Short Communication

Phagocytic receptors regulate *Drosophila* larval growth

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Abstract

Drosophila melanogaster is a key model organism for biological research due to its genetic manipulability and high degree of evolutionary conservation with humans. Phagocytic receptors play a central role in apoptotic cell clearance, a fundamental process that is highly conserved across species. Previous studies have identified two major phagocytic receptors in *Drosophila*: integrin αPS3βν and Draper, both of which contribute to apoptotic cell removal. However, the physiological significance of these receptors under normal developmental conditions remains unclear. Therefore, the aim of this study was to investigate the role of these receptors in developmental timing. The results demonstrated that double mutants lacking both receptors exhibited significant developmental delays, especially during the larval stage (p<0.001). Moreover, tissue-specific knockdown experiments revealed that phagocytic receptors within the fat body are mainly involved in regulating developmental timing (p=0.028). Further results established that nutrient availability influenced the extent of growth delay, suggesting that these receptors may play a role in nutrient-dependent growth regulation. Taken together, these findings suggest that phagocytic receptors contribute to maintaining proper growth timing in *Drosophila* larvae, potentially through energy metabolism pathways.

Keywords: *Drosophila*, phagocytic receptor, development, growth, nutrition

Introduction

Drosophila melanogaster (lesser fruit fly) is a major model organism used in biological research, and its history traces back to early genetic experiments performed by T. H. Morgan. In 1907, when the cellular location of genetic material was still unknown, Morgan and his colleagues demonstrated that genes reside on chromosomes using *Drosophila* [1]. The continued widespread use of *Drosophila* in biological and genetic research can be attributed to its short life cycle, high fertility, and relative harmlessness to humans. Moreover, there exists a substantial body of accumulated genetic knowledge and advanced genetic tools that can be readily used to study *Drosophila*. Such tools include the GAL4-upstream activating sequence (UAS) system [2] and RNA interference (RNAi)-based gene suppression [3], and allow precise genetic manipulation on the organismal level, thereby making *Drosophila* an indispensable "living test tube." The *Drosophila* genome was decoded relatively early among model organisms, and it was discovered that approximately 60% of human genes [4], as well as more than 70% of disease genes [5], are conserved in *Drosophila*. Consequently, *Drosophila* is also extensively used as a disease model in medical research [6].

In this study, phagocytic receptors, identified as key mediators of apoptotic cell clearance *in vivo,* were the focus of investigation. Apoptosis, also known as physiological cell death, is distinct from necrosis, which is associated with cell rupture and the leakage of cellular contents [7-9].

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Specifically, apoptosis is induced in cells that are no longer needed, such as aging blood cells, cells that have completed their roles during development, and cells infected with viral pathogens. Moreover, apoptosis can lead to changes in molecular patterns on the cell surface, DNA fragmentation, and cell shrinkage. Professional phagocytes (such as macrophages) and epithelial cells surrounding apoptotic cells can recognize cell surface structures characteristic of apoptosis and respond by engulfing and degrading these cells; this process is known as phagocytosis [10]. Phagocytic receptors play a central role in recognizing apoptotic cells and initiating the signaling pathways that mediate this engulfment. Studies in mammals have identified diverse phagocytic receptors and their signaling pathways. Subsequent genetic analyses in the nematode *Caenorhabditis elegans* have revealed that these pathways converge into two major conserved pathways. This finding has also been confirmed in *Drosophila*, thus reinforcing the notion that apoptotic cell phagocytosis is a fundamental, evolutionarily conserved process throughout the animal kingdom, including mammals [10,11].

Previous research (in which participation occurred) identified two phagocytic receptors in *Drosophila*, integrin αPS3βν and Draper, along with associated signaling pathways [12-14]. Moreover, since these two pathways are highly conserved [15-17], they likely play critical roles in multicellular organisms. However, to date, a comprehensive elucidation of the physiological significance of phagocytosis mediated by these receptors has not yet been performed. It is known that mutants lacking both receptors show a marked suppression of apoptotic cell clearance. For example, double mutant flies developed into adults with normal morphology, but their development period was delayed by a couple of days. Interestingly, this delay was observed only in double mutants and not in single mutants [13]. Taken together, these findings suggest that nonspecific phagocytosis, independent of these receptors, is sufficient for survival under physiological conditions. However, under non-physiological conditions, such as tumor formation [18] and infection by pathogenic bacteria [19] and viruses [20], the absence of these receptors can impair homeostasis. For example, it may be associated with a higher frequency of tumor appearance and may cause infected individuals to be more likely to die [18-20]. In such cases, these phagocytic receptors would constitute an extremely important adaptation under non-physiological conditions. In this study, attention was focused on the growth retardation from the embryo to adult life history stages. The aim of this study was to investigate the role of these receptors in developmental timing. Differences observed under physiological conditions were recorded, and their causes were investigated, specifically examining at which developmental stage (i.e., embryo, larva, or pupa) growth was delayed and whether the mechanisms responsible could be identified.

Methods

Fly stocks and maintenance

The following lines of Drosophila were used in this study: w1118 on the X chromosome; Intgbn2 on the 2nd chromosome [21], used as a null mutant of Itgbn, which codes for the β subunit of integrin αPS3βν; drprΔ5 on the 3rd chromosome [22], used as a null mutant of drpr, which codes for Draper; tubP-GAL4 on the 3rd chromosome (Bloomington Drosophila Stock Center: stock number 5138), used to ubiquitously express GAL4; Pxn-GAL4 8.1 on the 2nd chromosome (graciously donated by Dr. Michael J. Galko) used to express GAL4 in hemocytes; rn-GAL4-5 on the 3rd chromosome (Bloomington Drosophila Stock Center: stock number 7405) and dll-GAL4 on the 2nd chromosome (graciously donated by Dr. Shigeo Hayashi), used to express GAL4 in imaginal discs; ppl-GAL4 on the 2nd chromosome (graciously donated by Dr. Alex Gould), used to express GAL4 in fat bodies; UAS-Itgbn-IR on the 3rd chromosome (National Institute of Genetics: stock ID 1762R-1), used to express precursors of double-stranded RNA for the RNAi knockdown of Itgbn; UAS-drpr-IR on the 3rd chromosome (Vienna Drosophila Resource Center: VDRC Transformant ID 4833), used to express precursors of double-stranded RNA for the RNAi knockdown of drpr. Finally, the fly lines Intgbn2; drprΔ5, and UAS-Itgbn-IR UAS-drpr-IR were generated using the fly stocks described above.

All flies were maintained at a constant temperature of 21°C under 60% humidity and a 12-hour light-dark cycle in an HPAV 210–20 test chamber (Isuzu Manufacturing Co., Ltd., Niigata,

Japan). Flies were fed a standard diet composed of 1–10% (w/v) dry yeast (YSC2-500G, Merck KGaA, Darmstadt, Germany), 1% (w/v) agar (S-7, Ina Food Industry Co., Ltd., Nagano, Japan), 10% (w/v) glucose, 7% (w/v) cornmeal, 0.4% (v/v) ethanol, 0.07% (w/v) butyl p-hydroxybenzoate, and 0.4% (v/v) propionic acid.

Developmental period quantification

Drosophila embryogenesis is categorized into 17 stages based on distinct morphological changes. To measure the embryonic period, eggs laid on agar plates (prepared using 80 mL grape juice, 5 mL ethanol, 5 mL acetic acid, 4.4 g agar, and 100 mL distilled water) were collected and dechlorinated with sodium hydrochloride, thereby allowing transparent visualization under a microscope. Stage 5 embryos, which are at a relatively early stage of development, were then collected and transferred to agarose plates maintained at 21°C at high humidity. Embryos were then monitored once an hour until they hatched. To measure the larval and pupal period, stage 16 embryos, which represent the late embryonic stage, were collected and transferred to vials containing the standard diet and maintained as above. Vials were monitored every 12 hours to record pupation and adult eclosion.

Statistics analyses

Statistical comparisons between group means were performed using two-tailed Student's *t*-tests through Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). The numbers of observed individuals are noted in the tables accompanying this study. Finally, differences with *p*-values<0.05 were considered to be statistically significant.

Results

Identification of the developmental stages affected by growth delay

Drosophila development is highly dependent on nutritional status and rearing temperature. Under optimal conditions (25°C, adequate nutrition), embryonic development is completed in 22–24 hours, followed by approximately four days of larval feeding, a 12-hour prepupal period, and 4–4.5 days of pupation. Development completes with adult emergence after approximately 220 hours in total [23]. However, under the 21°C condition adopted in this study, the life cycle is extended to approximately 14 days [23]. Given this well-defined timeline, a two- or three-day delay was observed in the double mutant *Itgbn²; drpr$^{\Delta 5}$*, representing a significant deviation.

To investigate the stage-specific timing of this delay, the developmental period was first examined to determine whether the delay occurred during the embryonic, larval, or pupal stages. Stage 5 embryos were collected, and the time required for development to the larval stage was measured. Mutant embryos were hatched one hour earlier than *w^{1118}* embryos, indicating that the cause of the growth delay in *Itgbn²*; *drpr$^{\Delta 5}$* was not related to the embryonic stage (**Table 1**). Subsequently, stage 16 embryos were collected, and the time required for development to pupae and adults was measured (**Table 1**). Mutant embryos were pupated 62 hours later and emerged as adults 42 hours later compared to *w^{1118}* embryos. The difference in the time to adult emergence was not greater than the time required for pupation. These results indicate that the growth delay in *Itgbn²; drpr$^{\Delta 5}$* occurred during the larval stage, rather than at the pupal stage.

Table 1. Duration from stage 5 embryos to larvae and stage 16 embryo to pupae and adults

Developmental stage	*w^{1118}*	*w; Itgbn²; drpr$^{\Delta 5}$*	Difference	*p*-value
Hatching (h)	27.7±1.1 (n=18)	26.7±1.0 (n=18)	+ 1.0	0.008*
Pupation (h)	173.3±19.1 (n=81)	235.0±57.7 (n=57)	+ 61.7	<0.001*
Eclosion (h)	310.0±12.9 (n=76)	352.1±18.8 (n=42)	+ 42.1	<0.001*

Data show mean±standard deviation
*Statistically significant at $p<0.05$

Tissue-specific growth regulation by phagocytic receptors

Next, to determine how phagocytic receptors regulate larval development, growth delays in flies with tissue-specific knockdown of *Itgbn* and *drpr* were examined using the GAL4-UAS system and RNAi. Briefly, the GAL4-UAS system involves introducing the yeast transcription factor GAL4 and its corresponding upstream activating sequence into *Drosophila*. Typically, tissue-specific GAL4 driver lines are crossed with UAS-linked target gene lines to facilitate localized gene expression [24]. RNAi is a form of post-transcriptional gene silencing initially discovered in nematodes [25]. RNAi systems feature endogenous (micro-RNA) or exogenous (short interfering RNAs or siRNAs) RNA sequences that inhibit gene transcription or translation. Since its identification, RNAi has been observed in a wide range of organisms, including mammals, insects, and plants. In *Drosophila*, libraries containing UAS lines encoding precursor sequences for gene-specific siRNAs that cover almost all genes have been released by the National Institute of Genetics in Japan and the Vienna Drosophila Resource Center in Austria. This resource provides a convenient tool for *Drosophila* researchers to apply reverse genetic approaches.

In this study, *UAS-Itgbn-IR* and *UAS-drpr-IR* lines were used. These lines permitted the selective suppression of receptor expression in specific tissues and allowed analysis of their effects on the duration of growth. Whole-body knockdown using *tubP-GAL4*, although not as in the case of the null mutant, resulted in a significant (14 h) developmental delay relative to the control condition (**Table 2**). The growth retardation of phagocytic receptor-deficient flies occurs only in the *Itgbn* and *drpr* double mutant [13]. Thus, the developmental delay caused by *UAS-Itgbn-IR UAS-drpr-IR* means that the UAS lines genetically functioned correctly. Next, the growth of individuals in which the expression of both phagocytic receptors was suppressed in a site-specific manner was examined. Tissue-specific knockdown in hemocytes using *Pxd-GAL4* unexpectedly led to a nonsignificant 11-hour shortening of developmental time, suggesting that the phagocytic receptors of hemocytes were not involved in growth regulation. Moreover, knockdown in imaginal discs using *rn-GAL4* resulted in a significant (6-hour) delay, although this difference was smaller than that of *tubP-GAL4*. Another GAL4 driver, *dll-GAL4*, also caused a 6-hour delay but this was not statistically significant. Taken together, these results suggest that if phagocytic receptors regulated the growth period, they did so only to a small degree. In contrast, knockdown in the fat body using *ppl-GAL4* resulted in a significant delay of 12-hour, comparable to whole-body knockdown, suggesting that the phagocytic receptors in the fat body may regulate growth duration.

Table 2. Duration from stage 16 embryos to adult

GAL4-line	w^{1118}	*UAS-Itgbn-IR UAS-drpr-IR*	Difference	*p*-value
tubP-GAL4	353.5±17.5 (n=51)	367.1±22.8 (n=43)	+ 13.6	<0.001*
Pxn-GAL4	358.3±39.3 (n=47)	347.6±17.5 (n=49)	– 10.7	0.086
rn-GAL4	342.0±9.1 (n=53)	348.4±13.1 (n=56)	+ 6.4	0.004*
dll-GAL4	350.8±23.4 (n=48)	356.4±12.6 (n=56)	+ 5.6	0.120
ppl-GAL4	349.6±27.2 (n=54)	361.4±24.0 (n=42)	+ 11.8	0.028*

Male GAL4-lines were crossed with female w^{1118} or *UAS-Itgbn-IR UAS-drpr-IR*, and the resulting embryos were collected. Embryos were raised on a standard diet containing 2% yeast. Data show mean±standard deviation.
*Statistically significant at $p<0.05$

Nutrient-dependent growth regulation by phagocytic receptors

The fat body, which is analogous to the liver and adipose tissue in mammals, plays important roles in nutrition storage and humoral function. Therefore, the effect of nutrient conditions on the regulation of growth duration by phagocytic receptors was also examined. Under standard diet conditions (5% yeast), the $Itgbn^{2}$; $drpr^{\Delta 5}$ mutants exhibited a 62-hour delay compared to w^{1118} (**Table 1**). Increasing the yeast concentration to 10% reduced this delay to 33 hours, while decreasing it to 1% extended the delay to 137 hours (**Table 3**). Taken together, these findings

indicate that the phagocytic receptors contribute to nutrient-dependent growth regulation, likely through a specific function in the fat body.

Table 3. Duration from stage 16 embryos to pupae

Yeast content	w^{1118}	w; $Itgbn^{2}$; $drpr^{\Delta 5}$	Difference	p-value
1%	411.8±74.9 (n=79)	548.6±71.5 (n=70)	+ 136.8	<0.001*
2%	220.7±17.0 (n=85)	269.3±37.3 (n=76)	+ 48.6	<0.001*
10%	163.1±7.3 (n=93)	196.5±23.0 (n=71)	+ 33.4	<0.001*

Data are presented as mean±standard deviation
*Statistically significant at $p<0.05$

Discussion

In this study, it was revealed that phagocytic receptors integrin αPS3βν and Draper in fat body play a crucial role in nutrient-dependent regulation of growth period. Factors known to delay *Drosophila* growth include both nutritional deficiencies and low temperatures [23]. In the phagocytic receptor-deficient mutants used in this study, only the larval stage was prolonged, while low-temperature conditions did not further extend the developmental period (data not shown). These findings align with the hypothesis that phagocytic receptors regulate growth duration in a nutrient-dependent manner. In holometabolous insects, when larvae reach a "critical weight" threshold, a switch to pupation is turned on. At this point, ecdysone is released from the prothoracic gland, the main endocrine organ, causing the onset of pupation [26]. Recently, a number of enzymes involved in ecdysone biosynthesize have been identified [27]. Phagocytic receptors may have some effect on the expression or function of these enzymes. Moreover, phagocytosis receptors are known to recognize multiple ligands. For instance, in mammals, scavenger receptor class B, type I has been found to act as a phagocytic receptor of apoptotic spermatogenic cells in Sertoli cells (a nurse cell) within the testis [28]. This receptor is also known as a receptor for high-density lipoprotein [29]. In *Drosophila*, integrin αPS3βν and Draper also recognize multiple ligands. Although both receptors in hemocytes recognize bacteria such as *Staphylococcus aureus*, the ligands they recognize are not the same; integrin αPS3βν recognizes peptidoglycan [14,19], while Draper recognizes lipoteichoic acid [30]. Given the role of the fat body in energy metabolism and the potential of these receptors to recognize multiple ligands, it is speculated that phagocytic receptors influence energy storage and utilization by recognizing some nutrient-related molecules.

It is known that impairment of imaginal discs in larvae delays pupation. Dilp8, a *Drosophila* insulin-like peptide that delays metamorphosis by inhibiting ecdysone biosynthesis has been found to be highly expressed in abnormally growing imaginal discs, where it disturbs pupation until its condition is restored [31,32]. This restoration includes both cell death and compensatory cell proliferation [33]. The results of this study show that knockdown of *Itgbn* and *drpr* in imaginal discs also tended to delay the onset of pupation relative to a control line, although this difference was small. The delay may reflect the role of phagocytic receptors under non-physiological conditions. Both integrin αPS3βν and Draper are active in cancer prevention [18], and they may, therefore, also be involved in tissue maintenance.

For a judicious interpretation, several limitations in this study should be considered. Although this study showed that phagocytic receptors in fat body and imaginal discs could control larval growth period in *Drosophila*, all evidence had come from genetic experiments, and no biochemical, molecular biological, or cell biological evidence. These approaches are needed to be clear the molecular function of the receptors in energy metabolism in fat body and removal of unwanted cells in imaginal discs.

Conclusion

The study found that the loss of two phagocytic receptors, integrin αPS3βν and Draper, results in a significant developmental delay, specifically during the larval stage. Knockdown of both receptors via tissue-specific RNAi revealed that the fat body is the primary site where these

receptors contribute to the regulation of developmental timing. Furthermore, the extent of the developmental delay was influenced by nutrient availability, suggesting that phagocytic receptors play a role in nutrient-dependent growth regulation. Taken together, these results highlight a novel function of phagocytic receptors beyond their established role in apoptotic cell removal. Moreover, given the central role played by the fat body in nutrition storage and humoral function, it is plausible that phagocytic receptors may contribute to processes that regulate larval growth and the timing of pupation. Overall, this study provides new insight into the physiological significance of phagocytic receptors in *Drosophila*, particularly regarding growth regulation under varying nutritional conditions. Further research is required to elucidate the molecular mechanisms (and ligands) by which these receptors influence energy metabolism and humoral signaling pathways.

Ethics approval

Not required.

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Competing interests

The author reports that no conflicts of interest exist.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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