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CONTROL OF CELL DIFFERENTIATION IN THE ACCESSORY REPRODUCTIVE GLANDS OF MEALWORM BEETLES

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Sexual reproduction requires not only maturation of the primary germ cells but also the coordinate maturation of various accessory organs. We are interested in the function and development of such accessory reproductive glands in mealworm beetles. In the present paper, we will concentrate upon the importance of the pupal ecdysterone peak to gland growth and maturation in the pupa and the adult.

At the time of pupal ecdysis in Tenebrio molitor, both male and female reproductive systems have undergone primary organogenesis and the accessory glands are readily distinguishable upon dissection. During the succeeding nine days of pupal life, the glands are exposed to a large ecdysterone surge, which lasts from day 3 to day 7 and reaches its peak at day 4[1].

The female spermathecal accessory gland is of ectodermal origin, like the spermatheca and median oviduct. It is an elongate structure with an axial cuticular duct down its center. From outpocketings of the walls of the axial duct, fine cuticular ductules diverge into the surrounding secretory cell mass. The secretory unit (or organule) is composed of a secretory cell, a a ductule cell, and a cell associated with the outpocketing of the axial duct. Protein products from the rough endoplasmic reticulum pass through the Golgi zone and are secreted into a central extra-cellular space bounded by the secretory cell. From this space, products move through the cuticular ductule and to the axial duct[2].

The development of the spermathecal accessory gland in the pupa can be divided into three phases: I - Cell Division from days 1-4; II - Cell Morphogenesis from days 4-6½; and III - Cuticle Deposition between days 6½ and 9. In Phase I, the apical microvilli facing the axial lumen tend to be elongate and irregular. The rate of cell division is constant until about 100 hours, when mitosis abruptly arrests. In Phase II, the three cells of the organule wrap around one another to form a concentric cluster. The centermost cell develops a pseudocilium on its apical surface, and retracts away from the axial lumen, leaving a cylindrical sleeve of extracellular space around the

pseudocilium. Microvilli become short and regularly spaced. As Phase III begins, the outer epicuticle of the efferent ductule is laid down along the axial lumen and within the spaces around the pseudocilia. The cuticle of the axial duct continues to thicken for the remainder of the pupal stage[3].

Three experimental approaches were used to ask whether the pupal ecdysterone peak was necessary for the development of the spermathecal accessory gland. In the first approach, animals at 0 or 2 days of pupal life were ligated between the thorax and the abdomen. Gland development was normal, although the time course was slower than in the intact animals. This result could be explained by the fact that some tissues in the isolated abdomen produce ecdysterone[4]. In the second approach, young pupae were injected with the peak dose of ecdysterone in an attempt to trigger precocious development. No precocious development was observed. Rather, when 0 day pupae were injected, development was normal. When 2 day pupae were used, no pseudocilia formed, but cuticulogenesis proceeded in both hormone-injected and saline-injected animals. We interpret this failure to make the pseudocilia as a result of surgical trauma.

In the third approach, spermathecal glands were explanted into Landureau's medium[5] (400 mOsm, 0.1% fetal calf serum) for *in vitro* culture. The organs survived well for 10-15 days in basal medium. When spermathecal glands from 2 day pupae were placed in Landureau's medium, the apical microvilli remained elongate and irregular and neither pseudocilia nor cuticles were formed in the succeeding week. Culture of such explants with ecdysterone (4μg/ml) led to epicuticle deposition at 6-7 days, but no pseudocilia formation. Explantation of glands from 4 day pupae lead to the normal development *in vitro*: pseudocilia formed and cuticulogenesis took place whether or not ecdysterone was added. We conclude that the ecdysterone rise between 2 and 4 days triggers the commitment toward cuticulogenesis, but that the presence of ecdysterone is not required during the subsequent expression of that commitment[6].

Two pairs of male accessory glands, the bean-shaped accessory glands (BAGs) and the tubular accessory glands (TAGs) are derived from a common mesodermal rudiment[7]. Each gland consists of a simple epithelium surrounded by a thin muscular coat. Cell division in both glands proceeds for 6-7 days of the pupal life and takes place in two bouts: the first at 1½ days and the second at 5 days. Both nuclear counts and mitotic indices (determined by colchicine arrest) show that for each bout of cell division, BAG cells divide once and TAG cells divide twice. When 0 day pupal glands, are explanted into the

Landureau's medium, the first mitotic bout takes place, but the second does not follow. However, the second mitotic bout occurs when ecdysterone ($4 \mu g/ml$) is added at 4 days.

In this mesodermal tissue of Tenebrio, the pupal ecdysterone peak triggers a wave of cell division[8]. The same peak arrests the cell cycle at G2 in the sternal epidermis of Tenebrio[9], in the Kc cells of Drosophila[10] and probably also in the spermathecal accessory gland of Tenebrio. In these cases, the ecdysterone effects on ectoderm and mesoderm are markedly different.

Is the pupal ecdysterone peak a prerequisite of the terminal differentiation which takes place in the post-ecdysical adult? In the BAG, that differentiation includes the appearance of eight distinct types of secretory cells which are distinguished from one another by the ultrastructure of their secretory granules and by the differing affinity of each cell type for Oil red O. The secretory epithelium consists of patches of each cell type; the pattern is repeated from one gland to the next. The borders between patches are fairly sharp and no intermediate cell types are found in the adult glands[11]. The cell types become patent over the 2 days just after adult ecdysis[12] and at the same time, the many protein spots characteristic of terminal differentiation appear on polyacylamide gels[13].

We have cultured BAGs in vivo (transplants) and in vitro to determine whether the pupal ecdysterone peak is required before the adult phenotype occurs. When BAGs were transplanted from 0 day pupae to 0 day adults, the glands did not grow in size nor did they differentiate morphologically or biochemically. When BAGs were transplanted from 5 day pupae (after the pupal peak) to 0 day adults, the glands increased in size and differentiated (biochemical criteria). In agreement with this result, BAGs explanted into Landureau's medium for the pupal period can grow when subsequently implanted into 0 day adults only when they have been exposed to ecdysterone in vitro. We suspect that the pupal ecdysterone peak (and perhaps the round of cell division which it triggers) is required for a change in competence in the secretory epithelium of BAGs. After this peak, the epithelium can undergo the post-ecdysial terminal differentiation.

In summary, the pupal ecdysterone peak is required for ongoing growth and differentiation in the ectodermal spermathecal gland and the mesodermal BAG and TAG. The rise in ecdysterone effects a commitment to cuticulogensis, and the peak itself arrests mitoses in the spermathecal gland. The peak of ecdysterone triggers a second round of mitoses in the BAG and the TAG. The rising levels of ecdysterone (for the female gland) or probably the

actual peak of hormone (for BAG and TAG) are prerequisite to later differentiation which can take place in the absence of ecdysterone.

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