Chapter 5
Ecdysteroids and Ecdysteroid Signaling Pathways During Insect Oogenesis

Luc Swevers and Kostas Iatrou

Abstract During insect oogenesis, the oocyte acquires nutrients and genetic determinants to support embryonic development (previtellogenesis and vitellogenesis) and subsequently becomes surrounded by a protective eggshell (choriogenesis). In many insects, ecdysteroids are synthesized during ovarian growth which is often followed by the accumulation of ecdysteroid conjugates into the eggs. The exact role of the ecdysteroids during oogenesis remains largely unclarified although functions as paracrine or autocrine regulators to signal the progression of follicle development or the resumption of meiosis in the oocyte have been proposed. In the silkmoth, *Bombyx mori*, although ecdysteroids are synthesized by the ovarian follicles, progression of follicle development towards choriogenesis requires down-regulation in ecdysteroid signaling. Using the regulation of silkmoth oogenesis by 20-hydroxyecdysone as a starting point, this review discusses the physiological roles of ecdysteroids and the function of the ecdysone regulatory pathway during insect oogenesis.

Keywords ecdysone • ecdysteroid • ecdysteroid conjugate • 20E • insect oogenesis • *Bombyx mori* • *Drosophila melanogaster* • *Aedes aegypti* • ecdysone regulatory pathway • *EcR* • *usp* • *E75* • *BR-C* • *E74* • *FTZ-F1* • Broad-Complex • ecdysone receptor • ecdysone biosynthesis • maternal ecdysteroids • vitellogenesis • choriogenesis • embryogenesis • Lepidoptera • Diptera • Orthoptera • Dictyoptera, Hymenoptera

5.1 Introduction

In insects, the function of ecdysteroids has been mostly investigated in the developmental processes that regulate molting and metamorphosis. In these processes, 20-hydroxyecdysone (20E) has been shown to activate the ecdysone receptor (EcR) complex, which consists of a heterodimer of two members of the nuclear receptor

L. Swevers and K. Iatrou
Insect Molecular Genetics and Biotechnology,
Institute of Biology, National Center for Scientific Research "Demokritos",
Aghia Paraskevi Attikis, Greece

G. Smagghe (ed.), *Ecdysone: Structures and Functions*

family, EcR and USP, the latter being the homolog of the vertebrate retinoid X receptor (Thomas et al., 1993; Yao et al., 1992, 1993). Binding of the hormone to the EcR complex initially results in the activation of a conserved hierarchical cascade of gene expression consisting of an interacting set of transcription factors, encoded by the so-called ecdysone-responsive early and early-late genes, including Ets domain-containing E74, the orphan nuclear receptors E75 and HR3, and the Broad-Complex (BR-C) zinc finger proteins (Riddiford, 1993a; Thummel, 1990, 1996, 1997; Henrich and Brown, 1995). Transduction and amplification of the hormonal signal by the conserved set of early gene products subsequently results in the regulation of numerous ecdysone-responsive late genes that define the phenotypic effects of 20E in a stage- and tissue-specific manner (Thummel, 2002; Bender, 2003).

However, the activation of the ecdysone regulatory cascade comprises only the first half of the developmental events in which ecdysteroids are involved. Prepupal *Drosophila* tissues require a decline in ecdysteroid signaling in order for development to progress and to respond to the next rise in hormone titer (Richards, 1976; Woodard et al., 1994). In lepidopteran larvae, application of strong ecdysone agonists such as tebufenozide initiate larval molting (cuticle apolysis) but failure to clear the hormone results in a developmental arrest in the middle of the molt (absence of ecdysis; Retnakaran et al., 1995). These observations support the existence of a signalling cascade that is triggered by a decline in ecdysteroid titer. Genetic studies in *Drosophila* have shown that the orphan nuclear receptor βFTZ-F1 likely functions at the initiation of this cascade since expression of βFTZ-F1 is induced by a decline in 20E titer and βFTZ-F1 mutants show developmental defects consistent with its role as a competence factor to prepare the prepupal-pupal transition during low ecdysteroid titers (Woodard et al., 1994; Broadus et al., 1999).

Besides its role in molting and metamorphosis where the molecular mechanism of its action has been studied most extensively, 20E has been implicated in the regulation of many other biological processes in insects, such as reproduction (oogenesis, vitellogenesis and spermatogenesis; Raikhel et al., 2005), embryogenesis (Kozlova and Thummel, 2003), diapause (Denlinger et al., 2005) and polyphenism (Hartfelder and Emlen, 2005). Recent studies in the silkworm, *Bombyx mori*, have indicated that the development of the ovary during the pupal and pharate adult stages is regulated through the 'classical' hierarchical cascade of gene expression mentioned above (Swevers et al., 2005). Furthermore, in the mosquito, *Aedes aegypti*, the regulation of vitellogenin synthesis in the fat body by ecdysteroids produced by the ovary occurs by the conserved set of ecdysone-responsive early and early-late genes (Raikhel et al., 1999; Li et al., 2000). Genetic studies also established that progression of oogenesis in *Drosophila* requires the function of genes implicated in the ecdysone regulatory hierarchy such as *EcR*, *BR-C* and *E75* (Buszczak et al., 1999) as well as genes involved in synthesis of ecdysteroids (Freeman et al., 1999).

These findings therefore suggest that the ecdysone-regulatory hierarchy is involved in the regulation of oogenesis in insects. This review will focus on the possible role of ecdysteroids to regulate the process of oogenesis in insects. Inevitably, the review will focus on the three insects for which most knowledge has accumulated (*Bombyx*, *Aedes*, *Drosophila*) while oogenesis in insects of other groups (mainly cockroaches, locusts and hymenopterans) will also be discussed.

5.2 The Silkmoth Paradigm: Control of Ovarian Development by Ecdysone

In the silkmoth, ovarian development is completely coupled to metamorphosis: it occurs almost exclusively during pharate adult and adult development (Tsuchida et al., 1987; Swevers and Iatrou, 2003). The dependence of ovarian development on ecdysteroids produced by prothoracic glands has been clearly demonstrated in experiments with pupae ligated between thorax and abdomen (Tsuchida et al., 1987; Swevers and Iatrou, 1999). In the isolated abdomens, the ovaries remain undeveloped; complete ovarian development is achieved following a single injection of microgram quantities of 20E.

Induction of ovarian development occurs via binding of 20E to the ecdysone receptor heterodimer consisting of BmEcR and BmUSP (Swevers et al., 1995; Tzertzinis et al., 1994) which is followed by the activation of the ecdysone regulatory cascade. Silkmoth homologs of the A- and C-isoforms of the E75 nuclear receptor (BmE75A and C; Swevers et al., 2002) are induced first which is followed by the induction of the B and C-isoforms of BmHR3 (Eystathioy et al., 2001). Concomitant with the induction of BmE75 and BmHR3 occurs the decline of the BmFTZ-F1 nuclear receptor (Sun et al., 1994; Swevers and Iatrou, 2003). The expression of other "classical" ecdysone-responsive genes, such as BR-C and E74 (*Bombyx* homologs of BR-C have been described recently (Uhlirova et al., 2003; Nishita and Takiya, 2004; Ijiro et al., 2004) while E74 was also described recently in *Manduca* (Stilwell et al., 2003), has not been described yet during induction of ovarian development in *Bombyx*, but it is expected that they play similar roles as in the ecdysone-response in tissues in other insects.

The expression of the yolk protein produced by the follicular epithelium, egg-specific protein (ESP; Sato and Yamashita, 1991), corresponds to a late event in the ecdysone regulatory cascade induced in the silkmoth ovary (Swevers and Iatrou, 2003). Interestingly, expression of the A-isoform of the BmHR3 receptor occurs concomitantly with ESP expression and BmHR3A has been proposed to function as a regulator of ESP expression (Eystathioy et al., 2001).

While the early stages of oogenesis and the initiation of vitellogenesis are dependent on the presence of active ecdysteroids in the hemolymph, experiments using dibenzoyl hydrazine ecdysone agonists such as tebufenozide (Dhadialla et al., 1998) have indicated that the completion of vitellogenesis and the process of egg shell synthesis (choriogenesis) require the absence of ecdysone signaling. Similar to 20E, tebufenozide induces early and late gene expression in ecdysone-responsive target tissues but because of its persistence in tissues gene expression that is dependent on down-regulation of ecdysone signalling does not occur (Retnakaran et al., 1995). When tebufenozide is injected in silkmoth isolated abdomens, ovarian development, including vitellogenesis, is initiated but becomes subsequently arrested (Swevers and Iatrou, 1999). Gene expression analysis in arrested *versus* developing ovarioles established that the developmental block occurs during middle vitellogenesis and that the orphan nuclear receptor BmFTZ-F1 is the earliest factor whose induction does not occur in arrested follicles (Swevers and Iatrou,

1999). Thus, in silkmoth ovarian follicles BmFTZ-F1 may function as a 'competence factor' that regulates the transition between developmental periods with high ecdysteroid titers and low ecdysteroid titers in similar fashion as was shown in genetic experiments in *Drosophila* (Woodard et al., 1994; Broadus et al., 1999).

The regulation of ovarian development by ecdysteroids produced by the prothoracic glands during pupation in silkmoths is summarized in Fig. 5.1. The figure illustrates that ovarian follicle development occurs in two phases: an early phase dependent on high ecdysteroid titers from previtellogenesis to middle vitellogenesis and a late phase dependent on low ecdysteroid titers from middle vitellogenesis

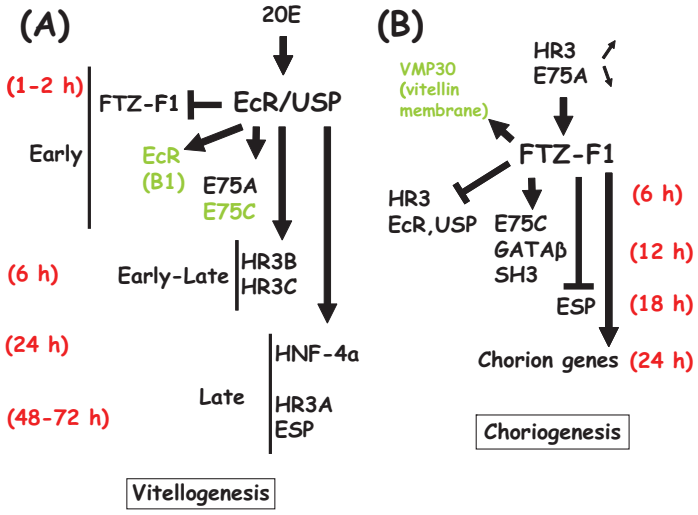

Fig. 5.1 Regulation of silkmoth (Bombyx mori) oogenesis during pharate adult and adult development by 20E produced by the prothoracic glands. **Panel a**: Induction of vitellogenesis by rising titers of 20E in the hemolymph. Indicated are different phases in the hormone response: early (repression of FTZ-F1, transient induction of B1-EcR and E75C, permanent induction of E75A), early-late (induction of HR3B and HR3C) and late (induction of HNF-4A, HR3A and ESP). Expression of ESP (egg-specific protein, a yolk protein precursor produced by the follicular epithelium; Sato and Yamashita, 1991) marks the initiation of vitellogenesis. **Panel b**: Induction of choriogenesis by declining titers of 20E in the hemolymph. At the top of the cascade is shown the nuclear receptor FTZ-F1, which plays a pivotal role in the regulation of developmental events during low titers of ecdysone (Broadus et al., 1999). As in Drosophila, induction of FTZ-F1 may be triggered by changes in the relative levels of the HR3 and E75 receptors (Swevers et al., 2002). Expression of FTZ-F1 is followed by the repression of HR3 and EcR/USP (at 6–12 h), induction of E75C, GATAβ and SH3 (at 12 h), repression of ESP (at 12–18 h) and induction of chorion gene expression (at 18–24 h). Because the vitellin membrane protein VMP30 is co-expressed with FTZ-F1, it was hypothesized that FTZ-F1 is a positive regulator of the expression of VMP30 (Kendirgi et al., 2002). Note that the deduction of the regulatory cascades that trigger vitellogenesis and choriogenesis in the silkmoth is based on expression patterns of mRNAs and remains to be investigated by functional analysis (*See Color Plates*)

until the end of choriogenesis. It can also be noted that silkmoth ovarian development is completely dependent on ecdysteroid signalling and that juvenile hormone (JH) does not play a role in silkmoth ovarian development (Izumi et al., 1984).

Besides the production of ecdysteroids by the prothoracic glands at early pupation that triggers ovarian development, the ovary itself also starts to produce ecdysteroids at day 4 after larval-pupal ecdysis (Ohnishi and Chatani, 1977). Ecdysteroids that were identified include ecdysone and 20E as well as several of their biosynthetic precursors and metabolites such as 2-deoxyecdysone, 2-deoxy-20E, 2, 22-bisdeoxy-20E, 3-epi-ecdysone and 3-epi-2-deoxy-ecdysone (Legay et al., 1976; Ohnishi et al., 1989; Sonobe and Yamada, 2004). Several lines of evidence indicate that the ecdysteroids produced by the ovary have no autocrine/paracrine or endocrine function to regulate the progression of oogenesis in the silkmoth. First, the ecdysteroids produced by the ovary are not secreted towards the hemolymph but accumulate in the eggs, mainly as ecdysteroid conjugates (C22 and C3 phosphate esters; Sonobe and Yamada, 2004). Second, as argued above, the experiments using the ecdysone agonist tebufenozide have clearly demonstrated that progression of vitellogenesis towards choriogenesis requires decline, not activation, of ecdysone signalling (Swevers and Iatrou, 1999). Third, suppression of the ecdysteroid content of ovarian follicles through the administration of the imidazole compound KK-42, a potent inhibitor of ecdysone synthesis, does not cause a disruption in ovarian follicle development (Kadono-Okuda et al., 1994). Application of KK-42 produced instead maternal defects on fertilization, embryogenesis and hatching of silkworm larvae.

These data argue for a role of ovarian ecdysteroids in progression of oocyte meiotic arrest and as ‘maternal’ ecdysteroids stored in the form of inactive ecdysteroid conjugates in the egg to regulate cuticulogenesis or morphogenetic events during embryogenesis (Lanot et al., 1989). In the silkmoth, however, contradictory results were obtained regarding correlation of ecdysteroid levels with cuticle formation in the embryo (Mizuni et al., 1981; Gharib and De Reggi, 1983; Gharib et al., 1983; discussed in Sonobe and Yamada, 2004). More recently, production of free 20E from pools of maternal ecdysteroid conjugates has been shown to be responsible for the developmental difference between diapausing and non-diapausing embryos (Makka et al., 2002).

Although an ‘autocrine’ or ‘paracrine’ role for ecdysteroids during ovarian follicle development is not likely in the case of the silkmoth, more recent experiments have pointed to such a role for prostaglandins. Application of non-steroidal anti-inflammatory drugs such as aspirin and indomethacin that block the production of prostaglandins by the cyclooxygenase enzyme, to ovarian follicles in culture results in arrest of follicle development (Machado et al., 2007). The arrest by aspirin and indomethacin can be reversed by exogenous application of prostaglandins and cAMP. Thus, as in mammalian models, prostaglandins may act through the activation of a membrane-bound G protein-coupled receptor (GPCR) and production of cAMP. However, prostaglandin signalling is unlikely to have a developmental role since they are required at all stages of follicle development investigated,

from middle vitellogenesis to late choriogenesis. Of note is the observation that exogenous prostaglandins or cAMP can not rescue the developmental arrest induced by tebufenozide. Prostaglandins may play a 'homeostatic' role in ovarian follicle development (Machado et al., 2007).

Recently, several genes that encode cytochrome P450 enzymes involved in the ecdysone biosynthetic pathway were identified in *Drosophila* and *Bombyx* (Warren et al., 2004; Gilbert and Warren, 2005; Namiki et al., 2005; Ono et al., 2006). It will be of interest to determine at which stages of oogenesis the cytochrome P450 genes are expressed in the silkmoth. Furthermore, FTZ-F1, besides its established role as a 'competence' factor in the regulation of developmental transitions from high to low ecdysteroid titers, is also known as the insect homolog of the nuclear receptor steroidogenic factor-1 (SF-1) which plays essential roles in the differentiation of steroidogenic organs (gonads and adrenals) in mammals (Val et al., 2003). Recently, a role for βFTZ-F1 was also proposed to regulate the ecdysteroidogenic activity in the prothoracic gland through modulation of the expression levels of ecdysteroidogenic cytochrome P450 enzymes (Parvy et al., 2005). It remains to be determined if a parallel situation exists also exists in the *Bombyx* ovary and that the expression of BmFTZ-F1 coincides with expression of ecdysteroidogenic genes such as *phantom* and *disembodied*.

5.3 The Silkmoth Paradigm: Applicability to Other Lepidopteran Insects?

In contrast to the silkmoth, ovarian development in many other lepidopteran insects is not initiated at the pupal stage in coordination with the metamorphic processes. In fact, ovarian development in lepidopteran insects is divided in four classes according to the extent of its coupling to metamorphic processes (review by Ramaswamy et al., 1997).

The first class, to which *Bombyx* (Bombycidae: Bombycoidea) belongs, comprises those species where oogenesis is initiated and completed during the larval and pupal stages in concert with metamorphic processes that are orchestrated by 20E. Oogenesis (vitellogenesis) in these species can be inhibited by JH (Davis et al., 1990). Other lepidopterans belonging to this class include *Hyalophora cecropia* (Saturniidae: Bombycoidea), *Malacosoma pluviale* (Lasiocampidae: Bombycoidea) and *Lymantria dispar* (Lymantriidae: Noctuoidea) (Ramaswamy et al., 1997).

In the second class of lepidopteran insects (example: *Plodia interpunctella* (Pyraloidea)), dependence of ovarian development on metamorphic events is reduced. In this moth, declining levels of ecdysteroids trigger vitellogenesis during pupation while completion of follicle development beyond vitellogenesis occurs before adult eclosion and is regulated by unknown factors (Shirk et al., 1992).

A further reduction on metamorphic events occurs in the third class, exemplified by *Manduca sexta* (Sphingidae: Bombycoidea), *Diatraea grandiosella* (Pyralidae: Pyraloidea) and *Choristoneura fumiferana* (Tortricoidea): in those lepidopterans,

vitellogenesis in the pharate adult occurs in the absence of ecdysteroids while completion of vitellogenesis and choriogenesis is regulated in the teneral adult by JH (Nijhout and Riddiford, 1974, 1979; Satyanarayana et al., 1994; Delisle and Cusson, 1999; Ramaswamy et al., 1997).

In the last (fourth) class, oogenesis takes place in the adult stage and is exclusively regulated by JH (Ramaswamy et al., 1997). In these cases, ecdysteroids can block the gonadotropic action of JH while mating stimulates the production of eggs (Satyanarayana et al., 1992). Species belonging to this class include *Pieris brassicae* (Pieridae: Papilionoidea), the nymphalids (Papilionoidea) *Polygonia* c-*aureum*, *Nymphalis antiopa* and *Vanessa cardui*, *Danaus plexippus* (Danaidae: Papilionoidea) and the noctuids (Noctuoidea) *Heliothis virescens*, *Helicoverpa zea* and *Pseudaletia unipuncta* (Ramaswamy et al., 1997).

From the examples described above, it appears that there is no clear correlation between phylogenetic relationships and class of ovarian development. Thus, the dependence (or independence) of ovarian development from metamorphic events orchestrated by 20E must have originated many times within the order of the Lepidoptera (Ramaswamy et al., 1997).

It is possible that the mechanism by which ovarian development in lepidopteran insects becomes dependent on 20E during metamorphosis involves heterochronic shifts in the expression of the ecdysone receptor EcR/USP. Thus, species belonging from class 3 to 1 are expected to show progressive shifts of expression of EcR/USP (and, consequently, dependence on regulation by 20E) to earlier stages of ovarian development or oogenesis. Such shifts in temporal expression of the ecdysone receptor have been described in gall midges (Diptera: Cecidomyiidae) where early (first larval) *versus* late (last larval) expression of EcR/USP directs paedogenetic or metamorphic ovarian development, respectively (Hodin and Riddiford, 2000). Also in *Drosophila* it was observed that alterations in the timing of expression of EcR in the ovary can uncouple the process of ovarian differentiation from tissue differentiation in the rest of the animal (Hodin and Riddiford, 1998).

In class 2 lepidopterans, such as *Plodia interpunctella*, it can be predicted that the regulatory cascade induced by a decline in ecdysteroid signalling occurs as in *B. mori*, but is shifted to earlier stages of oogenesis (vitellogenesis). On the other hand, it will also be interesting to investigate the expression pattern of the genes involved in the ecdysone regulatory cascade in representatives of classes 3 and 4 of lepidopteran ovarian development. Although oogenesis in these classes is independent of ecdysteroids produced by the prothoracic glands during metamorphosis, the ovaries synthesize ecdysteroids and ecdysteroid conjugates which accumulate in the eggs implicating a role in embryonic development as discussed above (Kaplanis et al., 1973; Bollenbacher et al., 1978; see further below). Investigation of the expression pattern of ecdysone-responsive genes during oogenesis in members of these classes could clarify whether ecdysteroids produced by the ovaries would also have an autocrine/paracrine function to regulate the progression of follicle development (as has been hypothesized in other insect species, most notably *Drosophila*, see further below).

If ecdysteroids play a role in the regulation of oogenesis of class 3 lepidopterans, it can be expected that ecdysone agonists influence ovarian development after adult eclosion. This is observed in the codling moth, *Cydia pomonella* (Tortricidae: Tortricoidea), where application of tebufenozide and methoxyfenozide to adults results in reduction in fecundity (Sun et al., 2003a). Reduction in fecundity and/or fertility after treatment by ecdysone agonists was also observed in other lepidopteran species such as *Helicoverpa zea*, *Platynota idaeusalis* and *Spodoptera exigua* (Smagghe and Degheele, 1994a, b; Carpenter and Chandler, 1994; Sun et al., 2003a). It was suggested that the reduced fecundity was due to interference with normal ovarian development such as failure of completion of choriogenesis (Sun et al., 2003a, b). EcR and USP are expressed in the adult ovary of *Cydia*, implicating the ovary as a direct target of ecdysone agonists, and the expression of EcR and USP is modulated by the ecdysone agonists (Sun et al., 2003a, b). However, the above studies did not investigate in detail which stages of oogenesis were affected by the ecdysone agonists and further work therefore needs to be done to elucidate the exact role of 20E in the regulation of oogenesis in *Cydia*.

In the most studied lepidopteran (besides *B. mori*), *Manduca sexta*, the main ecdysteroid found in ovaries and eggs is 26-hydroxyecdysone 26-phosphate (Thompson et al., 1984, 1985b). As also hypothesized in other insects, this conjugate could serve as precursor for the release of free ecdysteroids to regulate developmental events during embryogenesis. Peak levels of free ecdysteroids (mainly 26-hydroxyecdysone in early embryos and 20,26-hydroxyecdysone in late embryos) have been observed to coincide with the deposition of serosal and larval cuticle (Warren et al., 1986; Dorn et al., 1987) but it is not clear whether these represent the biologically active ecdysteroids (Lanot et al., 1989). *In vitro* experiments have shown that α-ecdysone, 20E and makisterone A promote elongation and segmentation of the embryonic germ band in *Manduca* although the concentrations of these ecdysteroids during *Manduca* embryogenesis always remained relatively low (Warren et al., 1986; Lanot et al., 1989).

Interestingly, *Manduca* eggs also contain a non-ecdysteroid conjugate, 5-pregnen-3β,20β-diol glucoside, of which the steroid moiety correspond to a C_{19} steroid one biosynthetic step away from pregnenolone, the precursor of steroid hormones in vertebrates (Thompson et al., 1985a). However, this compound is located in high quantities on the surface of the egg, where it probably serves a protective role as an antibacterial or antifungal reagent or as a feeding deterrent against predators (Thompson et al., 1985a; Meinwald et al., 1985).

In the noctuid *Spodoptera frugiperda* (class 4), ecdysteroids produced by the ovary seem to have acquired a function that is normally observed in dipteran insects. While JH regulates the formation of vitellogenic follicles in this species, it was also observed that injection of 20E into decapitated female adults results in the production of vitellogenin by the fat body (but not the uptake of vitellogenin by the ovary; Sorge et al., 2000). Thus, besides stimulation of vitellogenin uptake by the ovary, JH seems to stimulate the production of ecdysteroids which are released in the hemolymph and stimulate vitellogenin synthesis in the fat body, similar to dipteran insects (see further below).

5.4 Control of Oogenesis by the Ecdysone Regulatory Pathway in *Aedes aegypti* and *Drosophila melanogaster*

The two dipteran species from which most is known regarding the role of ecdysteroids in the regulation of oogenesis are the fruitfly, *Drosophila melanogaster*, and the yellow fever mosquito, *Aedes aegypti*. However, knowledge in both species differs regarding the processes that are affected by ecdysteroids. In *Drosophila*, the availability of a large array of genetic tools has allowed the analysis of the function of genes of the ecdysone regulatory hierarchy during follicle development in the ovary (Buszczak et al., 1999; Carney and Bender, 2000). In the mosquito, on the other hand, research has focused on the molecular mechanism of the regulation of the production of yolk protein precursors in the fat body by ecdysteroids produced by the ovary (Raikhel et al., 1999, 2005; Li et al., 2000). By contrast, relatively little is known regarding the role of the ecdysteroid regulatory cascade in follicle maturation in the ovary of the mosquito.

5.4.1 Aedes aegypti

In the anautogenous mosquito *Aedes aegypti*, vitellogenesis and follicle maturation occur synchronously following a blood meal (Raikhel and Lea, 1990). At the time of adult eclosion, each ovariole consists of the germarium and one follicle. JH is responsible for the maturation of the primary follicles in the ovary and the fat body to a resting stage at which they become competent to respond to a blood meal and initiate vitellogenesis and yolk protein precursor synthesis, respectively (Bownes, 1986; Pierceall et al., 1999). Following a blood meal, ecdysteroid synthesis, principally of α-ecdysone, is initiated in the ovary (Hagedorn, 1985), presumably in the follicular epithelium that surrounds the oocyte/nurse cell complex of the first follicle, and ecdysteroids accumulate in the hemolymph where they stimulate the first cycle of yolk protein precursor synthesis in the fat body (Deitsch et al., 1995; Zhu et al., 2000). Other functions associated with ecdysteroids during mosquito follicle maturation include the formation of the vitellin membrane envelope in the primary follicle (Lin et al., 1993) and the separation of the secondary follicle from the germarium (Beckemeyer and Lea, 1980).

Stimulation of ecdysone synthesis in the ovary following a blood meal occurs through the action of neurosecretory signals from the brain and the gut, including the ovary ecdysteroidogenic hormone (OEH), a neuroparsin homolog, and insulin-like peptides (Brown et al., 1998; Riehle and Brown, 1999; Badisco et al., 2007). The action of insulin-like peptides in the mosquito ovary has been shown to involve a conserved signaling pathway that includes an insulin receptor homolog, phosphatidyl-inositol 3-kinase and protein kinase B (Graf et al., 1997; Riehle and Brown, 1999, 2003; Wu and Brown, 2006). To which extent the transduction pathways of OEH and insulin-like peptides interact is unknown at present.

The classical ecdysteroid regulatory cascade that has been elucidated first during molting and metamorphosis in *Drosophila* seems to be reiteratively used during the vitellogenic cycle of the fat body in *Aedes* (Raikhel et al., 1999, 2003). Significant progress has been achieved in the understanding of the regulation of the transcription of the vitellogenin gene by the ecdysteroid regulatory cascade in the fat body as well as the mechanisms that regulate the competence of the fat body to respond to ecdysone and the termination of the vitellogenic response (Martín et al., 2001; Kokoza et al., 2001; Zhu et al., 2000, 2003a, b, 2006; Sun et al., 2005; review by Raikhel et al., 2005). What is less understood, however, is whether the same regulatory mechanisms are also operational in the primary follicles during the first vitellogenic cycle when they progress through previtellogenic growth, resting stage, vitellogenesis and choriogenesis concomitantly with fluctuating titers of JH and 20E. Nevertheles, although less extensively studied than the fat body, data exist with respect to the expression of ecdysone-responsive genes in the ovary of the mosquito and the possible functional significance of these will be discussed below. Although expression patterns were studied using whole ovaries, it can be inferred that these reflect primarily changes in the primary follicle as it undergoes dramatic changes during the first vitellogenic cycle.

Although the mosquito ovary traditionally is not considered a target for 20E (Bownes, 1986), several genes that are historically implicated in the ecdysone response such as *E75*, *HR3*, *E74* and *BR-C* are expressed in the ovary during the vitellogenic cycle (Pierceall et al., 1999; Kapitskaya et al., 2000; Sun et al., 2002; Chen et al., 2004). In addition, the expression of *EcR* mRNA increases at the initiation of vitellogenesis concomitantly with a switch in *usp* isoform expression (Cho et al., 1995; Kapitskaya et al., 1996, 2000; Wang et al., 2000). Besides the traditional ecdysone-responsive genes, also the B isoform of the mosquito orphan nuclear receptor HNF-4 is induced in ovarian tissue following a blood meal (Kapitskaya et al., 1998). Possible target genes for EcR/USP and the early ecdysone-responsive genes in the ovary include the components of the machinery for uptake of yolk protein precursor genes such as the vitellogenin receptor, clathrin heavy chain, and lipophorin receptor (Sappington et al., 1995; Kokoza et al., 1997; Cho and Raikhel, 2001; Cheon et al., 2001; Seo et al., 2003). In contrast to the regulation of the vitellogenin gene in the fat body, however, nothing is known whether EcR/USP and early ecdysone-responsive gene products bind to promoter elements of the target genes and how they interact with ovary-specific factors to stimulate gene expression during vitellogenesis. It can also be pointed out that the vitellogenin receptor, clathrin heavy chain and lipophorin receptor are expressed in the oocyte/nurse cell complex and that candidate target genes in the follicular epithelium remain to be identified.

Finally, one isoform of the classical ecdysone-responsive genes, *E74A*, is likely involved in the termination of vitellogenesis in both fat body and ovary. In the ovary, its expression coincides with the synthesis of the vitelline envelope in the terminal follicle (Sun et al., 2002).

In summary, the data indicate that genes historically involved in the regulation of the ecdysone response have an expression pattern in the ovary consistent with

those of regulators of vitellogenesis. The expression pattern also follows the rise and fall of ecdysteroid in the haemolymph and therefore could indicate regulation by ecdysteroids in similar fashion as in the fat body.

Recently, it was observed that the stimulation of expression of yolk protein precursor genes by 20E in the fat body requires the target-of-rapamycin (TOR) pathway that mediates nutrient (amino acid) signaling (Attardo et al., 2003; Hansen et al., 2004). Whether this pathway is also functional in the ovary and interacts with the 20E regulatory cascade remains to be investigated.

While ecdysone produced by the ovary in the mosquito is secreted in the hemolymph to act as a hormone to regulate vitellogenesis, the possible production of ecdysteroid conjugates by the ovary, as observed in other insect species, that accumulate in the oocytes and are used in egg development has not received much attention. The site of ecdysteroid synthesis in the vitellogenic ovary also remains to be determined. Since many ecdysteroidogenic enzymes have been identified in *Drosophila* and *Bombyx* (Gilbert and Warren, 2005) and the genome sequence of *Aedes* has become available recently (Nene et al., 2007), identification of *Aedes* homologs should also be straightforward and allow determination of their expression during follicle maturation in the ovary. Finally, it would be interesting to correlate the appearance of mRNAs of ecdysteroidogenic enzymes with the expression of putative transcriptional regulators such as FTZ-F1 (Parvy et al., 2005).

Control of vitellogenin synthesis in the fat body by ecdysteroids produced by ovarian tissue is conserved in anautogenous flies that develop eggs in batches and require a protein meal for egg development such as the house fly, *Musca domestica*, and the blowflies *Calliphora vicina*, *Neobellieria bullata* and *Phormia regina* (Adams et al., 1985; Huybrechts and De Loof, 1982; Briers and Huybrechts, 1984). Similar to the mosquito, JH is the predominant hormone after eclosion that stimulates previtellogenic follicle development in the ovary and prepares the fat body to respond to ecdysteroids to synthesize large quantities of yolk protein precursors (Adams et al., 1985). Protein meal uptake subsequently induces ovarian growth and production of ecdysteroids that stimulate vitellogenin synthesis in the fat body. Other roles for 20E produced by the ovary in flies include the control of parturition in the tsetse fly, *Glossina fuscipus* (Robert et al., 1986), and the production of pheromone in *Musca* (Adams et al., 1984). Interestingly, 20E can also induce vitellogenin synthesis in male *Neobellieria*, *Phormia* and *Lucilia* (Huybrechts and De Loof, 1977, 1982) and this has also been observed in *Drosophila* (Bownes et al., 1983).

5.4.2 Drosophila melanogaster

In species continuously laying eggs, such as *Drosophila*, follicle development in the ovarioles occurs asynchronously and its progression is modulated under the influence of external environmental factors such as food intake and mating (Spradling, 1993). Early physiological studies on *Drosophila* oogenesis focused on the regulation of vitellogenin synthesis in the fat body and vitellogenin uptake by

the ovary (Postlethwait and Handler, 1979; Schwartz et al., 1985; Bownes, 1989; Hagedorn, 1989). More recently, however, with the availability of genetic tools and the identification of genes involved in the biosynthesis as well as the transduction of 20E, it became possible to analyze the action of 20E within the developing ovary in a manner that has hitherto not been possible in other insects.

The discussion of oogenesis in *Drosophila* is divided in three parts. In the first part, experiments will be considered that relate to physiological and molecular effects of 20E on the process of oogenesis. The second part involves the phenotypic changes in ovarian follicle development following genetic manipulation of genes involved in the 20E biosynthetic and regulatory pathways. A final part will address the subject of the accumulation of maternal ecdysteroids in the developing oocytes of *Drosophila*.

5.4.2.1 Physiological Experiments

Ecdysteroids play both positive and negative roles in the regulation of oogenesis in *Drosophila* (Riddiford, 1993b; Soller et al., 1999). The positive effects of JH and 20E on the development of immature ovaries immediately after eclosion are probably related to their effects on the completion of metamorphosis. In isolated abdomens or decapicitated females prepared immediately after adult eclosion, JH stimulates yolk protein synthesis in both fat body and ovary while the stimulatory action of 20E is restricted to the fat body only (Jowett and Postlethwait, 1980; Postlethwait and Shirk, 1981). The action of JH on the fat body may be indirect since it stimulates ecdysteroid production in the ovary (Schwartz et al., 1989). On the other hand, the effects of 20E on yolk protein expression in the fat body also seem to be indirect and therefore may involve the classical ecdysteroid regulatory cascade as observed in the mosquito (Bownes et al., 1987, 1996).

Also in diapausing female adults, which are arrested at previtellogenic stages, termination of diapause and induction of vitellogenesis is achieved after injection of 20E (Richard et al., 1998). Based on this and other observations, it was proposed that ecdysteroids produced by the ovary or other sources (Bownes, 1989) stimulate yolk protein synthesis by the fat body as well as yolk protein uptake from the ovary immediately after eclosion when follicles are at the previtellogenic stages (Richard et al., 1998, 2001).

During later stages of oogenesis, alternatively, 20E has negative effects on ovarian follicle development. The critical period at which 20E exerts its negative effects is at stages 8 and 9 of follicle development which correspond to the initiation of vitellogenesis (Drummond-Barbosa and Spradling, 2001). In adverse physiological conditions that do not support oocyte maturation such as starvation, stress or absence of mating, ecdysteroid titers rise and cause nurse cell apoptosis and follicle degeneration in stage 8 and stage 9 follicles (Soller et al., 1999). In beneficial physiological conditions, on the other hand, the effects of 20E are counterbalanced by increased production of JH which stimulates initiation of vitellogenesis (Soller et al., 1999; Gruntenko et al., 2003).

In the case of mating, its positive effects on progression of follicle development are mediated by the transfer of the sex peptide, a product of the male accessory glands, to the female (Chen et al., 1988; Kubli, 1996). The sex peptide acts on the corpora allata to stimulate the production of JH (Moshitzky et al., 1996) which counteracts the apoptotic effect of 20E on vitellogenic follicles (Soller et al., 1999). Similarly, the availability of abundant nutritional resources results in the initiation of vitellogenesis, presumably through the stimulation of the insulin pathway (Leevers, 2001), while during starvation, apoptosis of stage 8 and 9 follicles is initiated (Terashima and Bownes, 2004, 2005). Nutritional stimulation of vitellogenesis is protected by JH and counteracted by 20E (Terashima et al., 2005). Thus, a correct balance between JH and ecdysteroids is essential for the progression of vitellogenic follicle development in *Drosophila* (Soller et al., 1999; Gruntenko et al., 2003; Terashima et al., 2005).

During the initiation of the apoptosis response by 20E or nutritional stress, switches occur in the expression of the ecdysone-responsive genes at stages 8 and 9 of follicle development (Terashima and Bownes, 2004, 2006). Increases in ecdysteroid concentrations result in increases in expression of the Z2 and Z3 isoforms of Broad-Complex in the cells of the follicular epithelium (Terashima and Bownes, 2004). The increase in Z2 and Z3 results in a decrease in E75B expression and an increase in E75A levels which induces apoptosis in the nurse cells of stage 8 and 9 follicles (Terashima and Bownes, 2006) (Fig. 5.2).

5.4.2.2 Genetic Analysis

The availability of mutants of genes involved in the ecdysone biosynthetic and regulatory pathways as well as the genetic tools to alter their expression in developing follicles has allowed an assessment of the role of ecdysteroids during oogenesis in *Drosophila* which hitherto has not been possible in other insects. As outlined, the discussion of genes of the ecdysone biosynthetic and regulatory pathways will be discussed separately. A third part discusses the activation of ecdysone 'sensors' during oogenesis in transgenic animals.

(a) Genes involved in ecdysteroid biosynthesis

Temperature-sensitive *ecdysoneless*^{1} (*ecd*^{1}) mutants that are characterized by reduced ecdysteroid levels at the restrictive temperature show a developmental arrest at the onset of vitellogenesis during ovarian follicle development, indicating that 20E may be required for the progression of oogenesis beyond stage 8 (Audit-Lamour and Busson, 1981; Walker et al., 1987). Analysis of ovaries with non-conditional *ecd*$^{+}$/*ecd*$^{-}$ clones show that *ecdysoneless* is required in the follicle cells for appropriate follicle formation in the germarium (Gaziova et al., 2004). On the other hand, *ecd*$^{+}$/*ecd*$^{-}$ germline clones show developmental arrest prior to vitellogenesis due to nurse cell degeneration. Molecular characterization of *ecdysoneless* shows that it encodes a conserved protein with unknown function that has broad expression in both ecdysteroidogenic and non-ecdysteroidogenic tissues (Gaziova et al., 2004). In ovaries, *ecd*

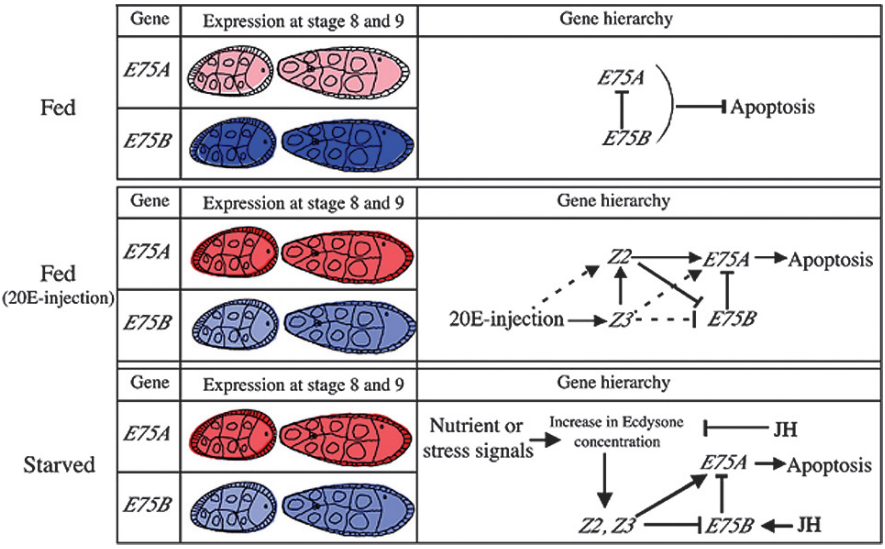

Fig. 5.2 Model to explain the hierarchy of ecdysone response genes regulating apoptosis of stage 8 and 9 follicles in *Drosophila melanogaster*. Upper Panel: Complete nutrition induces normal development of follicles during oogenesis. In this case, just the Z1 isoform of BR-C is expressed in the follicle cells at stage 8. E75B suppresses E75A expression to prevent apoptosis. Middle Panel: Injection of 20E induces apoptosis in stage 8 and 9 follicles. 20E injection results first in induction of the Z2 and Z3 isoforms of BR-C which in turn decrease E75B and increase E75A expression. While E75B is an apoptosis inhibitor, E75A is an apoptosis inducer. Lower Panel: Starvation induces apoptosis in stage 8 and 9 follicles. During starvation, ecdysone concentrations increase and the Z2 and Z3 isoforms of BR-C become expressed in the follicle cells to suppress E75B and activate E75A expression. The increase in E75A results in induction of apoptosis. JH can counteract the effects of starvation by interference with the increase in ecdysone concentration and by stimulation of the expression of E75B (Reprinted from Terashima and Bownes, (2006). E75A and E75B have opposite effects on the apoptosis/development choice of the *Drosophila* egg chamber. Cell Death Differ. **13**, 454–464. With permission from Macmillan.) (*See Color Plates*)

is expressed in both nurse cells and follicle cells (Gaziova et al., 2003). However, although it was proposed that the *ecdysoneless* gene product is involved in the regulation of ecdysteroid biosynthesis by facilitating the translocation of sterol precursors between different subcellar compartments (Warren et al., 1996), *ecd* clearly affects other cellular processes besides ecdysteroidogenesis. Thus, the observed phenotype of oogenesis arrest could be caused by the involvement of the gene in other cellular processes besides ecdysteroidogenesis.

Also mutations in the gene *dare*, which encodes the *Drosophila* homolog of adrenodoxin reductase, a mitochondrial protein that transports electrons to cytochrome P450 enzymes, including ecdysteroidogenic enzymes, cause developmental arrest of oogenesis at stages 8–9 (Buszczak et al., 1999; Freeman et al., 1999). Expression of *dare* occurs in the nurse cell complex (Freeman et al., 1999). Although it is tempting to speculate that the phenotypic effects in *dare* mutants are due to decreased

ecdysteroidogenesis, the disruption of other cellular processes caused by dysfunction of other cytochrome P450 enzymes, however, can not be discounted.

Recent molecular characterization of the Halloween gene family identified several members of the cytochrome P450 enzymes that are involved in 20E biosynthesis (Gilbert and Warren, 2005). These genes include *disembodied* (*dib*) that encodes the C22-hydroxylase, *shadow* (*sad*) encoding the C2-hydroxylase, *shade* (*shd*) corresponding to the C20-hydroxylase, *phantom* (*phm*) that produces the C25-hydroxylase and *spook* (*spo*) that probably catalyzes an early step in ecdysteroid synthesis (Chávez et al., 2000; Warren et al., 2002; Petryk et al., 2003; Warren et al., 2004; Namiki et al., 2005; Ono et al., 2006). For all of these genes, their expression in the ovary has been reported while for some mutant ovarian phenotypes have been described. Thus, the genes *shd*, *phm* and *sad* are expressed in both nurse cells and follicle cells while expression of *dib* and *spo* is restricted to the follicle cells. In all cases highest expression levels are observed in later stages of oogenesis, from stage 8 until stage 11. While it was observed that *dib* is not required in the germline for progression of oogenesis (Chávez et al., 2000), mutants of *shd* and *spo*, interestingly, are arrested in oogenesis at the initiation of vitellogenesis (Petryk et al., 2003; Ono et al., 2006). Because it is expected that the *spo* and *shd* gene products have very specific functions involved solely in ecdysteroid biosynthesis, these data therefore indicate a requirement for 20E production in ovarian tissue to regulate progression towards vitellogenesis. Moreover, in this case, 20E must act as a paracrine or autocrine factor since its effects are exerted within the same organ where it is produced.

Other genes involved in ecdysteroidogenesis are *Start1*, that encodes a putative cholesterol transporter and *neverland* (*nvd*), encoding a Rieske-domain protein that probably catalyzes the conversion from cholesterol to 7-dehydro-cholesterol, the first step of ecdysteroidogenesis (Roth et al., 2004; Yoshiyama et al., 2006). As detected by *in situ* hybridization, both genes are expressed in the nurse cells. In the case of *Start1*, it is speculated that its presence in the nurse cells corresponds to its production prior to transport and storage in the oocyte as maternal mRNA (Roth et al., 2004).

Transcription factors involved in the regulation of the expression of ecdysteroidogenic enzymes in the prothoracic glands include the products of the genes *without children* (*woc)* and *βFTZ-F1* (Warren et al., 2001; Parvy et al., 2005). However, their expression pattern or functional analysis during oogenesis has not been described so far.

(b) Genes involved in the ecdysone regulatory pathway

The components of the ecdysone receptor heterodimer, *EcR* and *usp*, as well as the early gene products *E75*, *E74* and *BR-C* are expressed during *Drosophila* oogenesis. EcR as well as USP protein can be detected from early stages in the germarium until the completion of oogenesis (Khoury-Christianson et al., 1992; Buszczak et al., 1999; Carney and Bender, 2000). High expression levels of EcR protein (B1 and B2 isoform) are observed in the border cells, a group of specialized cells that migrate from the anterior pole of the follicular epithelium through the nurse cell complex during stages 9 and 10 and constitute precursor cells of the micropyle channel (Buszczak et al., 1999; Bai et al., 2000). *In situ* hybridization

also detects expression of *E74* and *E75*, especially in the nurse cells and follicle cells of follicle stages 8 until 10 (Buszczak et al., 1999). A bimodal expression pattern is observed for BR-C protein, with an early expression pattern (stages 5–6) separated from a late pattern (stages 10–13) (Deng and Bownes, 1997; Buszczak et al., 1999; Tzolovsky et al., 1999).

Changes in ecdysteroid levels such as those induced by temperature shifts in *ecdysoneless*^{1} mutants or during ovarian cultures in the presence of 20E modulate the expression of *E75* and *BR-C*, indicating the activation of the ecdysone regulatory cascade during oogenesis (Buszczak et al., 1999). As noted above, it was observed also that administration of 20E can mimic nutritional stress in *Drosophila* resulting in changes in *BR-C* and *E75* isoform expression during stages 8 and 9 followed by induction of apoptosis (Terashima and Bownes, 2004, 2006) (Fig. 5.2).

However, it is clear that *E75* and *BR-C* expression during oogenesis can be regulated by other pathways besides the involvement of 20E. Spatial patterns of *E75* mRNA expression in the follicular epithelium are modulated by the epidermal growth factor receptor (EGFR) pathway (Buszczak et al., 1999). In the case of the late *BR-C* expression pattern, both EGFR and Decapentaplegic/transforming growth factor β (Dpp/TGFβ) pathways cooperate to restrict *BR-C* expression to two dorsolateral patches of follicle cells that correspond to the regions from which the dorsal appendages, two specialized eggshell structures, are derived (Deng and Bownes, 1997; Tzolovsky et al., 1999).

Germline clones of *EcR*, *E75* and *E74* cause developmental arrest and degeneration of follicles (stages 6–7 for *EcR*; stages 8–9 for *E75* and *E74*) (Buszczak et al., 1999; Carney and Bender, 2000). Because the similarity in phenotype with mutations that cause a defect in ecdysteroid synthesis, a model was proposed in which ecdysteroids produced at the beginning of vitellogenesis act in a paracrine or autocrine manner (i.e., within the developing follicle) to activate the ecdysone regulatory cascade and progression of oogenesis (Buszczak et al., 1999; Freeman et al., 1999). In addition, *EcR* (B1 and B2 isoforms) seems to be implicated in border cell migration since mutations in the *taiman* (*tai*) gene, that encodes a p160 transcriptional co-activator of EcR, cause defects in border cell migration (Bai et al., 2000; Montell, 2001).

Also *BR-C* plays a role at the initiation of vitellogenesis to control progression of oogenesis (Huang and Orr, 1992; Terashima and Bownes, 2004, 2006). Nutritional stress or high levels of ecdysteroids stimulate expression of the Z2 and Z3 isoforms of *BR-C* which in turn induce apoptosis through the modulation of expression levels of *E75A* and *E75B* (Terashima and Bownes, 2004, 2006) (Fig. 5.2). In addition, *BR-C* regulates other functions during late oogenesis such as chorion gene amplication, chorion gene expression and dorsal appendage formation but these functions are clearly independent of 20E signalling (Huang and Orr, 1992; Deng and Bownes, 1997; Tzolovsky et al., 1999). Interestingly, also mutations in the *Methoprene-tolerant* (*Met*) gene that encodes a bHLH-PAS transcription factor that could possibly function as a JH receptor (Wilson and Ashok, 1998), cause defects in vitellogenic development and genetic interactions were observed between alleles of *BR-C* and *Met*, indicating that they may operate in the

same genetic pathway (Wilson et al., 2006). Given the antagonism between JH and ecdysteroids to regulate progression of vitellogenesis or apoptosis, both pathways could converge on the regulation of the expression of particular BR-C isoforms to decide the developmental fate of early vitellogenic follicles (see discussion above; Terashima and Bownes, 2004, 2006).

In contrast to *EcR*, *E75*, *E74*, and *BR-C* mutants, *usp* mutants show no defects in progression of oogenesis at the initiation of vitellogenesis (Oro et al., 1992). On the other hand, genetic studies have shown that *usp* is required for fertilization and chorion formation (Perrimon et al., 1985; Oro et al., 1992). The fertilization defect is likely due to the failure of border cell migration in *usp* mutants (Bai et al., 2000; see also above). Regarding the chorion defect, it was shown that USP binds to essential elements in chorion gene promoters (Shea et al., 1990; Khoury-Christianson et al., 1992). However, genetic analysis has shown that the usp^{+} function is required in the germline, and not the follicle cells (where the chorion genes are produced) to produce normal chorion (Oro et al., 1992). Thus, it was proposed that USP may not act directly as a transcriptional of the chorion genes but could function in the germline to trigger the release of a signal that acts on the follicular cells to promote chorion gene expression (Oro et al., 1992).

(c) Detection of EcR/USP signalling during oogenesis

If active 20E signalling occurs at particular stages during oogenesis, it should be detected by ecdysone reporters at these particular stages such as EcRE-lacZ or EcRE-GFP in transgenic animals (Kozlova and Thummel, 2003). Using these constructs, reporter gene activity is restricted to the 'squamous' follicle cells, that overly the nurse cells, and the border cells at stages 10–14 of oogenesis (late vitellogenesis and choriogenesis; Hackney et al., 2007). Another reporter system consisting of heat-shock promoter-Gal4-EcR or heat-shock-promoter-Gal4-USP expression constructs in combination with a Gal4-lacZ reporter shows similar active EcR/USP signalling in subsets of follicular cells at the same late stages of oogenesis. Besides high activity in the 'squamous' follicular cells and the border cells, intermediate to low levels of activity are observed in the centripetally migrating and main body follicle cells, respectively, using the more sensitive Gal4-based detection system. However, no reporter gene activity was observed during earlier stages, despite the broad expression of *EcR* and *USP* at these stages (see above).

Interestingly, while EcR reporter activity is sensitive to exogenous ponasterone A, particularly during early stages of oogenesis, it is also negatively regulated by the EGFR-MAPK-Ras signalling pathway that defines the dorsal-anterior fate of the follicular epithelium during late oogenesis (Riechmann and Ephrussi, 2001). Expression of dominant negative EcR (Cherbas et al., 2003) in the follicular epithelium results in abnormal follicular cell migrations (including border cell migration, see above) during stage 10 and aberrant dorsal appendage formation, due to misregulation of genes that encode epithelial junction components, such as *DE-cadherin*, *discs large* and *armadillo*. Expression of dominant negative EcR results also in reduction of chorion gene expression and chorion gene amplification. Taken together, these data indicate a role for EcR signalling during late oogenesis

in the follicular epithelium to regulate its morphogenetic movements at the end of vitellogenesis and the synthesis of the chorion during choriogenesis (Hackney et al., 2007). The role for EcR signalling in dorsal appendage formation, chorion gene expression and chorion gene amplification coincides with the requirement for BR-C function (Huang and Orr, 1992; Deng and Bownes, 1997; Tzolovsky et al., 1999; see above). However, surprisingly, expression of dominant negative EcR does not alter expression of BR-C (Hackney et al., 2007). As noted above, the 'late' functions of BR-C are considered to be ecdysteroid-independent (Deng and Bownes, 1997).

In summary, *EcR*, *usp* and early genes such as *E74*, *E75* and *BR-C* seem to have both ecdysteroid-dependent and ecdysteroid-independent functions during *Drosophila* oogenesis. Ecdysteroid-dependent functions occur earlier during oogenesis at stages 8 and 9 when concentration-dependent expression of specific isoforms of E75 and BR-C will direct the developmental decision between progression through vitellogenesis or follicle degeneration. At later stages of oogenesis, on the other hand, *EcR*, *usp* and *BR-C* are involved in the regulation of the morphogenetic movements that mark the end of vitellogenesis and choriogenesis as well as the synthesis of the chorion (stages 10–14). At those stages, the activity of the EcR/USP complex and BR-C may be regulated by other signalling pathways such as EGFR-MAPK-Ras or Dpp/TGFβ.

5.4.2.3 Maternal Ecdysteroids and Regulation of Embryogenesis

The ecdysteroid content in freshly oviposited Drosophila eggs is low but increases after 2h and reaches a maximum before mid-embryogenesis (Maróy et al., 1988). Analogous to the function of ecdysteroids stored in eggs of locusts (Lanot et al., 1989; see further below), it was proposed that the rise in ecdysteroids during *Drosophila* embryogenesis could be achieved by the hydrolysis of maternal fatty acid ecdysteroid conjugates (Bownes et al., 1988). A key role in this process would be played by the yolk proteins which have significant sequence similarity to part of triacylglycerol lipase and were shown to bind ecdysteroid fatty acid conjugates. In this model, release of ecdysteroids at a specific period of embryogenesis would be achieved by breakdown of yolk proteins (Bownes et al., 1988). However, it was also observed that expression of the ecdysteroidogenic enzymes encoded by the genes *sad* (C2-hydroxylase) and *dib* (C22-hydroxylase) is induced in the embryonic epidermis prior to the differentiation of the prothoracic gland, indicating that *de novo* ecdysteroid synthesis contributes to the rise of ecdysteroid titers prior to mid-embryogenesis (Warren et al., 2002).

Whatever their source, ecdysone signalling in the *Drosophila* embryo is involved in the regulation of the morphogenetic movements such as germ-band retraction, head-involution and dorsal closure during mid-embryogenesis as well as the deposition of the cuticle during late embryogenesis. This has been shown recently through over-expression of dominant-negative EcR during early stages of embryogenesis (Kozlova and Thummel, 2003). Similar phenotypes are observed in mutants for the genes *sad*, *dib* and *spo* that encode ecdysteroidogenic enzymes (Chávez et al., 2000; Warren et al., 2002).

5.5 Ecdysone and Oogenesis in Orthoptera and Dictyoptera

Locusts and cockroaches are well known as classic physiological models of ovarian ecdysteroid production and ecdysteroid-regulated reproductive processes. At the molecular level, on the other hand, the hemimetabolous insects have lagged behind the holometabolous insects represented by the lepidopterans *Manduca sexta* and *Bombyx mori* and the dipterans *Drosophila melanogaster and Aedes aegypti*. Only recently, homologs of *EcR* and *usp* or the classical early ecdysteroid-responsive genes *E75*, *BR-C* and *HR3* were isolated in representatives of orthopterans, dictyopterans and hemipterans (Hayward et al., 1999, 2003; Maestro et al., 2005; Cruz et al., 2006, 2007; Erezyilmaz et al., 2006). In the cockroach, *Blatella germanica*, the expression pattern of *EcR* during ovarian development was determined and functional studies established essential roles for the ecdysone regulatory pathway during oogenesis.

5.5.1 Locusts

Locusts such as *Locusta migratoria* and *Schistocerca gregaria* have served for a long time as a paradigm for the production of ovarian ecdysteroids, their storage in eggs and their reiterative use during embryonic development (Sall et al., 1983; Lanot et al., 1989; Dinan, 1997; Tawfik et al., 1999). In the classical model, ovarian development and vitellogenin synthesis in the fat body are dependent on JH secretion by the corpora allata while large amounts of ecdysteroids are synthesized by the ovary at the end of the gonadotropic cycle after the completion of vitellogenesis (Lagueux et al., 1977). The ecdysteroids are synthesized by the cells of the follicular epithelium (Glass et al., 1978; Goltzene et al., 1978) and converted mostly (>95%) to polar conjugates (Gande and Morgan, 1979; Dinan and Rees, 1981). Most of the ovarian ecdysteroids (>95%) are confined to the terminal oocytes and only low amounts accumulate in the hemolymph (Lagueux et al., 1977). Thus, in locusts, a clear role for ecdysteroids is implicated in the regulation of terminal oogenic events such as the reinitiation of meiosis and their accumulation in eggs indicates a functional role as maternal ecdysteroids involved in the regulation of embryonic events.

In *Locusta migratoria*, titers of free (unconjugated) ecdysone and 2-deoxyecdysone peak at the posterior pole of the eggs at the times when the nuclear events of reinitiation of meiosis occur (first and second reinitation at ovulation and egg-laying, respectively; Lanot et al., 1987). Furthermore, initiation of meiosis can be stimulated by 1–100 μM ecdysone both in immature oocytes and in oocytes obtained from animals with a modified sterol profile and reduced levels of endogenous ecdysteroids (Lanot et al., 1987, 1988; Costet et al., 1987).

In *Locusta*, the maternal conjugates of ecdysone and 2-deoxy-ecdysone are hydrolyzed during embryonic development resulting in distinct peaks in the free ecdysteroid titer (Lagueux et al., 1984; Rees and Isaac, 1984). In *Locusta*,

four peaks in free ecdysteroid titer, consisting mainly of ecdysone and 2-deoxy-ecdysone, are observed that can be correlated to the developmental events of appearance of coelomic somites and appendage anlagen (day 3), blastokinesis (day 4), dorsal closure (day 5) and appearance of pigmentation (day 9) (Lagueux et al., 1979). The ecdysteroid peaks also correlate with the deposition of the serosal cuticle and three embryonic cuticles by the embryonic tissues. Correlations between free ecdysteroid titer and similar embryonic developmental events were also made in *Schistocerca* eggs (Scalia et al., 1987; Sbrenna et al., 1989; Tawfik et al., 1999). Thus, embryonic ecdysteroids in locusts likely are involved in morphogenetic and cuticular events during embryogenesis as was observed in *Drosophila* (Kozlova and Thummel, 2003). However, maternal ecdysteroid conjugates are believed to serve as precursors of active ecdysteroids only during the first half of embryonic development, i.e. before the differentiation of the embryonic prothoracic glands (Lagueux et al., 1979).

The ecdysteroid conjugates that are deposited in the locust eggs are metabolized during embryonic development with changes occurring on both ecdysteroid genins and conjugating moieties (Sall et al., 1983). In *Locusta*, the main maternal ecdysteroid conjugates deposited in the egg are C22 adenosinemonophosphate (AMP) esters of 2-deoxyecdysone and C22 N^6-(isopentenyl)-AMP esters of ecdysone (Tsoupras et al., 1983; Sall et al., 1983). In the course of embryonic development, the release of free ecdysteroid seems to be a minor pathway while the major pathway consists of the replacement of the initial set of polar conjugates by new types such as C22 phosphate and C3 phosphate esters in combination with conversions on the ecdysteroid moiety such as the epimerization of the 3β-hydroxyl group (Sall et al., 1983). A similar pattern of metabolic conversions during embryonic development was also observed in *Schistocerca gregaria* where C22 phosphates are the major conjugates that accumulate initially in the oocyte and the egg (Isaac et al., 1983; Rees and Isaac, 1984). It was proposed that the physiological role of the change in conjugate type reflects the "status" of the ecdysteroid conjugate as precursor of active ecdysteroids (C22 AMP and C22 phosphate esters) or as final inactive end product (C3 phosphate and C3 epi-3-phosphate esters) (Hoffmann et al., 1980).

The deposition of maternal ecdysteroid conjugates is considered a determinant for the development of the gregarious character of the locust. Gregarious locusts accumulate larger amounts of ecdysteroids and differences are observed in the composition of the amounts of ecdysone, 20E, 2-deoxy-ecdysone and 26-hydroxy-ecdysone between solitary and gregarious embryos (Tawfik et al., 1999).

More recently, the role for ecdysteroids acting exclusively within the ovary or as maternal ecdysteroids in *Locusta* was challenged following the isolation of an ecdysiotropic factor from the brain, ovary maturating parsin (OMP), that stimulates both vitellogenesis and ecdysone synthesis by the ovary (Girardie and Girardie, 1996). In these studies, it was found that part of the stimulatory effect of OMP on vitellogenin synthesis by the fat body was probably mediated by its ability to stimulate the production of ecdysone by the ovary. Further studies established that *Locusta* OMP appears to have two distinct roles that seem to be mediated by distinct domains of the peptide: the N-terminal domain, directly acting on fat

body tissue, has a protective effect on the stability of vitellogenin mRNA while the ecdysteroidogenic effect on the ovary is carried out by the C-terminal domain (Girardie et al., 1998a). Thus, OMP, probably partially acting via 20E, may be necessary in conjunction with JH for complete vitellogenesis in *Locusta*. While the role of OMP and 20E in stimulating vitellogenesis seems to be conserved in *Schistocerca* (Girardie et al., 1998b), no stimulatory roles of ovarian ecdysteroids on ovarian growth or hemolymph vitellogenin titers were observed in the lubber grasshopper, *Romalea microptera* (Hatle et al., 2003). Thus, further investigations are necessary to establish the role of OMP-like peptides and ecdysteroids during vitellogenesis in other orthopterans.

Although homologs of both EcR and RXR/USP were isolated in *Locusta migratoria* (Hayward et al., 1999, 2003), their expression patterns or roles during oogenesis have not been investigated in detail.

5.5.2 Crickets

Studies on other orthopterans, such as crickets, have revealed surprising differences to the pattern found in locusts with respect to ecdysteroid synthesis by the ovary (Whiting et al., 1997). In the house cricket, *Acheta domesticus*, ecdysteroids accumulate in the ovary as apolar instead of polar conjugates (mainly C22 fatty acyl esters of ecdysone; Whiting and Dinan, 1988a, b). In contrast to *Locusta* and *Schistocerca*, a significant part of the ecdysteroids produced by the ovary is secreted in the hemolymph and distributed throughout the body and the amount of ecdysteroid conjugates that accumulate in the eggs is 100-fold lower than in *Locusta* and *Schistocerca* (Dinan, 1997; Whiting et al., 1997). Furthermore, in crickets ecdysteroids occur both in males and females while they are confined to females in locusts.

The increase in ecdysteroids in the ovary is correlated to the maturation of increasing numbers of terminal oocytes, indicating that ecdysteroid production occurs during the period of patency and vitellogenin uptake (Dinan, 1997; Whiting et al., 1997). The primary regulator of ovarian development in crickets is JH but ecdysteroids have been observed to have both inhibitory and stimulatory roles, depending on their concentration (Chudakova et al., 1982; Behrens and Hoffmann, 1983). Ecdysteroid apolar conjugates also accumulate in the oocytes and predominate in newly-laid eggs. Hydrolysis of ecdysteroid conjugates could therefore serve as a source of active ecdysteroids that regulate embryonic processes (see above).

5.5.3 Cockroaches

As in locusts and crickets, the primary regulator of ovarian development in cockroaches is JH (Engelmann and Mala, 2000; Cruz et al., 2003; Raikhel et al., 2005).

During ovarian development, ecdysone, 20E and 2-deoxy-ecdysone, both as free ecdysteroids and as apolar conjugates, accumulate in the ovary and peak at choriogenesis (Zhu et al., 1983; Slinger and Isaac, 1988). The ecdysteroids are produced by the follicular epithelium of the synchronously developing primary follicles (Zhu et al., 1983). A significant portion of the ecdysteroids produced by the ovary accumulate in the hemolymph and an important physiological role proposed for the hemolymph ecdysteroids includes the downregulation of the JH production by the corpora allata that triggers the termination of the gonadotrophic cycle (Rankin and Stay, 1985). Autocrine paracrine roles that have been proposed for the ovarian ecdysteroids include the regulation of choriogenesis and oviposition and the programming of the developmental competence of the next generation of oocytes (Zhu et al., 1983). Experiments *in vitro* have shown that 20E is able to induce precocious choriogenesis in *Blatella germanica* (Bellés et al., 1993).

As in other insects, it is attractive to consider that the ecdysteroid esters accumulate in the egg and may serve as a reservoir of active hormone to be released by hydrolytic enzymes during embryogenesis. It was observed that, during embryogenesis of *Nauphoeta cinerea*, the quantity of free and highly polar ecdysteroids increases between ovulation and dorsal closure, i.e. before the differentiation of the prothoracic glands (Imboden and Lanzrein, 1982; Lanzrein et al., 1985). However, it is not clear whether in this species the ecdysteroids originate from maternal sources since only minimal amounts of ecdysteroids and their conjugates exist in newly-laid eggs (Zhu et al., 1983).

The cloning of the cDNA of *EcR* in *Blatella germanica* and the applicability of the RNAi technique in cockroaches has allowed the functional analysis of the role of the ecdysone regulatory pathway during oogenesis in this species (Maestro et al., 2005; Martín et al., 2006; Cruz et al., 2006, 2007). The mRNA of the A-isoform of *EcR* is detectable in the cells of the follicular epithelium but its expression is not regulated by 20E. RNAi-mediated inhibition of expression of *EcR-A* indicates a role in the proliferation and function of the cells of the follicular epithelium and normal choriogenesis (Cruz et al., 2006). On the other hand, cockroaches with reduced levels of EcR showed normal vitellin content in the ovary and normal patency of the follicular epithelium during vitellogenesis and loss of patency at the end of vitellogenesis. Also homologs of *RXR* (*usp*; two isoforms) and *HR3* (three isoforms) have been cloned in *Blatella* and essential roles were demonstrated in the regulation of the molt (Maestro et al., 2005; Martín et al., 2006; Cruz et al., 2007). The assessment of their role in oogenesis through RNAi techniques remains to be reported.

5.6 Ecdysone and Oogenesis in Hymenoptera

Reports regarding the roles of ecdysteroids during oogenesis in other insect orders are few and in general concern the occurrence of ecdysteroids in the ovary and eggs (e.g. Hemiptera: Kaplanis et al., 1975; Coleoptera: Gelman et al., 2000; Isoptera: Delbecque et al., 1978; Phasmida: Fournier and Radallah, 1988) or physiological

evaluation of the application of ecdysone agonist on ovarian development (e.g. *Tenebrio molitor* (Coleoptera): Taïbi et al., 2003).

Because of its status as a beneficial insect, the role of ecdysteroids during reproduction in the honeybee, *Apis mellifera*, has received more attention. In addition, with the availability of its annotated genome (Honeybee Genome Sequencing Consortium, 2006), cloning of genes of the ecdysone regulatory pathway is straightforward to allow determination of their expression pattern during oogenesis. Furthermore, the technique of RNAi has been used successfully in hymenopterans (Amdam et al., 2003) and therefore can be applied for functional studies that address the role of ecdysone during oogenesis.

In the honeybee, ovarian development is coupled to caste differentiation in which JH plays a crucial role while supporting roles have been hypothesized for ecdysteroids (Nijhout and Wheeler, 1982; Rachinsky et al., 1990; Robinson and Vargo, 1997). At the middle of the fifth instar larval stage, the larval ovaries become responsive to makisterone A, the principal ecdysteroid involved in moulting and metamorphosis in the honeybee (Feldlaufer et al., 1986a). Compared to workers, ecdysteroid titers in queen last instar larvae occur earlier and reach higher values, and therefore may trigger accelerated prepupal development, which includes ovarian morphogenesis (Rachinsky et al., 1990). While ovarian differentiation proceeds during metamorphosis in queen larvae and prepupae, apoptosis is induced in worker ovaries (Hepperle and Hartfelder, 2001). The higher levels of JH in queen larvae and prepupae may protect the ovary against apoptosis (Schmidt Capella and Hartfelder, 1998), as was observed in *Drosophila* adults (see above). Also differences in pupal ecdysteroid titers can be related to distinct modes of caste development (Pinto et al., 2002). In adults, ecdysteroid levels are higher in queens and egg-laying workers than in normal workers (Robinson et al., 1991) which probably reflects ecdysteroid synthesis by the growing ovary (Feldlaufer et al., 1986b). As in other insects, ecdysteroids may have autocrine/paracrine functions within ovarian tissue or may accumulate in the follicles/eggs as maternal determinants for embryonic development (see above). A hormonal role for ecdysteroids accumulating in the hemolymph seems unlikely because hemolymph ecdysteroid titers can not be correlated with reproductive status (Hartfelder et al., 2002). Application of 20E also can not stimulate vitellogenin synthesis in the fat body in vitro although it stimulates general protein synthesis (Engels et al., 1990).

The ecdysone regulatory genes *E74*, *E75* and *Broad-Complex* were cloned in *Apis* and their expression pattern during oogenesis was studied (Paul et al., 2005, 2006). During the adult stage, specific expression is observed in the queen ovaries, but not worker ovaries, implicating their role in the regulation of follicle maturation. In situ hybridization shows that *E74* is expressed in nurse cells and oocyte during vitellogenesis while it switches expression to the cells of the follicular epithelium during later stages. The presence of E74 (as well as E75 and Broad-Complex) in follicle cells at the end of oogenesis suggest a role in the trigger of apoptosis of the follicle cells during oviposition (Paul et al., 2005, 2006).

In primitively social bees and wasps, as well as the primitively eusocial bumblebee *Bombus terrestris*, JH acts as the main gonadotropic hormone of

ovarian development as in other insects (Bloch et al., 2000, 2002). Ovarian growth can be correlated with the accumulation of ecdysteroids that may have autocrine/paracrine functions or have a role as maternal determinants as discussed above. In the bumblebee, ecdysteroids have been proposed to play a secondary role to JH in the determination of social dominance (Geva et al., 2005).

In the parasitoid wasp *Eupelmus vuilleti*, ovarian development and concomitant ecdysteroid production are dependent on host availability and therefore can be reversed if unfavourable conditions may occur (Bodin et al., 2007). Host dependence of ovarian development represents an adaptive mechanism reminiscent of food dependence of oogenesis in anautogenous flies and mosquitoes. The role of ecdysteroids produced by *Eupelmus* ovaries (ecdysone and 2-deoxyecdysone) remains unknown.

5.7 Conclusion

The regulation of insect oogenesis by ecdysteroids is complex and can vary considerably from species to species. Following roles for ecdysteroids have been demonstrated or proposed:

1. Production of ecdysteroids by ovarian follicles may be involved in paracrine/autocrine regulation of follicle maturation beyond a control point determined by external factors such as food availability, environmental stress or mating. Furthermore, the concentration of ecdysteroids may determine the decision between developmental progression and follicle degeneration.
2. Secretion of ecdysteroids by ovarian tissue into the hemolymph can reflect their role as hormonal factors to regulate extra-ovarian reproductive processes such as vitellogenin synthesis by the fat body or release of gonadotropic factors from the brain. A role for ovarian ecdysteroids as hormonal factors has been demonstrated most clearly in dipteran insects.
3. Production of high levels of ecdysteroids can trigger processes that occur at the end of oogenesis such as the reinitiation of meiosis in the oocyte and the synthesis of the chorion or eggshell by the follicular epithelium.
4. Maternal ecdysteroids may accumulate as inactive ecdysteroids conjugates in the eggs and serve as a source of active ecdysteroids that regulate embryonic processes (morphogenetic movements and cuticulogenesis).
5. In some insects, such as *Bombyx mori*, ovarian development has become dependent of metamorphic processes regulated by ecdysteroids produced by the prothoracic glands. In such cases, the role for ovarian ecdysteroids as paracrine/autocrine or endocrine factors may have become less important than in other insects.

Close inspection of the regulation of oogenesis in insects (mainly *Bombyx*, *Aedes* and *Drosophila*) indicates that the exact role of ecdysteroids may be different in each of these cases and that a general pathway by which ecdysteroids regulate the progression of oogenesis remains to be discovered. In the silkmoth, the process of

oogenesis is coupled to the process of metamorphosis and therefore has become dependent on the large surge of ecdysteroid titers that occurs in the pupal stage (Ramaswamy et al., 1997; Swevers and Iatrou, 2003). The silkmoth model may therefore represent a special case that is not applicable to most insects. In the mosquito, while the role of the ecdysteroid signalling pathway to regulate vitellogenin synthesis in the fat body is well established (Raikhel et al., 2005), very little is known about the involvement of ecdysteroids to regulate oocyte development within the ovary. In the fruitfly, the regulation of oogenesis by ecdysteroids is complex as ecdysteroids can have both positive and negative effects on oocyte development (Riddiford, 1993b; Soller et al., 1999). Furthermore, it was established that the expression of genes classically implicated in the ecdysone regulatory pathway (*EcR*, *usp*, *E75*, *BR-C*) are dependent on other signalling pathways during oogenesis (Deng and Bownes, 1997; Buszczak et al., 1999; Hackney et al., 2007).

Much more effort may therefore be required to clarify the roles of ecdysteroids in insects, especially given the fact that research has focused mainly on a few dipteran and lepidopteran models as opposed to the diversity of other insects that remain uninvestigated.

Note

Recently, the technique of RNAi has been applied successfully to investigate the functional role of genes in many organisms, including insects (Marie et al., 1999; Amdam et al., 2003; Cruz et al., 2006). The technique, based on hemolymph injection of dsRNA fragments homologous to the target gene, promises to be an important tool to test the function of isolated genes in organisms that are not readily amenable to genetic analysis. However, it has become apparent that, for reasons that are not understood, large differences can exist among insects with respect to their sensitivity to gene silencing by injection of dsRNA (Tomoyasu et al., 2008). Regarding model insects, it became apparent that in two cases dsRNA-mediated gene silencing through simple injection in the hemolymph is very effective: the German cockroach *Blatella germanica* (Dictyoptera; Cruz et al., 2006) and the flour beetle *Tribolium castaneum* (Coleoptera; Konopova and Jindra, 2007; Tan and Palli, 2008). Because the RNAi technique is very straightforward in these insects, in comparison to other model insects, fast progress is predicted to occur in the genetic analysis of many developmental processes, including oogenesis and its regulation by ecdysone, in *Blatella* and *Tribolium*. Here, the latest results regarding the function of the ecdysone regulatory pathway during cockroach and beetle oogenesis, as assessed through RNAi, are discussed briefly.

In the cockroach, RNAi-mediated knockdown of two genes implicated in the ecdysone regulatory cascade, *BgE75* and *BgFTZ-F1*, was reported recently (Mané-Padros et al., 2008; Cruz et al., 2008). Besides effects on molting and development by causing degeneration of the prothoracic gland, *BgE75* deficiency also causes defects in ovarian follicle development: in the primary follicle, whose growth

parallels the increase of ecdysteroids in the haemolymph, proliferation of the cells of the follicular epithelium was reduced resulting in reduced size of the follicles (Mané-Padros et al., 2008). Remarkably, it was also observed that BgE75 dsRNA injected last instar nymphs do not molt to adults but nevertheless initiate the adult developmental program such as vitellogenin expression in the female fat body and its uptake by the ovary. Because of the documented persistence of dsRNA-mediated knockdown in the cockroach, the latter results may suggest that *BgE75* does not play a major role in the regulation of vitellogenesis in the cockroach (Mané-Padros et al., 2008). On the other hand, while knockdowns of *BgFTZ-F1* through dsRNA injection also resulted in molting defects, no effects on ovarian follicle development were hitherto reported (Cruz et al., 2008).

Until recently, no data existed with respect to the function of the ecdysone regulatory pathway during oogenesis in Coleoptera. During the 17th Ecdysone Workshop, the effects of dsRNA-mediated knockdown of genes involved in the ecdysone regulatory pathway on the development of the ovary in *Tribolium* was presented (Takaki et al., 2008). In this work, it was reported that *EcR* and usp are required for egg production. Interestingly, anomalies caused by *EcR* dsRNA in *Tribolium* differed from both ovarian phenotypes observed in *EcR* mutants in Drosophila and ovarian anomalies caused by *EcR* dsRNA in *Blatella*, which suggests that ecdysteroid signalling pathways carry out different processes during oogenesis in different insects. Because *Tribolium* is very sensitive to the RNAi technique, it is expected that the role of the ecdysone receptor, the genes of the ecdysone regulatory cascade as well as the genes involved in ecdysone biosynthesis during oogenesis will be revealed in detail in the near future.

Acknowledgements The research on silkmoth oogenesis by the laboratory of Insect Molecular Genetics and Biotechnology is in part supported by a PENED 2003 grant (03ED 124) of the General Secretariat for Research and Technology, Ministry of Development, in Greece.

References

Adams, T.S., Dillwith, J.W., and Blomquist, G.J. (1984). The role of 20 hydroxyecdysone in housefly sex pheromone synthesis. J. Insect Physiol. **30,** 287–294.

Adams, T.S., Hagedorn, H.H., and Wheelock, G.D. (1985). Haemolymph ecdysteroid in the housefly, *Musca domestica*, during oogenesis and its relationship with vitellogenin levels. J. Insect Physiol. **31,** 91–97.

Amdam, G.V., Simões, Z.L.P., Guidugli, K.R., Norberg, K., and Omholt, S.W. (2003). Disruption of vitellogenin gene function in adult honeybees by intra-abdominal injection of double-stranded RNA. BMC Biotechnol. **3,** 1.

Attardo, G.M., Higgs, S., Klingler, K.A., Vanlandingham, D.L., and Raikhel, A.S. (2003). RNA interference-mediated knockdown of a GATA factor reveals a link to anautogeny in the mosquito *Aedes aegypti*. Proc. Natl. Acad. Sci. USA **100,** 13374–13379.

Audit-Lamour, C., and Busson, D. (1981). Oogenesis defects in the *ecd-1* mutant of *Drosophila melanogaster*, deficient in ecdysteroid at high temperature. J. Insect Physiol. **27**, 829–837.

Badisco, L., Claeys, I., Van Loy, T., Van Hiel, M., Franssens, V., Simonet, G., and Vanden Broeck, J. (2007). Neuroparsins, a family of conserved arthropod neuropeptides. Gen. Comp. Endocrinol. **153,** 64–71.

Bai, J., Uehara, Y., and Montell, D.J. (2000). Regulation of invasive cell behaviour by Taiman, a *Drosophila* protein related to AIB1, a steroid receptor coactivator amplified in breast cancer. Cell **103,** 1047–1058.

Beckemeyer, E., and Lea, A. (1980). Induction of follicle separation in the mosquito by physiological amounts of ecdysterone. Science **209,** 819–821.

Behrens, W., and Hoffmann, K.H. (1983). Effects of exogenous ecdyste ecdysteroids on reproduction in crickets, *Gryllus bimaculatus*. Int. J. Invertebr. Reprod. **6,** 149–159.

Bellés, X., Cassier, P., Cerdá, X., Pascual, N., André, M., Rosso, Y., and Piulachs, M.D. (1993). Induction of choriogenesis by 20-hydroxyecdysone in the German cockroach. Tissue Cell **25,** 195–204.

Bender, M. (2003). Ecdysone action in insect development. In: Encyclopedia of Hormones, Volume 1, H.L. Henry and A.W. Norman (Eds.), pp. 438–446. Academic, San Diego, CA.

Bloch, G., Hefetz, A., and Hartfelder, K. (2000). Ecdysteroid titer, ovary status, and dominance in adult worker and queen bumble bees (*Bombus terrestris*). J. Insect Physiol. **46**, 1033–1040.

Bloch, G., Wheeler, D.E., and Robinson, G.E. (2002). Endocrine influences on the organization of insect societies. Horm. Brain Behav. **3,** 235.

Bodin, A., Jaloux, B., Mandon, N., Vannier, F., Delbecque, J.P., Monge, J.P., and Mondy, N. (2007). Host-induced ecdysteroids in the stop-and-go oogenesis in a synovigenic parasitoid wasp. Arch. Insect Biochem. Physiol. **65,** 103–111.

Bollenbacher, W.E., Zvenko, H., Kumaran, A.K., and Gilbert, L.I. (1978). Changes in ecdysone content during postembryonic development of the wax moth, Galleria mellonella: the role of the ovary. Gen. Comp. Endocrinol. **34,** 169–179.

Bownes, M. (1986). Expression of the genes coding for vitellogenin (yolk protein). Ann. Rev. Entomol. 31, 507–531.

Bownes, M. (1989). The roles of juvenile hormone, ecdysone and the ovary in the control of *Drosophila* vitellogenesis. J. Insect Physiol. **35,** 409–413.

Bownes, M., Blair, M., Kozma, R., and Dempster, M. (1983). 20-Hydroxyecdysone stimulates tissue-specific yolk protein gene transcription in both male and female *Drosophila*. J. Embryol. Exp. Morphol. **78,** 249–268.

Bownes, M., Scott, A., and Blair, M. (1987). The use of an inhibitor of protein synthesis to investigate the roles of ecdysteroids and sex determination genes on the expression of the genes encoding the *Drosophila* yolk proteins. Development **101,** 931–941.

Bownes, M., Shirras, A., Blair, M., Collins, J., and Coulson, A. (1988). Evidence that insect embryogenesis is regulated by ecdysteroids released from yolk proteins. Proc. Natl. Acad. Sci. USA **85,** 1554–1557.

Bownes, M., Ronaldson, E., and Mauchline, D. (1996). 20-hydroxyecdysone, but not juvenile hormone, regulation of *yolk protein* gene expression can be mapped to *cis*-acting DNA sequences. Dev. Biol. **173,** 475–489.

Briers, T., and Huybrechts, R. (1984). Control of vitellogenin synthesis by ecdysteroids in *Sarcophaga bullata*. Insect Biochem. **14,** 121–126.

Broadus, J., McCabe, J.R., Endrizzi, B., Thummel, C.S., and Woodard, C.T. (1999). The Drosophila βFTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone. Mol. Cell **3,** 143–149.

Brown, M.R., Graf, R., Swiderek, K.M., Fendley, D., Stracker, T.H., Champagne, D.E., and Lea, A.O. (1998). Identification of a steroidogenic hormone in female mosquitoes. J. Biol. Chem. **7,** 3967–3971.

Buszczak, M., Freeman, M.R., Carlson, J.R., Bender, M., Cooley, L., and Segraves, W.A. (1999). Ecdysone response genes govern egg chamber development during mid-oogenesis in *Drosophila*. Development **126,** 4581–4589.

Carney, G.E., and Bender, M. (2000). The Drosophila *ecdysone receptor* (*EcR*) gene is required maternally for normal oogenesis. Genetics **154,** 1203–1211.

Carpenter, J.E., and Chandler, L.D. (1994). Effects of sublethal doses of two insect growth regulators on *Helicoverpa zea (*Lepidoptera: Noctuidae) reproduction. J. Entomol. Sci. **29**, 428–435.

Chávez, V.M., Marqués, G., Delbecque, J.P., Kobayashi, K., Hollingsworth, M., Burr, J., Natzle, J., and O'Connor, M.B. (2000). The Drosophila disembodied gene controls late embryonic morphogenesis and codes for a cytochrome P450 enzyme that regulates embryonic ecdysone levels. Development **127,** 4115–4126.

Chen, L., Zhu, J., Sun, G., and Raikhel, A.S. (2004). The early gene *Broad* is involved in the ecdysteroid hierarchy governing vitellogenesis of the mosquito *Aedes aegypti*. J. Mol. Endocrinol. **33,** 743–761.

Chen, P.S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., and Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. Cell **54,** 291–298.

Cheon, H.-M., Seo, S.-J., Sun, J., Sappington, T.W., and Raikhel, A.S. (2001). Molecular characterization of the VLDL receptor homolog mediating binding of lipophorin in oocyte of the mosquito *Aedes aegypti*. Insect Biochem. Mol. Biol. **31,** 753–760.

Cherbas, L., Hu, X., Zhimulev, I., Belyaeva, E., and Cherbas, P. (2003). EcR isoforms in Drosophila: testing tissue-specific requirements by targeted blockade and rescue. Development **130,** 271–284.

Cho, K.-H., and Raikhel, A.S. (2001). Organization and developmental expression of the mosquito vitellogenin receptor gene. Insect Mol. Biol. **10,** 465–474.

Cho, W.-L., Kapitskaya, M.Z., and Raikhel, A.S. (1995). Mosquito ecdysteroid receptor: analysis of the cDNA and expression during vitellogenesis. Insect Biochem. Mol. Biol. **25,** 19–27.

Chudakova, I., Maslennikova, V., and Luchnikova, E. (1982). Effects of 20 hydroxyecdysone on *Acheta domestica* (L.) (Orthoptera) and *Drosophila melanogaster* (Meig.) (Diptera) reproduction. Zool. Jahrb. Physiol. **86,** 45–52.

Costet, M.F., El Achouri, M., Charlet, M., Lanot, R., Benveniste, P., and Hoffmann, J.A. (1987). Ecdysteroid biosynthesis and embryonic development are disturbed in insects (*Locusta migratoria*) reared on plant diet (*Triticum sativum*) with a selectively modified sterol profile. Proc. Natl. Acad. Sci. USA **84,** 643–647.

Cruz, J., Martín, D., Pascual, N., Maestro, J.L., Piulachs, M.D., and Bellés, X. (2003). Quantity does matter. Juvenile hormone and the onset of vitellogenesis in the German cockroach. Insect Biochem. Mol. Biol. **33,** 1219–1225.

Cruz, J., Mané-Padrós, D., Bellés, X., and Martín, D. (2006). Functions of the ecdysone receptor isoform-A in the hemimetabolous insect *Blatella germanica* revealed by systemic RNAi in vivo. Dev. Biol. **297,** 158–171.

Cruz, J., Martín, D., and Bellés, X. (2007). Redundant ecdysis regulatory functions of three nuclear receptor HR3 isoforms in the direct-developing insect Blattella germanica. Mech. Dev. **124,** 180–189.

Cruz, J., Nieva, C., Mane-Padros, D., Martin, D., and Belles, X. (2008). Nuclear receptor BgFTZ-F1 regulates molting and the timing of ecdysteroid production during nymphal development in the hemimetabolous insect *Blatella germanica*. Dev. Dyn. (In Press).

Davis, R.E., Kelly, T.J., Masler, E.P., Fescemeyer, H.W., Thyagaraja, B.S., and Borkovec, A.B. (1990). Hormonal control of vitellogenesis in the gypsy moth, *Lymantria dispar* (L.): suppression of haemolymph vitellogenin by the juvenile hormone analogue methoprene. J. Insect Physiol. **36,** 231–238.

Deitsch, K.W., Chen, J.-S., and Raikhel, A.S. (1995). Indirect control of yolk protein genes by 20-hydroxyecdysone in the fat body of the mosquito, *Aedes aegypti*. Insect Biochem. Mol. Biol. **25,** 449–454.

Delbecque, J.P., Lanzrein, B., Bordereau, C., Imboden, H., Hirn, M., O'Connor, J.D., Noirot, C., and Luscher, M. (1978). Ecdysone and ecdysterone in physogastric termite queens and eggs of Macrotermes bellicosus and Macrotermes subhyalinus. Gen. Comp. Endocrinol. **36,** 40–47.

Delisle, J., and Cusson, M. (1999). Juvenile hormone biosynthesis, oocyte growth and vitellogenin accumulation in Choristoneura fumiferana and C. rosaceana: a comparative study. J. Insect Physiol. **45,** 515–523.

Deng, W.-M., and Bownes, M. (1997). Two signalling pathways specify localised expression of the *Broad-Complex* in *Drosophila* eggshell patterning and morphogenesis. Development **124,** 4639–4647.

Denlinger, D.L., Yocum, G.D., and Rinehart, J.P. (2005). Hormonal control of diapause. In: Comprehensive Molecular Insect Science, Volume 3: Endocrinology, L.I. Gilbert, K. Iatrou and S.S. Gill (Eds.), pp. 615–650. Elsevier, Pergamon.

Dhadialla, T.S., Carlson, G.R., and Le, D.P. (1998). New insecticides with ecdysteroidal and juvenile hormone activity. Annu. Rev. Entomol. **43,** 545–569.

Dinan, L. (1997). Ecdysteroids in adults and eggs of the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). Comp. Biochem. Physiol. **116B,** 129–135.

Dinan, L., and Rees, H. (1981). The identification and titres of conjugated and free ecdysteroids in developing ovaries and newly-laid eggs of *Schistocerca gregaria*. J. Insect Physiol. **27**, 51–58.

Dorn, A., Gilbert, L.I., and Bollenbacher, W.E. (1987). Prothoracicotropic hormone activity in the embryonic brain of the tobacco hornworm, Manduca sexta. J. Comp. Physiol. B, **57,** 279–283.

Drummond-Barbosa, D., and Spradling, A.C. (2001). Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. Dev. Biol. **231**, 265–278.

Engelmann, F., and Mala, J. (2000). The interactions between juvenile hormone (JH), lipophorin, vitellogenin, and JH esterases in two cockroach species. Insect Biochem. Mol. Biol. **30,** 793–803.

Engels, W., Kaatz, H.-H., Zillikens, A., Simoes, Z.L.P., Trube, A., Braun, R., and Dittrich, F. (1990). Honey bee reproduction: vitellogenin and caste-specific regulation of fertility. Adv. Invertebr. Reprod. **5,** 495–502.

Erezyilmaz, D.F., Riddiford, L.M., and Truman, J.W. (2006). The pupal specifier broad directs progressive morphogenesis in a direct-developing insect. Proc. Natl. Acad. Sci. USA **103,** 6925–6930.

Eystathioy, T., Swevers, L., and Iatrou, K. (2001). The orphan nuclear receptor BmHR3A of *Bombyx mori*: hormonal control, ovarian expression and functional properties. Mech. Dev. **103,** 107–115.

Feldlaufer, M.F., Herbert, E.W., Svoboda, J.A., Thompson, M.J., and Lusby, W.R. (1986a). Makisterone A – The major ecdysteroid from the pupa of the honey bee, *Apis mellifera*. Insect Biochem. **15,** 597–600.

Feldlaufer, M.F., Svoboda, J.A., and Herbert, E.W. (1986b). Makisterone A and 24-methylenecholesterol from the ovaries of the honey bee, *Apis mellifera* L. Experientia **42,** 200–201.

Fournier, B., and Radallah, D. (1988). Ecdysteroids in *Carausius* eggs during embryonic development. Arch. Insect Physiol. Biochem. **7,** 211–224.

Freeman, M.R., Dobritsa, A., Gaines, P., Segraves, W.A., and Carlson, J.R. (1999). The *dare* gene: steroid hormone production, olfactory behaviour, and neural degeneration in *Drosophila*. Development **126,** 4591–4602.

Gande, A.R., and Morgan, E.D. (1979). Ecdysteroids in the developing eggs of the desert locust, *Schistocerca gregaria*. J. Insect Physiol. **25,** 289–293.

Gaziova, I., Bonnette, P.C., Henrich, V.C., and Jindra, M. (2004). Cell-autonomous roles of the *ecdysoneless* gene in *Drosophila* development and oogenesis. Development **131**, 2715–2725.

Gelman, D.B., Rojas, M.G., Kelly, T.J., Hu, J.S., and Bell, R.A. (2000). Ecdysteroid and free amino acid content of eggs of the Colorado potato beetle, *Leptinotarsa decemlineata*. Arch. Insect Physiol. Biochem. **44,** 172–182.

Geva, S., Hartfelder, K., and Bloch, G. (2005). Reproductive division of labor, dominance, and ecdysteroid levels in hemolymph and ovary of the bumble bee *Bombus terrestris*. J. Insect Physiol. **51,** 811–823.

Gharib, B., and De Reggi, M. (1983). Changes in ecdysteroid and juvenile hormone levels in developing eggs of *Bombyx mori*. J. Insect Physiol. **29,** 871–876.

Gharib, D., De Reggi, M., Conat, J., and Chaix, J. (1983). Ecdysteroid and juvenile hormone changes in *Bombyx mori* eggs, related to initiation of diapause. FEBS Lett. **160,** 119–123.

Gilbert, L.I., and Warren, J.T. (2005). A molecular genetic approach to the biosynthesis of the insect steroid molting hormone. Vitam. Horm. **73,** 31–57.

Girardie, J., and Girardie, A. (1996). Lom OMP, a putative ecdysiotropic factor for the ovary in *Locusta migratoria*. J. Insect Physiol. **42,** 215–221.

Girardie, J., Geoffre, S., Delbecque, J.-P., and Girardie, A. (1998a). Arguments for two distinct gonadotropic activities triggered by different domains of the ovary maturating parsin of *Locusta migratoria*. J. Insect Physiol. **44,** 1063–1071.

Girardie, J., Huet, J.-C., Atay-Kadiri, Z., Ettaouil, S., Delbecque, J.-P., Fournier, B., Pernollet, J.-C., and Girardie, A. (1998b). Isolation, sequence determination, physical and physiological characterization of the neuroparsins and ovary maturating parsins of *Schistocerca gregaria*. Insect Biochem. Mol. Biol. **28,** 641–650.

Glass, H., Emmerich, H., and Spindler, K.D. (1978). Immunohistochemical localisation of ecdysteroids in the follicular epithelium of locust oocyte. Cell Tissue Res. **194,** 237–244.

Goltzene, F., Lagueux, M., Charlet, M., and Hoffmann, J. (1978). The follicle cell epithelium of maturing ovaries of *Locusta migratoria*: a new biosynthetic tissue for ecdysone. H-S Z Physiol. Chem. **359,** 1427–1434.

Graf, R., Neuenschwander, S., Brown, M.R., and Ackermann, U. (1997). Insulin-mediated secretion of ecdysteroids from mosquito ovaries and molecular cloning of the insulin receptor homologue from ovaries of bloodfed *Aedes aegypti*. Insect Mol. Biol. **6,** 151–163.

Gruntenko, N.E., Bownes, M., Terashima, J., Sukhanova, M.Zh., and Raushenbach, I.Y. (2003). Heat stress affects oogenesis differently in wild-type *Drosophila virilis* and a mutant with altered juvenile hormone and 20-hydroxyecdysone levels. Insect Mol. Biol. **12,** 393–404.

Hackney, J.F., Pucci, C., Naes, E., and Dobens, L. (2007). Ras signaling modulates activity of the ecdysone receptor EcR during cell migration in the Drosophila ovary. Dev. Dyn. **236,** 1213–1226.

Hagedorn, H. (1985). The role of ecdysteroids in reproduction. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, Volume 8, G.A. Kerkut and L.I. Gilbert (Eds.), pp. 205–262. Pergamon Press, Oxford.

Hagedorn, H.H. (1989). Physiological roles of hemolymph ecdysteroids in the adult insect. In: Ecdysone, J. Koolman (Ed.), pp. 279–289. Thieme Verlag, Stuttgart.

Hansen, I.A., Attardo, G.M., Park, J.-H., Peng, Q., and Raikhel, A.S. (2004). Target of rapamycin-mediated amino acid signalling in mosquito anautogeny. Proc. Natl. Acad. Aci. USA **101,** 10626–10631.

Hartfelder, K., and Emlen, D.J. (2005). Endocrine control of insect polyphenism. In: Comprehensive Molecular Insect Science, Volume 3: Endocrinology, L.I. Gilbert, K. Iatrou and S.S. Gill (Eds.), pp. 651–703. Elsevier, Pergamon.

Hartfelder, K., Bitondi, M.M., Santana, W.C., and Simoes, Z.L. (2002). Ecdysteroid titer and reproduction in queens and workers of the honey bee and of a stingless bee: loss of ecdysteroid function at increasing levels of sociality? Insect Biochem. Mol. Biol. **32,** 211–216.

Hatle, J.D., Juliano, S.A., and Borst, D.W. (2003). Hemolymph ecdysteroids do not affect vitellogenesis in the lubber grasshopper. Arch. Insect Biochem. Physiol. **52,** 45–57.

Hayward, D.C., Bastiani, M.J., Trueman, J.W., Truman, J.W., Riddiford, L.M., and Ball, E.E. (1999). The sequence of Locusta RXR, homologous to Drosophila Ultraspiracle, and its evolutionary implications. Dev. Genes Evol. **209,** 564–571.

Hayward, D.C., Dhadialla, T.S., Zhou, S., Kuiper, M.J., Ball, E.E., Wyatt, G.R., and Walker, V.K. (2003). Ligand specificity and developmental expression of RXR and ecdysone receptor in the migratory locust. J. Insect Physiol. **49,** 1135–1144.

Henrich, V.C., and Brown, N.E. (1995). Insect nuclear receptors: a developmental and comparative perspective. Insect Biochem. Mol. Biol. **25,** 881–897.

Hepperle, C., and Hartfelder, K. (2001). Differentially expressed regulatory genes in honey bee caste development. Naturwissenschaften **88,** 113–116.

Hodin, J., and Riddiford, L.M. (1998). The ecdysone receptor and ultraspiracle regulate the timing and progression of ovarian morphogenesis during *Drosophila* metamorphosis. Dev. Genes Evol. **208,** 304–317.

Hodin, J., and Riddiford, L.M. (2000). Parallel alterations in the timing of ovarian ecdysone receptor and ultraspiracle expression characterize the independent evolution of larval reproduction in two species of gall midges (Diptera: Cecidomyiidae). Dev. Genes Evol. **210,** 358–372.

Hoffmann, J.A., Lagueux, M., Hetru, C., Charlet, M., and Goltzené, F. (1980). Ecdysone in reproduction competent female adults and embryos of insects. In: Progress in Ecdysone Research, J.A. Hoffmann (Ed.), pp. 431–465. Elsevier/North Holland, Amsterdam.

Honeybee Genome Sequencing Consortium (2006). Insights into social insects from the genome of the honeybee Apis mellifera. Nature **443,** 931–949.

Huang, R.-Y., and Orr, W.C. (1992). Broad-Complex function during oogenesis in *Drosophila melanogaster*. Dev. Genet. **13,** 277–288.

Huybrechts, R., and De Loof, A. (1977). Induction of vitellogenin synthesis in male Sarcophaga bullata by ecdysterone. Journal of Insect Physiology **23,** 1359–1362.

Huybrechts, R., and De Loof, A. (1982). Similarities in vitellogenin and control of vitellogenin synthesis within the genera *Sarcophaga*, *Phormia* and *Lucilia* (Diptera). Comp. Biochem. Physiol. B **72,** 339–344.

Ijiro, T., Urakawa, H., Yasukochi, Y., Takeda, M., and Fujiwara, Y. (2004). cDNA cloning, gene structure, and expression of Broad-Complex (BR-C) genes in the silkworm, Bombyx mori. Insect Biochem Mol. Biol. **34,** 963–969.

Imboden, H., and Lanzrein, B. (1982). Investigations on ecdysteroids and juvenile hormone and on morphological aspects during early embryogenesis in the ovoviviparous cockroach *Nauphoetu cinerea*. J. Insect Physiol. **28,** 37–46.

Isaac, R.E., Rose, M.E., Rees, H.H., and Goodwin, T.W. (1983). Identification of the 22-phosphate esters of ecdysone, 2-deoxyecdysone, 20-hydroxyecdysone and 2-deoxy-20-hydroxyecdysone from newly laid eggs of the desert locust, Schistocerca gregaria. Biochem. J. **213,** 533–541.

Izumi, S., Kiguchi, K., and Tomino, S. (1984). Hormonal regulation of biosynthesis of major plasma proteins in *Bombyx mori*. Zool. Sci. **1**, 223–228.

Jowett, T., and Postlethwait, J. H. (1980). Regulation of yolk polypeptide synthesis in *Drosophila* ovaries and fat body by 20-hydroxyecdysone and a juvenile hormone analog. Dev. Biol. **80,** 225–234.

Kadono-Okuda, K., Amornsak, W., and Yamashita, O. (1994). Controlled ecdysteroid accumulation in eggs of the silkworm, *Bombyx mori*, by an imidazole compound (KK-42), and embryogenesis in these eggs. Arch. Insect Biochem. Biophys. **25,** 121–135.

Kapitskaya, M., Wang, S., Cress, D.E., Dhadiallah, T.S., and Raikhel, A.S. (1996). The mosquito *ultraspiracle* homologue, a partner of ecdysteroid receptor heterodimer: cloning and characterization of isoforms expressed during vitellogenesis. Mol. Cell. Endocrinol. **121,** 119–132.

Kapitskaya, M.Z., Dittmer, N.T., Deitsch, K.W., Cho, W.-L., Taylor, D.G., Leff, T., and Raikhel, A.S. (1998). Three isoforms of a hepatocyte nuclear factor-4 transcription factor with tissue- and stage-specific expression in the adult mosquito. J. Biol. Chem. **273,** 29801–29810.

Kapitskaya, M.Z., Li, C., Miura, K., Segraves, W., and Raikhel, A.S. (2000). Expression of the early-late gene encoding the nuclear receptor HR3 suggests its involvement in regulating the vitellogenic response to ecdysone in the adult mosquito. Mol. Cell. Endocrinol. **160,** 25–37.

Kaplanis, J.N., Robbins, W.E., Thompson, M.J., and Dutky, S.R. (1973). 26-Hydroxyecdysone: new insect molting hormone from the egg of the tobacco hornworm. Science **180,** 307–308.

Kaplanis, J.N., Dutky, S.R., Robbins, W. E., Thompson, M. J., Lindquist, E.L., Horn, D.H., and Galbraith, M.N. (1975). Makisterone A: a 28-carbon hexahydroxy molting hormone from the embryo of the milkweed bug. Science **190,** 681–682.

Kendirgi, F., Swevers, L., and Iatrou, K. (2002). An ovarian follicular epithelium protein of the silkworm (*Bombyx mori*) that associates with the vitelline membrane and contributes to the structural integrity of the follicle. FEBS Lett. **524,** 59–68.

Khoury-Christianson, A.M., King, D.L., Hatzivassiliou, E., Casas, J.E., Hallenbeck, P.L., Nikodem, V.M., Mitsialis, S.A., and Kafatos, F.S. (1992). DNA binding and heterodimerization of the *Drosophila* transcription factor chorion factor 1/ultraspiracle. Proc. Natl. Acad. Sci. USA **89,** 11503–11507.

Kokoza, V.A., Snigirevskaya, E.S., and Raikhel, A.S. (1997). Mosquito clathrin heavy chain: analysis of protein structure and developmental expression in the ovary during vitellogenesis. Insect Mol. Biol. **6**, 357–368.

Kokoza, V.A., Martin, D., Mienaltowski, M.J., Ahmed, A., Morton, C.M., and Raikhel, A.S. (2001). Transcriptional regulation of the mosquito vitellogenin gene via a blood meal-triggered cascade. Gene **274,** 47–65.

Kozlova, T., and Thummel, C.S. (2003). Essential roles for ecdysone signalling during *Drosophila* mid-embryonic development. Science **301,** 1911–1914.

Konopova, B., and Jindra, M. (2007). Juvenile hormone resistance gene Methoprenetolerant controls entry into metamorphosis in the beetle *Tribolium castaneum*. Proceedings of the National Academy of Science USA **104**, 10488–10493

Kubli, E. (1996). The *Drosophila* sex-peptide: a peptide pheromone involved in reproduction. In: Advances in Developmental Biochemistry, Volume 4, P. Wassarman (Ed.), pp. 99–108. JAI Press, New York.

Lagueux, M., Hirn, M., and Hoffmann, J.A. (1977). Ecdysone during ovarian development in *Locusta migratoria*. J. Insect Physiol. **23,** 109–119.

Lagueux, M., Hetru, C., Goltzene, F., Kappler, C., and Hoffmann, J.A. (1979). Ecdysone titre and metabolism in relation to cuticulogenesis in embryos of *Locusta migratoria*. J. Insect Physiol. **25,** 709–723.

Lagueux, M., Hoffmann, J.A., Goltzené, F., Kappler, C., Tsoupras, G., Hetru, C., and Luu, B. (1984). Ecdysteroids in ovaries and embryos of Locusta migratoria. In: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, J.A. Hoffmann and M. Porchet (Eds.), pp. 168–180. Springer, Berlin.

Lanot, R., Thiebold, J., Lagueux, M., Goltzene, F., and Hoffmann, J.A. (1987). Involvement of ecdysone in the control of meiotic reinitation in oocytes of *Locusta migratoria*. Dev. Biol. **121,** 174–181.

Lanot, R., Thiebold, J., Costet-Corio, M.-F., Benveniste, P., and Hoffmann, J.A. (1988). Further experimental evidence for the involvement of ecdysone in the control of meiotic reinitiation in oocytes of *Locusta migratoria* (Insecta, Orthoptera). Dev. Biol. **126,** 212–214.

Lanot, R., Dom, A., Günster, B., Thiebold, J., Lagueux, M., and Hoffmann, J.A. (1989). Functions of ecdysteroids in oocyte maturation and embryonic development of insects. In: Ecdysone, J. Koolman (Ed.), pp. 262–270. Thieme Medical Publishers, New York.

Lanzrein, B., Gentinetta, V., Abegglen, H., Baker, F.C., Miller, C.A., and Schooley, D.A. (1985). Titers of ecdysone, 20-hydroxyecdysone and juvenile hormone III throughout the life cycle of a hemimetabolous insect, the viviviparous cockroach *Nauphoeta cinerea*. Experientia **41,** 913–917.

Leevers, S.J. (2001). Growth control: invertebrate insulin surprises. Curr. Biol. **11,** R209–R212.

Legay, J. M., Calvez, B., Hirn, M., and De Reggi, M. L. (1976). Ecdysone and oocyte morphogenesis in *Bombyx mori*. Nature **262,** 489–490.

Li, C., Kapitskaya, M.Z., Zhu, J., Miura, K., Segraves, W., and Raikhel, A.S. (2000). Conserved molecular mechanism for the stage specificity of the mosquito vitellogenic response to ecdysone. Dev. Biol. **224,** 96–110.

Lin, Y., Hamblin, M.T., Edwards, M.J., Barillas-Mury, C., Kanost, M.R., Knipple, D.C., Wolfner, M.F., and Hagedorn, H.H. (1993). Structure, expression, and hormonal control of genes from the mosquito, *Aedes aegypti*, which encode proteins similar to the vitellin membrane proteins of *Drosophila melanogaster*. Dev. Biol. **155,** 558–568.

Machado, E., Swevers, L., Sdralia, N., Medeiros, M.N., Mello, F.G., and Iatrou, K. (2007). Prostaglandin signaling and ovarian follicle development in the silkmoth, *Bombyx mori*. Insect Biochem. Mol. Biol. **37,** 876–885.

Maestro, O., Cruz, J., Pascual, N., Martín, D., and Bellés, X. (2005). Differential expression of two RXR/ultraspiracle isoforms during the life cycle of the hemimetabolous insect *Blatella germanica* (Dictyoptera, Blatellidae). Mol. Cell. Endocrinol. **238,** 27–37.

Makka, T., Seino, A., Tomita, S., Fujiwara, H., and Sonobe, H. (2002). A possible role for 20-hydroxyecdysone in embryonic development of the silkworm, *Bombyx mori*. Arch. Insect Biochem. Physiol. **51,** 111–120.

Mané-Padrós, D., Cruz, J., Vilaplana, L., Pascual, N., Bellés, X., and Martín, D. (2008). The nuclear hormone receptor BgE75 links molting and developmental progression in the direct-developing insect *Blattella germanica*. Dev. Biol. **315**, 147–160.

Marie, B., Bacon, J.P., and Blagburn, J.M. (1999). Double-stranded RNA interference shows that Engrailed controls the synaptic identify of identified sensory neurons. Curr. Biol. **10**, 289–292.

Maróy, P., Kaufmann, G., and Dübendorfer, A. (1988). Embryonic ecdysteroids of *Drosophila melanogaster*. J. Insect Physiol. **34,** 633–637.

Martín, D., Wang, S.-F., and Raikhel, A.S. (2001). The vitellogenin gene of the mosquito *Aedes aegypti* is a direct target of the ecdysteroid receptor. Mol. Cell. Endocrinol. **173,** 75–86.

Martín, D., Maestro, O., Cruz, J., Mané-Padrós, D., and Bellés, X. (2006). RNAi studies reveal a conserved role for RXR in molting in the cockroach *Blatella germanica*. J. Insect Physiol. **52,** 410–416.

Meinwald, J., Roach, B., Hicks, K., Alsop, D., and Eisner, T. (1985). Defensive steroids from a carrion beetle (Silpha americana). Experientia **41,** 516–519.

Mizuni, T., Watanabe, K., and Ohnishi, E. (1981). Developmental changes of ecdysteroids in the eggs of the silkworm, *Bombyx mori*. Dev. Growth Differ. **23**, 543–552.

Montell, D.J. (2001). Command and control: regulatory pathways controlling invasive behaviour of the border cells. Mech. Dev. **105,** 19–25.

Moshitzky, P., Fleischmann, I., Chaimov, N., Saudan, P., Klauser, S., Kubli, E., and Appelbaum, S.W. (1996). Sex-Peptide activates juvenile hormone biosynthesis in the *Drosophila melanogaster* corpus allatum. Arch. Insect Biochem. Physiol. **32,** 363–374.

Namiki, T., Niwa, R., Sakudoh, T., Shirai, K.-i., Takeuchi, H., and Kataoka, H. (2005). Cytochrome P450 CYP307A1/Spook: a regulator for ecdysone synthesis in insects. Biochem. Biophys. Res. Commun. **337,** 367–374.

Nene, V., Wortman, J.R., Lawson, D., Haas, B., Kodira, C., Tu, Z.J., Loftus, B., Xi, Z. et al. (2007). Genome sequence of Aedes aegypti, a major arbovirus vector. Science **316**, 1718–1723.

Nijhout, H.F., and Wheeler, D.E. (1982). Juvenile hormone and the physiological basis of insect polymorphism. Q. Rev. Biol. **57,** 109–133.

Nijhout, M.M., and Riddiford, L.M. (1974). The control of egg maturation by juvenile hormone in the tobacco hornworm moth, *Manduca sexta*. Biol. Bull. **146,** 377–392.

Nijhout, M.M., and Riddiford, L.M. (1979). Juvenile hormone and ovarian growth in *Manduca sexta*. Int. J. Invertebr. Reprod. Dev. **1,** 209–219.

Nishita, Y., and Takiya, S. (2004). Structure and expression of the gene encoding a Broad-Complex homolog in the silkworm, Bombyx mori. Gene **339,** 161–172.

Ohnishi, E., and Chatani, F. (1977). Biosynthesis of ecdysone in the isolated abdomen of the silkworm, *Bombyx mori*. Dev. Growth Differ. **19,** 67–70.

Ohnishi, E., Hiramoto, M., Fujimoto, Y., Kakinuma, K., and Ikekawa, N. (1989). Isolation and identification of major ecdysteroid conjugates from ovaries of *Bombyx mori*. Insect Biochem. **19,** 95–101.

Ono, H., Rewitz, K.F., Sinoda, T., Itoyama, K., Petryk, A., Rybczynski, R., Jarcho, M., Warren, J.T., Marqués, G., Shimell, M.J., Gilbert, L.I., and O'Connor, M.B. (2006). *Spook* and *Spookier* code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. Dev. Biol. **298**, 555–570.

Oro, A.E., McKeown, M., and Evans, R.M. (1992). The *Drosophila* retinoid X receptor homolog *ultraspiracle* functions in both female reproduction and eye morphogenesis. Development **115,** 449–462.

Parvy, J.-P., Blais, C., Bernard, F., Warren, J.T., Petryk, A., Gilbert, L.I., O'Connor, M.B., and Dauphin-Villemant, C. (2005). A role for βFTZ-F1 in regulating ecdysteroid titers during post-embryonic development in *Drosophila melanogaster*. Dev. Biol. **282,** 84–94.

Paul, R.K., Takeuchi, H., Matsuo, Y., and Kubo, T. (2005). Gene expression of ecdysteroid-regulated gene *E74* of the honeybee in ovary and brain. Insect Mol. Biol. **14,** 9–15.

Paul, R.K., Takeuchi, H., and Kubo, T. (2006). Expression of two ecdysteroid-regulated genes, *Broad-Complex* and *E75*, in the brain and ovary of the honeybee (*Apis mellifera* L.). Zool. Sci. **23,** 1085–1092.

Perrimon, N., Engstrom, L., and Mahowald, A.P. (1985). Developmental genetics of the 2C-D region of the Drosophila X chromosome. Genetics **111,** 23–41.

Petryk, A., Warren, J.T., Marqués, G., Jarcho, M.P., Gilbert, L.I., Kahler, J., Parvy, J.-P., Li, Y., Dauphin-Villemant, C., and O'Connor, M.B. (2003). Shade is the *Drosophila* P450 enzyme that mediates the hydroxylation of ecdysone to the steroid insect molting hormone 20-hydroxyecdysone. Proc. Natl. Acad. Sci. **100,** 13773–13778.

Pierceall, W.E., Li, C., Biran, A., Miura, K., Raikhel, A.S., and Segraves, W.A. (1999). E75 expression in mosquito ovary and fat body suggests reiterative use of ecdysone-regulated hierarchies in development and reproduction. Mol. Cell. Endocrinol. **150,** 73–89.

Pinto, L.Z., Hartfelder, K., Gentile Bitondi, M.M., and Paulino Simões, Z.L. (2002). Ecdysteroid titers in pupae of highly social bees relate to distinct modes of caste development. J. Insect Physiol. **48,** 783–790.

Postlethwait, J.H., and Handler, A.M. (1979). The roles of juvenile hormone and 20-hydroxyecdysone during vitellogenesis in isolated abdomens of *Drosophila melanogaster*. J. Insect Physiol. **25,** 455–460.

Postlethwait, J.H., and Shirk, P.D. (1981). Genetic and endocrine regulation of vitellogenesis in *Drosophila*. Am. Zool. **21,** 687–700.

Rachinsky, A., Strambi, C., Strambi, A., and Hartfelder, K. (1990). Caste and metamorphosis: hemolymph titers of juvenile hormone and ecdysteroids in last instar honeybee larvae. Gen. Comp. Endocrinol. **79,** 31–38.

Raikhel, A.S., and Lea, A.O. (1990). Juvenile hormone controls previtellogenic proliferation of ribosomal RNA in the mosquito fat body. Gen. Comp. Endocrinol. **77,** 423–434.

Raikhel, A.S., Miura, K., and Segraves, W.A. (1999). Nuclear receptors in mosquito vitellogenesis. Am. Zool. **39,** 722–735.

Raikhel, A.S., McGurk, L., and Bownes, M. (2003). Ecdysteroid action in insect reproduction. In: Encyclopedia of Hormones, Volume 1, H.L. Henry and A.W. Norman (Eds.), pp. 451–459. Academic, San Diego, CA.

Raikhel, A.S., Brown, M.R., and Bellés, X. (2005). Hormonal control of reproductive processes. In: Comprehensive Molecular Insect Science, Volume 3: Endocrinology, L.I. Gilbert, K. Iatrou and S.S. Gill (Eds.), pp. 433–491. Elsevier, Pergamon.

Ramaswamy, S.B., Shu, S., Park, Y.I., and Zeng, F. (1997). Dynamics of juvenile hormone-mediated gonadotropism in the Lepidoptera. Arch. Insect Biochem. Physiol. **35,** 539–558.

Rankin, S.M., and Stay, B. (1985). Ovarian inhibition of juvenile hormone synthesis in the viviparous cockroach, *Diploptera punctata*. Gen. Comp. Endocrinol. **59,** 230–237.

Rees, H.H., and Isaac, R.E. (1984). Biosynthesis of ovarian ecdysteroid phosphates and their metabolic fate during embryogenesis in *Schistocerca gregaria*. In: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, J.A. Hoffmann and M. Porchet (Eds.), pp. 181–195. Springer, Berlin.

Retnakaran, A., Hiruma, K., Palli, S.R., and Riddiford, L.M. (1995). Molecular analysis of the mode of action of RH-5992, a lepidopteran-specific, non-steroidal ecdysteroid agonist. Insect Biochem. Mol. Biol. **25,** 109–117.

Richards, G. (1976). Sequential gene activation by ecdysone in polytene chromosomes of *Drosophila melanogaster*. IV. The mid prepupal period. Dev. Biol. **54,** 256–263.

Richard, D.S., Watkins, N.L., Serafin, R.B., and Gilbert, L.I. (1998). Ecdysteroids regulate yolk protein uptake by *Drosophila melanogaster* oocytes. J. Insect Physiol. **44,** 637–644.

Richard, D.S., Gilbert, M., Crum, B., Hollinshead, D.M., Schelble, S., and Scheswohl, D. (2001). Yolk protein endocytosis by oocytes in Drosophila melanogaster: immunofluorescent localization of clathrin, adaptin and the yolk protein receptor. J. Insect Physiol. **47,** 715–723.

Riddiford, L.M. (1993a). Hormone receptors and the regulation of insect metamorphosis. Receptor **3,** 203–209.

Riddiford, L.M. (1993b). Hormones and *Drosophila* development. In: The Development of *Drosophila melanogaster*, Volume 2, M. Bate and A.M. Arias (Eds.), pp. 899–939. Cold Spring Harbor Laboratory Press, New York.

Riechmann, V., and Ephrussi, A. (2001). Axis formation during Drosophila oogenesis. Curr. Opin. Genet. Dev. **11,** 374–383.

Riehle, M.A., and Brown, M.R. (1999). Insulin stimulates ecdysteroid production through a conserved signalling cascade in the mosquito *Aedes aegypti*. Insect Biochem. Mol. Biol. **29,** 855–860.

Riehle, M.A., and Brown, M.R. (2003). Molecular analysis of the serine/threonine kinase Akt and its expression in the mosquito *Aedes aegypti*. Insect Mol. Biol. **12,** 225–232.

Robert, A., Strambi, A., Strambi, C., and Gonella, J. (1986). Opposite effects of ecdysone and 20-hydroxyecdysone on in vitro uterus motility of a tsetse fly. Life Sci. **39,** 2617–2622.

Robinson, G.E., and Vargo, E.L. (1997). Juvenile hormone in adult eusocial hymenoptera: gonadotropin and behavioral pacemaker. Arch. Insect Biochem. Physiol. **35,** 559–583.

Robinson, G.E., Strambi, C., Strambi, A., and Feldlaufer, M.F. (1991). Comparison of juvenile hormone and ecdysteroid titres in adult worker and queen honey bees. J. Insect Physiol. **37,** 929–935.

Roth, G.E., Gierl, M.S., Vollborn, L., Meise, M., Lintermann, R., and Korge, G. (2004). The *Drosophila* gene *Start1*: a putative cholesterol transporter and key regulator of ecdysteroid synthesis. Proc. Natl. Acad. Sci. USA **101,** 1601–1606.

Sall, C., Tsoupras, G., Kappler, C., Lagueux, M., Zachary, D., Luu, B., and Hofmann, J.A. (1983). Fate of maternal conjugated ecdysteroids during embryonic development in *Locusta migratoria*. J. Insect Physiol. **29,** 491–507.

Sappington, T.W., Hays, A.R., and Raikhel, A.S. (1995). Mosquito vitellogenin receptor: purification, developmental and biochemical characterization. Insect Biochem. Mol. Biol. **25,** 807–817.

Sato, Y., and Yamashita, O. (1991). Structure and expression of a gene coding for egg-specific protein in the silkworm, *Bombyx mori*. Insect Biochem. **21,** 495–505.

Satyanarayana, K., Yu, J.H., Bhaskaran, G., Dahm, K.H., and Meola, R. (1992). Regulation of vitellogenin synthesis by juvenile hormone in the corn earworm, *Helicoverpa zea*. Invertebr. Reprod. Dev. **21,** 169–178.

Satyanarayana, K., Bradfield, J.Y., Bhaskaran, G., and Dahm, K.H. (1994). Stimulation of vitellogenin production by methoprene in prepupae and pupae of *Manduca sexta*. Arch. Insect Biochem. Physiol. **25,** 21–37.

Sbrenna, G., Chicca, M., Bonora, M., and Sbrenna-Micciarelli, A. (1989). The response of embryonic epidermal tissue of *Schistocerca gregaria* (Orthoptera, Acrididae) to 20-OH- ecdysone in vitro. Acta Embryol. Morphol. Exp. n.s. **10,** 221–226.

Scalia, S., Sbrenna-Micciarelli, A., Sbrenna, G., and Morgan, E.D. (1987). Ecdysteroid titres and location in developing eggs of *Schistocerca gregaria*. Insect Biochem. **17,** 227–236.

Schmidt Capella, I.C., and Hartfelder, K. (1998) Juvenile hormone effect on DNA synthesis and apoptosis in caste-specific differentiation of the larval honey bee (*Apis mellifera* L.) ovary. J. Insect Physiol. **44,** 385–391.

Schwartz, M.B., Kelly, T.J., Imberski, R.B., and Rubenstein, E.C. (1985). The effects of nutrition and methoprene treatment on ovarian ecdysteroid synthesis in *Drosophila melanogaster*. J. Insect Physiol. **31,** 947–957.

Schwartz, M.B., Kelly, T.J., Woods, C.W., and Imberski, R.B. (1989). Ecdysteroid fluctuations in adult *Drosophila melanogaster* caused by elimination of pupal reserves and synthesis by early vitellogenic ovarian follicles. Insect Biochem. **19,** 243–249.

Seo, S.-J., Cheon, H.-M., Sun, J., Sappington, T.W., and Raikhel, A.S. (2003). Tissue- and stage-specific expression of two lipophorin receptor variants with seven and eight ligand-binding repeats in the adult mosquito. J. Biol. Chem. **278,** 41954–41962.

Shea, M.J., King, D.L., Conboy, M.J., Mariani, B.D., and Kafatos, F.C. (1990). Proteins that bind to *Drosophila* chorion *cis*-regulatory elements: a new C2H2 zinc finger protein and a C2C2 steroid receptor-like component. Gene. Dev. **4,** 1128–1140.

Shirk, P.D., Zimowska, G., Silhacek, D.L., and Shaaya, E. (1992). Initiation of vitellogenesis in pharate adult females of the Indianmeal moth, *Plodia interpunctella*. Arch. Insect Biochem. Physiol. **21,** 53–63.

Slinger, A.J., and Isaac, R.E. (1988). Synthesis of apolar ecdysone esters by ovaries of the cockroach Periplaneta americana. Gen. Comp. Endocrinol. **70,** 74–82.

Smagghe, G., and Degheele, D. (1994a). Action of the nonsteroidal ecdysteroid mimic RH 5849 on larval development and adult reproduction of insects of different orders. Invertebr. Reprod. Dev. **25,** 227–236.

Smagghe, G., and Degheele, D. (1994b). Action of a novel nonsteroid mimic, tebufenozide (RH-5992), on insects of different orders. Pestic. Sci. **42,** 85–92.

Soller, M., Bownes, M., and Kubli, E. (1999). Control of oocyte maturation in sexually mature *Drosophila* females. Dev. Biol. **208,** 337–351.

Sonobe, H., and Yamada, R. (2004). Ecdysteroids during early embryonic development in silkworm *Bombux mori*: metabolism and functions. Zool. Sci. **21,** 503–516.

Sorge, D., Nauen, R., Range, S., and Hoffmann, K.H. (2000). Regulation of vitellogenesis in the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Insect Physiol. **46,** 969–976.

Spradling, A.C. (1993). Developmental genetics of oogenesis. In: The Development of *Drosophila melanogaster*, M. Bate & A. Martinez Arias (Eds.), Vol. 1, pp 1–70. Cold Spring Harbor Laboratory Press, New York.

Stilwell, G.E., Nelson, C.A., Weller, J., Cui, H., Hiruma, K., Truman, J.W., and Riddiford, L.M. (2003). E74 exhibits stage-specific hormonal regulation in the epidermis of the tobacco hornworm, Manduca sexta. Dev. Biol. **258,** 76–90.

Sun, G., Zhu, J., Li, C., Tu, Z., and Raikhel, A.S. (2002). Two isoforms of the early E74 gene, an Ets transcription factor homologue, are implicated in the ecdysteroid hierarchy governing vitellogenesis of the mosquito, *Aedes aegypti*. Mol. Cell. Endocrinol. **190,** 147–157.

Sun, G.-C., Hirose, S., and Ueda, H. (1994). Intermittent expression of BmFTZ-F1, a member of the nuclear hormone receptor superfamily during development of the silkworm *Bombyx mori*. Dev. Biol. **162,** 426–437.

Sun, G.-Q., Zhu, J., Chen, L., and Raikhel, A.S. (2005). Synergistic action of E74B and ecdysteroid receptor in activating a 20-hydroxyecdysone effector gene. Proc. Natl. Acad. Sci. USA **102,** 15506–15511.

Sun, X., Song, Q., and Barrett, B. (2003a). Effect of ecdysone agonists on vitellogenesis and the expression of EcR and USP in codling moth (*Cydia pomonella*). Arch. Insect Biochem. Physiol. **52,** 115–129.

Sun, X., Song, Q., and Barrett, B. (2003b). Effects of ecdysone agonists on the expression of EcR, USP and other specific proteins in the ovaries of the codling moth (*Cydia pomonella* L.). Insect Biochem. Mol. Biol. **33,** 829–840.

Swevers, L., and Iatrou, K. (1999). The ecdysone agonist tebufenozide (RH-5992) blocks progression of the ecdysteroid-regulated gene expression cascade during silkmoth oogenesis and causes developmental arrest at mid-vitellogenesis. Insect Biochem. Mol. Biol. **29,** 955–963.

Swevers, L., and Iatrou, K. (2005). The ecdysone regulatory cascade and ovarian development in lepidopteran insects: insights from the silkmoth paradigm. Insect Biochem. Mol. Biol. **33,** 1285–1297.

Swevers, L., Drevet, J.R., Lunke, M.D., and Iatrou, K. (1995). The silkmoth homolog of the *Drosophila* ecdysone receptor (B1 isoform): cloning and analysis of expression during follicular cell differentiation. Insect Biochem. Mol. Biol. **25,** 857–866.

Swevers, L., Eystathioy, T., and Iatrou, K. (2002). The orphan nuclear receptors BmE75A and BmE75C of the silkmoth *Bombyx mori*: hormonal control and ovarian expression. Insect Biochem. Mol. Biol. **32,** 1643–1652.

Swevers, L., Raikhel, A.S., Sappington, T.W., Shirk, P., and Iatrou, K. (2005). Vitellogenesis and post-vitellogenic maturation of the insect ovarian follicle. In: Comprehensive Molecular Insect Science, Volume 1: Reproduction and Development, L.I. Gilbert, K. Iatrou and S.S. Gill (Eds.), pp. 87–155. Elsevier, Pergamon.

Taïbi, F., Smagghe, G., Amrani, L., and Soltani-Mazouni, N. (2003). Effect of ecdysone agonist RH-0345 on reproduction of mealworm, Tenebrio molitor. Comp. Biochem. Physiol. C Toxicol. Pharmacol. **135C,** 257–267.

Takaki, K., Konopova, B., and Jindra, M. (2008). EcR is required for oocyte development in the telotrophic ovary of *Tribolium castaneum*. Abstract Ecdysone-Workshop 2008, July 20th-24th Ulm, Germany.

Tan, A., and Palli, S.R. (2008. Edysone receptor isoforms play distinct roles in controlling molting and metamorphosis in the red flour beetle, *Tribolium castaneum*. Molec. Cell. Endocr. **291**, 42–49.

Tawfik, A.I., Vedrová, A., and Sehnal, F. (1999). Ecdysteroids during ovarian development and embryogenesis in solitary and gregarious *Schistocerca gregaria*. Arch. Insect Biochem. Physiol. **41,** 134–143.

Terashima, J., and Bownes, M. (2004). Translating available food into the number of eggs laid by *Drosophila melanogaster*. Genetics **167,** 1711–1719.

Terashima, J., and Bownes, M. (2005). A microarray analysis of genes involved in relating egg production to nutritional uptake in *Drosophila melanogaster*. Cell Death Differ. **12,** 429–440.

Terashima, J., and Bownes, M. (2006). *E75A* and *E75B* have opposite effects on the apoptosis/development choice of the *Drosophila* egg chamber. Cell Death Differ. **13,** 454–464.

Terashima, J., Takaki, K., Sakurai, S., and Bownes, M. (2005). Nutritional status affects 20-hydroxyecdysone concentration and progression of oogenesis in *Drosophila melanogaster*. J. Endocrinol. **187,** 69–79.

Thomas, H.E., Stunnenberg, H.G., and Stewart, A.F. (1993). Heterodimerization of the *Drosophila* ecdysone receptor with retinoid X receptor and *ultraspiracle*. Nature **362,** 471–475.

Thompson, M.J., Svoboda, J.A., and Weirich, G.F. (1984). Ecdysteroids in developing ovaries and eggs of the tobacco hornworm. Steroids **43,** 333–341.

Thompson, M.J., Svoboda, J.A., Lusby, W.R., Rees, H.H., Oliver, J.E., Weirich, G.F., and Wilzer, K.R. (1985a). Biosynthesis of a C21 steroid conjugate in an insect – the conversion of [^{14}C] cholesterol to 5-[^{14}C]pregnen-3β,20β-diol glucoside in the tobacco hornworm, *Manduca sexta*. J. Biol. Chem. **260,** 15410–15412.

Thompson, M.J., Weirich, G.F., Rees, H.H., Svoboda, J.A., Feldlaufer, F., and Wilzer, K.R. (1985b). New ecdysteroid conjugate: isolation and identification of 26-hydroxyecdysone 26-phosphate from eggs of the tobacco hornworm, *Manduca sexta* (L.). Arch. Insect Biochem. Physiol. **2,** 227–236.

Thummel, C.S. (1990). Puffs and gene regulation–molecular insights into the Drosophila ecdysone regulatory hierarchy. Bioessays **12,** 561–568.

Thummel, C.S. (1996). Flies on steroids – Drosophila metamorphosis and the mechanisms of steroid hormone action. Trends Genet. **12,** 306–310.

Thummel, C.S. (1997). Dueling orphans–interacting nuclear receptors coordinate Drosophila metamorphosis. Bioessays **19,** 669–672.

Thummel, C.S. (2002). Ecdysone-regulated puff genes 2000. Insect Biochem. Mol. Biol. **32,** 113–120.

Tomoyasu, Y., Miller, S.C., Tomita, S., Schoppmeier, M., Grossmann, D., and Bucher, G. (2008). Exploring systemic RNA interference in insects: a genome-wide survey for RNAi genes in *Tribolium*. Genome Biology **9**, R10.

Tsoupras, G., Luu, B., and Hoffmann, J.A. (1983). A cytokinin (Isopentenyl-adenosyl-mononucleotide) is bound to ecdysone in newly-laid eggs of *Locusta*. Science **220,** 507–509.

Tsuchida, K., Nagata, M., and Suzuki, A. (1987). Hormonal control of ovarian development in the silkworm, *Bombyx mori*. Arch. Insect Biochem. Biophys. **5,** 167–177.

Tzertzinis, G., Malecki, A., and Kafatos, F.C. (1994). BmCF1, a *Bombyx mori* RXR-type receptor related to the *Drosophila ultraspiracle*. J. Mol. Biol. **238,** 479–486.

Tzolovsky, G., Deng, W.-M., Schlitt, T., and Bownes, M. (1999). The function of the *Broad-Complex* during *Drosophila melanogaster* oogenesis. Genetics **153,** 1371–1383.

Uhlirova, M., Foy, B.D., Beaty, B.J., Olson, K.E., Riddiford, L.M., and Jindra, M. (2003). Use of Sindbis virus-mediated RNA interference to demonstrate a conserved role of Broad-Complex in insect metamorphosis. Proc. Natl. Acad. Sci. USA **100,** 15607–15612.

Val, P., Lefrançois-Martinez, A.M., Veyssière, G., and Martinez, A. (2003). SF-1 a key player in the development and differentiation of steroidogenic tissues. Nucl. Recept. **1,** 8.

Walker, L.L., Watson, K.L., Holden, J.J.A., and Steel, C.G.H. (1987). Vitellogenesis and fertility in *Drosophila* females with low ecdysteroid titers; the *L(3)3^{DTS}*. J. Insect Physiol. **33,** 137–143.

Wang, S.-F., Li, C., Zhu, J., Miura, K., Miksicek, R.J., and Raikhel, A.S. (2000). Differential expression and regulation by 20-Hydroxyecdysone of mosquito Ultraspiracle isoforms. Dev. Biol. **218,** 99–113.

Warren, J.T., Steiner, B., Dorn, A., Pak, M., and Gilbert, L.I. (1986). Metabolism of ecdysteroid during the embryogenesis of *Manduca sexta*. J. Liq. Chromatogr. **9,** 1759–1782.

Warren, J.T., Bachmann, J.S., Dai, J.D., and Gilbert, L.I. (1996). Differential incorporation of cholesterol and cholesterol derivatives into ecdysteroids by the larval ring glands and adult ovaries of Drosophila melanogaster: a putative explanation for the l(3)ecd1 mutation. Insect Biochem. Mol. Biol. **26,** 931–943.

Warren, J.T., Wismar, J., Subrahmanyam, B., and Gilbert, L.I. (2001). Woc (without children) gene control of ecdysone biosynthesis in Drosophila melanogaster. Mol. Cell. Endocrinol. **181,** 1–14.

Warren, J.T., Petryk, A., Marqués, G., Jarcho, M., Parvy, J.-P., Dauphin-Villemant, C., O'Connor, M.B., and Gilbert, L.I. (2002). Molecular and biochemical characterization of two P450 enzymes in the ecdysteroidogenic pathway of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **99,** 11043–11048.

Warren, J.T., Petryk, A., Marqués, G., Parvy, J.-P., Shinoda, T., Itoyama, K., Kobayashi, J., Jarcho, M., Li, Y., O'Connor, M.B., Dauphin-Villemant, C., and Gilbert, L.I. (2004). Phantom encodes the 25-hydroxylase of *Drosophila melanogaster* and *Bombyx mori*: a P450 enzyme critical in ecdysone biosynthesis. Insect Biochem. Mol. Biol. **34,** 991–1010.

Whiting, P., and Dinan, L. (1988a). Formation of apolar ecdysteroid conjugates by ovaries of the house cricket *Acheta domesticus in vitro*. Biochem. J. **252,** 95–103.

Whiting, P., and Dinan, L. (1988b). The occurrence of apolar ecdysteroid conjugates in newly-laid eggs of the house cricket, *Acheta domesticus*. J. Insect Physiol. **34,** 625–631.

Whiting, P., Sparks, S., and Dinan, L. (1997). Endogenous ecdysteroid levels and rates of ecdysone acylation by intact ovaries in vitro in relation to ovarian development in adult female house crickets, *Acheta domesticus*. Arch. Insect Biochem. Physiol. **35,** 279–299.

Wilson, T.G., and Ashok, M. (1998). Insecticide resistance resulting from an absence of target-site gene product. Proc. Natl. Acad. Sci. USA **95,** 14040–14044.

Wilson, T.G., Yerushalmi, Y., Donnell, D.M., and Restifo, L.L. (2006). Interaction between hormonal signalling pathways in *Drosophila melanogaster* as revealed by genetic interaction between *Methoprene-tolerant* and *Broad-Complex*. Genetics **172,** 253–264.

Woodard, C.T., Baehrecke, E.H., and Thummel, C.S. (1994). A molecular mechanism for the stage specificity of the Drosophila prepupal genetic response to ecdysone. Cell **79,** 607–615.

Wu, Q., and Brown, M.R. (2006). Signaling and function of insulin-like peptides in insects. Annu. Rev. Entomol. **51,** 1–24.

Yao, T.-P., Segraves, W.A., Oro, A.E., McKeown, M., and Evans, R.M. (1992). Drosophila ultraspiracle modulates ecdysone receptor function via heterodimer formation. Cell **71,** 63–72.

Yao, T.-P., Forman, B.M., Jiang, Z., Cherbas, L., Chen, J.-D., McKeown, M., Cherbas, P., and Evans, R.M. (1993). Functional ecdysone receptor is the product of *EcR* and *Ultraspiracle* genes. Nature **366,** 476–479.

Yoshiyama, T., Namiki, T., Mita, K., Kataoka, H., and Niwa, R. (2006). Neverland is an evolutionary conserved Rieske-domain protein that is essential for ecdysone synthesis and insect growth. Development **133,** 2565–2574.

Zhu, J., Miura, K., Chen, L., and Raikhel, A.S. (2000). AHR38, a homolog of NGFI-B, inhibits formation of the functional ecdysteroid receptor in the mosquito *Aedes aegypti*. EMBO J. **19,** 253–262.

Zhu, J., Chen, L., and Raikhel, A.S. (2003a). Posttranscriptional control of the competence factor βFTZ-F1 by juvenile hormone in the mosquito *Aedes aegypti*. Proc. Natl. Acad. Sci. USA **100,** 13338–13343.

Zhu, J., Miura, K., Chen, L., and Raikhel, A.S. (2003b). Cyclicity of mosquito vitellogenic ecdysteroid-mediated signalling is modulated by alternative dimerization of the RXR homologue *Ultraspiracle*. Proc. Natl. Acad. Sci. USA **100,** 544–549.

Zhu, J., Chen, L., Sun, G., and Raikhel, A.S. (2006). The competence factor βFtz-F1 potentiates ecdysone receptor activity via recruiting a p160/SRC coactivator. Mol. Cell. Biol. **26,** 9402–9412.

Zhu, X.X., Gfeller, H., and Lanzrein, B. (1983). Ecdysteroids during oogenesis in the ovoviviparous cockroach *Nauphoeta cinerea*. J. Insect Physiol. **29,** 225–235.