Published 2006 Wiley-Liss, Inc. genesis 44:396–400 (2006)

LETTER

Fat-Body Remodeling in *Drosophila melanogaster*

Archana Nelliot, Nichole Bond, and Deborah K. Hoshizaki*

Department of Biological Sciences and The Cancer Center, University of Nevada Las Vegas, Las Vegas, Nevada

Received 2 May 2006; Accepted 16 June 2006; Accepted 24 June 2006

Summary: The remodeling of the larval fat body is observed in many insects during metamorphosis, but little is known about the physiological importance or the regulation of this process. In *Drosophila melanogaster*, fat-body remodeling involves the dissociation of the fat body into individual fat cells, which persist throughout pupal development but are later removed by cell death in the young adult. Inhibition of fat-body dissociation is associated with pharate adult lethality and thus is likely to be an essential developmental event. As a start toward understanding the role of fat-body remodeling in the life history of insects, we carried out a detailed study of fat-body disassociation in *D. melanogaster* using fluorescent microscopy, and tested whether this process is mediated by hemocytes as proposed for fat-body remodeling in *Sarcophaga peregrina*. We identified and correlated stereotypic events in fat-body dissociation with developmental changes during metamorphosis, and have demonstrated by cell ablation studies that fat-body remodeling in *D. melanogaster* is a hemocyte independent process. genesis 44:396–400, 2006. Published 2006 Wiley-Liss, Inc.†

Key words: fat body remodeling; tissue dissociation; *Drosophila melanogaster*; hemocytes; *Sarcophaga peregrina*

Insect metamorphosis is characterized by extensive tissue remodeling and proliferation of adult progenitor cells. In *Drosophila melanogaster*, part of this remodeling entails the destruction of larval tissues through a two-step steroid-hormone induced regulatory cascade that culminates in the coordinated induction of cell death genes (Thummel, 2001; Yin and Thummel, 2005). In sharp contrast, the larval fat body is remodeled by undergoing tissue dissociation, resulting in the redistribution of individual fat cells throughout the body of the pupa. Inhibition of fat-body remodeling is associated with pharate adult lethality and is likely to be essential for completion of pupal development (Cherbas *et al.*, 2003; Hoshizaki, unpublished). In *D. melanogaster*, the detached fat cells persist throughout metamorphosis and are present in the newly eclosed adult as freely floating single, as well as, small clumps of cells (reviewed in Hoshizaki, 2005; Rizki, 1978; Rizki and Rizki, 1970). In the pupa and newly eclosed adult, the larval fat cells are the likely source of metabolic reserves (Aguila *et al.*, 2005; Aguila and Hoshizaki, unpublished). In the young adult, the larval fat cells eventually undergo cell death to be replaced by the adult fat body, which arises from a distinct pool of progenitor cells (Hoshizaki *et al.*, 1995).

In *Sarcophaga peregrina,* pupal hemocytes were thought to mediate fat-body dissociation through the production and storage of an inactive form of the cysteine protease Cathepsin B (Aronson and Barrett, 1978; Kurata *et al.*, 1990, 1992a,b; Takahashi *et al.*, 1993). It has been proposed that during metamorphosis, hemocytes interact with the fat body through a 200 kDa hemocyte-specific surface recognition protein that triggers the localized release of Cathepsin B through an unknown mechanism. Cathepsin B in its activated form is thought to degrade the fat-body extracellular matrix (Hori *et al.*, 1997; Kobayashi *et al.*, 1991; Natori *et al.*, 1999). Experimental data to support this model, however, has not been forthcoming; the putative 200 kDa surface recognition protein is myosin heavy chain derived from degraded larval muscle and not from pupal hemocytes (Hori *et al.*, 1997; Natori *et al.*, 1999). In *D. melanogaster*, hemocytes have also been implicated in fat-body remodeling based on genetic epistatic interaction between *croquetmort* (*crq*), which encodes a scavenger receptor found in hemocytes, and the fat-body gene *adenosine deaminase-growth-factor a* (*adgf-a*) (Dolezal *et al.*, 2005; Franc *et al.*, 1996).

To begin to understand the final developmental fate of the larval fat body and its remodeling, and how this impacts on the physiological role of the fat cells in the pupa and young adult, we have begun by detailing the stereotypic changes that take place during fat-body remodeling in *D. melanogaster* and have directly tested the hypothesis that dissociation is hemocyte mediated.

* Correspondence to: D.K. Hoshizaki, Department of Biological Sciences, University of Nevada Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154-4004.

E-mail: dkhosh@clark.nscee.edu

Contract grant sponsors: Nevada Biomedical Research Infrastructure Network (BRIN) Core Use Incentive Grants, UNLV Office of Research and Graduate Studies.

Published online in
Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/dvg.20229

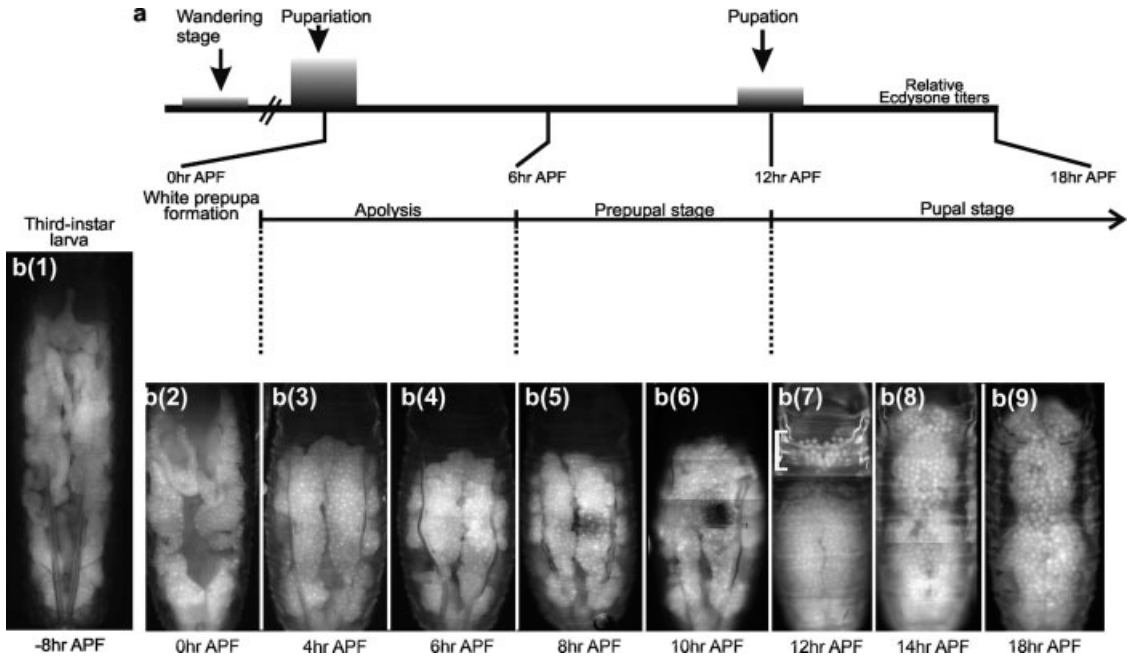

FIG. 1. Fat-body remodeling in *D. melanogaster*. (**a**) Relative ecdysone titer from whole animals during the early stages of metamorphosis (based on Riddiford, 1993). **b**(1–9) Corresponding changes in fat-body morphology in whole-mount animals. b(1) Late third-instar larva. b(2) White prepupa. At puparium formation, the fat body retains its larval morphology and is extended throughout the body cavity. b(3) Early-stage and b(4) Late-stage apolytic animal. The fat body retracts from the anterior part of the animal. b(5) Early-stage and b(6) Late-stage prepupa. After completion of apolysis (6 h APF), the fat cells begin to round up (and see Fig. 2). b(7) Early-stage pupa. Immediately after pupation individual fat cells are easily detected entering the head capsule (bracket). b(8) 14 h pupa and b(9) 18 h pupa. Fat-body cells were visualized by GFP expression in living animals carrying *Lsp2-GAL4*, *UAS-GFPnls*. (APF is time after puparium formation at 25°C.)

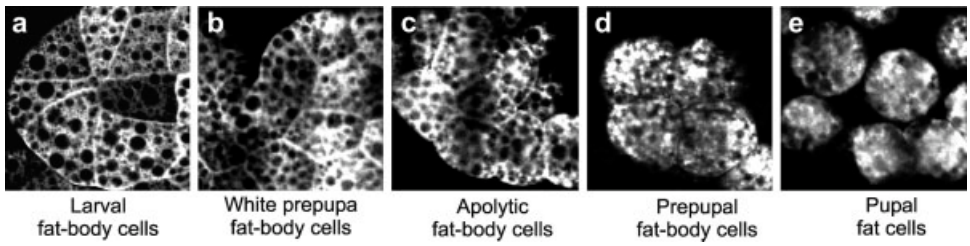

FIG. 2. Changes in fat-cell shape during fat-body remodeling. During the disassociation of the fat body, the fat cells undergo stereotypic cell-shape changes where the flat, polygonal larval fat cells are transformed into spherical cells that are detached from each other. Groups of fat cells from (**a**) third-instar larva; (**b**) white prepupa; (**c**) apolytic animal; (**d**) prepupa; and (**e**) early-stage pupa. Fat-body cells were visualized by confocal microscopy in whole-mount animals carrying *Lsp2-GAL4*, *UAS-GFP T10*. GFP protein is localized to the cytoplasm. The lipid droplets are visible as vesicles lacking GFP.

The remodeling of the fat body takes place during the early stages of metamorphosis. We have correlated this process with morphological events previously described in detail for the whole animal (see Fig. 1) (Ashburner, 1989; Bodenstein, 1950; Riddiford, 1993; Robertson, 1936). To visualize the dissociation process, we expressed green fluorescent protein (GFP) specifically in the larval fat body using the Gal4/UAS system (Brand and Perrimon, 1993). Using fluorescent microscopy and confocal imaging, we have followed fat-body remodeling in live animals.

Prior to metamorphosis, the larval fat body is composed of single-cell layers of white, translucent cells, which form sheets of tissue floating in the hemolymph between the body wall and the midgut (Fig. 1b(1)). The fat cells are flat and polygonal in shape, and appear tightly associated with each other (Fig. 2a). At the end of larval development, an increase in the ecdysone titer initiates puparium formation; the larva ceases movement and contracts into a white shortened animal, i.e., the white prepupa. At this stage, the gross morphology of the fat body is unchanged; the fat body extends into the head area and fills most of the peripheral space between the body wall and the gut, and the fat cells remain tightly associated with each other (Figs. 1b(2) and 2b). The fat body remains extended for a limited time (ca. 15–30 min). Over the next 3½ h, the larval cuticle hardens

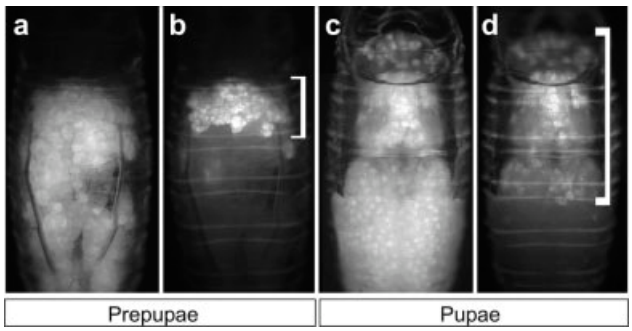

FIG. 3. Fat-body remodeling does not result in extensive mixing of cells. Fat cells in whole-mount prepupa (**a**,**b**) or pupa (**c**,**d**) were detected either by GFP (*Lsp2-GAL4, UAS-GFP T10*) in the larval fat cells (a,c) or by autofluorescence (anterior fat-body cells, bracket in b,d).

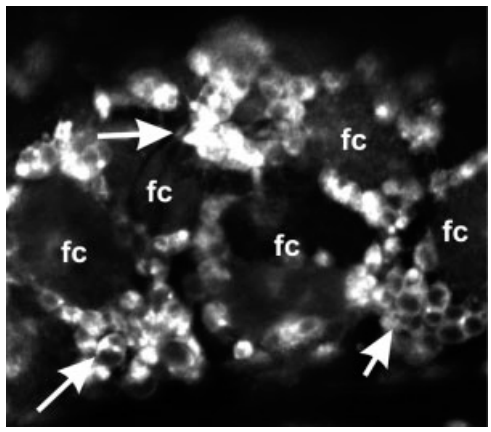

FIG. 4. Hemocytes are intimately associated with the fat body during early metamorphosis. Fat cells (fc) are surrounded by hemocytes (arrows). Hemocytes were visualized in a late prepupa by expression of the hemocyte driver *srpHemoGAL4*, *UAS GFP*.

and tans to form the puparium cuticle (pupal case). These animals have yet to undergo apolysis (Ashburner, 1989), but during this time, the fat body begins to retract from the anterior region. Apolysis occurs from 4 to 6 h after pupariation formation (APF) as the animal separates its larval epidermis from the tanned puparium cuticle (Fig. 1b(3,4)). At this time, the fat cells begin to change shape and take on a slightly rounded appearance (Fig. 2c). Completion of apolysis at 6 h APF marks the beginning of prepupal development and consistent with the observation of Rizki (1978), the fat body is completely retracted from the anterior region of the prepupa (Fig. 1b(4)). We define white prepupae formation (0 h APF) through apolysis (6 h APF) is as the retraction phase.

In the next phase, during prepupal development, distinct changes are detected at the cellular level (see Fig. 2). Starting from the anterior fat body and progressing toward the posterior, individual fat cells begin to lose their tight associations with each other (Fig. 2d). This phase is referred to as the disaggregation stage. Upon completion of prepupal development and in response to a brief rise in the ecdysone titer that peaks at ca. 12 h APF (Fig. 1a), the animal undergoes pupation, an event that is marked by the eversion of the head capsule (Handler, 1982; Sliter and Gilbert, 1992). Following this transition, anterior fat-body cells become spherical and begin to physically detach from each other (Fig. 2e). After head eversion, fat cells are readily visible as individual cells that are propelled into the head region by abdominal muscular contractions (Fig. 1b(7)). As the head capsule fills with cells, the remaining fat cells detach in a progressive wave in the anterior to posterior direction. By 14 h APF, the fat cells are freely packed in the open interior space of the pupa (Fig. 1b(8)).

Serendipitously, we discovered that anterior fat cells autofluoresce when viewed at the wavelength designed to visualize DAPI-stained material (359/461 μm absorption/emission) (see Fig. 3). We have used this observation to follow the anterior fat cells and find that there is little mixing of fat cells during disassociation such that the anterior fat cells fill the head capsule and contribute primarily to the fat cells in the anterior half of the animal (see Fig. 3).

The detailed description of fat-body remodeling provides necessary information to test the hypothesis that fat-body dissociation in *D. melanogaster* is hemocyte-mediated. To test the role of hemocytes, we genetically ablated these cells and looked for disruption of cell detachment from the fat body in the early pupa. The hemocyte population is made up primarily of plasmatocytes, which are phagocytic cells that differentiate into pupal macrophages during metamorphosis and are thought to be important for phagocytosis of apoptotic cells (Meister and Lagueux, 2003). At the onset of pupation, plasmatocytes are intimately associated with the fat body and accumulate at the boundaries between the cells (see Fig. 4). This association is consistent with the phagocytic nature of the macrophages and their role in the degradation and removal of extracellular matrix. We genetically ablated the hemocytes by using the *domino* (*dom*) mutation, which causes massive cell death of hemocytes in the lymph glands of third-instar larvae (Braun *et al.*, 1997). The loss of hemocytes was confirmed by visual examination of dissected mutant larvae (data not shown). To assess whether fat-body remodeling has taken place, live animals were examined for detachment of cells from the anterior fat body and normal displacement into the head capsule. In early-stage *dom* pupae, the fat body undergoes normal remodeling although in many cases the head capsule ruptures during head eversion, allowing individual fat cells to be released throughout the anterior half of the animal (Fig. 5a,b). These data suggest that fat-body dissociation does not depend upon hemocytes.

Because the surviving *dom* mutant larvae die shortly after the prepupal/pupal transition, it is possible that the detachment of fat cells is a general defect associated with the dying animal. We, therefore, employed a second strategy to ablate the hemocytes by ectopically

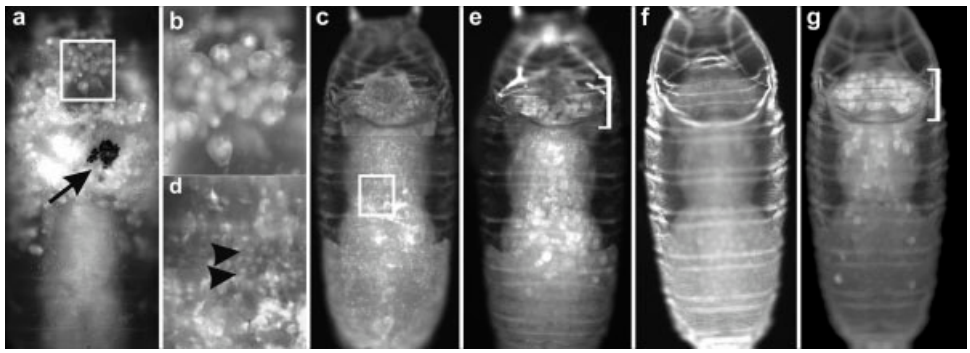

FIG. 5. Fat-body remodeling does not require hemocytes. (**a**) In the *dom* mutant pupa, the fat cells have detached from the fat body and are present as individual cells. Note the blackened lymph glands (arrow) characteristic of *dom* larvae. Fat cells are marked by expression of *Lsp2-GAL4*, *UAS-GFsyn.* (**b**) Higher magnification of inset in (a). (**c–e**) Early-stage *srphemo-GAL4*, *UAS-GFP* pupa. (c) Hemocytes visualized by the GFP expression. (d) Higher magnification of inset of (c) (arrowheads, hemocytes). (e) Individual fat cells have detached from the fat body and are distributed into the head capsule (bracket). (**f**,**g**) Heat-shocked treated *srphemo-GAL4*, *UAS-GFP/UAS-hid*$^{+}$; +/*tub-Gal80*^{ts} early-stage pupa. (f) Hemocytes are absent. (g) Individual fat cells have detached from the fat body and are distributed into the head capsule (bracket). Anterior fat cells in (e) and (g) are visualized by their autofluorescence.

expressing the cell death gene *head involution defective* (*hid*) (Grether *et al*., 1995). The loss of hemocytes was established by the absence of GFP positive hemocytes (Fig. 5c compared to 5f) and the anterior fat cells were visualized by their autofluorescence (Fig. 5e,g). Loss of hemocytes did not affect the normal detachment of the anterior fat cells from the fat body nor their redistribution into the head capsule (Fig. 5f,g).

Dolezal *et al*. (2005) have supported the idea that fat-body dissociation in *D. melanogaster* is a hemocyte-mediated event based on genetic studies of the fat body gene *crq* and the hemocyte gene *adgf-a.* The *adgf-a* mutant has a pleiotrophic phenotype culminating in pupal lethality. The larvae have elevated levels of adenosine and deoxyadenosine, exhibit multiple melanotic tumors, and have an increase in the number of hemocytes. The fat body undergoes an aberrant disintegration in the larva which Dolezal *et al.* suggest is premature metaphoric fat-body dissociation (compare Dolezal, Fig. 1b to Fig. 1b(7)). In support of this suggestion, they report that aberrant fat-body disintegration in the larva is blocked in the *adgf-a*, *crq* double mutant even though there is a decrease in lamellocytes. On the basis of these observations, interaction between fat body and hemocytes, through *crq* and *adgf-a*, respectively, are thought to be necessary for normal metamorphic fat-body dissociation. We have directly tested *crq* for a role in fat-body dissociation and find *crq*^{KG01679} mutant pupae undergo normal fat-body remodeling (see Fig. 6). We conclude that fat-body disintegration in *adgf-a* mutant larva is not a reflection of premature metamorphic fat-body remodeling but might be due to an acute tissue response to elevated levels of adenosine and deoxyadenosine that is mediated through the Croquemort receptor.

In this report, we have presented a detailed description of fat-body remodeling in *D. melanogaster* and several lines of evidence that lead to the conclusion that fat body remodeling in *D. melanogaster* is independent of hemocytes. Supporting this conclusion are the observations that in *Plodia interpunctella* (Indian meal worm), exposure of isolated fat body to the steroid-hormone ecdysone induces dissociation in a dose-dependent manner (Oberlander, 1976), while tissue-specific disruption of ecdysone signaling in the fat body of *D. melanogaster* inhibits the fat-body disassociation (Cherbas *et al*., 2003; Hoshizaki, unpublished data). Thus, in *D. melanogaster*, fat-body remodeling is likely to be hormone-regulated and cell autonomous.

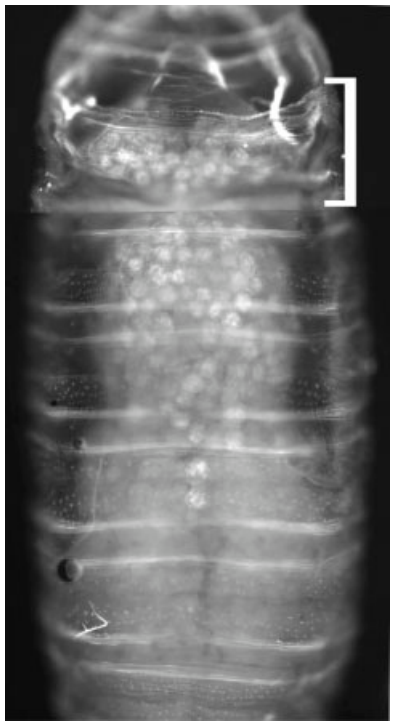

FIG. 6. Fat-body dissociation does not require Croquemort receptor. In a *cqr*^{KG01679}pupa fat-body cells detach are distributed into the head capsule (bracket). Fat cells are detected by their autofluorescence.

METHODS

Drosophila Stocks and Manipulations

The *UAS-GFPnls*, *UAS-GFPsyn*, *UAS-GFP T10, tub-gal80^{ts}*, *domino^{k08108}*, and *croquemort^{KG01679}* stocks were provided by the Drosophila Stock Center, Bloomington IN, while the *Lsp2-GAL4*, *srpHemoGAL4*, and *UAS-hid^{+}* lines were kindly provided by L. Cherbas, N. Perrimon, and R. Davis, respectively.

To identify *dom* mutant animals, a *dom^{k08108}*; *Lsp2-GAL4 UAS-GFPnls/T(2;3) Cy Tb* stock was constructed. *Lsp2-GAL4*, *UAS-GFPnl*s specifically marks fat-cell nuclei with GFP. *dom* mutant animals were selected as non-*Tb* animals and further examined for the characteristic blackened lymph glands of the *dom* mutant. Ablation of hemocytes by cell death was achieved by crossing *UAS-hid^{+}*; *tub-gal80^{ts}/T(2;3) Cy Tb* to the hemocyte-specific driver *srphemo-GAL4*, *UAS-GFP. Tb^{+}* late third-instar larvae or white prepupae were selected, heat-shocked at 29°C, and incubated on wet filter paper until examined for loss of hemocytes and alterations in fat-body dissociation. To identify *crq* mutant animals, *crq^{KG01679}* was balanced over *T(2;3) Cy Tb* and non-*Tb* pupae examined.

Microscopy and Imaging

Staged animals were rinsed in deionized water, and mounted on bridged slides in Gel/Mount (Biomedia). Both fluorescence and confocal imaging were carried out in the Department of Biological Sciences Imaging Center using a Zeiss Axioplan 2 microscope. Fluorescence images were captured with the Zeiss Axiocam using the Zeiss Axiovision software. LSM 510 software was used to procure the confocal images. All images were compiled in Corel Draw®.

ACKNOWLEDGMENTS

A.N. and N.B. were supported by NIH Nevada BRIN Undergraduate Core internships and A.N. was also supported by a Nevada EPSCoR Undergraduate Summer Research fellowship. We gratefully acknowledge technical support by Jenifer Utz in the early stages of this work.

LITERATURE CITED

Aguila JR, Suszko JW, Hoshizaki DK. 2005. Resistance to starvation: The role of larval-derived fat cells in the adult. A Dros Res Conf 46: 1028B.

Aronson NN Jr, Barrett AJ. 1978. The specificity of cathepsin B. Hydrolysis of glucagon at the C-terminus by a peptidyldipeptidase mechanism. Biochem J 171:759–765.

Ashburner M. 1989. Drosophila, a laboratory handbook. Plainview, NY: Cold Spring Harbor Laboratory Press.

Bodenstein D. 1950. The postembryonic development of Drosophila. In: Demerec M, editor. Biology of Drosophila. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. pp 275–375.

Brand AH, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415.

Braun A, Lemaitre B, Lanot R, Zachary D, Meister M. 1997. Drosophila immunity: Analysis of larval hemocytes by P-element-mediated enhancer trap. Genetics 147:623–634.

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.

Dolezal T, Dolezelova E, Zurovec M, Bryant PJ. 2005. A role for adenosine deaminase in Drosophila larval development. PLoS Biol 3: e201.

Franc NC, Dimarcq JL, Lagueux M, Hoffmann J, Ezekowitz RA. 1996. Croquemort, a novel Drosophila hemocyte/macrophage receptor that recognizes apoptotic cells. Immunity 4:431–443.

Grether ME, Abrams JM, Agapite J, White K, Steller H. 1995. The head involution defective gene of *Drosophila melanogaster* functions in programmed cell death. Genes Dev 9:1694–1708.

Handler AM. 1982. Ecdysteroid titers during pupal and adult development in *Drosophila melanogaster*. Dev Biol 93:73–82.

Hori S, Kobayashi A, Natori S. 1997. Monoclonal antibodies against pupa-specific surface antigens of *Sarcophaga peregrina* (flesh fly) hemocytes. Biochem Biophys Res Commun 236:497–501.

Hoshizaki DK. 2005. Fat-cell development. In: Gilbert LI, Iatrou K, Gill S, editors. Comprehesive molecular insect science. Oxford, England: Elsevier B.V. pp 315–345.

Hoshizaki DK, Lunz R, Ghosh M, Johnson W. 1995. Identification of fat-cell enhancer activity in *Drosophila melanogaster* using P-element enhancer traps. Genome 38:497–506.

Kobayashi H, Kurata S, Natori S. 1991. Purification of the 200kDa hemocyte membrane protein of *Sarcophaga peregrina* and its specific interaction with fat body. Insect Biochem 21:517–522.

Kurata S, Saito H, Natori S. 1990. Participation of hemocytes proteinase in dissociation of the fat body on pupation of *Sarcophaga peregrina* (flesh fly). Insect Biochem 20:461–465.

Kurata S, Saito H, Natori S. 1992a. The 29-kDa hemocyte proteinase dissociates fat body at metamorphosis of Sarcophaga. Dev Biol 153: 115–121.

Kurata S, Saito H, Natori S. 1992b. Purification of a 29-kDa hemocyte proteinase of *Sarcophaga peregrina*. Eur J Biochem 204:911–914.

Meister M, Lagueux M. 2003. Drosophila blood cells. Cell Microbiol 5:573–580.

Natori S, Shiraishi H, Hori S, Kobayashi A. 1999. The roles of Sarcophaga defense molecules in immunity and metamorphosis. Dev Comp Immunol 23:317–328.

Oberlander H. 1976. Dissociation and reagregation of fat body cells during insect metamorphosis. In: Invertebrate tissue culture: Applications in medicine, biology, and agriculture. New York: Academic Press. pp 241–246.

Riddiford LM. 1993. Hormone receptors and the regulation of insect metamorphosis. Receptor 3:203–209.

Rizki TM. 1978. Fat body. In: Ashburner M, Wright TRF, editors. The gentics and biology of Drosophila. New York: Academic Press. pp 561–601.

Rizki TM, Rizki RM. 1970. The genetic basis of a cell pattern homology in Drosophila species. In: Hecht MK, Steere WC, editors. Essays in evolution and genetics in honor of Theodosius Dobzhansky. New York: Appleton-Century-Crofts. pp 289.

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

Sliter TJ, Gilbert LI. 1992. Developmental arrest and ecdysteroid deficiency resulting from mutations at the dre4 locus of Drosophila. Genetics 130:555–568.

Takahashi N, Kurata S, Natori S. 1993. Molecular cloning of cDNA for the 29 kDa proteinase participating in decomposition of the larval fat body during metamorphosis of *Sarcophaga peregrina* (flesh fly). FEBS Lett 334:153–157.

Thummel CS. 2001. Steroid-triggered death by autophagy. Bioessays 23:677–682.

Yin VP, Thummel CS. 2005. Mechanisms of steroid-triggered programmed cell death in Drosophila. Semin Cell Dev Biol 16:237–243.