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Hormonal Control of Reproduction in Female Pyralid Moths Infesting Stored Products: Regulation of Yolk Protein Synthesis and Uptake

D. Silhacek, P. Shirk, E. Shaaya

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Investigators' Names:

D. Silhacek

P. Shirk

E. Shaaya

Investigators' Institutions:

USDA/ARS, Insect Attractants,
Behavior and Basic Biology
Laboratory

A.R.O. The Volcani Center

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Don L. Silhacek
Signature
Principal Investigator

Herbert Obukadey
Signature
Institution's Authorizing
Official

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**HORMONAL CONTROL OF REPRODUCTION
IN FEMALE PYRALID MOTHS INFESTING STORED PRODUCTS:
REGULATION OF YOLK PROTEIN SYNTHESIS AND UPTAKE**

**D. Silhacek, E. Shaaya and P. Shirk
with
G. Zimowska**

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Abstract

Our data indicate that ovarian development progresses in two distinct, ecdysteroid coordinated steps. The first occurs during the first three days of the pupal period and is initiated by an increasing titer of ecdysteroid (identified as α -ecdysone) starting eight hours after the molt. Once ovarian development is initiated, specialized cell types, e.g. nurse cells, follicle cells and oocytes, differentiate and develop to a point just prior to yolk protein synthesis and uptake by the oocyte. The second stage in ovarian development is signaled by the reattainment of a baseline level of ecdysteroid during the final three days of the pupal period. Follicle development and oocyte maturation are the predominant activities occurring during this stage. These two stages of ovarian development are triggered by the initial secretion of ecdysteroid, an event that may be influenced by photoperiod. Subsequent developmental events appear to proceed sequentially without further regulatory intervention by ecdysteroids; the inhibition of second stage development by elevated ecdysteroid titers probably provides a mechanism for synchronizing the events associated with pharate adult development. After eclosion, the oocytes are chorionated and the mature eggs are laid. Mating accelerates the final stages of egg maturation and egg laying, an effect that can be mimicked by juvenile hormone treatments.

Objectives

An important area of ecological-based control methods in stored product entomology is the development of procedures that disrupt the reproductive cycle of pest insects. The purpose of this study was to determine the role of endocrine factors regulating ovarian development, yolk protein synthesis by fat body and ovary and its uptake by the oocyte in the Indianmeal moth, Plodia interpunctella, a major pest of stored commodities. The focus of our investigation was to differentiate the endocrine regulation of metamorphosis from the endocrine regulation of ovarian development. In this study we have examined yolk protein synthesis and export by ovarian follicle cells, vitellogenin (Vg) synthesis and secretion by fat body cells and uptake and storage of these proteins by maturing oocytes during pupal and pharate adult development. Key features of development that occurred during this period were correlated with changes in ecdysteroid titer. These pioneering experiments provide a fundamental understanding of reproductive development in moths and how it is integrated with metamorphosis and the endocrine secretions that regulate it.

Summary of Research Results

Our initial experiments indicated that the differentiation and development of functional ovaries was largely confined to the first half of the pupal stage and the maturation of the oocytes occurred during the latter half. Our first objective was to determine the temporal sequence of ovarian development, keying specific developmental events in the ovary to specific morphogenic changes in the pupa. At pupation, the ovaries are still largely undeveloped with only undifferentiated clusters of germ cells (cystocytes) occurring at the anterior end of the ovarioles. For the first eight hours after pupation, the level of ecdysteroid remains at ≤ 300 pg/mg of wet weight (Figure 1). In each ovary, the cystocytes begin to differentiate into oocytes and nurse cells, from poorly organized aggregates or clusters of developing germ cells at the anterior end of each ovariole. The germ cells contain round nuclei that are still associated with synaptonemal complexes and generally appear to have low metabolic activity.

A ten-fold increase in the ecdysteroid (identified in this study as α -ecdysone) titer in the whole animal during the remainder of the first day following pupation signals the initiation of events leading to pharate adult development. As the titer of α -ecdysone rises, the fat body cells dissociate from one another, but are still confined within the basal membrane that surrounds the organ. In the ovaries, the oocyte-nurse cell clusters (each cluster consisting of 1 oocyte and 7 nurse cells) become organized in single file along the ovariole with the most posterior cluster being the most developed; the oocyte is oriented as the most posterior cell within each cluster. The oocyte

Figure 1. Changes in ecdysteroid titers during pupal and pharate adult development when white pupae of Indianmeal moths were placed at 30° C in either total darkness (DD) or in a 16L:8D photoperiod (LD); P, pupation; E, eclosion; — , scotophase.

Figure 2. Changes in the size of follicle cell and nurse cell mitochondria during pupal and pharate adult development; P, pupation; E, eclosion.

nuclei retain their round shape and remain associated with the synaptonemal complexes. In contrast, the nurse cell nuclei become ramiform with evenly dispersed chromatin and several nucleoli, indicating increased metabolic activity within these cells. Also, a unicellular layer of follicular epithelial cells is assembled for the first time along the ovariole walls.

The elevated level of ecdysteroid is maintained for about 8-10 hours before subsiding to ≤ 400 pg/mg tissue at 72 hours after the pupal molt (Figure 1). As the ecdysteroid titer declines, there is a rapid formation and development of follicles; 50% of the follicles are formed by 56 hours after pupation. The more developed (most posterior) oocyte-nurse clusters are separated from one another by a layer of interfollicular connective cells. Two additional follicular cell types, squamous and columnar, are differentiated to enclose the nurse cells and oocyte, respectively. Some of the squamous follicular epithelial cells invaginate to partially separate the oocyte from the nurse cells in the cluster. The nurse cells start to increase in size, increasing the size of the nurse cap. Within the nurse cells the nuclei begin to undergo endopolyploidization. The mitochondria rapidly increase over 3 fold in size between 32 and 48 hours after pupation (Figure 2). After peaking at 48 hours, the size of the nurse cell mitochondria decrease to their original size by 82 hours; at the same time there is a rapid endopolyploidization of nurse cell nuclei.

At 82 hours, the fat body cells have reaggregated forming a discrete adult organ that begins to produce and secrete vitellogenin (YP1 and YP3) into the hemolymph (Figure 3). In the ovaries, the nurse cells begin to increase in size (as well as the nurse cell cap) and the cells of the follicular epithelium show signs of increased metabolic activity.

Between 92 and 96 hours, the brush border between the oocyte and the follicle cells begins to develop. Endocytotic vesicles are seen around the cortex area of the oocyte indicating uptake of extracellular protein through the brush border region. Yolk bodies appear for the first time in the cytoplasm of the oocyte (Figure 4); they contain a proteinaceous material that has been identified by immunological methods to be largely YP2, the major yolk protein produced by the columnar follicular epithelial cells. The follicular epithelial cells show signs of increased metabolic activity such as an extensively developed rough endoplasmic reticulum and large mitochondria. Most likely this reflects the increased activity associated with the biosynthesis and export of proteins, such as YP2. Even though cellular activity is increasing, the follicular epithelial cells

Figure 3. The titers of vitellogenin and total protein in the hemolymph during vitellogenesis and egg laying.

Figure 4. Changes in the size of yolk granules and lipid droplets in maturing oocytes during vitellogenesis.

remain tightly attached to each other by the zonula adherens and show no signs of patency. This probably accounts for the absence of YP1 in the oocyte even though YP1 has been circulating in the hemolymph since 82 hours after pupation (Figure 4). Between 96 and 105 hours, the yolk bodies increase in numbers, but not in size. The only detectable yolk protein in the yolk bodies is YP2. Lipid droplets also appear in the oocyte cytoplasm and rapidly increase in size during this period. The brush border between the oocyte and follicle cells continues its rapid development, facilitating the translocation of increasing amounts of YP2 from the follicle cells into the oocyte.

The beginning of patency is marked by the appearance of small interfollicular spaces adjacent to the basal membrane at 96 hours after pupation. The interfollicular spaces, which contain proteinaceous material, continue to increase in size for the next several hours, culminating in the breakdown and disappearance of the zonula adherens after 105 hours. The follicle cells are completely separated from one another but retain attachments to the oocyte and to the basal membrane. The opening of the interfollicular spaces provides a clear channel for the movement of extraovarial proteins and lipids from the hemolymph to the oocyte without entering the follicle cells. During the ensuing 31 hours, the yolk proteins produced in the fat body, YP1 and YP3, and probably YP4 (produced by the follicle cells) are transferred to the oocyte via the interfollicular spaces where all three proteins (but not YP2) have been detected. Upon entering the oocyte, these three proteins are distributed evenly by accretion into existing yolk granules.

With the uptake of the extraovarial proteins starting at 105 hours, the yolk granules, as well as the lipid droplets, begin to rapidly increase in size. This uptake results in a >10 fold increase in the size of the oocyte during the 24-hour period following the initiation of extraovarial protein uptake (Figure 5). A further increase in oocyte size of almost two fold occurs during the subsequent eight-hour period when water and nurse cell cytoplasm are rapidly taken up by the oocyte along with the yolk protein and lipid. The nurse cells reach maximum size at about 120 hours after eclosion (Figure 5). During the remaining 16 hours of pharate adult development the nurse cell cytoplasm is transferred to the oocyte via the intercellular bridges, leading to a diminution in nurse cell size and, finally, their complete degeneration in the newly emerged adult. During the first 3-4 hours after eclosion, the electron dense layer of the vitelline membrane is secreted by the follicular epithelium, surrounding the yolk protein containing translucent layer that encloses the oocyte. Subsequently, the remaining nurse cell cytoplasm is transferred to the oocyte which is then followed by closure of the cytoplasmic bridges, the collapse of the oocyte microvilli and the complete

Figure 5. Changes in the size of terminal oocytes and nurse cells during pupal and pharate development; P, pupation; E, eclosion; —, scotophase.

degeneration of the nurse cells. The squamous cells situated between the oocyte and the degenerating nurse cells undergo further differentiation into the micropylar-forming cells. The columnar follicular epithelial cells flatten to provide a continuous layer around the oocyte; the cells and their nuclei are now arranged with their long axes parallel to the surface of the oocyte. The latter follicular cells produce the proteins used in chorion formation which begins at about 6 hours post eclosion in unmated females. Unmated females will chorionate most of their eggs during the first day after eclosion but none are layed until they are close to death (ca 9 days after eclosion) when they will lay about 15% of their full complement of ca 175 eggs. This entire process is accelerated by mating.

In mated females, chorionated eggs can be layed as soon as 2 hours after eclosion with the full complement of eggs being layed within 3 days after mating. The life span of females is ca 5 1/2 days when mating occurs shortly after eclosion. These effects of mating can be mimicked by topically applying juvenile hormone or an IGR to virgin females. IGR-treated females respond by initiating egg laying as quickly as mated controls and laying their full complement of eggs within 3 days of treatment. The life span of treated females is comparable to that of mated females.

Research Achievements and Conclusions

The current study has provided considerable insight into the control mechanisms that regulate the development of ovaries and oocytes during the course of metamorphosis in the Indianmeal moth. We found that follicular development is initiated shortly after pupation by the same secretion of ecdysteroid that initiates other metamorphic events such as fat body dissociation. The timing of this initial hormonal secretion appears to be dependent upon photoperiodic events. The major ecdysteroid that is released has been identified as α -ecdysone, whose origin is not in the ovary. Throughout this stage of ovarian development, α -ecdysone titers remain elevated, preventing further ovarian development beyond a point just prior to vitellogenesis. We speculate that this inhibition serves to coordinate developmental events in the ovaries and in ancillary tissues, e.g. fat body, so that all vitellogenic processes supporting oocyte maturation can be synchronously implemented. The reattainment of basal ecdysteroid levels at the midpupal period provides the cue for the resumption of pharate adult development which includes the reassociation of fat body cells and the beginning of vitellogenesis. Oocyte maturation proceeds to completion without any further hormonal intervention although we suspect that intraovariole factors may regulate the sequential development of follicles within an ovariole and that factors associated with eclosion may initiate chorionation. Juvenile hormone (and mating) accelerates egg laying by virgin females.

Cooperation

The major participants on this project have contributed to all aspects of this investigation. The unselfish cooperation exhibited by the collaborating scientists has been a major factor in achieving the objectives of this project. Dr. H. Rees has provided valuable assistance in identifying α -ecdysone as the ecdysteroid regulating the developmental events studied in this investigation.

Publications

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Zimowska, G., Shirk, P., Silhacek, D. and Shaaya, E. Initiation of yolk protein synthesis and development of patency by follicular epithelial cells. In preparation.