Programmed cell death: A destructive and constructive process in insects

Jabez Raju B, Karthik S, and Anil G

Abstract: Programmed Cell Death (PCD) is a basic phenomenon which can be seen in plants, animals and even some unicellular organisms. In insects, events of post-embryonic development such as metamorphosis occur. During metamorphosis, the insect loses several of its body components during larval-pupal, pupal-adult transition. Response of how the body components disintegrate in insects reclines on the concept of PCD. When we talk about programmed cell death we discuss two important phenomena i.e., Apoptosis and Autophagy. PCD can be seen in insects during embryogenesis, metamorphosis where juvenile tissues are destroyed in order to form adult body parts. Current article is an attempt to provide an insight into mechanism of programmed cell death in insects and to promote cytological study of insects to understand basic cell biology, topics concerned with human health such as cancers.

Key words: Programmed cell death, insects, apoptosis, autophagy, cancer

Post-embryonic development is a gradual process occurring in insects after embryogenesis, and it is under hormonal control. The process of post-embryonic development starts from the eclosion of the egg and proceeds until the final adult is formed through a process called metamorphosis. During the course of post-embryonic development, an insect undergoes many structural changes *viz.* development of segmentation, moulting, growth and metamorphosis, accompanied by a non-structural change *i.e.*, development of reproductive maturity (Gillot, 2005; Minelli and Fusco, 2013).

Now, have you ever wondered what happens to body parts in immature insects which are lost during metamorphosis? Consider a lepidopteran larva which is way heavier than its adult moth and how it changes its structure during metamorphosis. A phenomenon called Programmed Cell Death (PCD) occurs during the events of metamorphosis. When we talk about cell death, it is a basic part of life and maintaining homeostasis in living organisms. Cells die due to several factors both external and internal such as physiological stress, invasion of pathogens, toxins, ageing etc. But in the case of PCD, cells die due to an internal triggering mechanism that activates the cytolysis and disintegration of tissues. Apoptosis and autophagy are the two significant phenomena of PCD in insects.

PCD was first described in the silk moth, *Bombyx mori* intersegmental muscles during metamorphosis by Kuwana in 1936. From then on scientists have documented the occurrence of PCD in insect muscular system, nervous system, ovarian follicles, salivary glands, midgut of lepidopteran larvae (Terashima *et al.*, 2000; Gaino and

Rebora, 2003; Tettamanti *et al.,* 2007; Fahrbach *et al.* 2012; Lee and Park, 2020).

Physiological changes during PCD

As mentioned earlier, PCD is under the control of endocrine regulation. Certain enzymes called caspases (cysteine aspartate-specific proteinases) are present in insects as well as in many plants and animals. They are responsible for the initiation and execution of apoptosis (Cooper *et al*. 2009). However, PCD is not compulsorily caused by caspases. The Programmed cell death is classified into two types *viz.,* caspase-dependent and caspase-independent (Iga *et al.* 2007).

During PCD, several changes including structural and physiological changes take place. The hormonal concentrations in the body of insects vary accordingly with PCD. The cells undergoing apoptosis show distinctive modifications. Apoptosis is characterized by caspase activation, cell shrinkage, cytoplasmic blebbing, nuclear and DNA fragmentation and phagocytosis of the dying cells (Tracy and Baehrecke, 2013). In contrast, autophagy is characterized by formation of an isolation membrane separating cytoplasmic materials, followed by the formation of a double membrane vesicle called autophagosome. Lysosomes fuse with the autophagosome's outer membrane, release the lysosomal enzymes which digest cellular material and organelles (Poyraz *et al.* 2021).

Role of haemolymph in PCD and its regulation

The haemocytes of insect haemolymph are of prominence for insect immunity. However, they are also known to be involved in PCD during metamorphosis. It was found out that a certain type of haemocytes called granulocytes was increased during metamorphosis. These granulocytes enter the tissues to be degraded like fat bodies and transform into macrogranulocytes which undergo apoptosis in target tissues, releasing Cathepsin L which degrades the target tissues like fat bodies. The transformation of granulocytes into macrogranulocytes is regulated by 20-hydroxyecdysone and the expression of Cathepsin L (Zhai and Zhao, 2012). It was also found that Cathepsin B is associated with the programmed cell death (PCD) of the fat body cells, and Cathepsin B, D, and O are involved in metamorphosis (Pan *et al.* 2021).

Genetic signals involved in PCD

Drosophila melanogaster is used as a model to understand the process and mechanism of PCD. In *Drosophila*, Dronc is primary apoptotic caspase apart from Dredd and Strica (they are also initiator caspases) which is homologous to mammalian caspase-9. It is the initiator caspase that activates effector caspases like Drice, Dcp-1, Decay and Damm. The initiator caspases are activated following the transmission of cell death signal. After the perception of death signals from cells, genes coding for proteins such as Apaf-1/Ced-4 are expressed followed by their production triggering a cascade of caspase activity. The genome of *Drosophila* codes for proteins such as IAPs (Inhibitors of apoptosis proteins) which reduce the activity of caspases by binding to them thereby terminating Apoptosis. IAP antagonists such as Reaper, Hid, and Grim inhibit the IAP binding with caspases, thereby promote apoptosis (Cooper *et al.*

2009; Fahrbach *et al.* 2012). The schematic regulation of apoptosis in *Drosophila* is depicted in Fig 1.

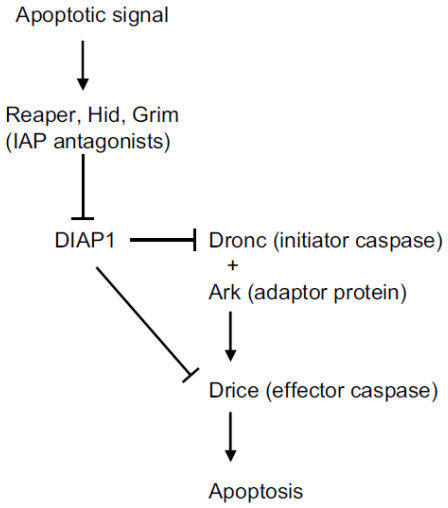

Fig. 1. Regulatory mechanism of Apoptosis (Fahrbach *et al.* 2012)

Other factors responsible for PCD

PCD occurs in all insects during metamorphosis however, there are several other factors which could lead to PCD in insects. Inducing oxidative stress in Sf9 cells by application of H_2O_2 produced apoptosis in cultured cells (Hasnain *et al.* 1999). Midgut cells of citrus psyllid (*Diaphorina citri*) undergo apoptosis when exposed to *Candidatus Liberibacter asiaticus* by an unknown mechanism (Ghanim *et al.* 2016). Harmine an alkaloid derived from *Paganum harmala* induced apoptosis in the cultures Sf9 cells (Cui *et al.* 2020). Alphaviruses are known to cause cell death in their mosquito vectors and the cell death was found to be important the propagation of the viruses in the vector (Cappuccio and Maisse, 2020). It was reported that Acetylcholineesterase promotes apoptosis of insect neurons (Knorr *et al.* 2020). It was showed that *Wolbachia* induces apoptosis of nurse cells in the ovaries of *Laodelphax striatellus* and it is associated with increase in fecundity (Guo *et al.* 2018). It was also reported that Phytohaemagglutinin induced apoptosis in the epithelial cells of *Sitobion avenae* gut, the cells showed the hall marks of apoptosis after the insects were fed with diet containing Phytohaemagglutinin (Sprawka *et al.* 2013). The presence of Zinc in higher concentrations in the diet induced the apoptosis of haemocytes in the larvae of *Spodoptera litura* (Xia *et al.* 2005).

Reports of PCD

a. Ametabolans:

It was reported that apoptosis, autophagy and sometimes necrosis will occur in the degeneration of midgut epithelium during molting in a proturan *Filientomon takanawanum* (Rost-Roszkowska *et al.* 2010a). In *Lepismachilis notata* and *Machilis hrabei* (Thysanura), the midgut epithelial cells surrounded with cisterns of endoplasmic reticulum form the autophagosomes and degenerate by necrosis. Necrosis intensifies just before each moult. Degenerating cells' basal lamina persists as a covering over the underlying regenerative cells. Apoptosis also occurs where condensation of chromatin takes place, nucleus assumes a lobular shape and fragmentation occurs. The apoptotic cells eventually separate from the basal lamina and are secreted into the lumen of midgut where they disintegrate (Rost-Roszkowska *et al.* 2010b). Also, in *Atelura formicaria* (Ateluridae), necrosis is the most common form cell death in midgut during metamorphosis however apoptosis also occurs (Rost-Roszkowska, 2010c). In *Allacma fusca* (Collembola) apoptosis is

common in younger forms but however as the insects age, apoptosis becomes less frequent and at the end of their life, necrosis occurs in the midgut epithelial cells (Rost-Roszkowska, 2008).

b. Paurometabolans:

In *Acheta domesticus* (Gryllidae), the midgut epithelium in the posterior region degenerates as the cells undergo necrosis. They are discharged into the lumen of the midgut where they disintegrate. The cell organelles are destroyed by autophagy (Rost-Roszkowska *et al.* 2010d). The Til pioneer neurons in a grasshopper, *Schistocerca americana* embryo undergo programmed death which is found to be triggered by the change in ecdysteroid titres in the haemolymph (Kutsch and Bentley, 1987). It was proved that the antennal cells during embryonic development undergo apoptosis in *Schistocerca gregaria* (Boyan *et al.* 2018).

c. Hemimetabolans:

It was reported that the follicular epithelial cells undergo apoptosis in nymphs of *Ecdyonurus venosus* (Ephemeroptera) during the developmental phase characterized by pale wing pads (Gaino and Rebora, 2003). Studies on the female accessory glands of *Aeshna juncea* and *A. grandis* (Odonata) revealed that PCD is initiated at the early reproductive phase and is almost disintegrated at the late reproductive phase by apoptosis (Abro, 2005). The labial musculature of *Anax junius* (Odonata) was degenerated during metamorphosis (Maloeuf, 1935).

d. Holometabolans:

Extensive studies on PCD were conducted in holometabolous insects like Lepidopteran caterpillars and *Drosophila*. In Lepidopterans, caspase-1 is the first caspase and was first identified in *Spodoptera frugiperda* and triggers apoptosis which is regulated by 20-hydroxyecdysterone and Juvenile Hormone. Both apoptosis and autophagy occur simultaneously however, there might be a cross-talk between apoptosis and autophagy. In *Drosophila*, Dronc is the initiator caspase and can trigger apoptosis. In *Apis mellifera*, autophagy and apoptosis are activated in tandem for destruction of salivary glands, larval gut and malpighian tubules during larva-pupa transition (Tettamanti and Casartelli, 2019).

PCD in Insects

PCD occurs in insects not only during growth and metamorphosis but there are several other instances like when cells which fulfilled their function undergo PCD. Also, insects use apoptosis as a defense against viral pathogens invading them (Clarke and Clem, 2003). In symbiotic association between aphid (*Acyrthosiphon pisum*) and the endosymbiont *Buchnera aphidicola*, modified host cells called bacteriocytes serve as the habitat for the symbiotic bacteria. These bacteriocytes are degenerated by a novel mechanism which is non-apoptotic and non-autophagic whereas, the endosymbionts are killed by a lysosomal-dependent mechanism in the adults (Simonet *et al.* 2018). In *Sitobion avenae*, the winged adults migrate to new host plants. It was reported that the flight muscles are degenerated after the migration through apoptosis (Feng *et al.* 2019).

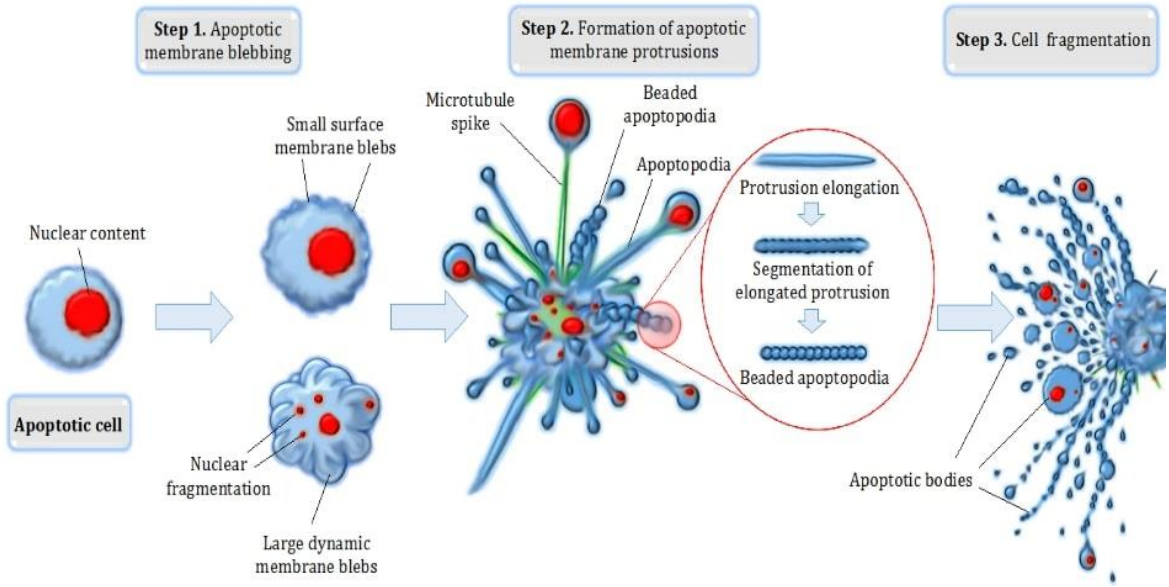

Fig. 2. Plasma membrane blebbing or zeosis (Smith et al. 2017)

Apoptosis

Apoptosis is a process where the cells will not grow and divide, but the cells and their contents undergo programmed death without any spillage of the cell contents (D'Arcy, 2019). During apoptosis, cells shrink and form plasma membrane blebs. The cytoskeleton structure of the cell is disrupted and the cytoplasmic content flows through these disruptions and forms outward bulges known as blebs carrying cytoplasm (Van der *et al.* 2016). These apoptotic blebs (Fig 2) are consumed by the phagocytes. The nucleus of apoptotic cells condenses, and electron dense chromatin gets aligned along the inner margin of the nuclear envelope (Fahrbach *et al.* 2012).

Autophagy

Autophagy is a process where the contents of the cells are sequestered into lysosomes for degradation which are then digested and then reabsorbed (D'Arcy 2019). Unlike apoptosis, cells undergoing autophagy do not from electron dense chromatin and instead they form autophagosomes. The Autophagosomes eventually fuse with lysosomes and the contents of the cell are recycled (Xie *et al.* 2007). Autophagy plays an active role in PCD in several tissues in *Drosophila* including the salivary gland and ovary (Fahrbach *et al.* 2012). Programmed cell death always has been a topic of interest and is studied in various organisms ranging from unicellular organisms, invertebrates to mammals. Among insects, exclusive studies were conducted on *Manduca sexta*, *Drosophila melanogaster*, *Bombyx mori* and *Helicoverpa virescens* (Table 1).

The *Manduca* model: *Manduca sexta* (Tobacco hornworm) attracts the attention of insect neurobiologists and endocrinologists because of its large size and ease of rearing and facultative diapause in their lifecycle. The Inter Segmental Musculature (ISM) of *M. sexta* is studied for elucidating PCD of muscles during metamorphosis. ISMs are prominent abdominal muscles found in

larva, pupa and pharate adults. They are divided into separate pairs of bilaterally symmetric bundles, each of which attaches to the cuticle at inter segmental boundaries. The ISMs are helpful in hatching and subsequent larval locomotion. After pupation, the muscles in the 1st, 2nd, 7th and 8th abdominal segments die and rapidly disappear. The muscles in the middle four segments persist throughout metamorphosis and are used for the defensive and respiratory movements of the pupa. Following adult eclosion, the remaining ISMs undergo PCD during the subsequent 30 hours. Under laboratory conditions, metamorphosis in *Manduca* is complete in 18 days, with adult eclosion taking place late on day 18. On day 15 of adult development the mass of the ISMs begins to decline, and during the next 3 days ISMs lose 40% of their mass. Ultra structural studies revealed that autophagy is the mode of PCD of ISMs (Fig 3).

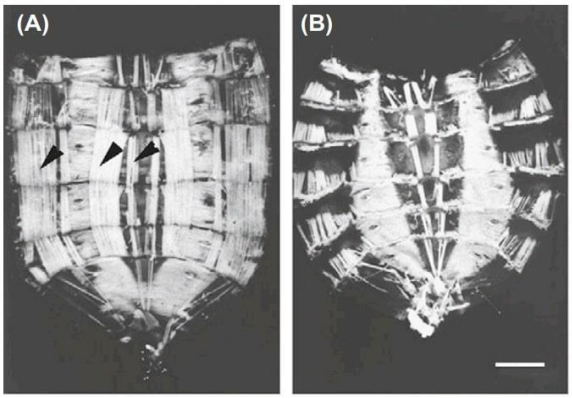

Fig. 3. ISMs in the abdomen of newly eclosed adults (A) and 30 hour-old adults (B) (Fahrbach *et al.* 2012)

The *Drosophila* model: PCD studies on *Drosophila melanogaster* (Diptera) indicated that the steroid hormone 20-hydroxyecdysone influences the PCD during metamorphosis. PCD is studied in the midgut and salivary glands of *Drosophila.* Three death genes have been identified in ~300kb region in its genome. There is two-way mechanism to control the apoptosis using the death genes *rpr*, *hid*, *grim* which trigger apoptosis mediated by caspases and a death regulatory gene baculovirus p35 gene which inhibits caspase activity. Besides this several baculovirus IAPs (Inhibitors of Apoptosis) are discovered in *Drosophila* genome. Titer changes in the ecdysone concentrations trigger the metamorphic events. At the end of larval development, a pulse of ecdysone triggers the formation of puparium and prepupal development, followed by another pulse after 10 hours signals for pupation. The salivary glands are degenerated by ~ 15 hours after puparium formation (Fig 4). PCD in the salivary glands of larva is ecdysone-triggered and is genetically regulated using early and late genes defined by the puffing pattern of salivary gland polyene chromosomes (Jiang *et al.* 1997).

The Silkworm model:

The salivary glands of the silkworm larvae are of prominent importance as they are used to spin silk. However, they are specific structures seen only in larvae. They are lost during metamorphosis from last instar larva to adult. The silk gland consists of an anterior, middle, and a posterior division. The anterior silk gland is a duct surrounded by a single layer of approximately 300 large, flat cells and lined with a thick cuticular intima at the internal surface. As in case of *Drosophila*, the PCD in *B. mori* is also triggered by the hormone 20-

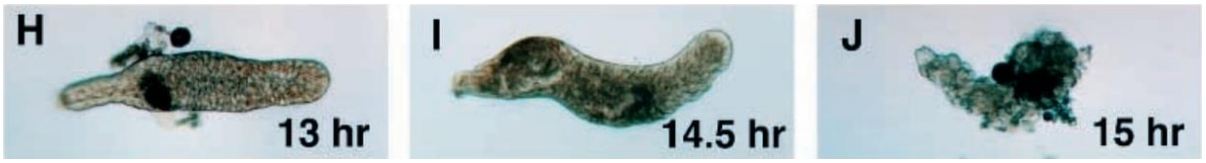

Fig. 4. PCD in salivary glands of Drosophila pupa. (H) Normal salivary glands seen in 13 hour pupae, (I) degenerating salivary glands in a 14.5 hour pupae, (J) completely degenerated salivary glands after 30 min (Jiang et al. 1997)

hydroxyecdysterone. The anterior salivary gland began to exhibit signs of PCD in vivo 2 days after gut purge and completed PCD by 48 hours as shown in Fig 5. Posterior silk glands are degenerated by apoptosis and autophagy (Montali *et al.* 2017).

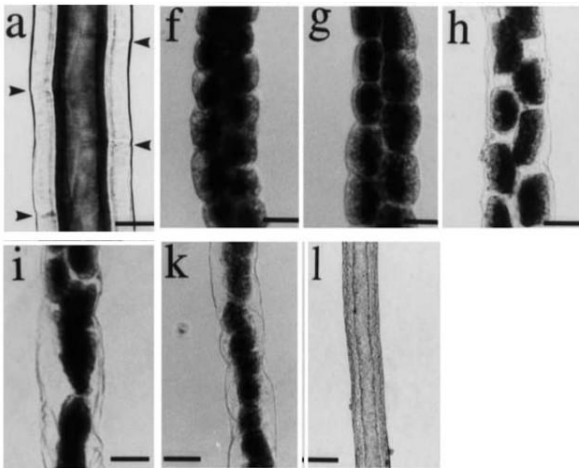

Fig. 5. In vivo progression of PCD in *B. mori* anterior salivary gland (a) salivary gland of last instar larvae, (f) at pupation, (g) 12h after pupation, (h) 24h after pupation, (i) 36h after pupation, (k) 42h after pupation, (l) 48h after pupation. (Terashima *et al.* 2000)

The *Helicoverpa* model: In the tobacco budworm (*Helicoverpa virescens*), the larval midgut epithelium undergoes a renewal process in pre-pupae. During this process of renovation, the old larval epithelium is destroyed by PCD mechanisms meanwhile the regenerative cells (Nidi) proliferate giving rise to a new epithelium. The old larval midgut undergoes PCD through co-occurrence of apoptosis and autophagy. For ease of study the larvae from 5th instar to pupa are arranged in phases1, 2, 3 and pupal phase, where phases 1, 2, 3 are from ecdysis to digging, digging phase, pupal cell formation to pharate pupa. The changes in nuclear structure attributable to apoptosis occur at late phase 1 especially in goblet cells and few columnar cells of the monolayered epithelium. The autophagic events occur in early phase 2 where feeding stops, so as to supplement halted food intake and continue throughout phase 3 (Tettamanti *et al.* 2007).

Future prospects in pest management:

The phenomenon of PCD could be a potential target for pest management as it is required for growth and development during metamorphosis. As of now pesticides were developed as growth regulators, molting disruptors but in the near future there is scope to develop novel pesticide molecules which actually target the PCD phenomenon in insects. For example, fenoxycarb a growth regulator inhibited PCD and remodeling of the fat bodies in *Galleria mellonella* (Poyraz *et al.* 2021). Similarly, novel molecules could be developed to act specifically on the PCD during metamorphosis. Also, harmine based molecules could be developed as pesticides as harmine induced apoptosis in Sf9 cells as

shown by Cui *et al*. 2020. As reported by Xia *et al*. 2005, breeding for resistance to *Spodoptera litura* could be done by elevating Zinc levels in crop plants. Gene silencing of iap (Inhibitor of apoptosis proteins) genes using RNAi could be a potential pest management strategy (Yoon *et al.* 2020).

Conclusion

Programmed cell death in insects can be triggered during growth and development of insects. Although having several genes which regulate PCD, insect hormone ecdysone triggers PCD during metamorphosis in almost all insects. PCD is also seen during embryogenesis of the insects. In insects PCD occurs through apoptosis, autophagy and necrosis, sometimes they co-occur simultaneously. The larval structures are destroyed and recycled during metamorphosis using PCD. Further exploration of PCD in insects can pave ways to understand basic cellular biology, explore new targets at molecular level for insect pest management and prepare new formulations of insecticides which target the PCD during growth and development of insects, study cancers in insects, and devise strategies for molecular therapeutics which can be used against cancers in humans.

References

Åbro A. 2005. The accessory glands of the female genital tract in *Aeshna juncea* (L.) and A. grandis (L.) (Anisoptera: Aeshnidae). Odonatologica 34(2): 103-110.

Boyan G, Graf P, Ehrhardt E. 2018. Patterns of cell death in the embryonic antenna of the grasshopper *Schistocerca gregaria*. Development Genes and Evolution 228(2): 105-118.

Cappuccio L, Maisse C. 2020. Infection of Mammals and Mosquitoes by Alphaviruses: Involvement of Cell Death. Cells 9(12): 2612.

Clarke T E, Clem R J. 2003. Insect defenses against virus infection: the role of apoptosis. International Reviews of Immunology 22(5-6): 401-424.

Cooper D M, Granville D J, Lowenberger C. 2009. The insect caspases. Apoptosis 14(3): 247-256.

Cui G, Sun R, Veeran S, Shu B, Yuan H, Zhong G. 2020. Combined transcriptomic and proteomic analysis of harmine on *Spodoptera frugiperda* Sf9 cells to reveal the potential resistance mechanism. Journal of Proteomics 211: 103573.

D'Arcy M S. 2019. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biology International 43(6): 582-592.

Fahrbach S E, Nambu J R, Schwartz L M. 2012. Programmed cell death in insects. Insect Molecular Biology and Biochemistry pp.419-449.

Feng H, Guo X, Sun H, Zhang S, Xi J, Yin J, Cao Y, Li K. 2019. Flight muscles degenerate by programmed cell death after migration in the wheat aphid, *Sitobion avenae*. BMC Research Notes 12(1): 1-7.

Gaino E, Rebora M. 2003. Detection of apoptosis in the ovarian follicle cells of *Ecdyonurus venosus* (Ephemeroptera, Heptageniidae). Italian Journal of Zoology 70(4): 291-295.

Ghanim M, Fattah-Hosseini S, Levy A, Cilia M. 2016. Morphological abnormalities and cell death in the Asian citrus psyllid (*Diaphorina citri*) midgut associated with *Candidatus Liberibacter asiaticus*. Scientific Reports 6(1): 1-11.

Gillott C. 2005. Postembryonic Development. Entomology, pp.623-652.

Guo Y, Hoffmann A A, Xu X Q, Zhang X, Huang H J, Ju J F, Gong J T, Hong X Y. 2018. Wolbachia-induced apoptosis associated with increased fecundity in *Laodelphax striatellus* (Hemiptera: Delphacidae). Insect Molecular Biology 27(6): 796-807.

Hasnain S E, Taneja T K, Sah N K, Mohan, M, Pathak N, Sahdev S, Athar M, Totey S M and Begum R. 1999. Invitro cultured *Spodoptera frugiperda* insect cells: Model for oxidative stress-induced apoptosis. Journal of Biosciences 24(1): 13-19.

Iga M, Iwami M, Sakurai S. 2007. Nongenomic action of an insect steroid hormone in steroid-induced programmed cell death. Molecular and Cellular Endocrinology 263(1-2): 18-28.

Jiang C, Baehrecke E H, Thummel C S. 1997. Steroid regulated programmed cell death during *Drosophila* metamorphosis. Development 124(22): 4673-4683.

Knorr D Y, Georges N S, Pauls S, Heinrich R. 2020. Acetylcholinesterase promotes apoptosis in insect neurons. Apoptosis 25(9): 730-746.

Kutsch W, Bentley D. 1987. Programmed death of peripheral pioneer neurons in the grasshopper embryo. Developmental Biology 123(2): 517-525.

Lee G, Park J H. 2020. Programmed cell death reshapes the central nervous system during metamorphosis in insects. Current Opinion in Insect Science, 43: 39-45.

Maloeuf N R. 1935. The postembryonic history of the somatic musculature of the dragonfly thorax. Journal of Morphology 58(1): 87-115.

Minelli A, Fusco G. 2013. Arthropod post-embryonic development. In Arthropod Biology and Evolution (pp. 91-122). Springer, Berlin, Heidelberg.

Montali A, Romanelli D, Cappellozza S, Grimaldi A, de Eguileor M, Tettamanti G. 2017. Timing of autophagy and apoptosis during posterior silk gland degeneration in *Bombyx mori*. Arthropod Structure & Development 46(4): 518-528.

Pan G, Zhang K, Li C, Hu X, Kausar S, Gu H, Yang L, Cui H. 2021. A hemocyte-specific cathepsin L-like cysteine protease is involved in response to 20-hydroxyecdysone and microbial pathogens stimulation in silkworm, *Bombyx mori*. Molecular Immunology 131: 78-88.

Poyraz T E, Goncu E, Koc K. 2021. Apoptotic and autophagic characteristics of perivisceral fat body remodeling of the greater wax moth *Galleria mellonella* and effects of juvenile hormone analog, fenoxycarb, on these processes. Archives of Insect Biochemistry and Physiology, pp. 21780.

Rost-Roszkowska M M. 2008. Degeneration of the midgut epithelium in *Allacma fusca* L. (Insecta, Collembola, Symphypleona):

apoptosis and necrosis. Zoological science, 25(7): 753-759.

Rost-Roszkowska M M, Machida R, Fukui M. 2010a. The role of cell death in the midgut epithelium in *Filientomon takanawanum* (Protura). Tissue and Cell 42(1): 24-31.

Rost-Roszkowska M M, Jansta P, Vilimova J. 2010b. Fine structure of the midgut epithelium in two *Archaeognatha*, *Lepismachilis notata* and *Machilis hrabei* (Insecta), in relation to its degeneration and regeneration. Protoplasma 247(1-2): 91-101.

Rost-Roszkowska M M, Vilimova J, Chajec L. 2010c. Fine structure of the midgut epithelium in *Atelura formicaria* (Hexapoda, Zygentoma, Ateluridae), with special reference to its regeneration and degeneration. Zoological Studies 49: 10-18.

Rost-Roszkowska M M, Poprawa I, Chachulska-Zymełka A. 2010d. Apoptosis and autophagy in the midgut epithelium of *Acheta domesticus* (Insecta, Orthoptera, Gryllidae). Zoological Science 27(9): 740-745.

Simonet P, Gaget K, Balmand S, Lopes M R, Parisot N, Buhler K, Duport G, Vulsteke V, Febvay G, Heddi A, Charles H. 2018. Bacteriocyte cell death in the pea aphid/Buchnera symbiotic system. Proceedings of the National Academy of Sciences 115(8): E1819-E1828.

Smith A, Parkes M A, Atkin-Smith G K, Tixeira R, Poon I K. 2017. Cell disassembly during apoptosis. WikiJournal of Medicine 4(1): 1.

Sprawka I, Goławska S, Parzych T, Goławski A, Czerniewicz P, Sytykiewicz H. 2013. Induction of apoptosis in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) under the influence of phytohaemagglutinin PHA. Applied Entomology and Zoology 48(4): 525-532.

Terashima J, Yasuhara N, Iwami M, Sakurai S, Sakurai S. 2000. Programmed cell death triggered by insect steroid hormone, 20-hydroxyecdysone, in the anterior silk gland of the silkworm, *Bombyx mori*. Development Genes and Evolution 210(11): 545-558.

Tettamanti G, Grimaldi A, Casartelli M, Ambrosetti E, Ponti B, Congiu T, De Eguileor M. 2007. Programmed cell death and stem cell differentiation are responsible for midgut replacement in *Heliothis virescens* during prepupal instar. Cell and Tissue Research 330(2): 345-359.

Tettamanti G, Casartelli M. 2019. Cell death during complete metamorphosis. Philosophical Transactions of the Royal Society B 374(1783): 20190065.

Tracy K, Baehrecke E H. 2013. The role of autophagy in *Drosophila* metamorphosis. Current Topics in Developmental Biology 103: 101-125.

Van der P E, Böing A N, Gool E L, Nieuwland R. 2016. Recent developments in the nomenclature, presence, isolation, detection and clinical impact of extracellular vesicles. Journal of Thrombosis and Haemostasis 14(1): 48-56.

Xia Q, Sun H, Hu X, Shu Y, Gu D, Zhang G. 2005. Apoptosis of *Spodoptera litura* larval hemocytes induced by heavy metal zinc. Chinese Science Bulletin 50(24): 2856-2860.

Xie Z, Klionsky D J. 2007. Autophagosome formation: core machinery and adaptations. Nature Cell Biology 9(10): 1102-1109.

Yoon J S, Koo J, George S, Palli S R. 2020. Evaluation of inhibitor of apoptosis genes as targets for RNAi-mediated control of insect pests. Archives of Insect Biochemistry and Physiology 104(4): 21689.

Zhai X, Zhao X F. 2012. Participation of haemocytes in fat body degradation via cathepsin L expression. Insect Molecular Biology 21(5): 521-534.

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Table 1. Programmed Cell Death (PCD) studies in different Insects

Insect	Location of PCD	Mechanism of PCD	References
Manduca sexta	Inter Segmental Musculature	Autophagy	Fahrbach *et al.* 2012
Drosophila melanogaster	Salivary glands	Apoptosis	Jiang *et al.* 1997
Bombyx mori	Silk glands and Salivary glands	Apoptosis and Autophagy	Terashima *et al.* 2000; Montali *et al.* 2017
Helicoverpa virescens	Larval midgut	Apoptosis and Autophagy	Tettamanti *et al.* 2007
Filientomon takanawanum	Midgut epithelium	apoptosis, autophagy and necrosis	Rost-Roszkowska *et al.* 2010a
Lepismachilis notata and Machilis hrabei	Midgut epithelium	Apoptosis and necrosis	Rost-Roszkowska *et al.* 2010b
Atelura formicaria	Midgut	Apoptosis and necrosis	Rost-Roszkowska *et al.* 2010c
Allacma fusca	Midgut	Apoptosis and necrosis	Rost-Roszkowska *et al.* 2008
Acheta domesticus	Midgut	autophagy and necrosis	Rost-Roszkowska *et al.* 2010d
Schistocerca gregaria	Embryonic antennal cells	Apoptosis	Boyan *et al.* 2018
Ecdyonurus venosus	Follicular epithelial cells	Apoptosis	Gaino and Rebora, 2003
Aeshna juncea and *A. grandis*	Female accessory glands	Apoptosis	Abro, 2005
Apis mellifera	Malpighian tubules, Larval gut, Salivary glands	Apoptosis and Autophagy	Tettamanti and Casartelli, 2019
Acyrthosiphon pisum	Bacteriocytes	Non-apoptotic and non-autophagic mechanism	Simonet *et al.* 2018
Sitobion avenae	Flight musculature	Apoptosis	Feng *et al.* 2019