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MATURATION OF THE MALE REPRODUCTIVE SYSTEM AND ITS ENDOCRINE REGULATION

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INTRODUCTION

The production of spermatozoa in the male insect and the transfer of the seminal mass to the female require significant investments of biochemical resources and the temporal coordination of development in several organs. Maturation of the gonads and the secondary reproductive systems must be synchronized with the metamorphic acquisition of energetic, sensory, motor, and central nervous adaptations for mating. The developmental hormones that commit cells to new programs and that trigger and modulate their expression in molting cycles also affect the primary and secondary reproductive systems. Many studies have addressed the endocrine regulation of female reproductive maturation and physiology. In comparison, investigations of the male insect systems have lagged. But in the past few years, more laboratories have focused their activities on analyses of male reproduction and its control (9, 57, 58).

A recent chapter in this series reviewed the male accessory gland systems (9). This review discusses some of the recent studies that enlarge our knowledge of the special physiology of the male. Selected examples summarize investigations that show that the major hormones that control growth and metamorphosis of the epidermis also control the growth, differentiation, and physiological modulation of the male reproductive system. In addition, male

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systems provide challenging new models for the exploration of endocrine action at the cellular and molecular level because hormones affect basic growth phenomena such as cell cycling as well as cardinal metamorphic events such as commitment.

MALE REPRODUCTIVE SYSTEMS

In insects, the male tract is typically comprised of the testes, the efferent ducts, and glands (17). A complex blend of secretions from the walls of the vasa deferentia, the seminal vesicles, the ejaculatory duct, and the accessory glands facilitates the smooth passage and insures the viability of the sperm. In many species, male secretions include not only the seminal fluids that bathe the spermatozoa but also the macromolecules that are the building blocks of the spermatophore, an elaborate sperm sac that packages the sperm and seminal fluids (59).

Accessory glands of insects differ markedly in gross shape and size, in cellular ultrastructure, and in histochemical staining features (9, 17). The secretory systems in any one species often show marked regional specializations along the length of the male tract. As a result, the blend of products shifts in composition and viscosity as the sperm pass through the male efferent ducts. The secretory products are biochemically complex. Only a very few of the protein constituents in seminal and paraseminal secretions have been characterized, and their functional significance requires much further study. Notable among the secretions are those that give rise, through a carefully orchestrated sequence of secretion and assembly, to organized spermatophores that are vehicles for sperm transfer.

The male systems of four taxa provide points of reference for our discussion of development and physiology: (*a*) the yellow mealworm *Tenebrio molitor* (accessory gland proteins); (*b*) two lepidopterans, the oriental silkmoth *Bombyx mori* (seminal proteins of the spermatophore) and the gypsy moth *Lymantria dispar* (sperm release from the testes); (*c*) the fruit fly, *Drosophila melanogaster* (paragonial proteins); and (*d*) the saltatory orthopteroids *Locusta migratoria* and *Acheta domesticus* (accessory gland proteins). I begin with an overview of the anatomy and biochemistry in each and then summarize information on the effects of endocrine factors on growth and cell cycling, on differentiation, and on physiological modulation in the adult.

The Accessory Glands of Tenebrio molitor

Figure 1*a* shows the male reproductive system of the yellow mealworm beetle *(Tenebrio molitor),* which includes two anatomically distinct accessory glands—the tubular accessory glands (TAGs) and the bean-shaped accessory glands (BAGs). Both are paired and mesodermal in origin. Each is composed

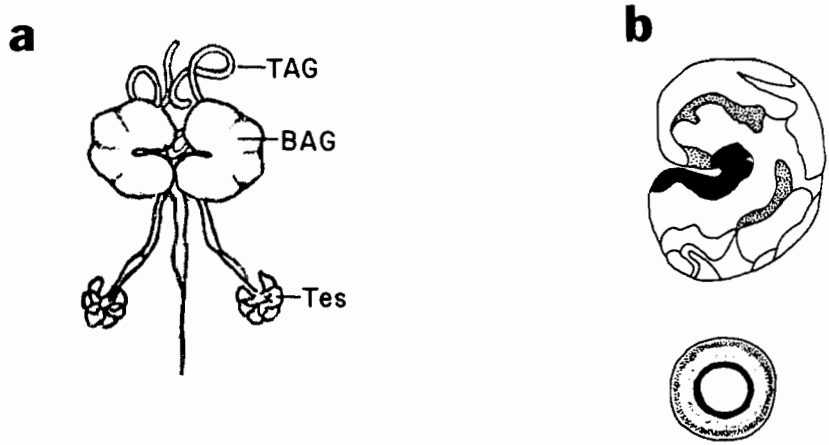

Figure 1 The reproductive system of *Tenebrio molitor*. (*a*) The complete male system: BAG, bean-shaped accessory gland; TAG, tubular accessory gland; Tes, testis. (*b*) The bean-shaped accessory gland (*top*) with the boundaries of each cell type. Secretions from cell-type 3 (*stipple*) and cell-type 7 (*solid*) are incorporated into specific layers of the spermatophore (shown in cross section, *bottom*).

of a secretory epithelium surrounded by a muscular coat. Their secretions flow into the grand junction where the paired seminal vesicles converge into the ejaculatory duct (25, 29, 70).

The TAGs contain one major type of secretory cell that produces a watery proteinaceous fluid that mixes with the sperm (5, 40, 47). Their mobility on SDS-polyacrylamide gels indicates that these soluble proteins have apparent molecular weights from 14–29 kilodaltons (kd) and fall into three size ranges: the A/B class (under 18 kd), the C class (about 21 kd), and the D class (25–29 kd) (47). Incorporation of ^{3}H-leucine into all three classes of secretory proteins increases rapidly during the first 2 days after adult ecdysis and reaches a peak at 4–6 days, when the male is reproductively mature (47).

The D proteins are polymorphic; at least five alleles have been reported (29). A monoclonal antibody produced by K. Grimnes (unpublished data) recognizes an epitope specific to the D proteins. Immunocytochemical experiments with colloidal gold show the D antigens residing in the secretory vesicles of cells of the TAG, in the lumen of the gland, and in the lumen of the spermatophore where they are mixed with the spermatozoa (F. Weyda, unpublished data).

An expression library of complementary DNAs from TAG was screened using the monoclonal antibody to D antigens. The amino acid sequences of two D proteins (D1, D2) have been inferred from three immunopositive clones. With the aid of reverse-phase high-performance liquid chromatogra-

phy (HPLC), a major D protein was purified and its N-terminal sequence determined. The partial direct sequence is in agreement with that from one of the cDNA clones (G. Paesen & G. M. Happ, unpublished data). Clones D1 and D2 code for proteins with three major domains, designated A, A', and B, each of which occupies approximately one-third of the polypeptide chain. Domains A and A' are similar to each other and are predicted to be largely α-helical; the B domain contains the putative major antigenic sites. All three domains contain a concensus sequence of amino acids that is similar to LEAR(W/K)APDDD. The concensus sequence is repeated 19 (D2) or 20 times (D1) along the length of the molecule. The physiological role of these intriguing proteins with tandem repeats has yet to be established.

The BAGs of the male mealworm beetle produce a semisolid product, with the consistency of springy toothpaste, that is largely transformed into the insoluble wall and core of the spermatophore (15, 16). The secretory epithelium of the BAG contains eight morphologically distinct cell types, each of which is confined to specific monotypic patches. Every cell type manufactures a different secretory product, and the secretory products of any one cell type collect in a coherent mass in the lumen.

Immunocytochemical experiments with monoclonal antibodies have identified at least four cell-specific and adult-specific secretory products of the BAGs (39, 41, 77). They are present in very low concentrations at adult ecdysis and accumulate to high levels in the ensuing five days. Three of these antigens have been traced from their cells of origin to precise zones of the plug in the BAG, to areas of the prespermatophoric mass in the ejaculatory duct, and to regions of the spermatophore (Figure 1*b*). The significant conclusion is that the order in the final sperm sac results from the placement of the secretory products in the lumen of the BAG.

Many of the proteins secreted by the BAGs are spermatophorins, the structural proteins of the spermatophore. One with an apparent molecular mass of 23 kd has been purified and partially characterized (77). This protein, designated SP23, is rich in proline (25%) and glutamic acid (15%). On pore-limiting native gels, the size is approximately 370 kd, suggesting a 16-mer. It was recently isoiated using HPLC. The sequence of the protein has been inferred from the corresponding cDNA; unlike the more soluble D proteins from the TAG, this proline-rich structural protein shows no conspicuous tandem repeats. Preliminary comparison of this protein with others in databases suggests a significant similarity with the plant cell-wall protein extensin (M. Schwartz & G. Paesen, unpublished data).

Researchers have studied the development of the male accessory glands in the pupal and adult stages of *T. molitor* using morphological techniques (light and electron microscopy) (16, 25, 38, 43), immunochemical procedures (5, 39, 41), and by examining the results of electrophoresis of protein con-

stituents and the relative rates of leucine incorporation into these proteins (46, 47), as well as patterns of cell division in vivo and in vitro (38, 45, 46, 86). Mitosis occurs for the first 6 days of the 9-day pupal stage. Based on all criteria for differentiation, major production of the secretory proteins characteristic of a mature reproductive adult begins at the time of adult ecdysis.

The Paragonia of Drosophila melanogaster

P. S. Chen thoroughly described the reproductive system of male *Drosophila* in a previous *Annual Review of Entomology* (9). The paragonia (accessory glands) are long simple sacs with two types of secretory cells; both cell types are fully differentiated when the fly emerges from its puparium. The glands mature and become turgid with secretion over the following week.

The paragonia of *Drosophila* synthesize many proteins. Over 85 spots greater than 14 kd were detected on two-dimensional gels (13, 80). Over half of those seen on one-dimensional SDS gels show genetic polymorphism (82). The amino acid sequences of five interesting paragonial peptides have been described. Chen and his group have isolated two: the "sex peptide" and a serine protease inhibitor. M. F. Wolfner and her coworkers recently reported three additional gland-specific proteins (19, 62, 63).

The sex peptide is synthesized within paragonia and is passed to the female at copulation; it represses female receptivity for 6–9 days and accelerates oviposition (reviewed in 9). Chen and his colleagues (10) have employed reverse-phase HPLC to isolate this peptide in a physiologically active form. The pure peptide contains only 36 amino acids and is encoded by an organ-specific mRNA.

The protein msP 355a has 264 amino acids and a calculated molecular weight of 28 kd. However, the major msP 355a band detected on Western blots migrates like that of a protein of 37 kd; this increase in apparent molecular mass probably results from glycosylation. The amino acid sequence of msP 355a suggests that it could function as a prohormone because one region strongly resembles that of the egg-laying hormone (ELH) of the mollusc *Aplysia californica* (63). At the time of mating, msP 355a is transferred to the female, and within 10 minutes later a portion of this antigen is found in the hemolymph. The msP 355a remaining in the female tract undergoes rapid and specific proteolytic cleavage to yield first a 29-kd fragment and then a 22-kd fragment that predominates 30 minutes later. The ELH-like sequence is retained in the 22-kd fragment. Cleavage does not occur in the hemolymph. Within 2–3 h after mating, the females expel unstored sperm and secretions, and the 22-kd fragment is lost from the female tract (62).

The gene *mst 355b* codes for an acidic protein, msP 355b, that is 90 amino

acids in length. The corresponding band recognized on Western blots is apparently 12 kd. Like msP 355a, msP 355b is found in the hemolymph of the female shortly after copulation, but msP 355b is not cleaved to specific smaller fragments (62, 63). Both msP 355a and msP 355b are first detected only after adult eclosion, although their transcripts are present in late pupae. Copulation leads to increased transcription and translation for both proteins.

The gene *mst 316* codes for a small basic protein of 52 amino acids (19). The sequence suggests that cleavage of a signal peptide occurs at Ala-23; the 29–amino acid product resulting from such a cleavage has two single arginines that may be sites for proteolytic cleavage to yield final products of 11, 6, and 12 amino acids. These putative small peptides are of unknown physiological significance. The pattern of expression of *mst 316* was elegantly demonstrated using germ-line transformation with a *mst 316–lacZ* hybrid gene (19). The expression in transgenic flies indicates that the native gene product is specific to the main cells of the accessory gland, that the transcript increases with maturation, and that mating induces increased transcription and then increased translation (19).

A serine protease inhibitor of 63 amino acids has been isolated from paragonia and seminal fluid of *Drosophila funebris* (73). This small protein inhibits the activity of a variety of proteases including acrosin, an enzyme associated with the acrosome of mammalian sperm. Acrosin inhibitors have been reported from mammalian seminal plasma, and this serine protease inhibitor in *D. funebris* might play an analagous role. We know nothing of the expression of this inhibitor during development.

An enzyme, esterase 6, is produced by the cells of the anterior ejaculatory duct, is present in the seminal fluids, and is transferred to the female at mating (72, 74). It appears to have long-term effects on female remating and sperm use (34). Within minutes after mating, the enzyme is translocated from the female reproductive tract to her hemolymph, where it continues to be detectable by Western blot analysis for as long as four days after mating (61).

The Accessory Reproductive System of Bombyx mori

The reproductive systems of Lepidoptera were among the first to be studied by insect morphologists. The testes, the spermatophore, and the bursa copulatrix of the oriental silkmoth, *Bombyx mori,* were first described by Malpighi in 1669. Omura (65) carefully investigated the histology of the male system. Figure 2*a* shows the male system of *Bombyx* and its spermatophore. The vasa deferentia and accessory glands converge on the median ampulla and median seminal vesicle. From this point, the bolus of semen then passes through specialized secretory areas of the ejaculatory duct and into the spermatophore created largely from the secretions of that duct.

B. mori, as do most moths, has two types of sperm—the anucleate apyrene

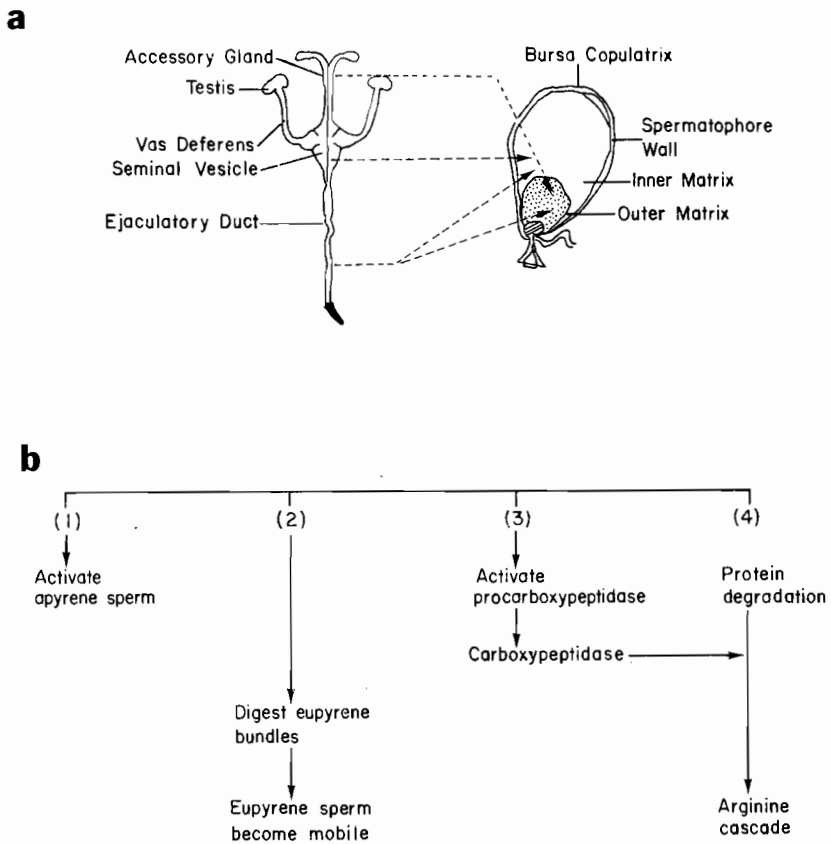

Figure 2 Reproductive system of *Bombyx mori*. (*a*) Secretions from various regions of the male system are found in specific zones of the spermatophore [after Osanai et al (66)]. (*b*) The four roles of initiatorin, an endopeptidase from the glandula prostatica (posterior region of the ejaculatory duct) [after Osanai et al (66)].

sperm and the nucleated eupyrene sperm that fertilize eggs. In the seminal vesicles, eupyrene sperm are present in bundles while apyrene sperm have already dissociated to free single cells. During passage through the distal portion of the ejaculatory duct, the previously immobile apyrene sperm are rendered mobile as they are deposited in the spermatophore.

Almost 20 years ago, Shepherd showed that a secretion of the male tract in saturniid moths is responsible for sperm activation (75). The recent work of Osanai and his colleagues in Tokyo has greatly expanded our knowledge of this process in which the apyrene sperm become mobile and the eupyrene sperm undergo final maturation. The bulk of these reactions occur within the

spermatophore (Figure 2*a*), a complex structure formed as secretions from increasingly distal portions of the male tract pass into the bursa copulatrix of the female (65, 68). The spermatophore functions not merely as a passive receiver for the sperm mass but also as a reaction vessel for sperm maturation (66).

The energy required for sperm maturation in the spermatophore is made available by digestion of proteins (Figure 2*b*). The key factor that begins this process is an endopeptidase, named initiatorin, contained in the secretion of the glandula prostatica—the terminal segment of the ejaculatory duct (1). Initiatorin is a glycoprotein endopeptidase of the serine protease type weighing 30 kd and with an optimum basic pH (pH 9.2). Initiatorin activates the apyrene spermatozoa by gently digesting their surface coats (67) and causing a marked increase in their motility (69). The highly motile apyrene spermatozoa act as micro stir-bars within the viscous internal mileau of the spermatophore. Initiatorin simultaneously digests the intercellular matrix of the eupyrene bundles, liberating and activating these true sperm (69). Finally, it activates a carboxypeptidase (produced in the ampulla) and cleaves proteins on the C-side of arginine residues (1). Arginine, liberated from proteins (50) by the carboxypeptidase, passes into an arginine degradation cascade that produces substrates (notably α-glutamate) that can support sperm motility.

The enzymes and their substrates are synthesized within different parts of the male system and remain segregated from one another before copulation (Figure 2*a*). Only upon ejection of the male secretions and the sperm mass during the formation of the spermatophore do substrates begin to mix with the corresponding enzymes. At this time, proteolysis begins and sperm activation takes place (Figure 2*b*) (66). The kinetic characterization of the enzymes within the spermatophore and the stoichiometry in the silkmoth of the processes remain to be investigated. Evidence also supports proteolytic cleavage in the spermatophores of *Lymantria dispar*, in which a 90-kd protein associated with the soluble spermatophore components and a 45-kd protein associated with the insoluble spermatophore components undergo stepwise degradations over the first hour after formation of the spermatophore (M. Kiuchi & H. Kasuga, unpublished data). This proteolytic cascade is rather like that described for msP 355a from the paragonia of *Drosophila* species.

The Accessory Gland Systems of Saltatoria

The accessory reproductive glands of male grasshoppers and crickets are two bilaterally symmetrical masses composed of specialized secretory tubules. In *Acheta domesticus*, each mass consists of several hundred delicate tubules (52), while in *Melanoplus sanguinipes*, each side has only 16 tubules. Also, the tubules in this species differ markedly in gross morphology: 4 are whitish and opaque, 10 are short and hyaline, 1 is long and hyaline, and 1 is a seminal

vesicle (11). When the proteins in each of these types are separated on SDS-polyacrylamide gels, each mature tubule type has a characteristic banding pattern. During maturation in the young adult, changes in staining intensity and in rates of leucine incorporation show that specific proteins appear and accumulate according to a fixed developmental schedule (35).

In the long hyaline gland of *M. sanguinipes,* the major product is a glycoprotein, called LHP1, with a M_r of 36 on SDS gels and a native molecular mass estimated from Sephadex G-100 gel filtration to be 72 kd (7, 8). The 36-kd subunit is found in the viscous secretions that coat the spermatophore and apparently lubricate its passage through the male and female ducts. LPH1 is found at very low quantities at ecdysis and increases to peak levels at 10–15 days when reproductive maturation has been achieved.

ENDOCRINE CONTROL OF PREIMAGINAL CELL CYCLING

Growth of male systems is rapid in the preadult instar (17), and sudden growth can often be correlated with peaks of ecdysteroid activity. In some species, for example *Bombyx mori,* experiments have demonstrated the importance of ecdysteroids in accessory gland growth and differentiation (76), and it seems likely that the pattern is almost universal.

Many authors have linked ecdysteroid action to turns of the cell cycle (e.g. 53, 83). In male reproductive systems, the pioneering work of Dumser & Davey (21, 22) showed that injection of ecdysone into fourth-instar *Rhodnius prolixus* caused a doubling of the mitotic index in the testes and that juvenoids counter the effects of applied ecdysone. Dumser (20) found a similar ecdysteroid-driven acceleration when locust testes were cultured in vitro, and he suggested that the hormone removed a block between the G_2 phase of the cycle and entry into mitosis (20). Subsequently, the action of ecdysteroids on cell cycling became less clear. Studies of cell cycling in the epidermis of moths (23), beetles (4), and flies (37) and in putative ectodermal derivatives such as Kc cell lines of *Drosophila melanogaster* (14, 79) indicated that edysteroids provoked arrest rather than stimulating mitoses. The conclusions from the studies on epidermis seemed to conflict with Dumser's earlier results on testes. Further research on beetle accessory glands and on moth epidermis has largely resolved the conflict (51). Apparently, a short term effect of ecdysteroid is to stimulate cell cycling while arrest and terminal differentiation often follow that stimulation.

In the BAGs and TAGs of *Tenebrio molitor,* primary organogenesis is accomplished before the onset of pupal ecdysis (48). Over the 9-day pupal instar, ecdysteroid levels remain low (10^{-8} M) in the first few days, begin to rise at day 3, reach peak levels at day 5 (nearly 10^{-5} M), and decline again to

low levels by day 7–8 (18). Mitotic activity in the accessory glands is likewise low at pupal ecdysis and then rises. In both TAGs and BAGs, two mitotic maxima occur: at 1–2 days and at 4–5 days (38, 45, 46). DNA synthesis occurs throughout the mitotic bout and persists a bit longer, such that the secretory cells of the BAGs and the TAGs are all arrested in the tetraploid state by the eighth day of the pupal instar (38).

To test directly the hypothesis that ecdysteroids stimulate cell cycling in the BAGs and the TAGs, 20-hydroxyecdysone was applied to intact glands cultured in vitro (81, 86) and to dissociated cells from the BAGs (44). Addition of physiological doses of ecdysteroids to the cultures increased the mitotic index (81), and the effect persisted in the presence of hydroxyurea, an agent that blocks DNA synthesis in the S phase (86). The movement of cells through the cycle was also monitored using flow cytometry, after administration of hormone to cultures in vitro. Addition of hormone accelerated flow into the S phase (86) and also increased accumulation during G_2 in the presence of colchicine (44). Ecdysteroids likewise increased the accumulation of cells in G_1 in the presence of hydroxyurea. Thus, ecdysteroids must act in both gap phases of the cell cycle to stimulate cell cycling. According to dose-response data acquired using flow cytometry, the hormone acted within 30 min of its application and at an ED_{50} of 5×10^{-7} M, the physiological level at day 3 when mitotic activity in the accessory glands is beginning to rise toward the major midpupal peak.

The regulation of cell cycling by ecdysteroids is not limited to mitosis; meiotic progression is also sensitive to these hormones [as well as other uncharacterized factors (21, 32)]. In the European corn borer, *Ostrinia nubialis,* physiological doses of ecdysteriods stimulate apyrene spermiogenesis in vitro (28). In development of the eupyrene primary spermatocytes of *Manduca sexta,* progression from meiotic prophase into meiotic metaphase in day-2 wandering larvae is induced by the postwandering peak in the level of 20-hydroxyecdysone (24). Abdomens isolated before the peak show the spermatocytes blocked in meiotic prophase while those after the peak show spermatocytes blocked in meiotic metaphase. The block can be broken by implantation of prothoracic glands or injection of 20-hydroxyecdysone. The effect is dose dependent and appears to occur at a lower dose than that required for pupation. A parallel role for 20-hydroxyecdysone in removing a meiotic block has been reported for the oocytes of *Locusta migratoria* (54–56).

The demonstration that ecdysteroids control meiotic progression in insects is particularly interesting because there are parallels in other phyla, most notably in amphibians, where the steroid hormone progesterone breaks a block at meiotic prophase in the oocytes of *Xenopus laevis*. This led to the identification of a responsible factor, named MPF (maturation promoting

factor) (60). Considerable recent work shows an almost universal set of gene products that regulate cell cycling in eukaryotes, from yeast to *Drosophila* to *Xenopus* to humans (reviewed in 64). Whether these regulatory proteins are linked to the action of ecdysteroids in the G_2 phase remains unknown.

ENDOCRINE CONTROL OF PREIMAGINAL DIFFERENTIATION AND COMMITMENT

The repeated pulses of ecdysteroids during predult instars affect both cell cycles in the testes and differentiation of the germ cells. These periodic hormone surges may also promote early development of the rudiments that give rise to the secondary reproductive system. However, the most dramatic effect of ecdysteroids on accessory gland development is in the preadult instar when juvenile hormone levels have declined. At the time of the major pupal ecdysteroid peak, the specialized secretory cells of accessory glands become committed to produce adult-specific proteins. The expression of that commitment may be delayed until after the imaginal molt.

The influence of ecdysteroids on development of accessory glands of *Locusta migratoria* has been investigated by Gallois (26, 27), who implanted accessory glands from immature males of various ages into the thoracic musculature of adults. In this species, the accessory glands are fully differentiated only after the fifteenth day of the adult instar. Implants from early instars do not differentiate after implantation in an adult, while those from males close to the adult ecdysis do differentiate. The glands become competent for terminal differentiation at a critical period at day 2–3 of the final (fifth) larval instar, when there is a drop in juvenile hormone and a transient rise in ecdysteroids (2).

Ecdysteroids are likewise important in the pupal growth and commitment to the adult pattern of protein synthesis for the male accessory glands of lepidopterans. For *Bombyx mori,* ecdysteroid was required both for early pupal growth and for the appearance of an adult-specific antigen in the accessory glands. The effect on growth, demonstrated in vitro, was dose dependent (76). In *Spodoptera litura,* the injection of hormone and the addition of ecdysteroid to organ culture were correlated with increased leucine incorporation (78).

In mealworm pupae, the ecdysteroid peak at day 5 renders accessory glands competent to make adult-specific proteins (42, 85). The adult synthetic pattern begins to be expressed only after adult ecdysis (4 days after the pupal ecdysteroid peak) and full differentiation is achieved at day 6–8 of the adult instar.

The most convenient index of differentiation in the BAGs has been the activity of the enzyme trehalase, which is secreted by the BAGs and is found

in the spermatophore (84). By transplanting pupal BAGs of various ages into adult hosts and allowing further development, investigators showed that the capacity to make trehalase was acquired during the first half of the pupal period, at the time of the ecdysteroid surge (85).

To test the direct involvement of ecdysteroids in the change in competence, BAGs from day 0 pupae were cultured in vitro for 24 h with and without 20-hydroxyecdysone. After implantation into adults, they were allowed to develop for 8 days. Glands exposed to hormone in vitro showed high levels of trehalase while controls cultured in basal media before implantation did not. The hormone dose required for this change in competence in vitro was close to the physiological peak levels and at least 10-fold higher than that required to accelerate cell cycling. Clearly, the two ecdysteroid effects are distinct: at lower hormone levels, ecdysteroids affect cell cycling, and at higher levels, they affect commitment. The change in competence occurred in the presence of hydroxyurea (an inhibitor of DNA synthesis) and therefore probably in the absence of cell cycling (85).

GROWTH, DIFFERENTIATION, AND MODULATION IN THE POSTECDYSIAL ADULT

Peak differentation of male accessory glands is correlated with differences in reproductive strategies of different species. In the oriental silkmoth, the glands are terminally differentiated at ecdysis (77), while in locusts, the process of maturation takes 15 days (27). Little is known about the biochemical correlates of competence that is not yet expressed. For example, are transcripts for the adult-specific proteins accumulated in the pharate adult? This seems to be the case for the msP 355a and msP 355b proteins of *Drosophila melanogaster*. The mRNAs for these paragonial proteins first occur in pupae and increase to their adult levels at the time of eclosion (6).

Many studies have reported that, in a variety of young adult insects, juvenile hormone accelerates the maturation of accessory glands or is required for the renewal of secretory products after depletion during mating (9, 12, 17, 57, 71). Recent work on the transparent reproductive accessory gland of *Rhodnius prolixus* showed that the effect appears to be direct, because application of juvenile hormone I to glands cultured in vitro increases the incorporation of ^{3}H-leucine (36). Research on *R. prolixus* points to the involvement of a peptide both from the brain and from the corpora cardiaca (3).

Mating stimulates the synthesis of msP 355a and msP 355b proteins and of the corresponding mRNAs in the accessory glands of *Drosophila melanogaster* (62). Similar results were reported for the mst 316–LacZ fusion protein and its message (19). The recent work of Pellegrini and her coworkers on

accessory gland function in *D. melanogaster* has linked that effect of copulation to the action of juvenile hormone. At physiological concentrations (10^{-9} M), juvenile hormone III increased threefold the incorporation of radioactive methionine into trichloracetic acid precipitable proteins of paragonia cultured in vitro; in contrast, 20-hydroxyecdysone had little effect (87). Stimulation by juvenile hormone III required the presence of calcium. Stimulation was also affected by phorbol esters—tumor-promoting agents known to stimulate the production of the enzyme protein kinase C. No increase in methionine incorporation was seen in mutants deficient in protein kinase C. Because protein kinase C is an integral membrane protein involved in signal transduction, the result suggests that juvenile hormone acts at the membrane level. This conclusion appears to complement that of Ilenchuk & Davey (49), who reported that juvenile hormone acts on membranes of follicle cells of the *R. prolixus* ovary to stimulate a transport ATPase.

Once the male system is mature, sperm release from the testes may be hormonally regulated in some species, as shown by the work of Giebultowicz and coworkers (30–33) on *Lymantria dispar*. In the pharate adult, mature sperm are released according to a circadian rhythm (30). Once it has begun in situ, the rhythm persists within isolated complexes of testes and seminal ducts cultured in vitro in constant darkness. Because the phase of the rhythm can be reset by shifting the light-dark cycle of the organ cultures, the circadian pacemaker is probably within these isolated organs (33). The rhythmic release occurs only after decline of the midpupal ecdysteroid peak. Infusion of 20-hydroxyecdysone into male pupae inhibits that decline, and thus the drop in hormone titer appears to be essential for the onset of sperm release (31).

CONCLUDING COMMENTS

Male reproductive systems are functional only in the adult, but the accessory system and the testis generally mature along somewhat different time lines. Usually, accessory gland rudiments grow slowly and differentiate little until the preimaginal stage when ecdysteroid levels are high and juvenile hormone levels are low. Development then involves defined episodes of cell multiplication followed by acquisition of the competence to make adult-specific and cell-specific proteins. Coincident with the metamorphic molt, the adult competence begins to be strongly expressed and cells grow rapidly as full reproductive maturation is reached. If the adult instar lasts more than a few days, the secretory cycles will probably modulate with changing physiological demands.

Testicular growth proceeds in spurts over several preadult instars and is followed by maturation in the preimaginal instar and in the adult. The endocrine control of spermatogenesis is initially the control of cell cycling:

spermatogonia multiply by mitosis within the testes and spermatocytes divide in two meiotic divisions to yield spermatids. The spermatids then differentiate.

Sperm-cell maturation, storage, and transfer are stimulated and modulated by a host of internal and external factors, including hormones and endocrine factors. In this chapter, I have reviewed some of the recent work on the male systems with particular attention to the hormonal control of maturation. The biochemistry and the functions of the proteins of male accessory glands will likely provide us with new ways to monitor differentiation that follows after hormone action. The recent work on spermatogenesis and on accessory gland maturation is providing models not only for reproductive processes in insects but also for the actions of hormones on such fundamental developmental phenomena as regulation of cell cycling and commitment to new cellular programs.

Male reproductive development is of obvious importance to the growth of insect populations. A better understanding of the general regulatory mechanisms affecting reproductive maturation and of the specific ones peculiar to particular taxa may be of future utility in many fields of entomology, including evaluating mass rearing protocols or developing strategies for insect control.

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