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Identifying Genes Involved in Larval Fat Body Remodeling in *Drosophila melanogaster*

by

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for eight credits.

For my family

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ABSTRACT

Tissue remodeling is an essential process that occurs in multicellular organisms and is essential for the growth, development, and health of any organism. *Drosophila melanogaster* is an important organism for the study of this process as tissue remodeling is crucial for proper metamorphosis, during which the larval fat body remodels from a sheet of connected, polygonal cells into single, spherical cells which can then move throughout the body and head cavity of the fly. In this study, complementation tests were performed on lines of flies that each had a single mutation on the third chromosome that resulted in both abnormal fat body morphology and pharate adult lethality. The F_1 progeny were scored for fat body morphology and adult lifespan post-eclosion in order to elucidate the relationship between the two phenotypes and better understand the role of potential novel genes. Abnormal fat body morphology was found to result in a reduced lifespan post-eclosion, where the degree of remodeling shows a slightly positive correlation with lifespan. In addition, I have begun to linkage map some of the mutations using pairs of dominant markers to identify the region of the third chromosome where each mutation is present.

INTRODUCTION

Tissue Remodeling

Tissue remodeling, a process that occurs in all multicellular organisms, is essential for growth and development as well as a requirement for responding to the surrounding physiochemical environment. As tissues grow, their structures change, so being able to undergo tissue remodeling is necessary for growth and development. In addition, an organism may need to change the structure of a tissue in response to environmental changes or injury. Wound healing is one of the important functions of tissue remodeling, and an understanding of this process has significant medical applications. Tumor metastasis is another process that relies on tissue remodeling. A better understanding of tumor metastasis can lead to the discovery of therapeutic targets for potential cancer drugs.

The overall process of tissue remodeling is conserved across different cell types. With the help of adhesion proteins, cells attach to each other through cell-cell junctions or the extracellular matrix (ECM). When tissues remodel, the cell-cell junctions and ECM are targeted for degradation by proteases such as matrix metalloproteinases (MMPs). Breakdown of the ECM and thus cell-cell junctions enhances the mobility of individual cells because space opens up between the cells (Sternlicht and Werb 2009). The ECM plays a major role in the individual environment of the cell and its components are constantly being modified (Lu *et*

al. 2011), and the ECM differs between cell types. Understanding the role of the ECM in tissue remodeling gives insight into other essential processes such as stem cell maintenance and wound healing (Lu *et al.* 2011). In addition, understanding the role of MMPs in tissue remodeling, which can digest non-ECM molecules as well, can give insight into tumor metastasis, wound healing delay, and other medical issues that can arise when tissue remodeling goes awry, potentially due to problems with MMPs (Krejner *et al.* 2016). Wound healing is the process by which cells respond to injury by a series of different signaling cascades. Wound healing has three main stages – inflammation, proliferation, and remodeling. The constitution of the proteins in the ECM of wounded cells changes rapidly as the wound heals. Specifically, collagen is degraded and produced at different rates in order for tissues to remodel. In wound healing, cells that do not respond by remodeling undergo programmed cell death (Son and Harijan 2014). Changes in the ECM might also play a role in tumor metastasis. Altered MMP expression and activity in the ECM has been implicated in the metastasis of cancer cells because cleaving ECM components allows cells to detach and travel throughout the body (Cox and Erler 2011).

Drosophila melanogaster is an ideal model organism to study tissue remodeling processes, as tissue remodeling plays a major role in metamorphosis. When the fly transitions from its juvenile form to its adult form, many of the larval tissues die, while the larval fat body remodels. This process is essential for

the fly to complete its metamorphosis, and studying the underlying mechanisms of larval fat body remodeling can shed light on the overall process of tissue remodeling.

Drosophila melanogaster as a Model Organism

Drosophila melanogaster is a holometabolous insect that has been used extensively as a model organism for genetics research. During development, *Drosophila* undergoes a complete metamorphosis that carries it from the pupal stage into a fully formed adult. During this process, many of the larval tissues undergo programmed cell death, and new adult tissues emerge in their place. However, some larval tissues, most notably the larval fat body, do not undergo programmed cell death and instead remodel. Understanding the process of larval fat body remodeling is interesting and important for understanding similar processes in higher organisms, including humans, as tissue remodeling has medical relevance from wound healing to tumor metastasis. The remodeling of the larval fat body and other metamorphic processes are controlled by the steroid hormone 20-hydroxyecdysone (also called “ecdysone” or “20E”). Understanding this process as a whole, and the implications of mistakes in these pathways, will give insight into the overall mechanisms of fat body remodeling.

Drosophila is an invaluable model organism for biological research. In use as early as 1901, *Drosophila* as a model organism gained popularity after Thomas

Hunt Morgan, often touted as the father of *Drosophila* research, was able to enhance Mendel's theory of inheritance by using fruit flies to elucidate the role of chromosomes in heredity. For this work, Morgan won a Nobel Prize in Physiology or Medicine in 1933 (Jennings 2011). Initially, the popularity of the fruit fly came from its short life cycle, easily recognized and manipulated phenotypes, simple maintenance, and lack of ethical and safety concerns. Once comparisons between the genomes of fruit flies and humans revealed that approximately 75% of known human disease genes have a fruit fly ortholog, its relevance in human disease and genetics research increased and *Drosophila* are now recognized as one of the most important model organisms in current research (Jennings 2011). Conservation of cellular processes and signaling pathways between fruit flies and vertebrates such as mice and humans has kept *Drosophila* front and center as a research tool in genetics. For this reason, it is an important organism to help expand our understanding of the process of tissue remodeling.

Drosophila **Life Cycle**

In all higher organisms, development from the juvenile to mature adult is a strictly controlled response to hormonal cascades. Because fruit flies are holometabolous insects, their development includes a complete metamorphosis and they have four distinct life stages: egg, larva, pupa, and adult (Figure 1). The pupal stage, where the organism undergoes complete metamorphosis, is unique to

holometabolous insects and is an essential characteristic that makes them useful as an organism to study development. In contrast, hemimatabolous insects do not spend as much time in development because they do not undergo complete metamorphosis which results in their larval form looking similar to their adult form (Bainbridge and Bownes 1981).

The first stage in the *Drosophila* life cycle is embryogenesis, the development from fertilized egg to larva, which takes about one day. The larva undergoes two molts to move from first instar to second and then third instar before it moves into the pupal stage. The molting process occurs as the organism rids itself of the old cuticle by biting through it with mouth hooks and then rupturing it, leaving behind not only the cuticle but the mouthparts and spiracles, which are all reformed in the following larval stage. While the first and second instars each last about one day, the third instar larva lasts for about two days and ends with puparium formation and transition into the pupual stage and the beginning of metamorphosis (Riddiford and Truman 1993). During the larval stages the larva feeds in order to grow and to build up stores of essential nutrients it will need to successfully undergo metamorphosis. Eggs are usually laid on decaying fruit on which the larvae will feed and build up nutrient stores. This feeding stage is characterized by a 200-fold increase in mass (Church and Robertson 1966), which is required to provide nutrients for the wandering stage, metamorphosis, and post-eclosion. Growth happens only in the larval stages; after

metamorphosis, the adult fly does not change in size (Teleman 2010). After feeding, the larva will wander for 12-24 hours until it finds a pupariation site where puparium formation begins and with it, the beginning of metamorphosis (Riddiford and Truman 1993). The prepupal stage lasts about 10 hours, until an ecdysone titer that signals the prepupal-to-pupal transition (Robertson 1936). In the pupal stage, which lasts between five and seven days, the larva undergoes complete metamorphosis to give rise to the adult form. The resulting adult looks nothing like the larva. Most of the larval structures are destroyed by autophagy and apoptosis in the early stages of metamorphosis and are replaced by adult tissues that develop from imaginal discs and other imaginal cells (Thummel 2004).

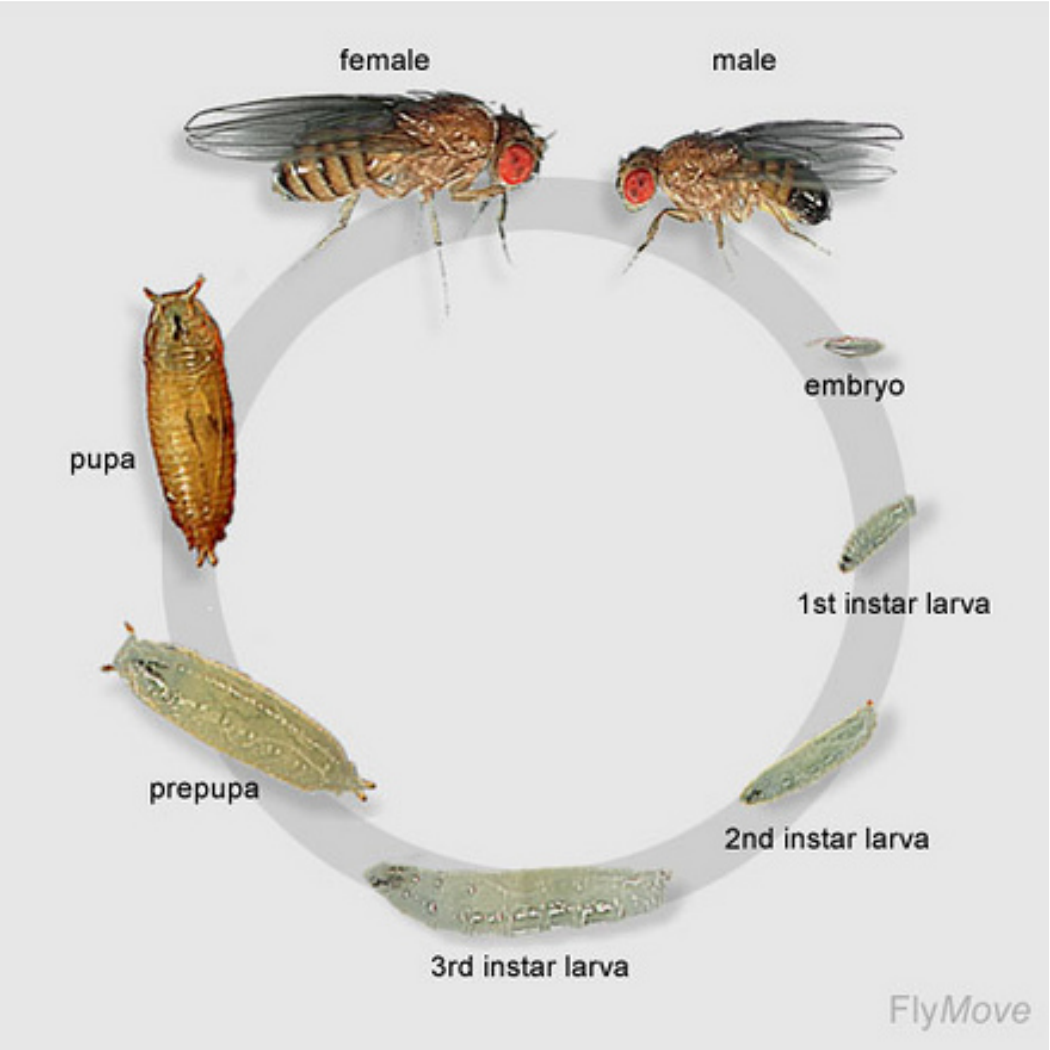


Figure 1. Life cycle of *Drosophila melanogaster.* The life cycle of *Drosophila melanogaster* takes approximately 12 days from embryo to adult. After spending one day as an embryo, the fly hatches and proceeds through the larval stages which include 1st, 2nd, and 3rd instar, where it feeds and grows. It proceeds into the wandering stage, looking for a place to attach and proceed into the pupal stage, where it undergoes metamorphosis. After undergoing a full metamorphosis, it ecloses from its pupal case as a fully formed adult fly (Weigmann *et al.* 2003).

The metamorphosis of *Drosophila* can be broken down into 52 distinct stages (Bainbridge and Bownes 1981). All of these different processes are

regulated by the steroid hormone ecdysone. The timing of events in the prepual and pupal stages is very specific. The pupal stage begins approximately 120 hours after the beginning of embryonic development. At zero hours after puparium formation (APF), the larval cuticle forms the puparium, which is the hard shell that encloses the organism throughout the pupal stage. In the first 3.5 hours APF, the cuticle darkens to a brown color and stiffens. At approximately 4-6 hours APF, apolysis occurs and the epidermis separates from the cuticle; this is the first step of fat body remodeling, as the cells begin to change shape (Nelliot *et al.* 2006). Head eversion happens by 12 hours APF and the organism undergoes pupation, moving from the prepupal to the pupal stage, induced by a peak of ecdysone. The remainder of the pupal stage occurs over the next four to five days, during which most remaining larval tissues are destroyed and adult structures form. Once the adult structures are fully formed, the fly ecloses from its pupal case and remains inactive for approximately 8 hours while the wings expand and the cuticle tans (Chiang 1963; Hoshizaki 2005; Nelliot *et al.* 2006).

Larval Fat Body

The larval fat body, derived from the mesoderm, consists of a single layer of polygonal cells, floating in the hemolymph, that forms a wide, bilateral ribbon of connected tissue layer between the body wall and the midgut (Hoshizaki 2005; Nelliot *et al.* 2006). During metamorphosis, the cells remodel and take on a

spherical shape and detach from each other in order to distribute themselves throughout the body of the fly for nutrients and energy (Nelliot *et al.* 2006). These cells persist into the adult fly until about 3-4 days post-eclosion, by which point all the larval cells have undergone programmed cell death and have been replaced by the adult fat body (Aguila *et al.* 2007). Due to its role in metabolic processes and signaling, the larval fat body is often thought to be the insect equivalent of the mammalian liver. However, this comparison misrepresents the role of the larval fat body, since many functions of the mammalian liver, such as detoxification, are also carried out in insect midgut (Locke 2003). In addition, the larval fat body is derived from the mesoderm while mammalian liver is derived from the endoderm (Hoshizaki 2005).

As previously mentioned, the larva spends its time growing and feeding before it transitions into the prepupal stage and begins metamorphosis. Similarly to the mammalian liver, the larval fat body can function as an endocrine organ, which is essential for monitoring the nutritional status of the larva and coordinating its growth by monitoring the animal's need and altering its feeding patterns and other behavior accordingly (Hoshizaki 2005). The larval fat body is a nutrient reserve that can be drawn upon during and after metamorphosis. However, the exact mechanisms by which larval tissues, such as the fat body, are saved from programmed cell death and then released to be used for later developmental processes are still not well understood (Aguila *et al.* 2013). The fat

body synthesizes a group of proteins which serve as a reservoir of amino acids that are an essential resource in later developmental stages (Haunerland 1996). These proteins are then secreted into the hemolymph, only to be taken up by the fat body again a few days later; this group of proteins is known as storage proteins (Haunerland 1996). The fat body produces two different classes of growth factors: imaginal disc growth factors (IDGFs) and adenosine deaminase-related growth factors (ADGFs)(Kawamura *et al.* 1999; Zurovec *et al.* 2002; Hoshizaki 2005). The role of the fat body as a nutrient sensor allows it to play a role in energy homeostasis. The larval fat body functions to monitor the growth of larval tissues as well as to remotely inhibit the Inr/P13K pathway in response to local nutrient conditions, specifically amino acid levels (Colombani *et al.* 2003). It is the Inr/P13K pathway that allows the fat body to respond to local conditions as well as signal to remote tissues (Colombani *et al.* 2003).

The larval fat body is derived from the mesoderm in the embryo, a completely different cell line from the adult fat body. The embryonic fat body develops into the larval fat body, beginning at stage 10/11 of embryogenesis and finishing at stage 16 so that at the completion of embryogenesis, the larva has its developed fat body (Figure 2). The embryonic fat body at stage 16 has three sections: the ventral commissure, the dorsal fat cell projection, and the lateral fat body. The ventral commissure is a group of two fat cell bridges that spreads from the anterior region of the lateral fat body and spans the ventral midline. Extending

from the posterior region of the lateral fat body are a pair of projections that flanks the dorsal vessel; this is the dorsal fat cell projection (Hoshizaki 2005).

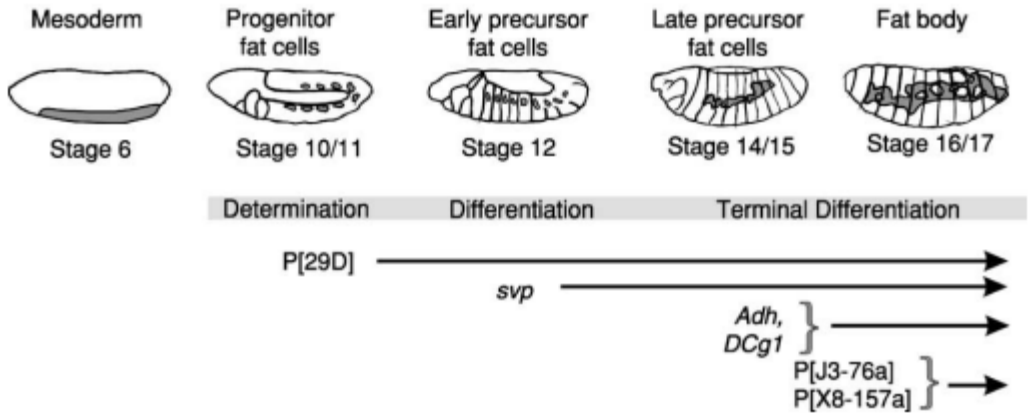


Figure 2. Summary of the development of the larval fat body during embryogenesis. The stages of fat cell development are determined by the time-specific expression of genes and enhancer trap lines. The first identified cells that will become the larval fat body are in stage 10/11 as nine bilateral clusters of mesodermal cells reach determination. These cells expand and begin to differentiate through late stage 15. The fully formed larval fat body is present by stage 16/17 (Hoshizaki 2005).

In stage 6, the ventral region of the embryo holds the cells that will be involved in the specification of the mesoderm (Figure 2). The initial expression of the P-element enhancer-trap line [29D] in nine bilateral clusters of mesodermal cells in stage 10/11 marks the progenitor fat cells of the lateral fat body and the beginning of determination (Figure 2). Enhancer-trap lines are used to monitor gene expression by fusing a reporter gene to a minimal promoter that needs local enhancer elements to drive expression (Springer 2000). The differentiation of fat

body cells begins in stage 12 with the expression of the transcriptional activator *seven up (svp),* an orphan steroid hormone receptor, in the nine bilateral cell clusters (Figure 2). Expression in the developing fat body of *Alcohol dehydrogenase (Adh)* and *collagen type IV (DCg1)*, genes involved in the cellular function of the fat-cell, begins at stage 15 and marks the beginning of differentiation of the terminal fat cells (Figure 2). Finally, the activity of the enhancer-trap lines P[J3-76a] and P[X8-157a] towards the end of stage 15 marks terminal differentiation and the fully formed larval fat body (Figure 2). The genes associated with the P-element enhancer-trap lines have not yet been identified (Hoshizaki *et al.* 1994; Hoshizaki 2005).

Remodeling of the Larval Fat Body

While the organism undergoes metamorphosis, the larval fat body remodels from a sheet of connected, polygonal cells to individual, spherical cells (Figure 3). This is unusual for larval tissues, the majority of which undergo programmed cell death by autophagy and apoptosis before the completion of metamorphosis. The individual spherical cells spread themselves throughout the pupa and float freely in the body cavity (Hoshizaki *et al.* 1994). The larval fat cells persist in the adult body cavity until four days post-eclosion, although 90% of them disappear by the second day (Aguila *et al.* 2007). By day four the adult fat body has formed (Hoshizaki 2005). Inhibition of fat body dissociation has

many affects, including lethality at different stages in the life cycle (Nelliot *et al.* 2006).

The remodeling of the fat body occurs in distinct stages, temporally regulated by the steroid hormone ecdysone (Figure 3). The first stage, referred to as retraction, happens at 6 hours APF, where the fat body is completely retracted from the anterior region and the individual cells are beginning to change shape. The next stage is called disaggregation, as the individual cells begin to lose their association to one another. This happens in a spatially specific manner, starting from the anterior and moving to the posterior. The final stage happens by 14 hours APF, when the individual cells detach and move throughout the body cavity. Initially, they are propelled into the head cavity after head eversion, but detach to move throughout the rest of the body in a wave that moves anterior to posterior (Nelliot *et al.* 2006).

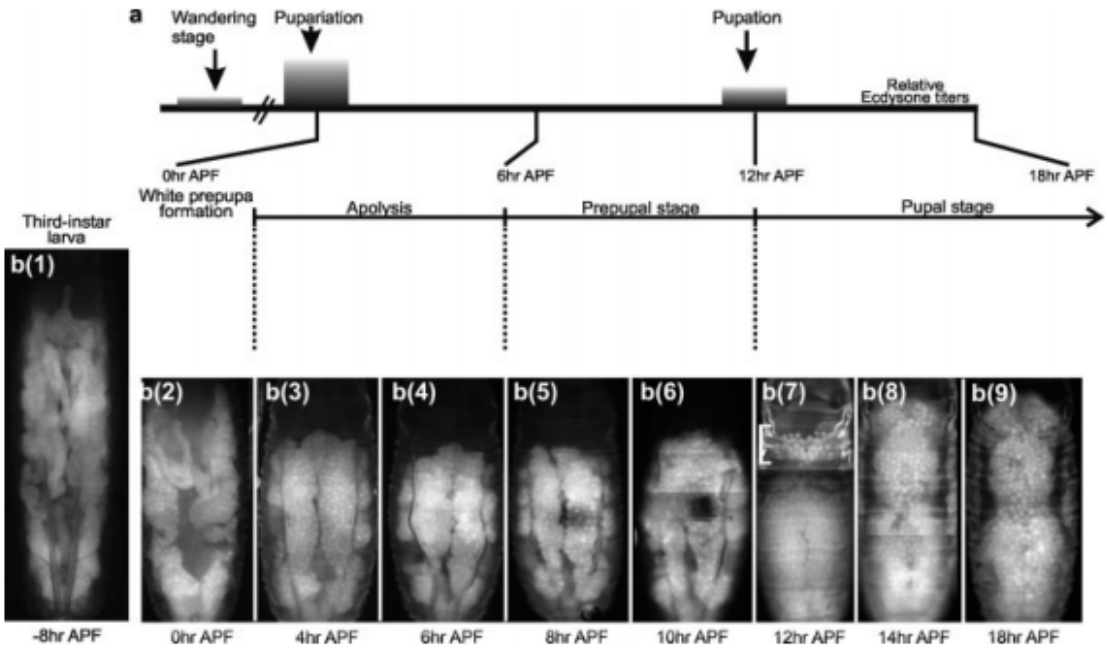


Figure 3. Stages of Larval Fat Body Remodeling. Relative ecdysone titers during the early stages of metamorphosis and the corresponding changes in the larval fat body. Starting with the larvae before puparium formation through the 18 hour pupa where the larval fat body has completely remodeled and the individual cells have spread throughout the body cavity and into the head cavity (Nelliot *et al.* 2006).

Role of Larval Fat Cells in the Adult

Once the fat body has remodeled, the cells persist in the adult fly. In nature, the adult fly may need to seek a new food source because the one available to the larva may no longer be available to the adult. Thus, the adult needs to have a nutrient and energy source available while it searches for a new, more permanent one. This nutritional role is thought to be the reason the larval fat body cells survive into the adult post-eclosion. It has been shown that the presence of

these fat cells can improve both stress-and-starvation-resistance (Aguila *et al.* 2007). While larval fat cells persist into the adult fly, they eventually undergo apoptosis and give way to the adult fat body. It has been proposed that they undergo programmed cell death in order to release nutrients to the fly. However, starvation does not affect the rate at which they break down, which suggests that their use as energy reservoirs is not solely contingent on the bulk recycling of the components they release during apoptosis (Aguila *et al.* 2007). Further research has shown that apoptosis allows the larval fat body cells to reallocate their energy stores to the gonads, specifically the ovaries in the female adult fly (Aguila *et al.* 2013).

20-hydroxyecdysone

The steroid hormone 20-hydroxyecdysone (20E), which is the active form of ecdysone (all forms will be collectively referred to as ecdysone hereafter) plays an important role in metamorphosis (Woodard *et al.* 1994). Pulses of ecdysone regulate and coordinate the developmental transition known as metamorphosis by inducing signaling cascades and regulating gene transcription. The fly goes through a series of checkpoints to ensure everything is in place for successful metamorphosis into an adult fly before producing ecdysone to move it through metamorphosis (Moeller *et al.* 2013). Prothoracicotropic hormone (PTTH)

initiates pupariation and the resulting upregulation of ecdysone biosynthesis in the prothoracic gland (PG) (McBrayer *et al.* 2007).

In fact, ecdysone regulates half of the known nuclear-receptor superfamily members in *Drosophila* (Thummel 2004). The nuclear-receptor superfamily members all have both a highly conserved DNA binding domain (DBD) in addition to a less conserved C-terminal ligand-binding and dimerization domain (LBD) (King-Jones and Thummel 2005). The ecdysone receptor is the only ligand-dependent nuclear receptor to have been identified in *Drosophila,* and although it is a steroid receptor, it consists of a heterodimer that closely resembles the retinoic acid receptor in vertebrates (Thummel 2004). Functioning as a heterodimer, one half of the receptor is encoded by the *EcR* gene, a gene induced directly by ecdysone in a positive autoregulatory loop (Thummel 2004). *EcR* encodes three protein isoforms with different sequences at their amino terminus but identical DBDs and LBDs. Two of these isoforms are expressed in patterns that associate with the divergent developmental fates of larval and adult tissues, suggesting that these isoforms may play a role in the tissue specificity of ecdysone responses. All three isoforms heterodimerize with Ultraspiracle (USP) in order to bind DNA and the ligand. Ligand binding stabilizes the heterodimer as well as increases its affinity for ecdysone responsive elements (EcREs). While USP functions in abdominal cuticle synthesis during mid-embryogenesis in addition to larval cuticle molting, its absence does not prevent adult thoracic and

abdominal metamorphosis (Thummel 2004). The EcR-USP heterodimer induces the transcription of genes encoding different members of the nuclear-receptor superfamily. These genes include *DHR3*, *DHR4*, *DHR39*, *E75*, *E78*, and *ftz* transcription factor 1, which then activate a set of secondary response genes (King-Jones and Thummel 2005).

Titers of ecdysone drive the development of larvae into pupae, as well as being responsible for key stages in metamorphosis (Figure 3). The ecdysone concentration varies in different tissues during metamorphosis and peaks during key developmental changes (Riddiford and Truman 1993; Thummel 2004). Peaks of ecdysone cause an increase in the binding between the steroid hormone and its receptors, which induces different signaling cascades necessary for metamorphosis (Figure 4) (Bond *et al.* 2011). In some tissues, ecdysone peaks begin programmed cell death, while in others, they correspond to tissue remodeling (Riddiford and Truman 1993). During the end of the larval stages and the beginning of metamorphosis there are two key peaks in hormone concentration; these occur at the onset of puparium formation and then again around the 10-12 hour APF mark (Figure 4) (Woodard *et al.* 1994; Agawa *et al.* 2007). The ecdysone pulse that occurs at the end of the third instar stage, referred to as the late-larval pulse, triggers a group of regulatory "early genes" which play roles in puparium formation and the entrance into the prepupal stage and initiate the beginning of fat body remodeling (Figure 3, 4) (Nelliot *et al.* 2006; Agawa *et*

al. 2007). In addition to beginning fat body remodeling, this pulse of ecdysone can also initiate apoptosis and autophagy (Rusten *et al.* 2004). The "early genes" have both negative autoregulatory function and induce "late genes" that are important for tissue development during metamorphosis (Thummel 2004).

The second pulse, at 10-12 hours APF, referred to as the prepupal pulse, triggers transcriptional cascades that induce the prepupal to pupal shift (Figure 4) (Bond *et al.* 2011). This shift includes salivary gland death as well as movement of remodeled fat body cells into the head cavity (Woodard *et al.* 1994; Nelliot *et al.* 2006). Between these two pulses, the concentration of ecdysone drops. This period is referred to as the "mid-prepupal period," and genes that are transcribed during this time are referred to as mid-prepupal genes (Woodard *et al.* 1994; Yamada *et al.* 2000).

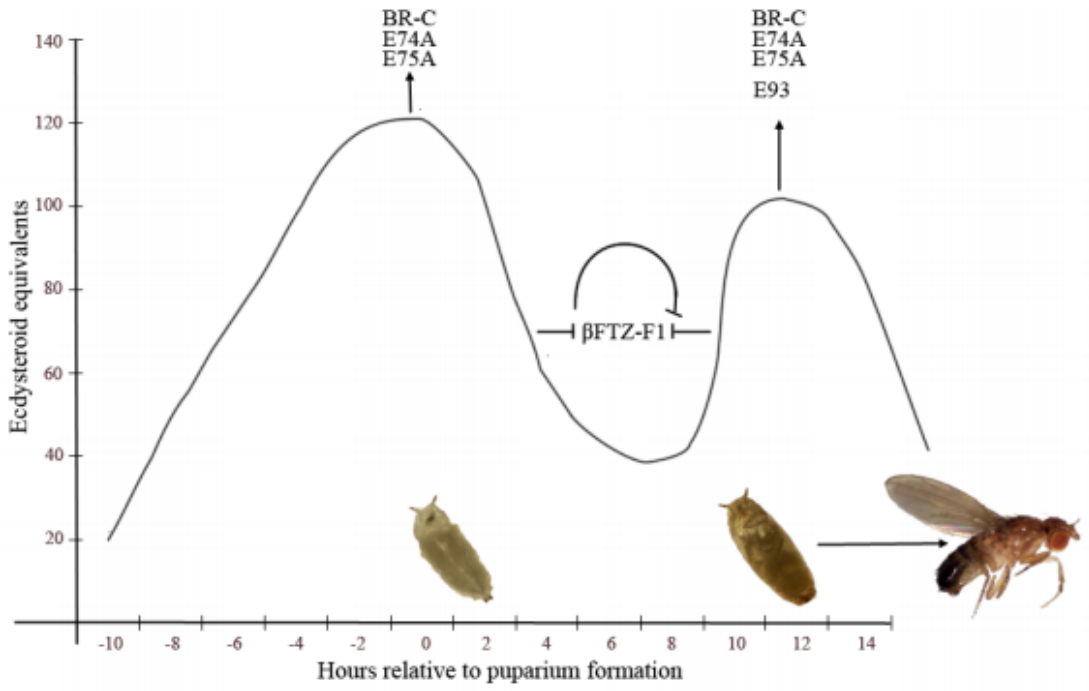


Figure 4. Ecdysone concentration and gene expression through the first 14 hours of *Drosophila* metamorphosis. During the first 14 hours of metamorphosis, two pulses of ecdysone occur. The first induces the transcription of the early genes including *BR-C, E74A*, and *E74A*. After the first pulse, a decline in concentration allows the nuclear receptor *βftz-f1* to be expressed which confers competence to the larval tissues to respond to the second pulse, which induces the transcription of the late genes (Notarangelo 2014).

βFTZ-F1

Between the two ecdysone pulses, there is a decrease in ecdysone titer, which allows mid-prepupal genes to be activated. One of the mid-prepupal genes encodes the nuclear receptor βFTZ-F1 (Figure 4). Ecdysone represses *βftz-f1* transcription (Woodard *et al.* 1994). *βftz-f1* expression during the ecdysone drop is regulated by the resulting transcription of *DHR3* and *DHR4* and the elimination of the repressive effects of the dBlimp-1 protein, both corresponding to the drop

in ecdysone concentration (King-Jones and Thummel 2005; Agawa *et al.* 2007). *βftz-f1* encodes the competence factor that enables the early genes to respond to the prepupal pulse and in turn allows the late genes to be expressed. Expression of βFTZ-F1 is necessary for the induction of fat body remodeling. Ecdysone induced transcription of *βftz-f1* is also necessary for programmed cell death and autophagy, two other important processes in metamorphosis (Woodard *et al.* 1994). The time-specific manner in which *βftz-f1* is expressed is governed by ecdysone, which makes it part of a class of unique transcription factors (Yamada *et al.* 2000). Because premature expression of *βftz-f1* at specific times in postembryonic development is lethal, the temporally specific expression is an important part of the function of the βFTZ-F1protein (Yamada *et al.* 2000). βFTZ-F1 is likely able to provide competence by directly regulating the promoters of the early genes as studies show that loss-of-function mutations do not change the levels of the ecdysone receptors (Broadus *et al.* 1999).

The prepupal ecdysone pulse plays a role in directing larval fat body remodeling, which is why the expression of *βftz-f1* is essential at the correct time. Premature *βftz-f1* expression results in premature larval fat body remodeling, and repressing *βftz-f1* expression results in the failure of larval fat body remodeling (Bond *et al.* 2011). This indicates that when ecdysone levels are high, βFTZ-F1 is both necessary and sufficient for the larval fat body to remodel. In addition to larval fat body remodeling, transcription of *βftz-f1* is important for the regulation

of cuticle proteins which must be expressed in a stage-specific manner during the prepupal stage in order for cuticle formation to occur (Yamada *et al.* 2000).

DHR3 and *DHR4* are both induced by ecdysone and promote the expression of *βftz-f1*. It has been suggested that DHR3 acts on the promoter region of *βftz-f1,* as three binding sites have been identified downstream of the *βftz-f1* transcriptional start site (Lam *et al.* 1997). Also induced by ecdysone is the protein dBlimp1, which represses the expression of *βftz-f1,* also by binding to its promoter region. The expression of *dBlimp1* is very temporally specific, as are many ecdysone-induced genes. Thus, *βftz-f1* transcription is also temporally specific (Agawa *et al.* 2007). In addition, *Mmp2* is a target of the signaling cascade involving *βftz-f1* expression and ecdysone (Bond *et al.* 2011).

MMPs

Matrix metalloproteinases (MMPs) are responsible for protein cleavage in different membranes, and they can cleave the proteins in the ECM, specifically collagen and laminin (Page-McCaw *et al.* 2003, 2007). MMPs play a complex and important role in disease pathogenesis, especially tumor development and metastasis. MMPs are involved in a regulatory network that also involves tissue inhibitors of metalloproteinases (TIMPs). *Drosophila melanogaster* have two MMPs and one TIMP, a simpler system when compared to that of humans and mice. Both *Drosophila* MMPs are involved in the degradation of basement

membrane (BM) components throughout the lifecycle (Jia *et al.* 2014). The one TIMP in *Drosophila* is able to inhibit both MMP1 and MMP2 activity. While neither MMP is essential for embryonic development, MMP2 plays a role in the histolysis of larval tissues during metamorphosis (Page-McCaw *et al.* 2003). The MMPs and TIMP both play a role in the ability of the larval fat body to remodel. It is thought that MMP1 acts to degrade the ECM that forms the cell-cell junctions and MMP2 degrades the BM components to release the cells from their connected form and allow them to move throughout the body cavity of the fly – an essential step in both the late phases of metamorphosis and the early life of the adult fly. TIMP is a competitive inhibitor of both *Mmp1* and *Mmp2*. If *Timp* is misexpressed in such a fashion that it inhibits the function of MMP2 in the fat body, the fat body cells will no longer dissociate (Bond *et al.* 2011). However, if *Mmp2* is overexpressed, fat body cells will dissociate early, leading to larval lethality (Jia *et al.* 2014). Transcription of *Mmp2* is upregulated by βFTZ-F1, which makes it a potential target of the βFTZ-F1-mediated ecdysone signaling pathway (Bond *et al.* 2011).

Insulin/IGF Signaling

As previously mentioned, the larval fat body acts as a nutrient sensor. Nutrient sensing is controlled by the insulin/IGF pathway (Teleman 2010). The *Drosophila* pathway, while surprisingly complex, is still a simpler version of its

mammalian counterpart. *Drosophila* has an insulin-like receptor (InR) that is the initiation site for the insulin pathway upon binding of the appropriate ligand. Insulin-like peptides (ILPs) which regulate feeding behavior are part of a homeostatic feedback loop that is regulated by nutrient conditions. Upon ligand binding, InR autophosphorylates, which recruits PI3K to the cell membrane and activates it. PDK1 and Akt, both kinases, are recruited to the plasma membrane and activated by phosphorylation (Teleman 2010). Signaling by P13K and TOR in response to ecdysone is responsible for the control of autophagy in the larval fat body (Rusten *et al.* 2004). The P13K signaling pathway is responsible for inhibiting autophagy. The suppression of this pathway, likely by ecdysone, leads to apoptosis in the larval fat body, which is one of the ways ecdysone is responsible for regulating autophagy (Rusten *et al.* 2004). TOR signaling is responsible for the regulation of cellular metabolism. The kinase TOR exists in two complexes, TOR-C1 and TOR-C2. TOR-C1 is the main regulator of cellular metabolism and as such is responsible for the use and conservation of energy and nutrients, and the loss of its function causes the aggregation of lipid vesicles in the fat body, an effect similar to larvae subject to amino acid deprivation (Zhang *et al.* 2000; Teleman 2010).

TGFβ pathway

Transforming growth factor β (TGFβ) is involved in a signaling pathway that may also play a role in larval fat body remodeling. The signaling pathway works by secreting a polypeptide and the receptor ligand interacts to phosphorylate a SMAD transcription factor. Studies show that a disruption in this pathway causes a premature dissociation of the fat body cells, meaning that this pathway somehow plays a role in regulating the genes that are responsible for larval fat body remodeling. These genes likely have downstream effects on adhesion molecules, proteases, and other proteins that play a role in connecting fat body cells (Massagué and Chen 2000; Massague and Lo 2000; Hoshizaki 2005).

Pharate Adult Lethality

Pharate adult lethality is the phenomenon that occurs when the organism is able to undergo complete metamorphosis but unable to eclose from its pupal case and therefore dies at the pharate adult stage. The inhibition of fat body remodeling during metamorphosis causes pharate adult lethality (Nelliot *et al.* 2006). Due to the complexity of the mechanism of fat body remodeling, there could be multiple different underlying causes that can lead to this phenotype.

Principles of a Broad Genetic Screen

A broad genetic screen is an attempt to identify certain genes that cause specific phenotypes by employing a mutagenesis. The type of screen utilized here allows for a non-biased identification of mutated genes because the mutagenesis did not target specific genes but instead identified specific phenotypes. In this screen I looked for the pharate adult lethal phenotype and the corresponding abnormal fat body remodeling phenotype. Exploration of the relationship between these two phenotypes and the identification of the genes involved can help elucidate novel genes involved in larval fat body remodeling and their roles. This specific screen uses flies that have unknown mutations on the third chromosome that correspond to abnormal fat body remodeling and pharate adult lethality.

Hypothesis and Aims

The goal of this study was to identify novel genes that are involved in the remodeling of the larval fat body in *Drosophila melanogaster.* The mutagenesis done for this study produced lines of *Drosophila* with mutations on the third chromosome. In this study I looked at lines of flies that have a single mutation on the third chromosome but display two phenotypes: abnormal fat body remodeling and pharate adult lethality. This study aimed to identify genes involved in the process of fat body remodeling and elucidate their role through an understanding of the effect of the mutation on the two phenotypes. Because these two

phenotypes are linked to a single gene, I expected to find a link between fat body morphology and pharate adult lethality. The fat body is used for energy storage and signaling. Thus when it cannot fully remodel, it cannot provide the energy and signaling necessary for the adult to eclose from the pupal case. This relationship is essential for the survival of the adult fly.

MATERIALS AND METHODS

Drosophila Maintenance

Stocks of each mutant line were cultured using Nutri-Fly™ Bloomington Formulation medium prepared with the addition of propionic acid and tegosept in plastic vials and bottles and kept at 25 ºC at 50% humidity. Stocks were transferred to new food every other week. Lines were expanded from vials into bottles and kept at 25 ºC. Virgins and males were collected from these expansions and kept at 18 ºC until they were needed for crosses. Mutant lines were obtained from Wang *et al.* (2008). Due to the health of some of the stocks, not all the lines were able to be expanded or crossed for these experiments.

EMS Mutagenesis and Screen

The mutagenesis was performed by Wang *et al.* (2008). Newly eclosed w^{1118} males isogenized for the third chromosome were aged for 3-4 days and then starved for 8-12 hours before being fed 10mM EMS in 5% sucrose for 12-24 hours. Males were allowed to recover on food not containing EMS before being mated to virgin females carrying a reporter transgene, markers, and balancers. Single F_1 males were crossed to 3-5 multiply marked virgin females so that each of the F_2 stocks created carried a single mutagenized third chromosome; these stocks were labeled with a unique number and scored for lethality (Wang *et al.*

2008). Lines for this screen were selected where the mutants die during metamorphosis, and those that arrested during or after head eversion were considered pupal lethal mutants. These were crossed to the *TM6B, Hu, Tb* third chromosome balancer to select for lines in which more than 75% of the homozygous mutant progeny die as pupae (Wang *et al.* 2008). Fifty of these lines were taken from this screen to be used in the current genetic screen to identify genes involved in fat body remodeling.

Crosses

In order to collect virgin females for crosses, adults were cleared and then all newly eclosed females were collected every 8 hours from the 25ºC incubator or 18 hours from the 18ºC incubator. Virgin females were stored in the 25ºC incubator for 3-5 days after being collected to check for the presence of larvae before being used in crosses, to ensure that all the females were virgin.

Crosses in vials were set up by putting 5-8 males from one line and 8-10 virgin females from another line into vials of fly food with yeast and then put in the 25ºC incubator. These crosses were then transferred to new vials every 2-3 days, after eggs had been laid, to obtain as much progeny as possible for dissection and aging. Crosses set up in bottles with yeast and fly food used 10-12 males from one line and 10-15 virgin females from another.

Dissections

Prepupae were identified as displaying the *Tubby* phenotype or not to determine the genotype. They are taken from their vial at 0 hours APF and staged on a petri dish with a wet filter paper to keep a moist environment. 0 hours APF was identified by a motionless white pupal case. They were then placed in a closed container with a wet paper towel and stored in the 25 ºC incubator for 12 or 14 hours. Then, they were placed on a slide in 1X phosphate buffered saline (PBS) and dissected to look at the fat body. Fat body was visualized by using a Nikon SMZ1500 stereomicroscope. Images were taken using a SPOT Insight QE camera and SPOT Imaging Solutions 5.0 software. Scale bars were added using ImageJ software and images were brightened and text was added using Adobe Photoshop CS5.

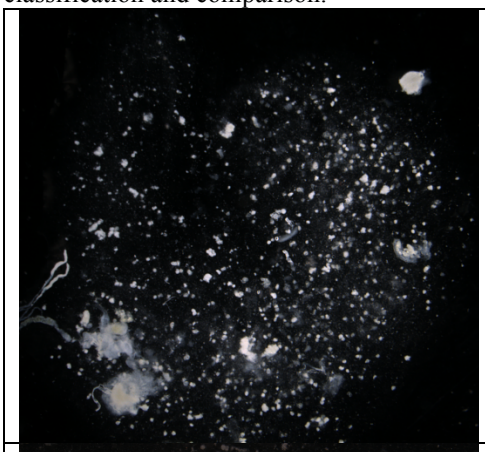
The parental strains were initially all dissected at 12 hours to classify fat body morphology by Patricia Walchessen, Constance Fontanet, and Lissette DeLeon (Walchessen 2016). The F_1 progeny were all dissected at 14 hours to ensure that I was dissecting at a time point when the fat body should have remodeled completely, even if the mutation resulted in some sort of delay.

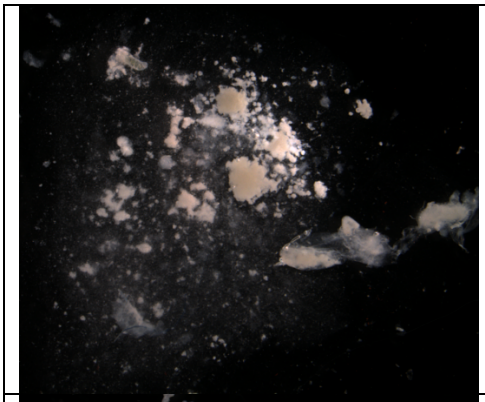
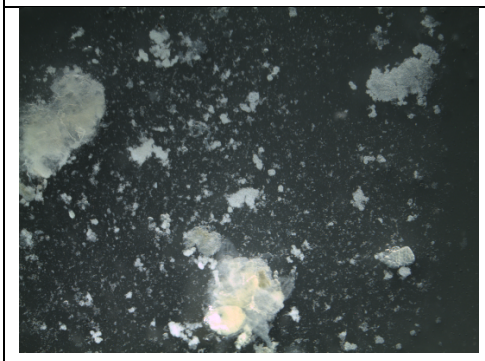
Fat Body Morphology Characterization

Initially, fat body morphology was characterized as similar to wild-type, partially remodeled, no remodeling, and abnormal cell appearance (Walchessen

2016). The complementation tests done for this study were done with the help of Danielle Arshinoff and Uswa Iqbal and characterized using a scoring system based on a set rubric (Table 1), due to the variety in intermediate phenotypes. All scoring of F_1 progeny of the no remodeling crosses was done using photos that had been randomized and three scorers, to ensure accurate and replicable results.

Table 1. Rubric for scoring degree of fat body remodeling at 14 hours APF. Images were taken 14 hours APF. Key differences between the images were identified in order to help classification and comparison.

	**Score: 1** – Wild-Type Remodeling • almost all cells are clearly rounded • almost all cells are dissociated and not part of a clump • clumping is minimal, and individual cells can be seen in the clump • "looks like a bag of marbles was spilled"
	**Score: 2** – Partial Remodeling • Majority of cells are clearly rounded • Majority of cells are dissociated and not part of a clump • Clumping is visible and present • Some of the cells may not be rounded in the clump

	**Score: 3** – Partial Remodeling • About half the cells are individual and half the cells are in clumps • Multiple groups of clumping can be seen • Some individual, rounded cells are present
	**Score: 4** – Partial Remodeling • Majority of cells are in clumps instead of individual • Majority of cells are in one big clump • Rounded, individual cells can be distinguished in the clumps • Some smaller clumps are present • Some individual, rounded cells are present
	**Score: 5** – No Remodeling • Majority of cells are in one big clump • Some individual rounded cells are present, but very few • Hard to identify individual cells in the clump • Note: what look like individual cells are mostly miscellaneous debris

Pharate Adult Lethality

Pupae that had not been collected for dissection or longevity were observed for pharate adult lethality. Two days after the F_1 from each cross started eclosing, I no longer collected prepupae for dissection. I kept the vials to note whether the F_1 were eclosing.

Longevity

In addition to dissecting pupae 14 hours APF, I also took 0 hour APF flies that did not display the *Tubby* phenotype and staged them similarly to the flies I was taking for dissection. Instead of being dissected at 14 hours, these were left in the petri dishes and aged at 25 ºC and then marked for pharate adult lethality or survival. The time and date of their eclosion was marked as was the time and date of their death, and then this phenotype was compared to their fat body phenotype.

Linkage Mapping

We obtained mapping stocks from Sapiro *et al.* (2013) which were originally obtained from the Bloomington *Drosophila* Stock Center, and used the same mapping method that they used. The point of this mapping method was not to identify the exact map position of the mutation but to identify its position relative to two markers with known positions and then use that information to direct subsequent mapping and sequencing techniques.

Sapiro *et al.* (2013) had four pairs of dominant markers to span the third chromosome. In the parental cross, the mutants were crossed to a stock containing two dominant markers on the same chromosome and the female F_1 progeny were selected for the presence of the markers but did not have a balancer, so they were backcrossed to mutant males and the viable nonbalancer progeny, F_2, were then scored for the loss of the markers. The presence of the markers then indicated where a recombination event may have happened. The ratio of the recombination "splits" between the markers reflects the position of the mutation relative to these markers. Once the relative position is identified, that specific part of the chromosome can be sequenced, and the specific chromosomal locus of the mutation can be identified (Sapiro *et al.* 2013).

Males were selected from the no remodeling lines I(3)LL-13567: L 04 PA, I(3)LL-15413: L 04 PA, I(3)LL-7275: L 04 PA, and I(3)LL-11075: L 04 PA to cross to mapping stocks and map both arms of the third chromosome using the technique above.

To map the left arm of the third chromosome, BL1689 mapping flies were used. These flies have *Roughened* and *Diachete* as their dominant markers. Virgin females from this stock were crossed to males from the mutant lines. F_1 virgin females with both of these markers and without the *Humoral* phenotype were selected to back cross to males from the mutant lines. The F_2 progeny were scored

for the presence of the markers, and then the location of crossing over was used to identify chromosomal location (Appendix).

To map the right arm of the third chromosome, BL516 mapping flies were used. These flies have *Hairless* and *Prickly* as their dominant markers. Virgin females from this stock were crossed to males from the mutant lines. Pupal cases that are not *Tubby* were isolated, and virgins that eclosed from these with both of these markers were back crossed to males from the mutant lines. The F_2 progeny were scored for the presence of the markers, and then the location of crossing over was used to identify chromosomal location (Appendix).

RESULTS

Initial Complementation Tests

Previous analyses had determined different fat body morphology classifications for the individual mutant lines, of which there were 50. The initial analyses found 29 lines that had abnormal fat body morphology in conjunction with pharate adult lethality (Walchessen 2016) (Table 5, Appendix). The initial complementation tests were performed at random, due mostly to the health of the stocks, and the F_1 generation phenotypes were assessed (Table 2). While all the adult flies were able to eclose, the morphology of their larval fat bodies at 14h APF was very different. To scale down the experiments, the next set of complementation tests were focused on only the lines that had displayed no fat body remodeling (Table 3).

Table 2. Results from the initial round of complementation tests. After crossing multiple lines and observing the fat body phenotype and the pharate adult phenotype of the F1 generation, there is a consistent trend with regards to pharate adult phenotype but not fat body phenotype.

Cross	Fat Body Phenotype	Pharate Adult Phenotype
I(3)LL-13567: L 04 PA x I(3)LL-15241: L 04 PA	Failure to Complement	Eclose
I(3)LL-15241: L 04 PA x I(3)LL-7275: L 04 PA	Failure to Complement	Eclose
I(3)LL-14641: L 04 PA x I(3)LL-7275: L 04 PA	Failure to Complement	Eclose
I(3)LL-14641: L 04 PA x I(3)LL-13567: L 04 PA	Failure to Complement	Eclose

I(3)LL-15241: L 04 PA x I(3)LL-15413: L 04 PA	Failure to Complement	Eclose
I(3)LL-15413: L 04 PA x I(3)LL-15600: L 04 PA	Failure to Complement	Eclose
I(3)LL-15413: L 04 PA x I(3)LL-1524: L 04 PA	Failure to Complement	Eclose
I(3)LL-15413: L 04 PA x I(3)LL-1464: L 04 PA	Failure to Complement	Eclose
I(3)LL-15600: L 04 PA x I(3)LL-7275: L 04 PA	Failure to Complement	Eclose
I(3)LL-2310: L 04 PA x I(3)LL-15600: L 04 PA	Failure to Complement	Eclose
I(3)LL-6992: L 04 PA x I(3)LL-7275: L 04 PA	Failure to Complement	Eclose
I(3)LL-13567: L 04 PA x I(3)LL-15370: L 04 PA	Failure to Complement –	N/A
I(3)LL-13567: L 04 PA x I(3)LL-17656: L 04 PA	Failure to Complement –	N/A
I(3)LL-13567: L 04 PA x I(3)LL-16768: L 04 PA	Failure to Complement –	N/A
I(3)LL-17656: L 04 PA x I(3)LL-16768: L 04 PA	Failure to Complement –	N/A

No Remodeling Complementation Tests

There were multiple lines that had been assigned the no fat body remodeling phenotype (Walchessen 2016; Iqbal, unpublished). These lines are 13567, 15413, 7275, 2310, 11075, 17770, and 15370 (Table 5). The next round of complementation tests was performed between these lines (Table 3). Due to the health of the stocks, not all lines could be expanded enough to set up crosses between all of the lines. The fat body of the F_1 was dissected and photographed at 14 hours APF (Figures 5, 6; Appendix), and the vials were observed for eclosion of adults. The pharate adult lethal phenotype complemented in all of the crosses,

but the fat body morphology did not. All the parental lines had shown no remodeling, and the F_1 generation of the crosses showed a new phenotype that was neither complete remodeling nor lack of remodeling (Figures 5,6; Appendix). Photos were randomized and scored using the three-scorer and rubric system, and the average of these scores was calculated for each line (Table 3).

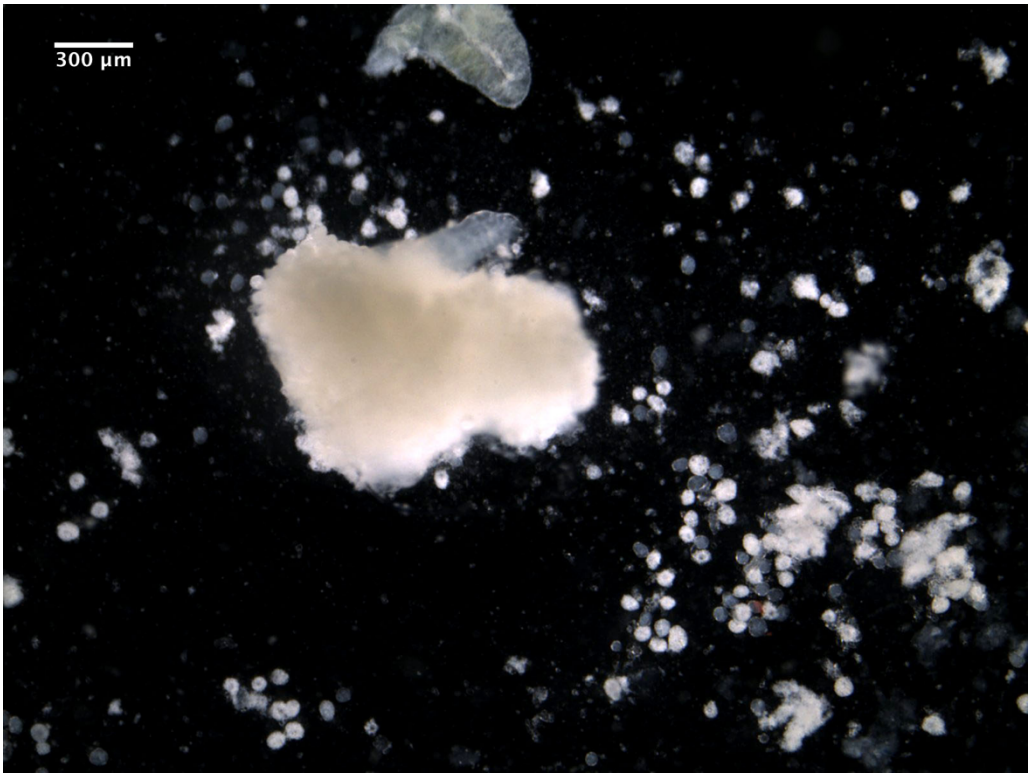


Figure 5. I(3)LL-13567: L 04 PA x I(3)LL-15413: L 04 PA F1 progeny dissected at 14 hours APF. Phenotype is a clear intermediate between no remodeling and wild-type remodeling as there are clearly dissociated cells as well as multiple clumps.

Figure 6. I(3)LL-15413: L 04 PA x I(3)LL-11075: L 04 PA F1 progeny dissected at 14 hours APF. Phenotype is a clear intermediate between no remodeling and wild-type remodeling as there are clearly dissociated cells as well as individual clumps.

Table 3. No Remodeling Complementation Tests. After crossing multiple lines and observing the fat body phenotype and the pharate adult phenotype of the F1 generation, there is a consistent trend with regards to pharate adult phenotype but not fat body phenotype.

Cross	Fat Body Phenotype	Pharate Adult Phenotype
I(3)LL-11075: L 04 PA x I(3)LL-15413: L 04 PA	Score: 3.3	Eclose
I(3)LL-11075: L 04 PA x I(3)LL-13567: L 04 PA	Score: 2	Eclose
I(3)LL-11075: L 04 PA x I(3)LL-2310: L 04 PA	Score: 2.93	Eclose

I(3)LL-13567: L 04 PA x I(3)LL-11075: L 04 PA	Score: 3	Eclose
I(3)LL-13567: L 04 PA x I(3)LL-15413: L 04 PA	Score: 2.5	Eclose
I(3)LL-13567: L 04 PA x I(3)LL-17770: L 04 PA	Score: 3.2	Eclose
I(3)LL-15413: L 04 PA x I(3)LL-13567: L 04 PA	Score: 2.71	Eclose
I(3)LL-15413: L 04 PA x I(3)LL-11075: L 04 PA	Score: 3.3	Eclose
I(3)LL-15413: L 04 PA x I(3)LL-7275: L 04 PA	Score: 3.25	Eclose
I(3)LL-17770: L 04 PA x I(3)LL-11075: L 04 PA	Score: 3.08	Eclose
I(3)LL-17770: L 04 PA x I(3)LL-13567: L 04 PA	Score: 3.21	Eclose
I(3)LL-17770: L 04 PA x I(3)LL-7275: L 04 PA	Score: 3.33	Eclose
I(3)LL-2310: L 04 PA x I(3)LL-17770: L 04 PA	Score: 2.76	Eclose
I(3)LL-2310: L 04 PA x I(3)LL-7275: L 04 PA	N/A	Eclose
I(3)LL-2310: L 04 PA x I(3)LL-13567: L 04 PA	Score: 3.2	Eclose
I(3)LL-2310: L 04 PA x I(3)LL-15413: L 04 PA	Score: 2.5	Eclose
I(3)LL-7275: L 04 PA x I(3)LL-13567: L 04 PA	N/A	Eclose
I(3)LL-7275: L 04 PA x I(3)LL-11075: L 04 PA	Score: 3.13	Eclose
I(3)LL-7275: L 04 PA x I(3)LL-2310: L 04 PA	N/A	Eclose

Dissections of Heterozygotes

Initial fat body dissections were done with pupae that were homozygous for the mutation in order to categorize the phenotype that corresponded to pharate adult lethality. Heterozygous pupae were also dissected in order to determine if the mutations were dominant or not. Pupae were taken at 0 hours APF and aged to 12 hours APF to compare to the homozygotes from the original classification. Many dissections of heterozygotes from the no remodeling lines showed various intermediate phenotypes. There were no lines where the heterozygotes were dissected where the fat body had completely remodeled at 12 hours APF. Some lines showed varying degrees of remodeling between the flies that were dissected (Figure 8), while some lines showed phenotypes that were similar to the homozygotes (Figure 7). Initial classifications held that the heterozygotes were not pharate adult lethal. However, upon closer examination, while most heterozygous flies are able to eclose from their pupal cases, there are some lines, such as I(3)LL-7275:L 04 PA and I(3)LL-11075:L 04 PA, that appear to have some pharate adult lethality in the heterozygous phenotypes. These heterozygotes also show variability in fat body morphology (Appendix).

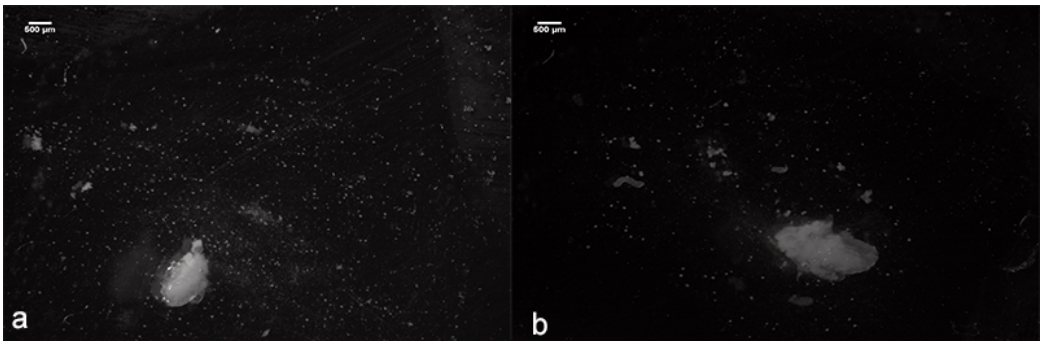


Figure 7. Side by side comparison of I(3)LL-2310: L 04 PA homozygote and heterozygote dissected at 12 hours APF. (a) homozygous mutant shows no remodeling phenotype. (b) heterozygote shows slightly more remodeling. This mutation may be dominant.

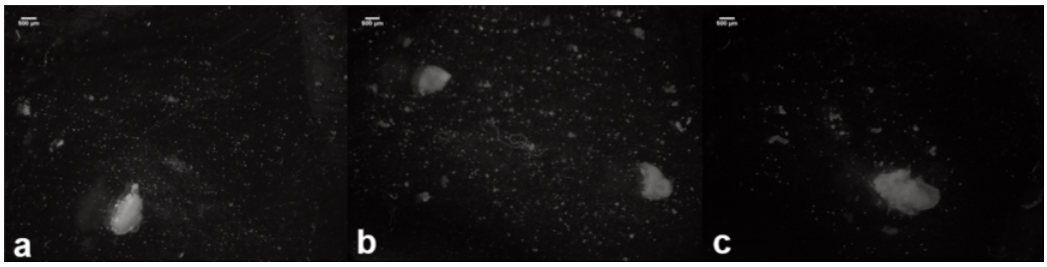


Figure 8. I(3)LL-2310: L 04 PA heterozygotes dissected at 12 hours APF. (a) Heterozygote shows partial remodeling (b) Heterozygote shows partial remodeling (c) Heterozygote shows partial remodeling. All are variable levels of remodeling which may be due to mutation penetrance.

Longevity Assay

Results from both rounds of complementation testing showed multiple different fat body phenotypes, but consistently showed a normal pharate adult phenotype. In order to better understand what the effect of fat body remodeling had on the pharate adult phenotype, the post-eclosion lifespan of the adults was compared to the wild-type life span (Table 4). To understand if the difference in

lifespan between the F_1 progeny and wild-type was significant, an independent t-test was performed to compare the average lifespan between the two lines. In addition, the average post-eclosion lifespan to the average amount of remodeling was compared to see if there was any correlation between the amount the fat body had remodeled by 14 hours APF and the length of time the adult fly lived post-eclosion (Figure 9). The linear regression model of this relationship shows that there is a generally positive correlation between amount of remodeling and post-eclosion lifespan ($R^2 = 0.15634$).

Table 4. Average Post-Eclosion Lifespan of F1 Generation. The no remodeling lines were crossed to each other and the pupae were selected at 0h to be aged until eclosion. The time of eclosion was marked, as was the time of death. The average lifespan was then calculated. An independent t-test was run to compare the F1 average lifespan of each cross to the average wild-type life span and determine if the results were significant.

* P-value is significant
**Weren't able to get 10 0 hour flies for assay

Cross	Average post-eclosion lifespan (hours)	p-value
w^{1118}	70.9	-
I(3)LL-13567: L 04 PA x I(3)LL-15413: L 04 PA	31.8	< 0.00001*
I(3)LL-15413: L 04 PA x I(3)LL-13567: L 04 PA	38.7	< 0.00001*
I(3)LL-7275: L 04 PA x I(3)LL-11075: L 04 PA	44.5	0.000508*
I(3)LL-17770: L 04 PA x I(3)LL-7275: L 04 PA	47.2	0.000013*
I(3)LL-11075: L 04 PA x I(3)LL-2310: L 04 PA	51.5	< 0.00001*
I(3)LL-2310: L 04 PA x I(3)LL-17770: L 04 PA	52.0	0.000132*
I(3)LL-13567: L 04 PA x I(3)LL-17770: L 04 PA	52.7**	0.001237*
I(3)LL-17770: L 04 PA x I(3)LL-13567: L 04 PA	51.3	< 0.00001*
I(3)LL-2310: L 04 PA x I(3)LL-13567: L 04 PA	50.0	< 0.00001*
I(3)LL-2310: L 04 PA x I(3)LL-15413: L 04 PA	45.3	< 0.00001*
I(3)LL-7275: L 04 PA x I(3)LL-2310: L 04 PA	58.8**	0.054499
I(3)LL-15241: L 04 PA x I(3)LL-2310: L 04 PA	65	0.09714
I(3)LL-15370: L 04 PA x I(3)LL-7275: L 04 PA	55.5**	0.028296*
I(3)LL-13567: L 04 PA x I(3)LL-17770: L 04 PA	69.5**	0.433136
I(3)LL-15370: L 04 PA x I(3)LL-11075: L 04 PA	61.8**	0.136999

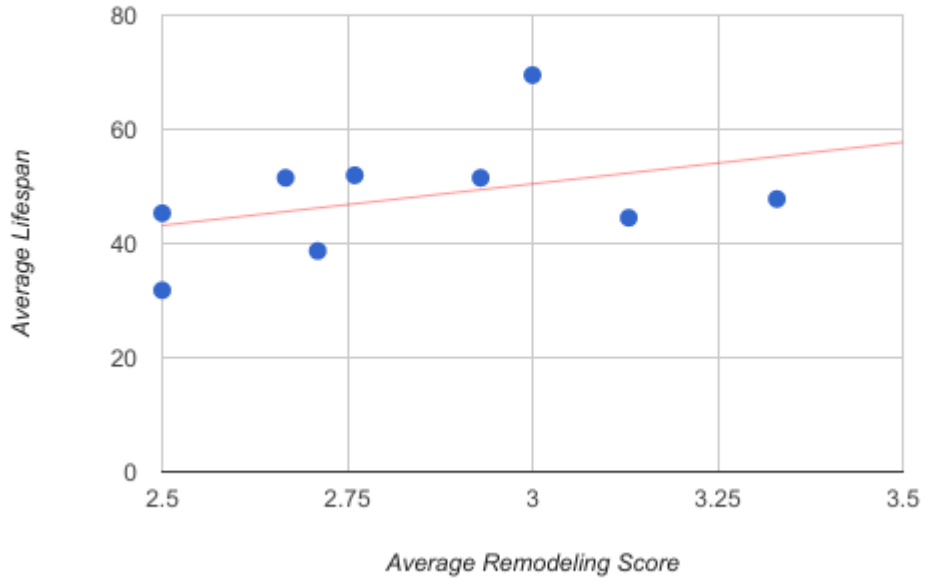


Figure 9. Scatter plot with trendline of mean post-eclosion life span and mean remodeling score for above crosses. The r value of this trend line is 0.3954 and the r^2 value is 0.15634, showing that there is a generally positive correlation between the length of time an adult fly lives post-eclosion and the amount its fat body has remodeled.

Linkage Mapping

The process of linkage mapping using the method in Sapiro *et al.,* (2013) was begun on four of the no remodeling lines: I(3)LL-13567: L 04 PA, I(3)LL-15413: L 04 PA, I(3)LL-11075: L 04 PA, and I(3)LL-7275: L 04 PA. A pair of dominant markers for each arm of the third chromosome was used in order to narrow down the location of each mutation. However, the method in Sapiro *et al.* (2013) used four pairs which spanned the entire chromosome, while this

preliminary mapping was done with two pairs of dominant markers in order to narrow down the location of each mutation.

For I(3)LL-13567:L 04 PA, a majority of the flies that were scored had lost the left marker of the dominant marker pair for the left arm of the chromosome, and therefore the mutation is located between these two markers. Using the equation in Sapiro *et al.,* (2013) (Appendix), the location of the mutation was calculated to be at 29.9 centimorgans. For I(3)LL-7275: L 04 PA, a majority of the flies that were scored had lost the left marker of the dominant marker pair for the right arm of the chromosome, and therefore the mutation is located between these two markers. Calculations for this location mapped it to 79.5 centimorgans. For I(3)LL-11075: L 04 PA and I(3)LL-15413: L 04 PA, the high number of flies that had either both of the dominant markers or neither suggested that the mutation was actually in a part of the chromosome that was between the two pairs of markers. Without information from other dominant marker pairs spanning this region of the chromosome, the map units for this location could not be calculated.

DISCUSSION

Screen

The lines from the original EMS mutagenesis that were initially selected for this study showed both abnormal fat body morphology and pharate adult lethality. Because the mutagenesis was assumed to have produced a single mutation on the third chromosome for all of these lines, it was hypothesized that fat body remodeling was impacting the pharate adult phenotype – that abnormal fat body remodeling lead to pharate adult lethality. A fully saturated screen is able to identify all genes that play a role in a specific biological function. The initial screen was fully saturated for the third chromosome, so that this screen should be able to identify every gene on the third chromosome that required for proper fat body remodeling. There are many known genes that may have been mutated in this initial screen that are on the third chromosome and are likely responsible for some of the phenotypes that have been observed. However, due to the amount of intermediate remodeling detected by the complementation tests, it is likely that there are other, novel genes that were mutated that play a role in the overall pathway of fat body remodeling.

The process of fat body remodeling involves multiple proteins and signaling cascades. In order for the fat body to remodel, the cells must change

from polygonal cells to spherical cells and then dissociate from each other. If there are abnormalities in the ECM, the adhesion proteins, or the proteases responsible for degradation, the fat body may fail to remodel completely (Jia *et al.* 2014). In addition, any abnormalities in the signaling cascades that signal for the fat body to remodel could contribute to an abnormal remodeling phenotype. Ecdysone signaling is important for cell shape change and dissociation (Bond *et al.* 2011); therefore any interruption in this signaling pathway could result in abnormal morphology. Previous studies have shown that disruptions in ecdysone signaling results in the aggregation of fat body cells (Cherbas *et al.* 2003) in a manner similar to some of the phenotypes we have seen in our dissections.

There are some known genes on the third chromosome that are involved in the process of fat body remodeling, and mutations in any of these genes would likely affect fat body morphology. One of the genes of interest is *βftz-f1*, which is located on the third chromosome (Bond *et al.* 2011). This gene is an important competence factor for tissues to be able to respond to ecdysone. Therefore mutations in this gene could have downstream effects that could result in abnormal fat body morphology. *TIMP* is another gene that is located on the third chromosome and has been implicated in playing a role in the ability of the fat body to remodel due to its interaction with *Mmp1* and *Mmp2* (Wei *et al.* 2003; Bond *et al.* 2011). The P13K pathway is regulated by ecdysone and could also play a role in fat body remodeling as well as autophagy in the fat body. There are

essential genes in the phosphorylation cascade of this pathway which are located on the third chromosome. These genes are the insulin-like peptide (dILP), the insulin receptor (dInR), the phosphoinositide 3-kinase (P3K), and the effector Akt (Li *et al.* 2010).

Complementation Tests

The results from the initial set of complementation tests show a lack of complementation for the fat body remodeling phenotype but complementation for the pharate adult lethal phenotype (Table 2). These results show that the mutations may all be in different genes and this screen could be looking at dozens of genes. These results lead me to try and target my approach in order to better understand the relationship of the two phenotypes.

The no remodeling phenotype is the most severe, so I wanted to better understand the mutations that were causing this phenotype. In each line, the homozygotes are pharate adult lethal, but heterozygotes are able to eclose. I wanted to perform a set of complementation tests on these lines in hopes of establishing complementation groups within these lines and identifying the number of genes. However, even though all of the crosses complemented each other with regards to the pharate adult phenotype, I still failed to see normal fat body morphology. The complementation tests showed various intermediate levels of remodeling in the progeny. A likely explanation for this result could be that the

genes affected are all in the same pathway, so that a mutation in any gene disrupts the pathway sufficiently to cause fat body morphology. Since most of the heterozygotes of the parental strains are able to eclose from their pupal cases, it can be inferred that one wild-type copy of each of these genes is enough to compensate for the pharate adult lethal phenotype.

The heterozygote phenotypes were different across the no remodeling lines. Even within some of the lines, the heterozygote fat body phenotype varied, which may be due to the penetrance of the mutation. In some heterozygotes, fat body morphology was similar to the homozygote morphology, suggesting that these mutations are dominant. There were also lines in which some of the heterozygotes were not able to eclose. These are likely flies where the mutation is stronger and results in no fat body remodeling.

Longevity Assay

The initial mutagenesis produced one mutation in each line that had two phenotypes – abnormal fat body morphology and pharate adult lethality. The results from the complementation tests and dissections in which the fat body abnormalities appeared less severe and the pharate adult lethal phenotype would complement, which lead me to try to better understand the relationship between the two phenotypes. The larval fat body cells persist in the adult fly for a few days post-eclosion because they may be a source of energy for the newly eclosed adult

fly (Nelliot *et al.* 2006). If remodeling of the fat body is essential for the fat body cells to be used as energy and nutrients, then it can be expected that the amount of remodeling will correspond with the lifespan of the adult fly post-eclosion. All of the F_1 progeny that showed some level of partial remodeling were able to eclose, yet they did not live as long as the wild-type flies (Table 4). For almost all of the F_1 progeny, lifespans were significantly reduced compared to the wild-type flies, providing evidence that the ability of the fat body to remodel plays a role in the ability of the adult fly to eclose and in adult lifespan post-eclosion.

When the average remodeling scores were compared to the lifespan in a linear model, the model showed a generally positive correlation which means that these two traits are likely related (Figure 9). However, the low R^2 value means that this specific model cannot accurately use the amount of remodeling to predict lifespan. The overall results from this set of experiments provide evidence that the ability of the fat body to remodel does have some effect on the adult's post-eclosion lifespan, although further studies and more data are needed to be able to identify whether and to what degree level of remodeling can predict lifespan.

Linkage Mapping

I was able to identify the general locations of the mutations in the four lines where mapping was begun: I(3)LL-13567: L 04 PA, I(3)LL-15413: L 04 PA, I(3)LL-11075: L 04 PA, and I(3)LL-7275: L 04 PA. I was able to identify if

the mutations were either within one of the dominant marker pairs I had used to map, or if they were in the region of the chromosome for which I was lacking the dominant marker pairs. However, sequencing will be needed to determine the exact locus of the mutation and the type of mutation. Once such sequencing is complete, a gene will be identified as having a role in fat body remodeling and the mutation can help elucidate gene function and also the role it may have in the pathway that I believe exists.

Future Directions

This study chose to focus on 7 of the initial 50 lines. In order to fully identify the number of genes that are involved, complementation tests should be done between all 50 lines. Longevity can also be compared to fat body morphology in all of these crosses to better understand the relationship between the two phenotypes. More data may be able to produce a better linear model which can be used to gain a better understanding of whether or not and with what level of accuracy fat body morphology can be used as a predictor of longevity.

The mapping of the 7 lines has not been finished, as the precise chromosomal locus and mutation type can be identified through sequencing to better understand the specific genes and gene functions that were causing the no remodeling phenotypes. In addition, once complementation tests have been run, all the genes can be mapped using the dominant marker system and then

sequenced to be able to gain a broader understanding of the genes and pathways involved in the process of fat body remodeling.

These complementation tests and mapping processes could be expanded to include a screen on all four *Drosophila* chromosomes to gain a comprehensive picture of all of the genes that are involved in fat body remodeling.

Summary

This study used a genetic screen to identify genes on the third chromosome involved in fat body remodeling, and to elucidate the relationship between the fat body and its function in the later stages of metamorphosis and into the early adult. While no specific genes were identified in this study, this study was able to provide insight into the relationship between the two phenotypes, which may help elucidate the role of the larval fat body and the importance of remodeling for the adult fly. In addition, this study made progress in identifying specific genes. This headway suggests greater implications for understanding the process of tissue remodeling in higher organisms.

APPENDIX

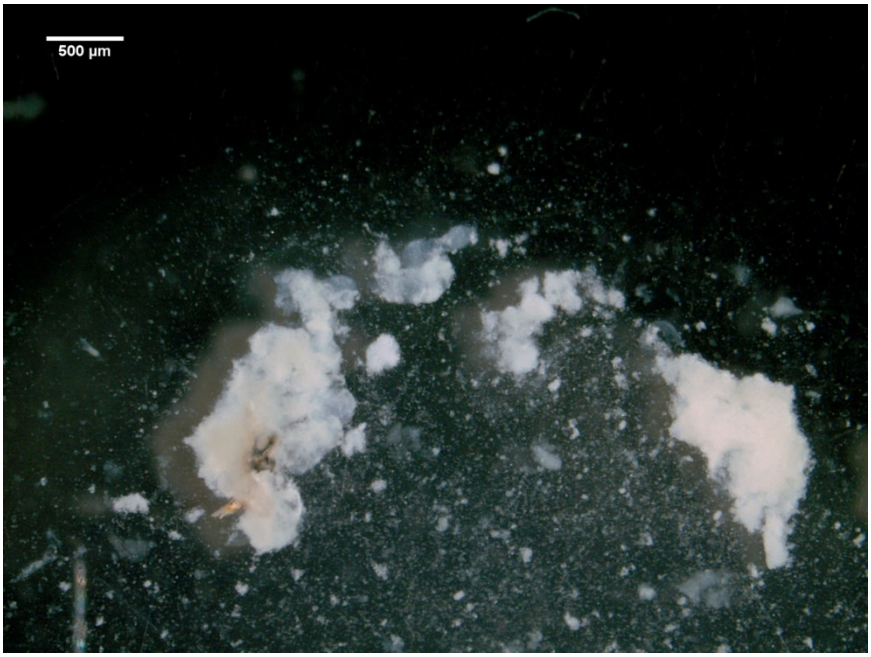


Figure 10. I(3)LL-2310: L 04 PA homozygous mutant dissected at 12 hours APF. This line was classified as the no remodeling phenotype (Walchesson 2016).

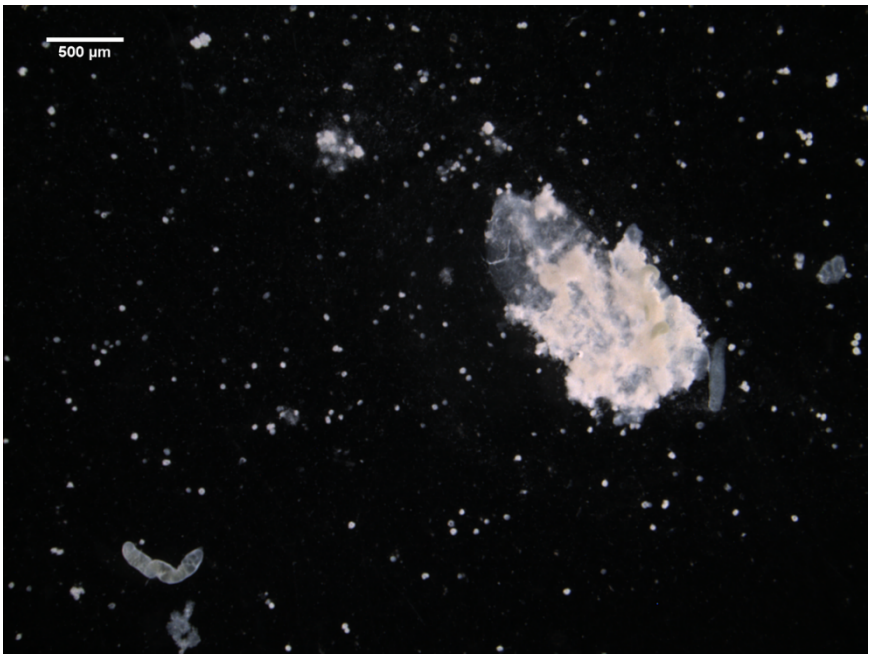


Figure 11. I(3)LL-7275: L 04 PA homozygous mutant dissected at 12 hours APF. This line was classified as the no remodeling phenotype (Walchesson 2016).

Figure 12. I(3)LL-11075: L 04 PA homozygous mutant dissected at 12 hours APF. This line was classified as no remodeling (Walchesson 2016).

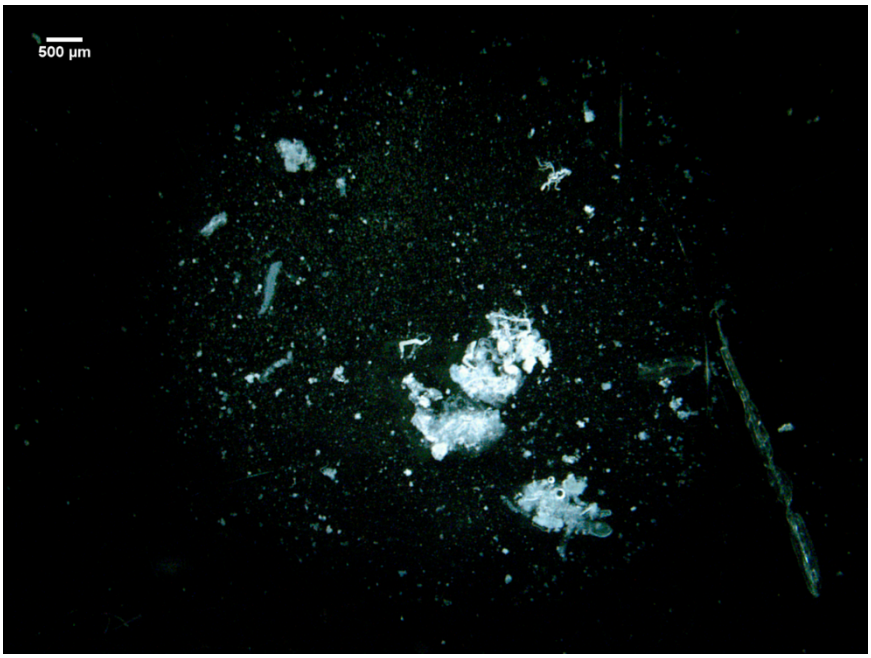


Figure 13. I(3)LL-13567: L 04 PA homozygous mutant dissected at 12 hours APF. This line was classified as no remodeling (Walchesson 2016).

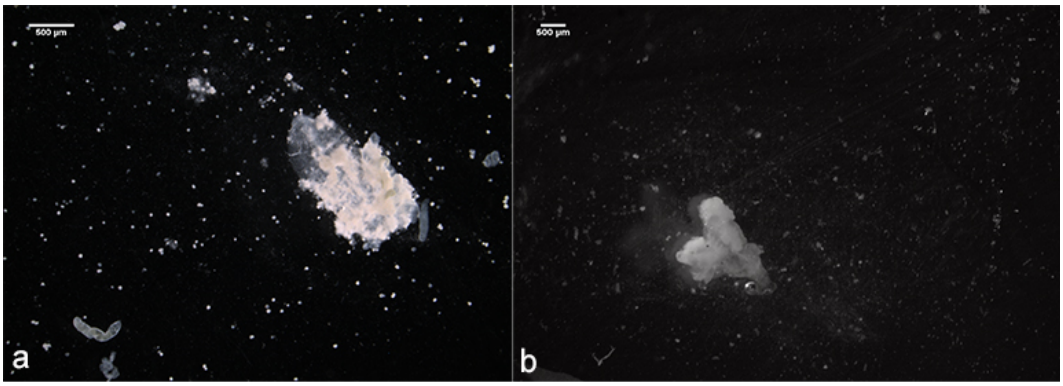


Figure 14. Side by side comparison of I(3)LL-7275: L 04 PA homozygote and heterozygote dissected at 12 hours APF. (a) homozygote and (b) heterozygote show different levels of remodeling.

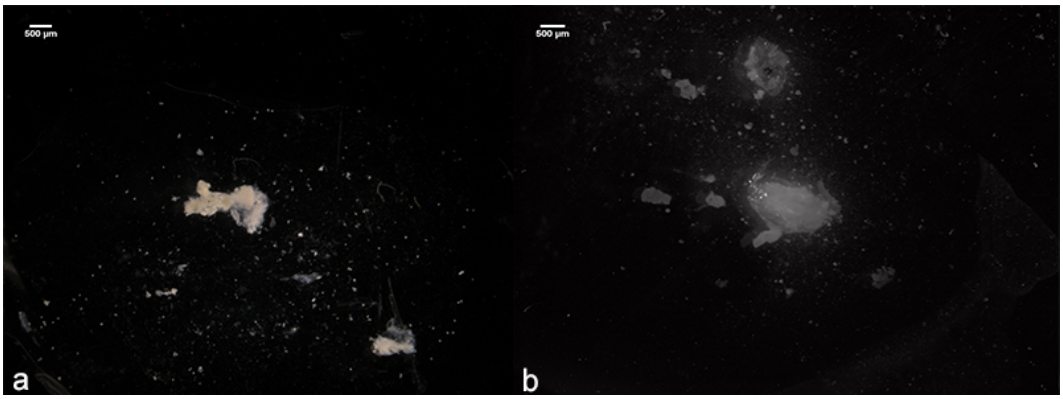


Figure 15. Side by side comparison of I(3)LL-11075: L 04 PA homozygote and heterozygote dissected at 12 hours APF. (a) homozygote and (b) heterozygote show different levels of remodeling.

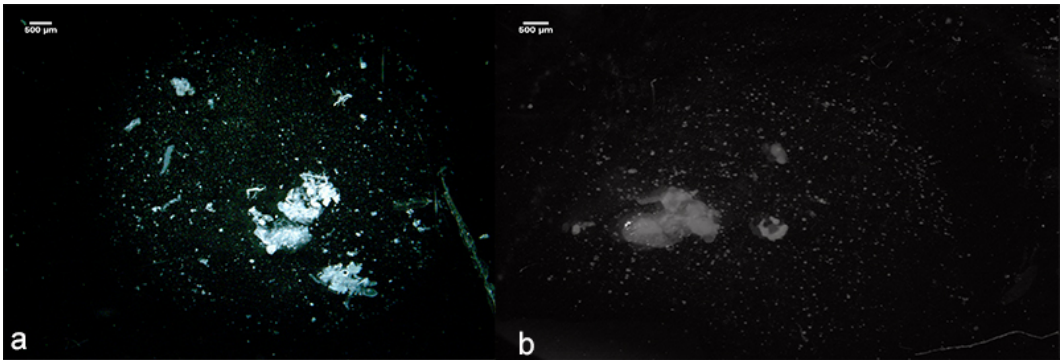


Figure 16. Side by side comparison of I(3)LL-13567: L 04 PA homozygote and heterozygote dissected at 12 hours APF. (a) homozygote and (b) heterozygote show different levels of remodeling.

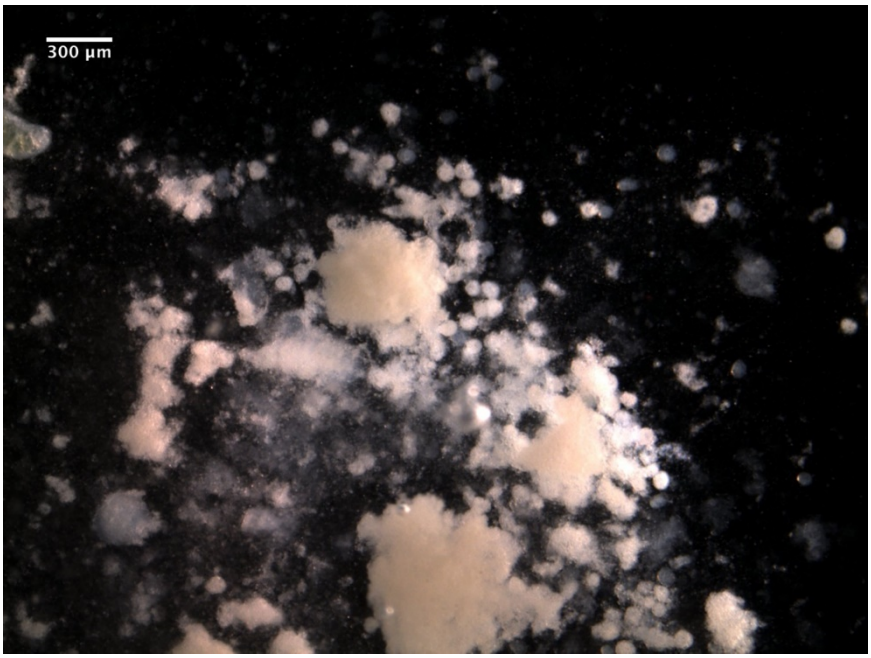


Figure 17. I(3)LL-2310: L 04 PA x I(3)LL-7275: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

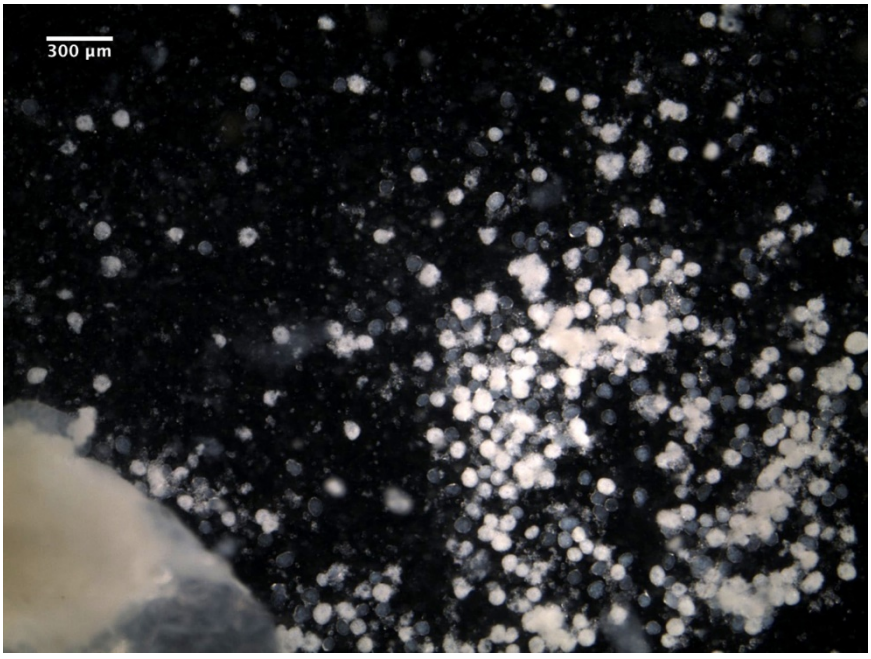


Figure 18. I(3)LL-2310: L 04 PA x I(3)LL-17770: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

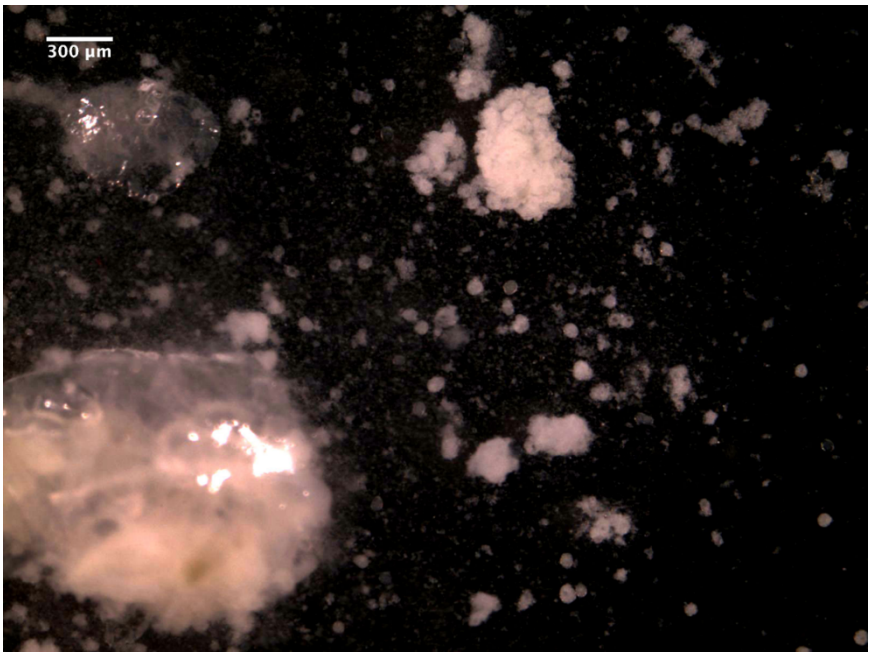


Figure 19. I(3)LL-2310: L 04 PA x I(3)LL-135367: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype

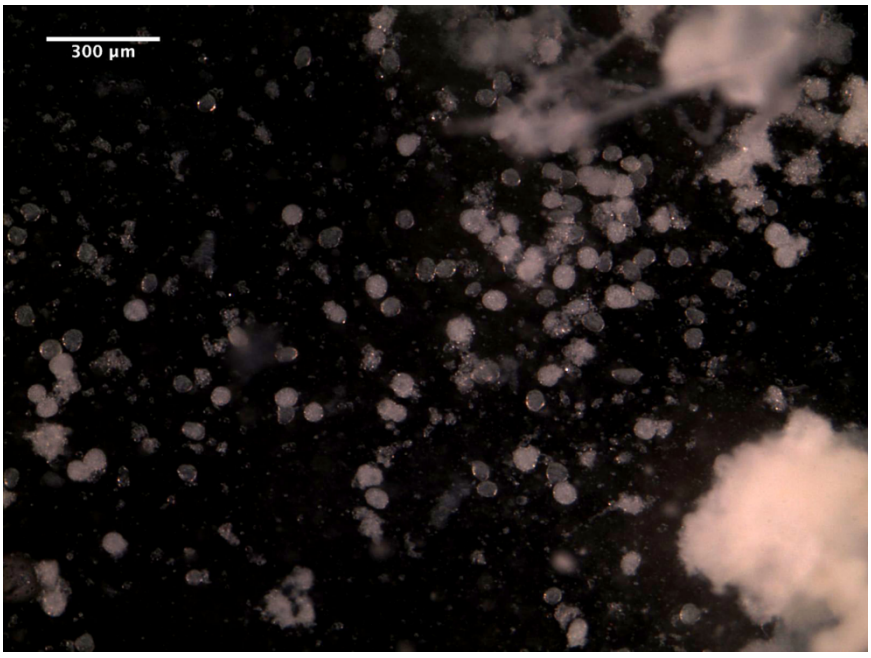


Figure 20. I(3)LL-2310: L 04 PA x I(3)LL-15413: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

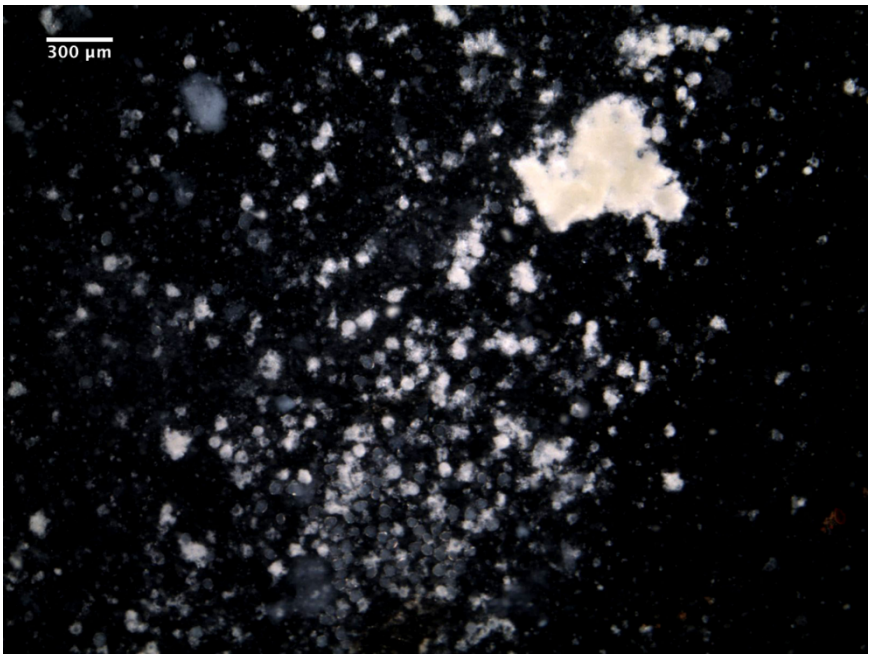


Figure 21. I(3)LL-7275: L 04 PA x I(3)LL-11075: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

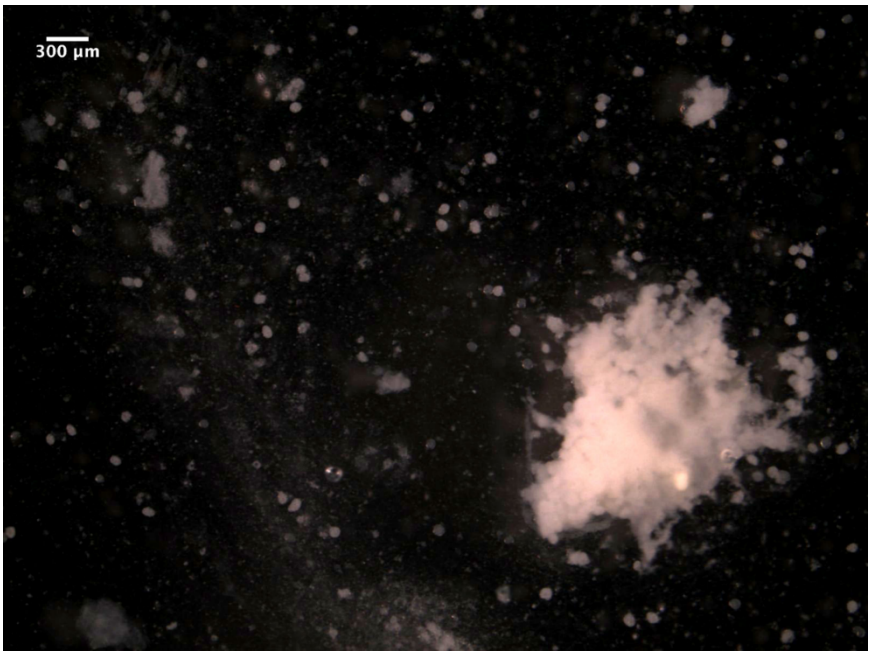


Figure 22. I(3)LL-7275: L 04 PA x I(3)LL-2310: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

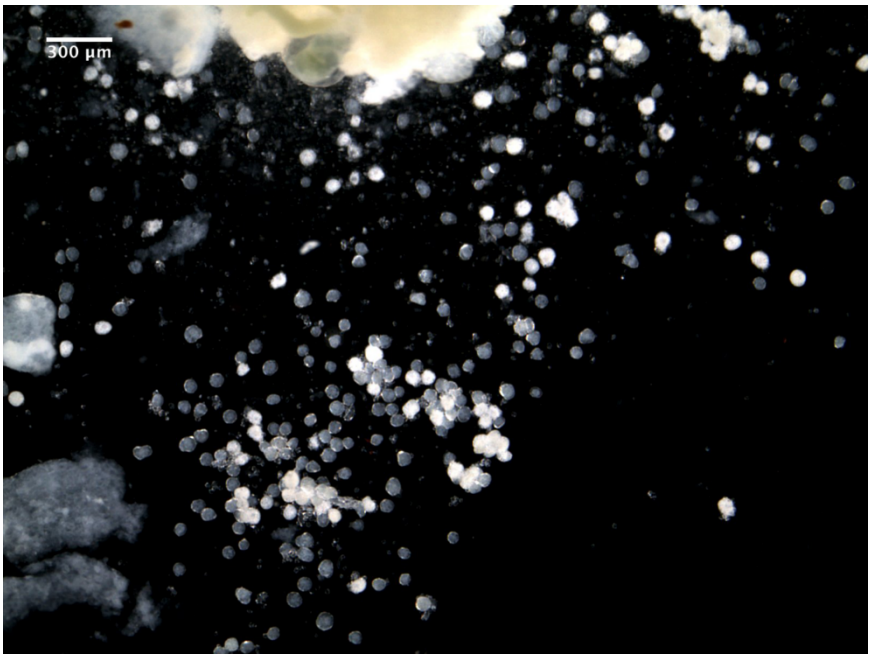


Figure 23. I(3)LL-17770: L 04 PA x I(3)LL-7275: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

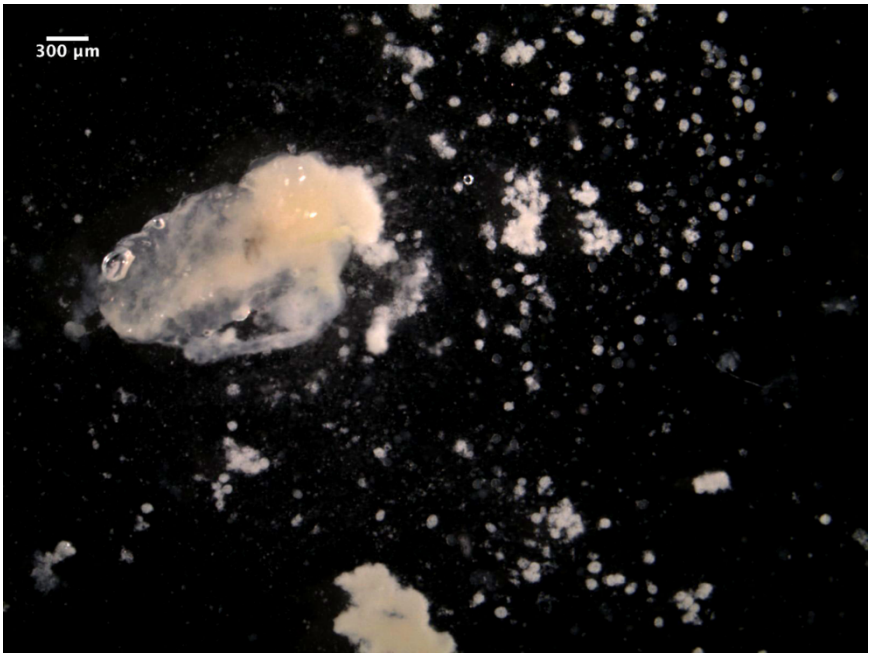


Figure 24. I(3)LL-17770: L 04 PA x I(3)LL-11075: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

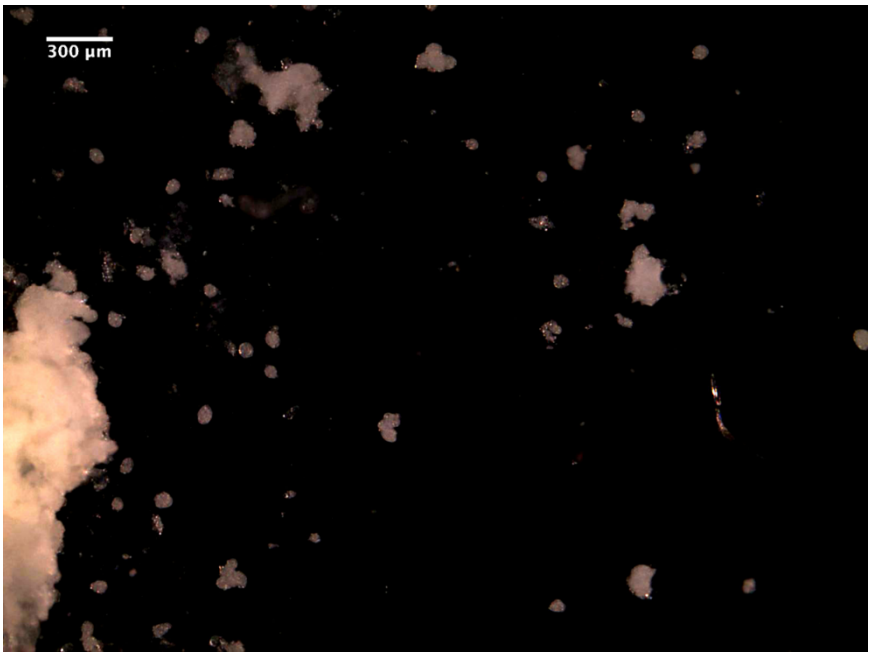


Figure 25. I(3)LL-17770: L 04 PA x I(3)LL-13567: L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

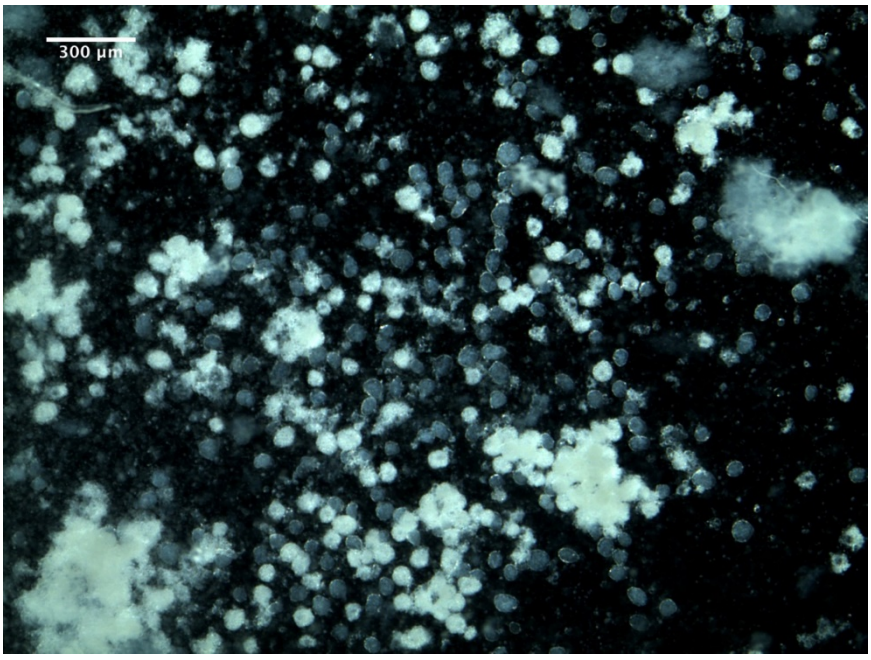


Figure 26. I(3)LL-7275:L 04 PA x I(3)LL-13567:L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

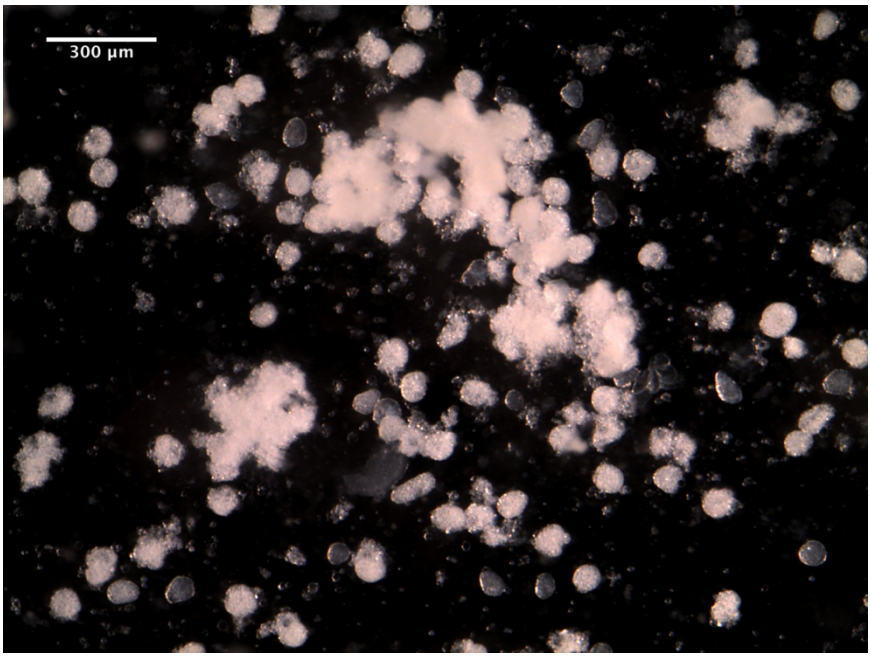


Figure 27. I(3)LL-11075: L 04 PA x I(3)LL-2310:L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

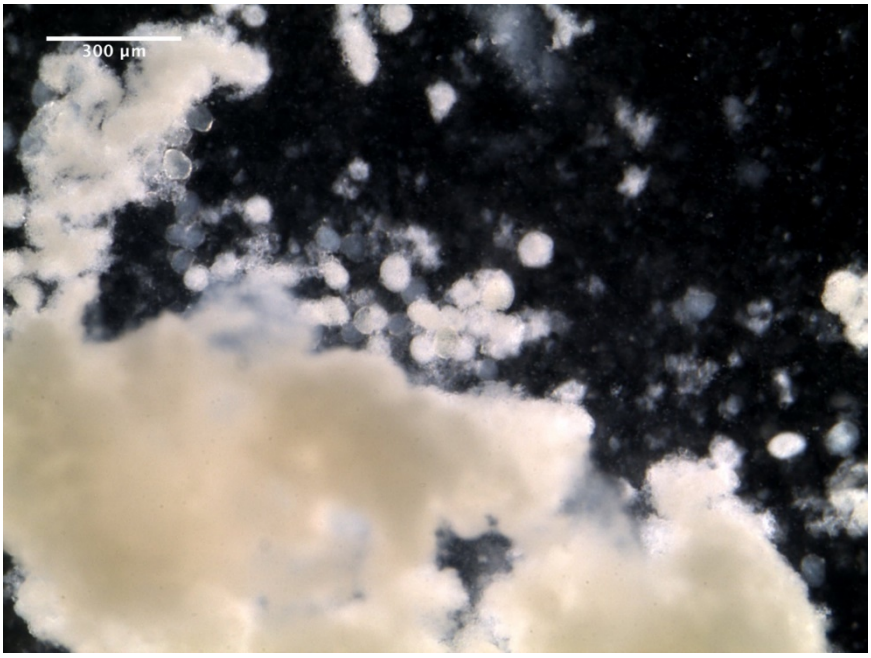


Figure 28. I(3)LL-13567:L 04 PA x I(3)LL-2310:L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

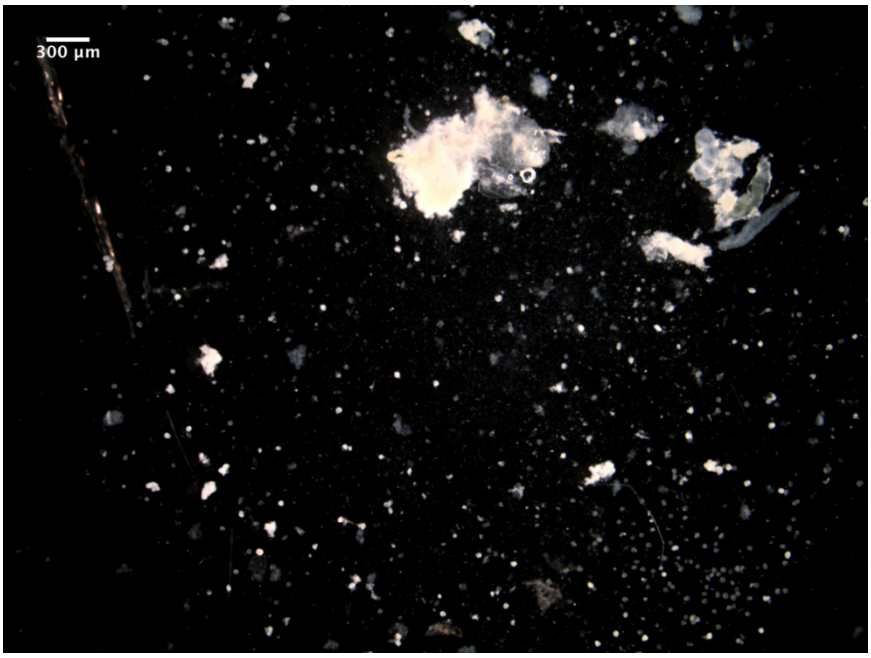


Figure 29. I(3)LL-13567:L 04 PA x I(3)LL-11075:L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

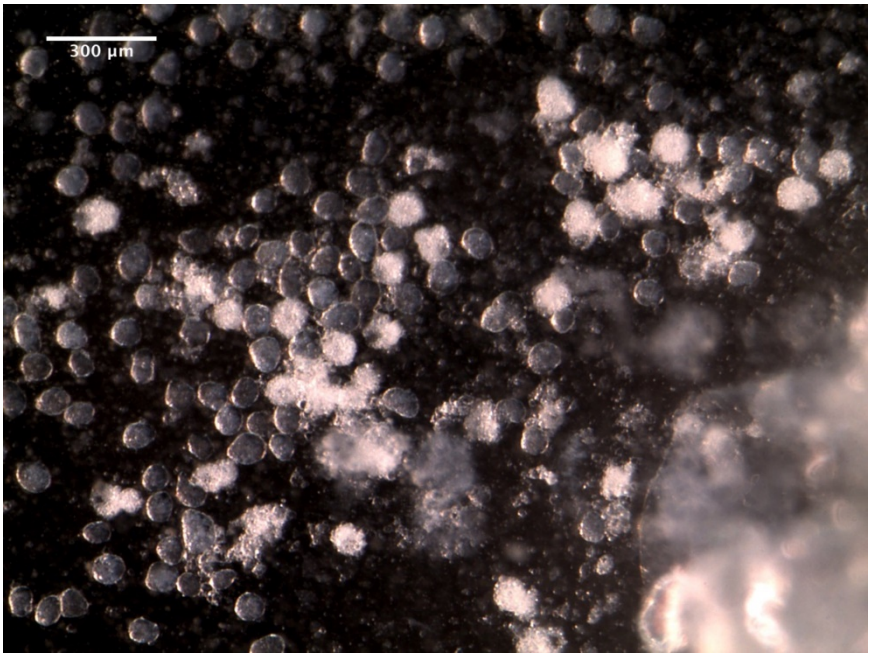


Figure 30. I(3)LL-15413:L 04 PA x I(3)LL-7275:L 04 PA F1 progeny dissected at 14 hours APF. Fat body shows partial remodeling phenotype.

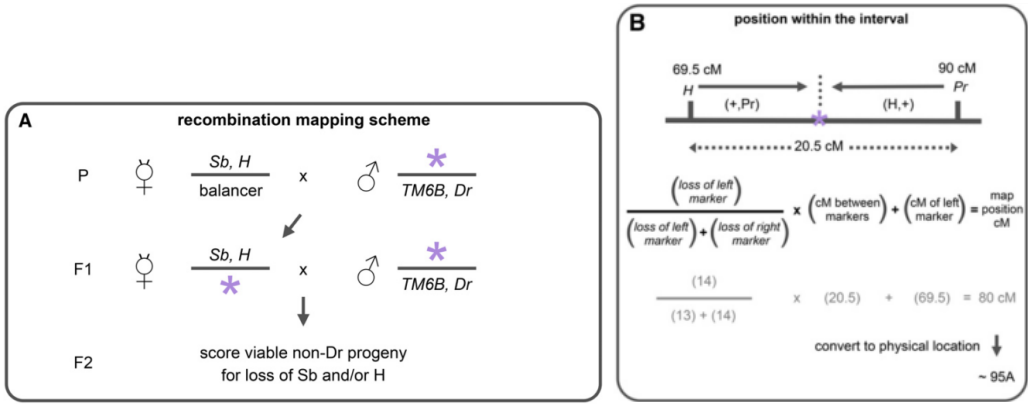


Figure 31. Recombination mapping scheme and chromosomal mapping equation for linkage mapping with dominant marker pairs. (a) Example of crosses used between mutagenized males and mapping virgin females to obtain F_2 generation with dominant markers for scoring to then determine the location of the mutation relative to the markers by number of recombination events. (b) Equation used to map chromosomal location after using recombination events to place the mutation between dominant markers (Sapir*o et al*., 2013).

Table 5. Initial categorization of fat body morphology of mutagenized lines. Parental strains were dissected at 12 hours APF and their fat body morphology was classified before the rubric and scoring system was developed (Walchessen 2016).

Normal Appearance	Partially Remodeled	Not Remodeled	Abnormal Appearance	Uncategorized
I(3)LL-11049: L 04 PA	I(3)LL-2536: L 04 PA	I(3)LL-2310: L 04 PA	I(3)LL-2658: L 04 PA	I(3)LL-620: L 04 PA
I(3)LL-11224: L 04 PA	I(3)LL-3284: L 04 PA	I(3)LL-11075: L 04 PA	I(3)LL-4234: L 04 PA	I(3)LL-3770: L 04 PA
I(3)LL-11961: L 04 PA	I(3)LL-4994: L 04 PA	I(3)LL-15370: L 04 PA	I(3)LL-5996: L 04 PA	I(3)LL-6031: L 04 PA
I(3)LL18835: L 04 PA	I(3)LL-6128: L 04 PA	I(3)LL-15413: L 04 PA	I(3)LL-7759: L 04 PA	I(3)LL-7275: L 04 PA
	I(3)LL-6992: L 04 PA	I(3)LL-17770: L 04 PA	I(3)LL-7827: L 04 PA	I(3)LL-7298: L 04 PA
	I(3)LL-7749: L 04 PA		I(3)LL-11074: L 04 PA	I(3)LL-8683: L 04 PA
	I(3)LL-11250: L 04 PA		I(3)LL-13204: L 04 PA	I(3)LL-9114: L 04 PA
	I(3)LL-13774: L 04 PA		I(3)LL-16768: L 04 PA	I(3)LL-9725: L 04 PA
	I(3)LL-14641: L 04 PA		I(3)LL-17656: L 04 PA	I(3)LL-17656: L 04 PA
	I(3)LL-11062: L 04 PA			I(3)LL-15600: L 04 PA
	I(3)LL-11640: L 04 PA			I(3)LL-13567: L 04 PA
	I(3)LL-17019: L 04 PA			I(3)LL-13757: L 04 PA
	I(3)LL-17514: L 04 PA			I(3)LL-14261: L 04 PA
	I(3)LL-18867: L 04 PA			I(3)LL-15174: L 04 PA
	I(3)LL-18942: L 04 PA			I(3)LL-15241: L 04 PA
	I(3)LL-19028: L 04 PA			I(3)LL-15882: L 04 PA
				I(3)LL-17821: L 04 PA
				I(3)LL-18724: L 04 PA
				I(3)LL-18805: L 04 PA

LITERATURE CITED

Agawa Y., Sarhan M., Kageyama Y., Akagi K., Takai M., *et al.*, 2007 *Drosophila* Blimp-1 is a transient transcriptional repressor that controls timing of the ecdysone-induced developmental pathway. Mol. Cell. Biol. 27: 8739–8747.

Aguila J. R., Suszko J., Gibbs A. G., Hoshizaki D. K., 2007 The role of larval fat cells in adult *Drosophila melanogaster*. J. Exp. Biol. 210: 956–63.

Aguila J. R., Hoshizaki D. K., Gibbs A. G., 2013 Contribution of larval nutrition to adult reproduction in *Drosophila melanogaster*. J Exp Biol 216: 399–406.

Bainbridge S. P., Bownes M., 1981 Staging the metamorphosis of *Drosophila melanogaster*. J. Embryol. Exp. Morphol. 66: 57–80.

Bond N. D., Nelliot A., Bernardo M. K., Ayerh M. A., Gorski K. A., *et al.*, 2011 ßFTZ-F1 and Matrix metalloproteinase 2 are required for fat-body remodeling in *Drosophila*. Dev. Biol. 360: 286–296.

Broadus J., McCabe J. R., Endrizzi B., Thummel C. S., Woodard C. T., *et al.*, 1999 The *Drosophila* beta FTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone. Mol. Cell 3: 143–9.

Cherbas L., Hu X., Zhimulev I., Belyaeva E., Cherbas P., 2003 EcR isoforms in *Drosophila*: testing tissue-specific requirements by targeted blockade and rescue. Development 130: 271–284.

Chiang H. C., 1963 Tactic Reactions of Young Adults of *Drosophila melanogaster.* Am. Midl. Nat. 70: 329–338.

Church, R.B. and Robertson F. ., 1966 A biochemical study of the growth of *Drosophila melanogaster*. J. Exp. Zool. 162: 337–351.

Colombani J., Raisin S., Pantalacci S., Radimerski T., Montagne J., *et al.*, 2003 A nutrient sensor mechanism controls *Drosophila* growth. Cell 114: 739–749.

Cox T. R., Erler J. T., 2011 Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis. Model. Mech. 4: 165–78.

Haunerland N. H., 1996 Insect storage proteins: Gene families and receptors. Insect Biochem. Mol. Biol. 26: 755–765.

Hoshizaki D. K., Blackburn T., Price C., Ghosh M., Miles K., *et al.*, 1994 Embryonic fat-cell lineage in *Drosophila melanogaster*. Development 120:

2489–2499.

Hoshizaki D. K., 2005 2.9 Fat-Cell Development.

Jennings B. H., 2011 *Drosophila*-a versatile model in biology & medicine. Mater. Today 14: 190–195.

Jia Q., Liu Y., Liu H., Li S., 2014 Mmp1 and Mmp2 cooperatively induce *Drosophila* fat body cell dissociation with distinct roles. Sci. Rep. 4: 7535.

Kawamura K., Shibata T., Saget O., Peel D., Bryant P. J., 1999 A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc cells. 219: 211–219.

King-Jones K., Thummel C. S., 2005 Nuclear receptors - A perspective from *Drosophila*. Nat. Rev. Genet. 6: 311–323.

Krejner A., Litwiniuk M., Grzela T., 2016 Matrix metalloproteinases in the wound microenvironment : therapeutic perspectives. Chronic Wound Care Manag. Res. 3: 29–39.

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.

Li Y., Ray P., Rao E. J., Shi C., Guo W., *et al.*, 2010 A *Drosophila* model for TDP-43 proteinopathy. Pnas 107: 3169–3174.

Locke M., 2003 SURFACE MEMBRANES , GOLGI COMPLEXES ,AND VACUOLAR SYSTEMS. Annu.Rev.Entomol. 48: 1–27.

Lu P., Takai K., Weaver V. M., Werb Z., 2011 Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol 3: 1–24.

Massague J., Lo R. S., 2000 TGF-β Signaling in Growth Control, Cancer, and Heritable Disorders. Cell 103: 295–309.

Massagué J., Chen Y. G., 2000 Controlling TGF-beta signaling. Genes Dev. 14: 627–44.

McBrayer Z., Ono H., Shimell M., Parvy J. P., Beckstead R. B., *et al.*, 2007 Prothoracicotropic Hormone Regulates Developmental Timing and Body Size in *Drosophila*. Dev. Cell 13: 857–871.

Moeller M. E., Danielsen E. T., Herder R., O'Connor M. B., Rewitz K. F., 2013 Dynamic feedback circuits function as a switch for shaping a maturation-inducing steroid pulse in *Drosophila*. Development 140: 4730–9.

Nelliot A., Bond N., Hoshizaki D. K., 2006 Fat-body remodeling in *Drosophila melanogaster.* Genesis 44: 396–400.

Notarangelo G., 2014 The Role of βFTZ-F1 and MMP2 in Regulating Hormone-Mediated Autophagy and Insulin Signaling in the *Drosophila* Fat Body.

Page-McCaw A., Serano J., Sante J. M., Rubin G. M., 2003 Are Required for Tissue Remodeling , but Not Embryonic Development. Situ 4: 95–106.

Page-McCaw A., Ewald A. J., Werb Z., 2007 Matrix metalloproteinases and the regulation of tissue. Nat Rev Mol Cell Biol. 8: 221–233.

Riddiford L. M., Truman J. W., 1993 Hormone Receptors and the Regulation of Insect Metamorphosis. 33: 340–347.

Robertson C. W., 1936 The metamorphosis of *Drosophila melanogaster,* including an accurately timed account of the prinicpal morphological changes. J. Morphol. 59: 351–399.

Rusten T. E., Lindmo K., Juhász G., Sass M., Seglen P. O., *et al.*, 2004 Programmed autophagy in the *Drosophila* fat body is induced by ecdysone through regulation of the PI3K Pathway. Dev. Cell 7: 179–192.

Sapiro A. L., Ihry R. J., Buhr D. L., Konieczko K. M., Ives S. M., *et al.*, 2013 Rapid recombination mapping for high-throughput genetic screens in *Drosophila*. G3 (Bethesda). 3: 2313–9.

Son D., Harijan A., 2014 Overview of surgical scar prevention and management. J. Korean Med. Sci. 29: 751–757.

Springer P. S., 2000 Gene Traps : Tools for Plant Development and Genomics. 12: 1007–1020.

Sternlicht M., Werb Z., 2009 How Matrix metalloproteinases regulate cell behavior. Annu REv Cell Biol: 463–516.

Teleman A. A., 2010 Molecular mechanisms of metabolic regulation by insulin in *Drosophila*. Biochem. J. 425: 13–26.

Thummel C. S., 2004 From Embryogenesis to Metamorphosis:The Regulation and Function of *Drosophila* Nuclear Receptor Superfamily MembersReview. 83: 1–7.

Walchessen P., 2016 A Genetic Screen for Genes Involved in Tissue Remodeling.

Wang L., Evans J., Andrews H. K., Beckstead R. B., Thummel C. S., *et al.*, 2008 A genetic screen identifies new regulators of steroid-triggered programmed cell death in *Drosophila*. Genetics 180: 269–281.

Wei S., Xie Z., Filenova E., Brew K., 2003 *Drosophila* TIMP Is a Potent Inhibitor of MMPs and TACE : Similarities in Structure and Function to TIMP-3 †. Biochemistry 42: 12200–12207.

Weigmann K., Klapper R., Strasser T., Rickert C., Technau G., *et al.*, 2003 FlyMove--a new way to look at development of *Drosophila.* Trends Genet. 19: 306–310.

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.

Yamada M., Murata T., Hirose S., Lavorgna G., Suzuki E., *et al.*, 2000 Temporally restricted expression of transcription factor βFTZ-F1: significance for embryogenesis, molting and metamorphosis in *Drosophila melanogaster*. Development 127: 5083–92.

Zhang H., Stallock J. P., Ng J. C., Reinhard C., Neufeld T. P., 2000 Regulation of cellular growth by the dTOR. Genes Dev. 14: 2712–2724.

Zurovec M., Dolezal T., Gazi M., Pavlova E., Bryant P. J., *et al.*, 2002 Adenosine deaminase-related growth factors stimulate cell proliferation in *Drosophila* by depleting extracellular adenosine. 99: 4403–4408.