	2021.5	658.7
	12	9	2.06	1.96	1962.6	556.2
w^{1118}	10	1	2.09	2.01	2041.7	1444.8
	10	2	1.97	1.90	769.2	551.7
	10	3	2.12	1.98	1205.8	737.5
	10	4	1.88	1.85	1277.8	1035.6
	10	5	1.96	1.88	898.6	566.6
	12	1	1.97	1.85	860.0	677.2
	12	2	2.11	2.01	1012.4	642.5
	12	3	2.10	1.97	1304.1	655.4
	12	4	2.07	2.00	867.9	513.3
	12	5	1.96	1.94	857.5	577.2
	12	6	2.03	1.97	807.2	577.0

Each set of samples included 5-8 animals. The sets were numbered from earliest date to latest date of larval fat body collection. The highlighted samples are considered my three best samples for each hour and genotype. The OD_{260}/OD_{280} ratios of the best samples ranged from 1.97 to 2.01 after DNase treatment. The nucleic acid concentrations after DNase treatment (to remove remaining genomic DNA) in this group range from 418.5 to 1444.8 ng/µl.

Gel electrophoresis

Electrophoresis was used to confirm *MMP2* expression by RT-PCR. RT-PCR and the *MMP2* primer set were used to test larval fat body samples from w^{1118} and *cg-Gal4/UAS-dBlimp-1* flies. Unfortunately, PCR was not successful for the 12 hour sets. There was only one visible band from the wild type *Actin* 5C control sample (Figure 9).

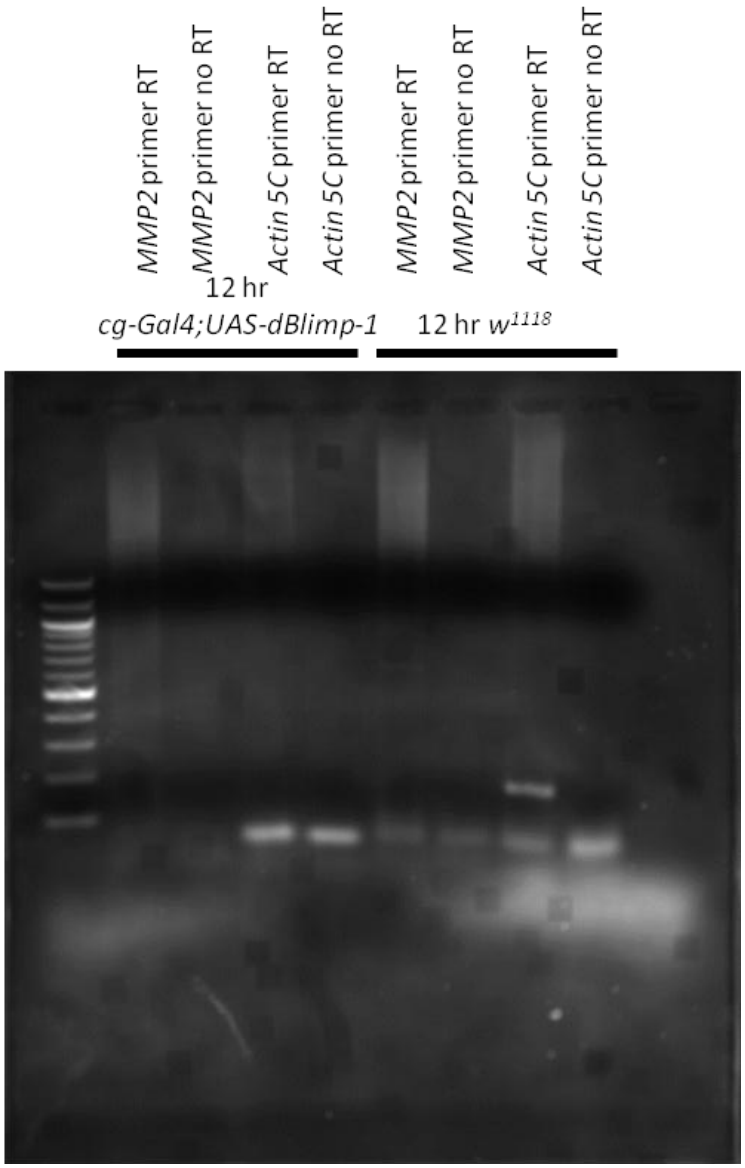

Figure 10. Gel electrophoresis of transgenic and wild type 12 hour samples. These PCR sets were synthesized from transgenic 12 hour set 1, and wild type 12 hour set 3. The gel shows that PCR was not successful. The 12 hour w^{1118} *Actin 5C* primer RT sample is the only band indicating successful amplification of the DNA previously synthesized.

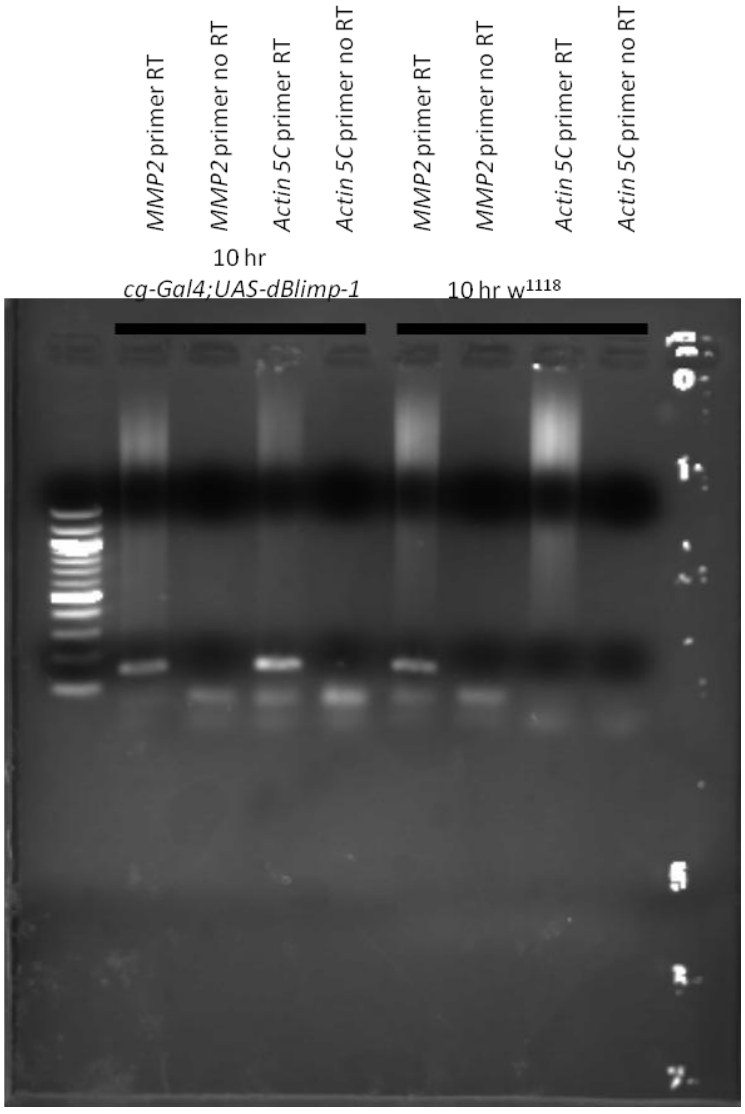

Figure 11. Gel electrophoresis of transgenic and wild type 10 hour samples. These PCR sets were synthesized from transgenic 10 hour set 1, and wild type 10 hour set 1. The gel shows that PCR was successful for these samples. The 10 hour *w*^{1118} *Actin 5C* primer RT and no RT samples are the only bands indicating no amplification of the DNA during PCR.

DISCUSSION

Many of my samples show potential based on their quality after DNase treatment (Table 4). I have enough samples to produce triplicate data in 10 hour and 12 hour samples, sufficient for statistical significance after qPCR. I successfully performed PCR on two sets of samples: 10 hour transgenic, and 10 hour wild type. Gel electrophoresis visualized the success of the polymerase chain reaction and qualitatively shows that the primers bonded and amplified the cDNA previously synthesized. The presence of the bands in the 10 hour transgenic fly is evidence that MMP2 is expressed in the genotype at 10 hours. qPCR will have to be performed to quantify to what extent MMP2 is expressed at 10 hours.

However, successful RT-PCR of 12 hour samples have not yet been performed (Figure 9). Possible reasons for this failure are described below. Since the control genotype and samples are also unsuccessful, it is possible that there are problems in the steps leading up to PCR during cDNA or PCR preparation procedures. Issues with the primers used in RT-PCR are also being considered.

Moving forward, completed, successful reverse-transcriptase polymerase chain reaction for transgenic samples collected at 12 hours APF is a main objective. Once RT-PCR is completed successfully, qPCR can be used to quantify the amount of PCR product present in the sample for each cycle of amplification. My hypothesis is that the pulse of *βftz-f1* at 6 hours APF controls the expression of *MMP2*, which is normally expressed around 10-12 hours APF. It was initially

predicted that we would see a significant reduction of *MMP2* expression in 12 hour larval fat body.

This research project aimed to focus on the first two pulses of 20E during metamorphosis. The first pulse of 20E encodes the ßFTZ-F1transcription factor, and the second pulse, at 10 hours APF, leads to the induction of transcriptional cascades by ßFTZ-F1(Bond et al., 2011). *MMP2* is known to have an important role in the larval fat body during the second pulse, when the prepupa transitions into a pupa. The role of MMP2 includes cleaving the ECM between the larval fat body cells, allowing the organ to dissociate and migrate to various parts of the prepupa for nutritional use. MMP2 expression is seen from 7 to 14 hours APF in the wild-type animal. It's activity is highest when 20E titer is at its highest point during the second pulse at 10 to 12 hours APF (Bond et al., 2011).

In our lab, the role of ßFTZ-F1 in fat body remodeling is also being investigated. Mount Holyoke College thesis student, Liz Perez studied *βftz-f1* transcription in the transgenic fly *cg-Gal4;UAS-dBlimp-1* (2014). In this genotype, dBlimp-1 is overexpressed in the fat body and transcriptionally represses *βftz-f1*. Liz found that fat body remodeling was not seen 6 hours APF. At 7 hours APF, less remodeling occurred in the transgenic animal than in the control animal. Liz's successful qPCR results showed that *βftz-f1*was down-regulated at 6 hours APF at a significance value of p=0.021. Although not statistically significant, she did find that *βftz-f1* was up-regulated at 7 hours APF. At 7 hours, the expression of *βftz-f1*was similar to expression levels in the wild

type animal (Figure 10.). Her conclusions suggest that over-expressing dBlimp-1 leads to delayed *βftz-f1*expression.

The *cg-Gal4;Uas-dBlimp-1* genotype was also used in my experiment for overexpression of dBlimp-1, and transcriptional repression of *βftz-f1* in hopes of regulating *MMP2* expression. Based on Liz's results, delayed *βftz-f1*expression in the genotype could have a minor to no effect on *MMP2* expression. This is possible because *βftz-f1*expression in the transgene is normalized at 7 hours APF, before *MMP2* expression at 10 to 12 hours APF. The normalized expression could potentially result in normal *MMP2* expression despite the initial repression of *βftz*-f1. The observations I made during dissection are also indicative of normal *MMP2* expression in the late prepupa. The fat body was harder to collect at 12 hours due to the dissociation of the larval fat cells. MMP2 mutants show a lack of dissociation of the fat body, resulting in death (Bond et al., 2011). Our transgenic *Drosophila* produce viable adult flies, suggesting that *MMP2* is being sufficiently expressed in the progeny.

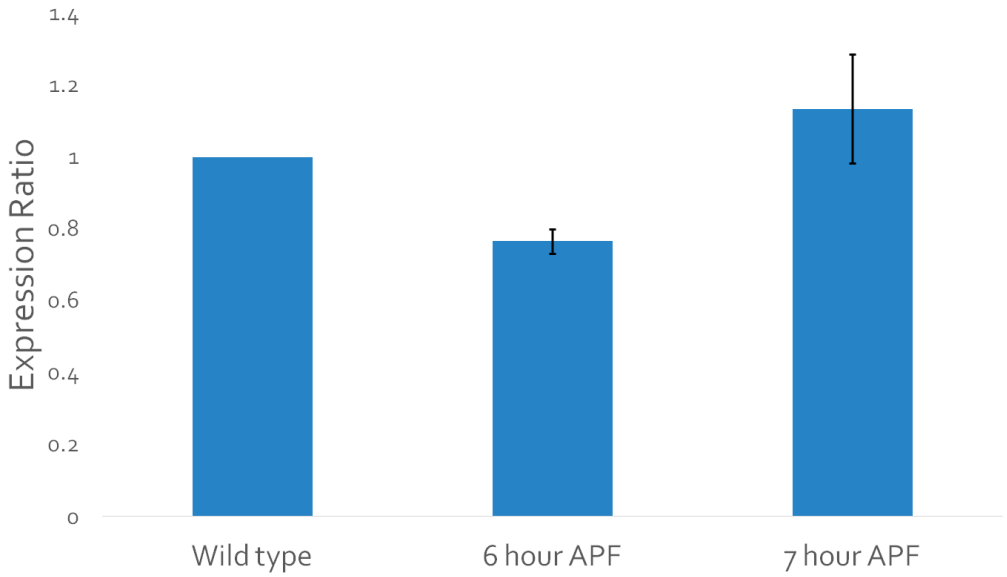

Figure 12. The log of *βftz-f1*expression levels found in qPCR results. 6 hours APF, expression levels of *βftz-f1* are down-regulation. At 7 hours APF, expression levels in the transgenic animal is similar to that in the wild type. Adapted from Perez (2014).

Future studies of fat body remodeling in *Drosophila*

It is possible that the transgenic genotype is not functioning as expected. Research in our lab shows delayed, and possibly reduced, expression of *βftz-f1*in the *cg-Gal4; UAS-dBlimp-1* animals. At 7 hours APF the expression levels seem to normalize to those seen in the control genotype. This could have implications for *MMP2* expression in the prepupa during later stages of metamorphosis. The fat body dissociation of the transgenic animal seen during dissection at 12 hours APF suggests a lack of *MMP2* regulation. If we see normal *MMP2* expression at 10 and 12 hours (quantified via qPCR), it is possible that the genotype is not working as planned or that *MMP2* is not entirely regulated by *βftz-f1*.

Completion of my project will shed light on the effects of the genotype on *MMP2* expression during the transition of *Drosophila* from prepupa to pupa. It will also be telling of the relationship between *βftz-f1* and *MMP2*. Liz's research should also be repeated, with *βftz-f1* expression in the transgenic genotype noted at various hours of metamorphosis. Having more information on the expression levels of *βftz-f1* through metamorphosis will help in designing future experiments on *Drosophila* fat body remodeling. A successful genotype is needed to efficiently assess the potential of *MMP2* as a down-stream target of *βftz-f1*.

APPENDIX

Moving forward with the experiment, the following qPCR method will be used

Quantitative real time polymerase chain reaction (qPCR)

qPCR will be used to quantify the amount of PCR product present in the sample for each cycle of amplification. The products used for this procedure will be from the PerfeCta™ SYBR® Green Supermix with ROX kit from Quanta Biosciences. The amount of PCR product is quantified with the use of a fluorescent reporter dye, which is measured during every extension phase in the reaction. These amounts are graphed against the PCR cycle count. The cDNA used for qPCR is initially single stranded, but becomes double stranded as product accumulates during PCR. SYBR Green functions by binding to the dsDNA amplicon during the extension phase.

Three phases of PCR accumulation are shown in this graph called exponential, linear and plateau. The phases are made using mathematical functions which describe the variability between samples. Since the linear phase describes high variability between samples, this phase is not used for quantification. However, the exponential phase has small variability between samples.

REFERENCES

Agawa, Y., Sarhan, M., Kageyama, Y., Akagi, K., Takai, M., Hashiyama, K., et al. (2007). *Drosophila* blimp-1 is a transient transcriptional repressor that controls timing of the ecdysone-induced developmental pathway. *Molecular and Cellular Biology, 27*(24), 8739-8747.

Aguila, J. R., Hoshizaki, D. K., & Gibbs, A. G. (2013). Contribution of larval nutrition to adult reproduction in *drosophila melanogaster*. *The Journal of Experimental Biology, 216*, 399-406.

Aguila, J. R., Suszko, J., Gibbs, A. G., & Hoshizaki, D. K. (2007). The role of larval fat cells in adult *drosophila melanogaster*. *The Journal of Experimental Biology, 210*, 956-963.

Bakker, K. (1959). Feeding period, growth, and pupation in larvae of *drosophila melanogaster*. *Entomologia Experimentalis Et Applicata, 2*, 171-186.

Bond, N. (2010). The role of ecdysone signaling in fat-body tissue remodeling and pupal metabolism. (Doctor of Philosophy, University of Nevada). (Public)

Bond, N., Nelliot, A., Bemardo, M. L., Ayerh, M. A., Gorski, K. A., Hoshizaki, D. K., et al. (2011). ßFTZ-F1 and matrix metalloproteinase 2 are required for fat-body remodeling in *drosophila* . *Developmental Biology, 360*, 286-296.

Britton, J. S., Loackwood, W. K., Li, L., Cohen, S. M., & Edgar, B. A. (2002). *Drosophila's* insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Developmental Cell, 2*, 239-249.

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., & Hafen, E. (2001). An evolutionarily conserved function of the *drosophila* insulin receptor and insulinlike peptides in growth control. *Current Biology, 11*, 213-221.

Colombiani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., & Leopold, P. (2003). A nutrient sensor mechanism controls *drosophila* growth. *Cell, 114*, 739-749.

Duffy, J. B. (2002). GAL4 system in *drosophila*: A fly geneticist's swiss army knife. *Genesis, 34*, 1-15.

Edgar, B. A. (2006). How flies get their size: Genetics meets physiology. *National Review of Genetics, 7*(12), 907-916.

Falkenstein, E., Tillman, H. C., Christ, M., Feuring, M., & Wehling, M. (2000). Multiple actions of steroif hormones: A focus on rapid, nongenomic effects. *Pharmacological Reviews, 52*(4), 513-556.

Géminard, C., Rulifson, E. J., & Leopold, P. (2009). Remote control of insulin secretion by fat cells in *drosophila*. *Cell Metabolism, 10*(3), 199-207.

Gendzhova, Y. (2011). *The role of inhibitor of apoptosis (DIAP1) in fat body remodeling of drosophila melanogaster.* Unpublished Bachelors of Arts, Mount Holyoke College,

Gill, S. E., & Parks, W. C. (2008). Metalloproteinases and their inhibitors: Regulators of wound healing. *The International Journal of Biochemistry & Cell Biology, 40*, 1334-1347.

Hennig, K. M., Colombiani, J., & Neufeld, T. P. (2006). TOR coordinates bulk and targeted endocytosis in the *Drosophila melanogaster* fat body to regulate cell growth. *The Journal of Cell Biology, 173*(6), 963-974.

Hoshizaki, D. K., Lunz, R., Ghosh, M., & Johnson, W. (1995). Identification of fat-cell enhancer activity in *drosophila melanogaster* using P-element enhancer traps. *Genome, 38*(3), 497-506.

King-Jones, K., & Thummel, C. S. (2005). Nuclear receptors: A perspective from *drosophila*. *Nature Reviews. Genetics., 6*(4), 311-323.

Lam, G. T., Jiang, C., & Thummel, C. S. (1997). Coordination of larval and prepupal gene expression by the DHR3 orphan receptor during *drosophila* metamorphosis. *Development, 124*, 1757-1769.

Liu, H., Wang, J., & Li, S. (2014). E93 predominantly transduces 20-hydroxyecdysone signaling to induce autophagy and caspase activity in *drosophila* fat body. *Insect Biochemistry and Molecular Biology, 45*, 30-39.

Moeller, M. E., Danielson, E. T., Herder, R., OConner, M. B., & Rewtiz, K. F. (2013). Dynamic feedback circuits function as a switch for shaping a maturation-inducing steroid pulse in *drosophila* . *Development, 140*, 4730-4739.

Musselman, L. P., Fink, J. L., Ramachandran, P. V., Patterson, B. W., Okunade, A. L., Maier, E., et al. (2013). Role of fat body lipogenesis in protection against the effects of caloric overload in *drosophila*. *The Journal of Biological Chemistry, 288*, 8028-8042.

Nelliot, A., Bond, N., & Hoshizaki, D. K. (2006). Fat-body remodeling in *drosophila melanogaster*. *Genesis, 44*(6), 396-400.

Nijhout, H. F. (2003). The control of body size in insects. *Developmental Biology, 261*, 1-9.

Page-McCaw, A. (2008). Remodeling the model organism: Matrix metalloproteinase functions in invertebrates. *Seminars in Cell & Developmental Biology, 19*, 14-23.

Page-McCaw, A., Ewald, A. J., & Werb, Z. (2007). Matrix metalloproteinases and the regulation of tissue remodeling. *National Review of Molecular Cell Biology, 8*, 221-233.

Page-McCaw, A., Serano, J., Santé, J. M., & Rubin, G. M. (2003). *Drosophila melanogaster* matriz metalloproteinases are required for tissue remodeling, but not embryonic development. *Developmental Cell, 4*(1), 95-106.

Perez, L. (2014). *The effects of ßftz-f1 in drosophlia melanogaster larval fat body remodeling.* Unpublished Bachelors of Arts, Mount Holyoke College,

Rewitza, K. F., Rybczynskib, R., Warrenb, J. T., & Gilbertb, L. I. (2006). Developmental expression of *manduca shade*, the P450 mediating the final step in molting hormone synthesis. *Molecular and Cellular Endocrinology, 247*, 166–174.

Riddiford, L. M. (1993). Hormones and *drosophila* development. In M. Bate, & A. M. Arias (Eds.), *The development of drosophila melanogaster* (pp. 899-939) Cold Spring Harbor Laboratory Press.

Rsuten, T. E., Lindmo, K., Juhász, G., Sass, M., Seglen, P. O., Brech, A., et al. (2004). Programmed autophagy in the *drosophila* fat body is induced by ecdysone through regulation of the P13K pathway. *Developmental Cell, 7*, 179-192.

Sato, H., Takino, T., & Miyamori, H. (2005). Roles of membrane-type matrix metalloproteinase-1 in tumor invasion and metastasis. *Cancer Science., 96*(4), 212-217.

Stephenson, R., & Metcalfe, N. H. (2013). *Drosophila melanogaster*: A fly through its history and current use. *Journal of the Royal College Physicians Edinburgh, 43*, 70-75.

Taguchi, A., & White, M. F. (2008). Insulin-like signaling, nutrient homeostasis, and life span. *Annual Review Physiology, 70*, 191-212.

Teleman, A. A. (2010). miR-200 de-FOGs insulin signaling. *Cell Metabolism, 11*(1), 8-9.

Tennesen, J. M., & Thummel, C. S. (2011). Coordinating growth and maturation: Insights from *drosophila*. *Current Biology, 21*, R750-R757.

Tracy, K., & Baehrecke, E. H. (2013). The role of autophagy in *drosophila* metamorphosis. *Current Top Developmental Biology, 103*, 101-125.

Weigmann, K., Klapper, R., Strasser, T., Rickert, C., Technau, G. M., Jackle, H., et al. (2003). FlyMove: A new way to look at development of *drosophila*. *Trends Genetics, 19*(6), 310-311.

White, K. P., Hurban, P., Watanabe, T., & Hogness, D. S. (1997). Coordination of *drosophila* metamorphosis by two ecdysone-induced nuclear receptors. *Science, 276*, 114-117.

Woodard, C. T., Baehrecke, E. H., & Thummel, C. S. (1994). A molecular mechanism for the stage specificity of the *drosophila* prepupal genetic response to ecdysone. *Cell, 79*, 607-615.

Zhou, X., Zhou, B., Truman, J. W., & and Riddiford, L. M. (2004). Overexpression of broad: A new insight into its role in the *drosophila* prothoracic gland cells. *The Journal of Experimental Biology, 207*, 1151-1161.