HHS Public Access

Author manuscript

Curr Opin Insect Sci. Author manuscript; available in PMC 2022 February 01.

Published in final edited form as:

Curr Opin Insect Sci. ; 43: 11–20. doi:10.1016/j.cois.2020.09.004.

Control of the insect metamorphic transition by ecdysteroid production and secretion

Xueyang Pan[1], **Robert P. Connacher**[2], **Michael B. O'Connor**[1,3]

[1]Dept. of Genetics, Cell Biology and Development, University of Minnesota, USA

[2]Dept. of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, USA

Abstract

Ecdysteroids are a class of steroid hormones that controls molting and metamorphic transitions in *Ecdysozoan* species including insects, in which ecdysteroid biosynthesis and its regulation have been extensively studied. Insect ecdysteroids are produced from dietary sterols by a series of reduction-oxidation reactions in the prothoracic gland and in Drosophila they are released into the hemolymph via vesicle-mediated secretion at the time of metamorphosis. To initiate precisely controlled ecdysteroid pulses, the prothoracic gland functions as a central node integrating both intrinsic and extrinsic signals to control ecdysteroid biosynthesis and secretion. In this review, we outline recent progress in the characterization of ecdysone biosynthesis and steroid trafficking pathways and the discoveries of novel factors regulating prothoracic gland function.

Keywords

Ecdysone; Metamorphosis; Steroid Synthesis; Steroid Secretion

Introduction

Steroid hormones, a group of systemic signaling molecules that are produced from cholesterol and thus share the steroid backbone, comprise an important class of messengers and exert a myriad of physiological functions in metazoans. A well-known example is to promote sexual maturation of animals. In mammals, sex hormones such as estradiol and testosterone circulate and act in multiple organs to promote the transition of a juvenile individual to a sexually reproductive adult, a process known as puberty [1]. Another well-characterized example exists in insects, where ecdysteroids control metamorphic transitions. In both mammals and holometabolous insects, the sexual maturation process is controlled by

[3]Corresponding author.

Conflict of interest statement 2020

This is acknowledge that Xueyang Pan, Robert Connacher and Michael B O'Connor, all authors of the manuscript entitled " **Control of the metamorphic transition by ecdysteroid production and secretion"**, have no conflicts of interest concerning the content of this manuscript.

a neuroendocrine pathway in which a steroid hormone functions as the final output signal. In mammals, the pathway operates within the hypothalamic-pituitary-gonadal (HPG) axis [1] and utilizes a cascade of neuropeptide signals that begin with Kisspeptin stimulating hypothalamic release of gonadotropin-releasing hormone (GnRH) which in turn promotes systemic release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland to increase gonadal production of the sex steroids [1,2]. These steroids bind to nuclear hormone receptors to form various types of transcriptional regulatory complexes that execute the developmental programs associated with sexual maturation and fertility.

In insects, a pair of neurons in each brain lobe produce prothoracicotropic hormone (PTTH), which stimulates ecdysone production in the prothoracic gland (PG), a major endocrine organ of the larva. A recent study in the fruit fly *Drosophila melanogaster* demonstrated that the PTTH producing neurons can be activated by a presynaptic Allastatin A (AstA) signal [3 **]. Surprisingly, AstA and the AstA receptor exhibit high levels of homology to the mammalian Kisspeptin and cognate receptor GPR54 which activate the HPG axis to initiate puberty [2]. Ecdysone also binds to a related nuclear hormone receptor (EcR/Usp) further highlighting the conserved aspects of the mechanism that underlies the juvenile to adult transition in both insects and man.

In insects, the effect of ecdysteroid on metamorphosis control has been studied in a plethora of species [4*]. In the pre-genomic/genetic era, Lepidopteran species such as tobacco hornworm *Manduca sexta* and silkworm *Bombyx mori* were commonly used as model organisms due to their large size and rapid life cycle which facilitated physiological and biochemical studies. More recently, *Drosophila* has become a predominant model system for these inquiries because of its unparalleled genetic toolkit [5]. This review will briefly summarize the current understanding of ecdysteroid synthesis and its regulation. First, we will outline the ecdysteroid biosynthetic pathway. Then, we will discuss recent progress on the signal inputs and intracellular regulatory mechanisms controlling ecdysone synthesis. Finally, we will highlight newly emerging evidence for vesicle mediated ecdysone secretion.

The ecdysteroid biosynthetic pathway: glimmer in the Black Box

Insects utilize dietary sterols as precursors for ecdysteroid biosynthesis [6]. Dietary cholesterol, found in laboratory food, is converted to ecdysone (E) through a series of oxygen additions to the steroid ring. Plant and yeast sterols are likewise converted to makisterone A and 24(28)-dehydromakisterone A, respectively, which differ from E at carbon 24. While all three ecdysteroids can support development, cholesterol is the preferential ecdysteroid precursor [6,7].

Despite some variations across insect species, the core ecdysteroid biosynthetic pathway appears to be very similar, as demonstrated by conservation of the principle biosynthetic enzymes and reaction intermediates (Fig. 1) [8]. Briefly, dehydrogenation of cholesterol at carbons 7 and 8 by the Reiske oxygenase Neverland forms 7-dehydrocholesterol (7dC) [9,10]. Subsequently, 7dC is modified by the “Black box” reactions, so named because the intermediates and enzymes responsible remain unclear. The output from the black box is the

intermediate 5β-ketodiol which is then subject to sequential hydroxylation on carbons 25, 22 and 2 to produce ecdysone [11–14]. After secretion from the PG and import into peripheral tissues, E is hydroxylated on carbon 20 by Shade to produce the active ecdysteroid 20-hydroxyecdysone (20E) [15]. In some species, such as *Manduca*, the pathway is slightly different in that the first post-Black Box compound is a 5β-diketol and the major PG-secreted ecdysteroid is 3-dehydroecdysone (3dE). 3dE is then processed into E in hemolymph [8].

To date, the Black Box reactions have not been fully elucidated. However, recent observations have provided important insights. Key intermediates such as 3-oxo-steroids and Δ^4-diketol have been confirmed in the Black Box [16–18]. The results unambiguously show that 7dC is first oxidized at carbon 3 to form 3-oxo-7dC, and the unstable 3-oxo-7dC is then isomerized into the more stable 3-oxo-$\Delta^{4,7}$C. Feeding Δ^4-diketol rescues *neverland* mutants, suggesting 3-oxo-$\Delta^{4,7}$C is converted to Δ^4-diketol by hydroxylation of carbon 14 and oxidation of carbon 6, though the intermediates remain a mystery [17,18]. The Δ^4-diketol is in turn reduced at carbon 5 and carbon 3. Rescue experiments suggest the order of these reactions may be flexible [17,18]. In *Drosophila*, the resulting 5β-ketodiol is the final Black Box product. In *Manduca*, the carbon 3 reduction is initially skipped, and 5β-diketol is subjected to the terminal hydroxylations to form 3dE.

Several enzymes have been identified and associated with the Black Box reactions including Non-molting glossy/Shroud [19], Spooky/Spookier/Spookiest [20–22], Cyp6t3 [23] and Cyp6u1 [24] although the exact reactions catalyzed by each remain to be determined. Downstream of 5β ketodiol, the enzymes encoded by *phantom* [11,12], *disembodied* [13] *shadow* [14] and *shade* [15], all cytochrome P450 monooxygenases, act sequentially to add OH groups to the 25, 22, 2 and 20 carbons, respectively, to produce the final active 20-E hormone. These genes have been historically classified as “Halloween genes” due to the characteristically “empty, and ghost-like” embryonic cuticles formed by zygotic loss-of-function mutants [25]. As in mammals, steroids are also important for fertility, and follicle cell produced 20E has been shown to be required for both oogenesis and ovulation [26]. Curiously. the putative Black box enzyme Spookier substitutes for Spook during 20E production in the follicle cells [21].

Extracellular signals: A network of ever-increasing complexity

As the major endocrine organ that produces ecdysone, the PG functions as a central node to integrate diverse physiological and environmental signals and converts them into E pulses that trigger molting and the metamorphic transition (Fig. 2). In this section we focus on the extracellular input signals that regulate ecdysteroidogenesis in the PG.

Prothoracicotropic hormone (PTTH)

The existence of a brain-derived ecdysteroidogenic factor was proposed almost a century ago [27]. The first neuropeptide identified in the brain was prothoraciotropic hormone (PTTH), whose activity has been demonstrated in many insect species [28]. PTTH is produced by specific neuroendocrine cells and reaches the PG either via the hemolymph (*Manduca*) [29] or through direct neural innervation (*Drosophila*) [30]. Once at the PG,

PTTH binds with the receptor tyrosine kinase (RTK) Torso and activates the Ras/Raf/Erk pathway [31]. How Ras/Raf/Erk regulates ecdysone biosynthesis is not fully understood, but several transcription factors including hormone receptor 4 (Hr4) and Pointed (Ptn) are involved in the pathway [23,32**].

Despite the demonstration that purified PTTH can stimulate PG glands to make ecdysone [33], recent studies using *ptth* mutants indicate that it is not strictly required for metamorphosis. In *Bombyx*, many *ptth* null mutant larvae manage to metamorphose at either the L4 or L5 stages [34*]. In *Drosophila,* ablation of PTTH producing neurons severely delays metamorphosis [30], but does not eliminate it, and *ptth* null mutants cause only a modest change in metamorphic timing [35*]. Recent studies have found three additional RTK receptors including epidermal growth factor receptor (Egfr), anaplastic lymphoma kinase (Alk), and PDGF- and VEGF-receptor related (Pvr) to be expressed in the PG and able to regulate E synthesis via the Ras/Raf/Erk pathway [32**,36]. Interestingly, the Alk ligand Jelly belly and the Pvr ligand Pvf3 are expressed in the PTTH producing neurons [36]. This may explain some of the phenotypic differences between the *ptth* null mutant and neuron ablation. In contrast, the EGFR ligands Spitz and Vein are expressed in the PG itself and act in an autocrine manner, which may explain why even PTTH neuron-ablated larvae are still able to undergo metamorphosis (see below) [32**].

Insulin/insulin-like growth factors

Another RTK mediated signal is produced by the insulin/insulin-like (IIS) growth factor ligand family. Systemically, the IIS pathway functions in various organs to couple nutrition with the overall growth and development of the animal [37]. In the PG, the insulin receptor (InR) and its downstream signal transduction components PI3K/Akt and target of rapamycin (TOR) are indispensable for PG tissue growth and ecdysone synthesis in both *Drosophila* and *Bombyx* [38–42]. However, the observations in *Manduca* appear controversial. Feeding TOR inhibitor Rapamycin to *Manduca* compromises PG growth, reduces ecdysone synthesis and delays metamorphosis [43,44]. However, treatment with PI3K inhibitors LY294002 and wortmannin does not attenuate E production [45]. Species differences are also evident upon injection of the insulin-like peptide Bombyxin [46], which triggers ecdysone synthesis and secretion in *Bombyx* [39,47] but not in *Manduca* [45,48]. Further investigation is needed to elucidate the differences in mechanisms that underlie these observations.

Neuropeptides and serotonin

Other than RTKs, G-protein coupled receptors (GPCRs) have also been proposed to mediate ecdysteroidogenic signals, since typical second messengers such as Ca^{2+} and cAMP are crucial factors regulating ecdysteroid synthesis in Lepidoptera [28]. In *Bombyx*, a series of neuropeptides have been shown to exert either prothoracicostatic or prothoracicotropic effects through GPCR receptors in the PG. The prothoracicostatic factors include prothoracicostatic peptides (PTSPs), Bommo-myosuppressin (BMS) and FMRFamide-related peptide (BRFa) , while the prothoracicotropic molecules include FXPRL-amide peptide, Orcokinin and pigment dispersing factor (PDF) [49,50]. Some of these neuropeptides are released into hemolymph, while others such as BRFa and Orcokinin are delivered to the PG by direct neural innervation [49]. Despite the intense study of

Drosophila neuropeptides [51], neuropeptide F is the only confirmed factor (other than PTTH and Ilps) shown to regulate ecdysteroidogenesis in the *Drosophila* PG [52]. Besides peptidergic neurons, a subset of serotoninergic neurons also regulate PG function in *Drosophila* [53].

Juvenile hormone and ecdysone

Juvenile hormone (JH) and ecdysone are the two key hormones that together coordinate molting and metamorphic activities. The systemic regulation of ecdysone synthesis by these two hormones involves multiple organs and complicated inter-organ communication, which cannot be fully discussed here (for review, see [54]). Instead, we will briefly introduce how the PG directly responds to each hormone.

JH is the best-known anti-metamorphic hormone which prevents larvae/nymphs from undergoing precocious metamorphosis. Accordingly, it is often referred to as the *status quo* hormone (for review, see [55] and this issue of Curr Opin). Although early evidence reported that JH suppressed ecdysteroidogenesis in *Bombyx* PGs [56], the direct regulation of PG cell function by JH was not verified until the JH receptors Methoprene-tolerant (Met)/Germ cell-expressed (Gce) were identified in *Drosophila* and subsequently in other insect models [55,57]. In Drosophila, PG specific knockdown of *Met*/*gce* causes precocious ecdysone production and accelerated pupariation, clearly demonstrating that the JH signal directly antagonizes ecdysteroid synthesis in the PG [58].

20E itself is also thought to exhibit both positive and negative feedback on its own production. Such a mechanism helps create a hormone pulse wherein a small amount of 20E stimulates its own synthesis through an EcR/Usp mediated positive feedback loop. At a certain hormone level, a negative feedback loop kicks in to bring the ecdysone level back to baseline thereby helping create a pulse. Additional mechanisms such as steroid hormone inactivation [59] also contribute to hormone removal and pulse control. Positive and negative feedback control in steroid hormone level modulation has been noted in both Lepidoptera and Drosophila [54,60] , and *Drosophila* genetic analysis has implicated both the ecdysone receptor (EcR) and the early responsive transcription factor Broad (Br) [60] in feedback control. Moreover, it appears that two isoforms of Br, Br-Z1 and Br-Z4, differentially mediate positive and negative feedback, respectively [60].

Transforming growth factor β

The TGFβ family is comprised of two branches of factors, the bone morphogenetic proteins (BMPs) and the Activins. Both pathways have been studied in *Drosophila* PGs and function in opposite ways. Loss of dSmad2/Smox, the primary transcriptional transducer of the Activin pathway, compromises E synthesis and causes a severe metamorphic delay. Although the identity and the source of the ligand that activates the pathway is not yet clear [61], Activin signaling appears to act as a competence pathway since it is required for normal expression of Torso and InR [61]. In contrast, BMP signaling functions as a gatekeeper to suppress precocious metamorphosis. The BMP ligand Decapentaplegic (Dpp) is released from imaginal discs during early L3 stage, which results in phosphorylation of Mad by the Thickvein (Tkv)-Punt/Wishful thinking (Wit) receptors in the PG to suppresses

ecdysone synthesis. When larvae grow beyond a certain size, Dpp “leakage/release” from imaginal discs terminates, allowing resumption of hormone synthesis by the PG [62**].

Hedgehog

Hedgehog (Hh) signaling is a highly conserved pathway that controls embryonic patterning and adult tissue homeostasis from *Drosophila* to mammals [63]. As a canonical morphogen and paracrine factor, secreted Hh diffuses within tissues such as imaginal discs to regulate differentiation and patterning. However, recent studies also identified Hh circulating in the hemolymph of *Drosophila* larvae, which it acts in an endocrine manner to regulate larval body growth and developmental timing [64]. The source of the circulating Hh is the midgut, and it signals to both the PG and fat body. The expression and secretion of Hh in the midgut is upregulated during starvation. Activation of Hh signaling in the PG suppresses expression of Halloween genes and ecdysone biosynthesis, while in fat body it promotes neutral lipid mobilization during starvation. These activities enable Hh to play an important role during starvation by delaying pupariation and mobilizing nutrient stores to support the survival of animals. Under fed conditions, however, the combinatory effects of Hh on slowing down both body growth and developmental timing results in normal sized pupae irrespective of gain or loss of circulating Hh [64].

Autocrine factors

The existence of uncharacterized autocrine factor(s) regulating ecdysteroid synthesis was first observed in *Bombyx* [65,66]. Recently, several autocrine factors have been identified in *Drosophila*, including β3-octopamine [67], the Egfr ligands Vein, and Spitz [32] and the Pvr ligands Pvf2, Pvf3 [36]. The β3-octopamine-induced pathway functions upstream of both IIS and PTTH signaling [67], while the Egfr and Pvr ligands activate Ras/Raf/Erk directly [32**,36]. Intriguingly, the expression of Vein and Spitz in the PG are induced by E feeding [32**], indicating a role of Egfr signaling as part of the ecdysone synthesis positive feedback loop described above.

Cell-autonomous factors: power of the -omics

In response to input signals, the PG cells undergo many intracellular modifications that help stimulate ecdysteroid production. Recent studies have uncovered an increasing number of intracellular factors that are essential to PG function, including transcription factors controlling Halloween gene expression [68,69*], iron metabolism regulators required for production of biosynthetic enzyme cofactors such as heme [70], cholesterol trafficking regulators [71,72], circadian cycle factors [73], glutathione [72,74], and nitric oxide (NO) [75] production. Among these factors, most were identified in *Drosophila* by PG-specific gene knockdown experiments. At the genome-wide level, PG-knockdown of an astonishing 1,906 genes (out of 12,504 total) cause various levels of developmental defects [71]. Although many of these “ecdysteroidogenic” genes have known or speculated functions relevant to hormone production, or are required for maintenance of basic cell functions, there are many whose potential role(s) in ecdysone synthesis and trafficking remain to be investigated.

In addition to traditional genetic screening, advancements in transcriptome and proteome profiling techniques and bioinformatics also provide powerful insights into the biological activities of the PG. To date, at least four RNA-seq [24,76–78], one microarray [76] and one proteomic dataset [78] have been obtained using the *Drosophila* ring gland or *Bombyx* PG tissues. Moreover, the samples have been prepared from tissues subject to different sorts of manipulations (e.g. early vs. late stage larvae [76], pre- vs. post-PTTH treatment [76,78], providing a better coverage of different biological scenarios. Analyses of these datasets have uncovered membrane receptor distributions [78], cholesterol trafficking components [71,79] and cytochrome P450 enzyme profiles [24,76] in the PG. Further mining of these data may lead to additional discoveries concerning regulation of E synthesis in the PG.

Steroid trafficking: an old question yields a new perspective

In addition to the enzymology, steroid hormone biosynthesis also appears to utilize complex trafficking mechanisms to move steroid molecules into, out of, and within cells. As strict cholesterol auxotrophs, insects fully rely on dietary sterol for viability. Upon ingestion, cholesterol is transported to the PG in lipophorins (insect lipoprotein) [80,81] and imported into PG cells using scavenger [82] or lipophorin receptors [83]. Subsequently, cholesterol translocates into the ER through the endo-lysosome system, during which cholesterol binding proteins such as Neimann-Pick complex 1 (NPC1), NPC2 and Start1 play essential roles [84]. Additional cholesterol trafficking factors influencing hormone production have been described in recent reports, including the fatty acid elongase Sit, the glutathione S-transferase Nopperabo [72,85], the deadenylase CCR4-NOT complex [86], and the autophagic machinery [87*,88*], but the mechanisms involved are not fully understood. During E biosynthesis the first step involving conversion of cholesterol to 7dC occurs in the ER. From there, the Black Box reactions continue to live up to their name with respect to the subcellular compartments in which they act. The microsomal localization of Spok indicates that they likely occur in the ER [21]. The Black Box product is further hydroxylated into 2,22-dideoxyecdysone in the ER and then into ecdysone within mitochondria [8]. Thus, ecdysone biosynthesis appears to involve shuttling of steroid intermediates between ER and mitochondria perhaps at mitochondrial associated membrane contact sites (MAMs). However, the mechanism and potential steroid shuttling proteins involved in this process are still unknown as are inferred hemolymphatic steroid carrier proteins [89].

Until recently, the discussion of steroid trafficking pathways stopped at the secretion point. It was, and still is, widely assumed that steroid hormones can freely diffuse across the plasma membrane due to the molecules' inherently lipophilic nature. Recently, this idea was challenged by the demonstration that in *Drosophila*, E exits PG cells via vesicle-mediated secretion [90]. In this scenario, in the presence of ATP, ecdysone is pumped into secretory vesicles by Atet, a specific member of the ABC transporter family. At the time of ecdysone secretion, the vesicles are released into the hemolymph in response to a Ca^{2+} signal [90]. Following this discovery, an ecdysone importer (EcI) was also identified, which is expressed in peripheral tissues and mediates the entry of E into responding cells from the hemolymph [91**]. These discoveries demonstrate that ecdysone secretion is distinct from its synthesis, and that both secretion and uptake could potentially serve as novel regulatory nodes.

Whether similar exporters and importers are employed by mammals for proper steroid trafficking and tissue response remains to be determined.

Future outlook

As efforts devoted to understanding the biology of ecdysteroids and their regulation during metamorphosis continue, many issues (re)emerge as the result of continuously accumulating knowledge. Old mysteries such as the chemistry and enzymatic steps of the Black Box remain to be fully elucidated, and new inquiries concerning the mechanisms of steroid trafficking, ecdysteroid secretion and its uptake are needed. As the number of signals regulating PG function rise, the cross talk between these signals and the mechanism(s) for integrating them into an appropriate temporal E production profile will require significant additional intellectual and experimental inquires. Studies utilizing other insect orders and species, besides Drosophila, are also essential to understand both the similarities and differences in how E production and release are regulated relative to the ecology of each species.

Acknowledgements:

The authors thank MJ Shimell and Aidan Peterson for comments on the manuscript. This work was funded by a grant R35GM118029 from the National Institute of Health to MBO. Support for R. C. was provided by National Institutes of Health, R01GM105707 to Aaron Goldstrohm.

References

1. Herbison AE: Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. Nat Rev Endocrinol 2016, 12:452–466. [PubMed: 27199290]

2. Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M: Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 2012, 92:1235–1316. [PubMed: 22811428]

3**. Deveci D, Martin FA, Leopold P, Romero NM: AstA Signaling Functions as an Evolutionary Conserved Mechanism Timing Juvenile to Adult Transition. Curr Biol 2019, 29:813–822 e814. [PubMed: 30799245] This study identifies a group of AstA expressing neurons that innervate the PTTH neurons and stimulate their activity via the AstA receptor. Intriguingly, AstA and AstAR exhibit high level of homology to Kisspeptin and GPR54, rescpectively, suggesting an evolutionarily conserved mechanism controlling the juvenile-to-adult transition in insects and mammals.

4*. Truman JW, Riddiford LM: The evolution of insect metamorphosis: a developmental and endocrine view. Philos Trans R Soc Lond B Biol Sci 2019, 374:20190070. [PubMed: 31438820] A good general review on insect metamorphosis.

5. Yamanaka N, Rewitz KF, O'Connor MB: Ecdysone control of developmental transitions: lessons from Drosophila research. Annu Rev Entomol 2013, 58:497–516. [PubMed: 23072462]

6. Lavrynenko O, Rodenfels J, Carvalho M, Dye NA, Lafont R, Eaton S, Shevchenko A: The ecdysteroidome of Drosophila: influence of diet and development. Development 2015, 142:3758–3768. [PubMed: 26395481]

7. Feldlaufer MF, Weirich GF, Imberski RB, Svoboda JA: Ecdysteroid production in Drosophila melanogaster reared on defined diets. Insect Biochem Mol Biol 1995, 25:709–712. [PubMed: 7627202]

8. Lafont R, Dauphin-Villemant C, Warren J, Rees H: Ecdysteroid chemistry and biochemistry. In Insect endocrinology. Edited by: Elsevier; 2012:106–176.

9. Yoshiyama T, Namiki T, Mita K, Kataoka H, Niwa R: Neverland is an evolutionally conserved Rieske-domain protein that is essential for ecdysone synthesis and insect growth. Development 2006, 133:2565–2574. [PubMed: 16763204]
10. Yoshiyama-Yanagawa T, Enya S, Shimada-Niwa Y, Yaguchi S, Haramoto Y, Matsuya T, Shiomi K, Sasakura Y, Takahashi S, Asashima M, et al.: The conserved Rieske oxygenase DAF-36/Neverland is a novel cholesterol-metabolizing enzyme. J Biol Chem 2011, 286:25756–25762. [PubMed: 21632547]
11. Niwa R, Matsuda T, Yoshiyama T, Namiki T, Mita K, Fujimoto Y, Kataoka H: CYP306A1, a cytochrome P450 enzyme, is essential for ecdysteroid biosynthesis in the prothoracic glands of Bombyx and Drosophila. J Biol Chem 2004, 279:35942–35949. [PubMed: 15197185]
12. Warren JT, Petryk A, Marques G, Parvy JP, Shinoda T, Itoyama K, Kobayashi J, Jarcho M, Li Y, O'Connor MB, et al.: Phantom encodes the 25-hydroxylase of Drosophila melanogaster and Bombyx mori: a P450 enzyme critical in ecdysone biosynthesis. Insect Biochem Mol Biol 2004, 34:991–1010. [PubMed: 15350618]
13. Chavez VM, Marques G, Delbecque JP, Kobayashi K, Hollingsworth M, Burr J, Natzle JE, O'Connor MB: The Drosophila disembodied gene controls late embryonic morphogenesis and codes for a cytochrome P450 enzyme that regulates embryonic ecdysone levels. Development 2000, 127:4115–4126. [PubMed: 10976044]
14. Warren JT, Petryk A, Marques G, Jarcho M, Parvy JP, Dauphin-Villemant C, O'Connor MB, Gilbert LI: Molecular and biochemical characterization of two P450 enzymes in the ecdysteroidogenic pathway of Drosophila melanogaster. Proc Natl Acad Sci U S A 2002, 99:11043–11048. [PubMed: 12177427]
15. Petryk A, Warren JT, Marques G, Jarcho MP, Gilbert LI, Kahler J, Parvy JP, Li Y, Dauphin-Villemant C, O'Connor MB: Shade is the Drosophila P450 enzyme that mediates the hydroxylation of ecdysone to the steroid insect molting hormone 20-hydroxyecdysone. Proc Natl Acad Sci U S A 2003, 100:13773–13778. [PubMed: 14610274]
16. Warren JT, O'Connor MB, Gilbert LI: Studies on the Black Box: incorporation of 3-oxo-7-dehydrocholesterol into ecdysteroids by Drosophila melanogaster and Manduca sexta. Insect Biochem Mol Biol 2009, 39:677–687. [PubMed: 19699302]
17. Ono H, Morita S, Asakura I, Nishida R: Conversion of 3-oxo steroids into ecdysteroids triggers molting and expression of 20E-inducible genes in Drosophila melanogaster. Biochem Biophys Res Commun 2012, 421:561–566. [PubMed: 22525676]
18. Saito J, Kimura R, Kaieda Y, Nishida R, Ono H: Characterization of candidate intermediates in the Black Box of the ecdysone biosynthetic pathway in Drosophila melanogaster: Evaluation of molting activities on ecdysteroid-defective larvae. J Insect Physiol 2016, 93–94:94–104.
19. Niwa R, Namiki T, Ito K, Shimada-Niwa Y, Kiuchi M, Kawaoka S, Kayukawa T, Banno Y, Fujimoto Y, Shigenobu S, et al.: Non-molting glossy/shroud encodes a short-chain dehydrogenase/reductase that functions in the 'Black Box' of the ecdysteroid biosynthesis pathway. Development 2010, 137:1991–1999. [PubMed: 20501590]
20. Namiki T, Niwa R, Sakudoh T, Shirai K, Takeuchi H, Kataoka H: Cytochrome P450 CYP307A1/Spook: a regulator for ecdysone synthesis in insects. Biochem Biophys Res Commun 2005, 337:367–374. [PubMed: 16188237]
21. Ono H, Rewitz KF, Shinoda T, Itoyama K, Petryk A, Rybczynski R, Jarcho M, Warren JT, Marques G, Shimell MJ, et al.: Spook and Spookier code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. Dev Biol 2006, 298:555–570. [PubMed: 16949568]
22. Rewitz KF, O'Connor MB, Gilbert LI: Molecular evolution of the insect Halloween family of cytochrome P450s: phylogeny, gene organization and functional conservation. Insect Biochem Mol Biol 2007, 37:741–753. [PubMed: 17628274]
23. Ou Q, Magico A, King-Jones K: Nuclear receptor DHR4 controls the timing of steroid hormone pulses during Drosophila development. PLoS Biol 2011, 9:e1001160. [PubMed: 21980261]
24. Christesen D, Yang YT, Somers J, Robin C, Sztal T, Batterham P, Perry T: Transcriptome Analysis of Drosophila melanogaster Third Instar Larval Ring Glands Points to Novel Functions and Uncovers a Cytochrome p450 Required for Development. G3 (Bethesda) 2017, 7:467–479. [PubMed: 27974438]

25. Gilbert LI: Halloween genes encode P450 enzymes that mediate steroid hormone biosynthesis in Drosophila melanogaster. Mol Cell Endocrinol 2004, 215:1–10. [PubMed: 15026169]

26. Belles X, Piulachs MD: Ecdysone signalling and ovarian development in insects: from stem cells to ovarian follicle formation. Biochim Biophys Acta 2015, 1849:181–186. [PubMed: 24939835]

27. Kopeć S: Studies on the Necessity of the Brain for the Inception of Insect Metamorphosis. Biological Bulletin 1922, 42:323–342.

28. Smith W, Rybczynski R: Prothoracicotropic hormone. In Insect endocrinology. Edited by: Elsevier; 2012:1–62.

29. Agui N, Bollenbacher W, Granger N, Gilbert L: Corpus allatum is release site for insect prothoracicotropic hormone. Nature 1980, 285:669–670.

30. McBrayer Z, Ono H, Shimell M, Parvy JP, Beckstead RB, Warren JT, Thummel CS, Dauphin-Villemant C, Gilbert LI, O'Connor MB: Prothoracicotropic hormone regulates developmental timing and body size in Drosophila. Dev Cell 2007, 13:857–871. [PubMed: 18061567]

31. Rewitz KF, Yamanaka N, Gilbert LI, O'Connor MB: The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis. Science 2009, 326:1403–1405. [PubMed: 19965758]

32**. Cruz J, Martín D, Franch-Marro X: Egfr Signaling Is a Major Regulator of Ecdysone Biosynthesis in the Drosophila Prothoracic Gland. Current Biology 2020.This study demonstrates the importance of autocrine versus extrinsic cues in stimulating the Ras/ERK pathway to regulate ecdysone biosynthesis.

33. Gilbert LI, Rybczynski R, Song Q, Mizoguchi A, Morreale R, Smith WA, Matubayashi H, Shionoya M, Nagata S, Kataoka H: Dynamic regulation of prothoracic gland ecdysteroidogenesis: Manduca sexta recombinant prothoracicotropic hormone and brain extracts have identical effects. Insect Biochem Mol Biol 2000, 30:1079–1089. [PubMed: 10989295]

34*. Uchibori-Asano M, Kayukawa T, Sezutsu H, Shinoda T, Daimon T: Severe developmental timing defects in the prothoracicotropic hormone (PTTH)-deficient silkworm, Bombyx mori. Insect Biochem Mol Biol 2017, 87:14–25. [PubMed: 28627423] This study describe the loss of function phenotype of ptth in Bombyx mori.

35**. Shimell M, Pan X, Martin FA, Ghosh AC, Leopold P, O'Connor MB, Romero NM: Prothoracicotropic hormone modulates environmental adaptive plasticity through the control of developmental timing. Development 2018, 145.This study describes the ptth loss of function phenotype in Drosophila and provides evidence of additonal signals origninating from the PG neurons in the regulation of metamorphic timing.

36. Pan X, O'Connor MB: PTTH producing neurons also release Jeb and Pvf2 to control Drosophila developmental timing via Ras/Erk and Jak/Stat signaling. BioXriv 2020.

37. Okamoto N, Yamanaka N: Nutrition-dependent control of insect development by insulin-like peptides. Curr Opin Insect Sci 2015, 11:21–30. [PubMed: 26664828]

38. Caldwell PE, Walkiewicz M, Stern M: Ras activity in the Drosophila prothoracic gland regulates body size and developmental rate via ecdysone release. Current Biology 2005, 15:1785–1795. [PubMed: 16182526]

39. Gu SH, Lin JL, Lin PL, Chen CH: Insulin stimulates ecdysteroidogenesis by prothoracic glands in the silkworm, Bombyx mori. Insect Biochem Mol Biol 2009, 39:171–179. [PubMed: 19049871]

40. Layalle S, Arquier N, Leopold P: The TOR pathway couples nutrition and developmental timing in Drosophila. Dev Cell 2008, 15:568–577. [PubMed: 18854141]

41. Mirth C, Truman JW, Riddiford LM: The role of the prothoracic gland in determining critical weight for metamorphosis in Drosophila melanogaster. Curr Biol 2005, 15:1796–1807. [PubMed: 16182527]

42. Colombani J, Bianchini L, Layalle S, Pondeville E, Dauphin-Villemant C, Antoniewski C, Carre C, Noselli S, Leopold P: Antagonistic actions of ecdysone and insulins determine final size in Drosophila. Science 2005, 310:667–670. [PubMed: 16179433]

43. Hatem NE, Wang Z, Nave KB, Koyama T, Suzuki Y: The role of juvenile hormone and insulin/TOR signaling in the growth of Manduca sexta. BMC Biol 2015, 13:44. [PubMed: 26108483]

44. Kemirembe K, Liebmann K, Bootes A, Smith WA, Suzuki Y: Amino acids and TOR signaling promote prothoracic gland growth and the initiation of larval molts in the tobacco hornworm Manduca sexta. PLoS One 2012, 7:e44429. [PubMed: 22984508]
45. Smith WA, Lamattina A, Collins M: Insulin signaling pathways in lepidopteran ecdysone secretion. Front Physiol 2014, 5:19. [PubMed: 24550835]
46. Mizoguchi A, Okamoto N: Insulin-like and IGF-like peptides in the silkmoth Bombyx mori: discovery, structure, secretion, and function. Front Physiol 2013, 4:217. [PubMed: 23966952]
47. Gu SH, Chen CH, Hsieh YC, Lin PL, Young SC: Modulatory effects of bombyxin on ecdysteroidogenesis in Bombyx mori prothoracic glands. J Insect Physiol 2015, 72:61–69. [PubMed: 25497117]
48. Walsh AL, Smith WA: Nutritional sensitivity of fifth instar prothoracic glands in the tobacco hornworm, Manduca sexta. J Insect Physiol 2011, 57:809–818. [PubMed: 21420972]
49. Tanaka Y: Recent topics on the regulatory mechanism of ecdysteroidogenesis by the prothoracic glands in insects. Front Endocrinol (Lausanne) 2011, 2:107. [PubMed: 22645515]
50. Iga M, Nakaoka T, Suzuki Y, Kataoka H: Pigment dispersing factor regulates ecdysone biosynthesis via bombyx neuropeptide G protein coupled receptor-B2 in the prothoracic glands of Bombyx mori. PLoS One 2014, 9:e103239. [PubMed: 25072638]
51. Nassel DR, Zandawala M: Recent advances in neuropeptide signaling in Drosophila, from genes to physiology and behavior. Prog Neurobiol 2019, 179:101607. [PubMed: 30905728]
52. Kannangara JR, Henstridge MA, Parsons LM, Kondo S, Mirth CK, Warr GG: Neuropeptide F receptor acts in the Drosophila prthoracic gland to regulate growth and developmental timing. BioRxiv 2019.
53. Shimada-Niwa Y, Niwa R: Serotonergic neurons respond to nutrients and regulate the timing of steroid hormone biosynthesis in Drosophila. Nat Commun 2014, 5:5778. [PubMed: 25502946]
54. Sakurai S: 3.8 - Feedback Regulation of Prothoracic Gland Activity. In Comprehensive Molecular Insect Science. Edited by Gilbert LI: Elsevier; 2005:409–431.
55. Goodman W, Cusson M: The juvenile hormones. In Insect Endocrinology. Edited by: Elsevier; 2012:310–365.
56. Sakurai S, Okuda M, Ohtaki T: Juvenile hormone inhibits ecdysone secretion and responsiveness to prothoracicotropic hormone in prothoracic glands of Bombyx mori. Gen Comp Endocrinol 1989, 75:222–230. [PubMed: 2806872]
57. Jindra M, Uhlirova M, Charles JP, Smykal V, Hill RJ: Genetic Evidence for Function of the bHLH-PAS Protein Gce/Met As a Juvenile Hormone Receptor. PLoS Genet 2015, 11:e1005394. [PubMed: 26161662]
58. Liu S, Li K, Gao Y, Liu X, Chen W, Ge W, Feng Q, Palli SR, Li S: Antagonistic actions of juvenile hormone and 20-hydroxyecdysone within the ring gland determine developmental transitions in Drosophila. Proc Natl Acad Sci U S A 2018, 115:139–144. [PubMed: 29255055]
59. Rewitz KF, Yamanaka N, O'Connor MB: Steroid hormone inactivation is required during the juvenile-adult transition in Drosophila. Dev Cell 2010, 19:895–902. [PubMed: 21145504]
60. Moeller ME, Danielsen ET, Herder R, O'Connor MB, Rewitz KF: Dynamic feedback circuits function as a switch for shaping a maturation-inducing steroid pulse in Drosophila. Development 2013, 140:4730–4739. [PubMed: 24173800]
61. Gibbens YY, Warren JT, Gilbert LI, O'Connor MB: Neuroendocrine regulation of Drosophila metamorphosis requires TGFbeta/Activin signaling. Development 2011, 138:2693–2703. [PubMed: 21613324]
62**. Setiawan L, Pan X, Woods AL, O'Connor MB, Hariharan IK: The BMP2/4 ortholog Dpp can function as an inter-organ signal that regulates developmental timing. Life Sci Alliance 2018, 1:e201800216. [PubMed: 30515478] The authors show that the BMP ligand Dpp produced by imaginal discs circulates in the hemolymph and acts on the PG to regulate the Critical weight checkpoint.
63. Jiang J, Hui CC: Hedgehog signaling in development and cancer. Dev Cell 2008, 15:801–812. [PubMed: 19081070]

64. Rodenfels J, Lavrynenko O, Ayciriex S, Sampaio JL, Carvalho M, Shevchenko A, Eaton S: Production of systemically circulating Hedgehog by the intestine couples nutrition to growth and development. Genes Dev 2014, 28:2636–2651. [PubMed: 25452274]

65. Gu SH: Autocrine activation of DNA synthesis in prothoracic gland cells of the silkworm, Bombyx mori. J Insect Physiol 2006, 52:136–145. [PubMed: 16266718]

66. Gu SH: Autocrine activation of ecdysteroidogenesis in the prothoracic glands of the silkworm, Bombyx mori. J Insect Physiol 2007, 53:538–549. [PubMed: 17442336]

67. Ohhara Y, Shimada-Niwa Y, Niwa R, Kayashima Y, Hayashi Y, Akagi K, Ueda H, Yamakawa-Kobayashi K, Kobayashi S: Autocrine regulation of ecdysone synthesis by beta3-octopamine receptor in the prothoracic gland is essential for Drosophila metamorphosis. Proc Natl Acad Sci U S A 2015, 112:1452–1457. [PubMed: 25605909]

68. Niwa YS, Niwa R: Transcriptional regulation of insect steroid hormone biosynthesis and its role in controlling timing of molting and metamorphosis. Dev Growth Differ 2016, 58:94–105. [PubMed: 26667894]

69*. Uryu O, Ou Q, Komura-Kawa T, Kamiyama T, Iga M, Syrzycka M, Hirota K, Kataoka H, Honda BM, King-Jones K, et al.: Cooperative Control of Ecdysone Biosynthesis in Drosophila by Transcription Factors Seance, Ouija Board, and Molting Defective. Genetics 2018, 208:605–622. [PubMed: 29187506] Describes an interesting transcritption network that regulates certain groups of Ecdysone biosynthtic enzymes.

70. Huynh N, Ou Q, Cox P, Lill R, King-Jones K: Glycogen branching enzyme controls cellular iron homeostasis via Iron Regulatory Protein 1 and mitoNEET. Nat Commun 2019, 10:5463. [PubMed: 31784520]

71. Danielsen ET, Moeller ME, Yamanaka N, Ou Q, Laursen JM, Soenderholm C, Zhuo R, Phelps B, Tang K, Zeng J, et al.: A Drosophila Genome-Wide Screen Identifies Regulators of Steroid Hormone Production and Developmental Timing. Dev Cell 2016, 37:558–570. [PubMed: 27326933]

72. Enya S, Yamamoto C, Mizuno H, Esaki T, Lin HK, Iga M, Morohashi K, Hirano Y, Kataoka H, Masujima T, et al.: Dual Roles of Glutathione in Ecdysone Biosynthesis and Antioxidant Function During Larval Development in Drosophila. Genetics 2017, 207:1519–1532. [PubMed: 29021278]

73. Di Cara F, King-Jones K: The Circadian Clock Is a Key Driver of Steroid Hormone Production in Drosophila. Curr Biol 2016, 26:2469–2477. [PubMed: 27546572]

74. Enya S, Daimon T, Igarashi F, Kataoka H, Uchibori M, Sezutsu H, Shinoda T, Niwa R: The silkworm glutathione S-transferase gene noppera-bo is required for ecdysteroid biosynthesis and larval development. Insect Biochem Mol Biol 2015, 61:1–7. [PubMed: 25881968]

75. Caceres L, Necakov AS, Schwartz C, Kimber S, Roberts IJ, Krause HM: Nitric oxide coordinates metabolism, growth, and development via the nuclear receptor E75. Genes Dev 2011, 25:1476–1485. [PubMed: 21715559]

76. Ou Q, Zeng J, Yamanaka N, Brakken-Thal C, O'Connor MB, King-Jones K: The Insect Prothoracic Gland as a Model for Steroid Hormone Biosynthesis and Regulation. Cell Rep 2016, 16:247–262. [PubMed: 27320926]

77. Nakaoka T, Iga M, Yamada T, Koujima I, Takeshima M, Zhou X, Suzuki Y, Ogihara MH, Kataoka H: Deep sequencing of the prothoracic gland transcriptome reveals new players in insect ecdysteroidogenesis. PLoS One 2017, 12:e0172951. [PubMed: 28257485]

78. Alexandratos A, Moulos P, Nellas I, Mavridis K, Dedos SG: Reassessing ecdysteroidogenic cells from the cell membrane receptors' perspective. Sci Rep 2016, 6:20229. [PubMed: 26847502]

79. Moulos P, Alexandratos A, Nellas I, Dedos SG: Refining a steroidogenic model: an analysis of RNA-seq datasets from insect prothoracic glands. BMC Genomics 2018, 19:537. [PubMed: 30005604]

80. Palm W, Sampaio JL, Brankatschk M, Carvalho M, Mahmoud A, Shevchenko A, Eaton S: Lipoproteins in Drosophila melanogaster--assembly, function, and influence on tissue lipid composition. PLoS Genet 2012, 8:e1002828. [PubMed: 22844248]

81. Igarashi F, Ogihara MH, Iga M, Kataoka H: Cholesterol internalization and metabolism in insect prothoracic gland, a steroidogenic organ, via lipoproteins. Steroids 2018, 134:110–116. [PubMed: 29410082]

82. Talamillo A, Herboso L, Pirone L, Perez C, Gonzalez M, Sanchez J, Mayor U, Lopitz-Otsoa F, Rodriguez MS, Sutherland JD, et al.: Scavenger receptors mediate the role of SUMO and Ftz-f1 in Drosophila steroidogenesis. PLoS Genet 2013, 9:e1003473. [PubMed: 23637637]

83. Ravikumar G, Vijayaprakash N: Lipophorin receptor: the insect lipoprotein receptor. Resonance 2013, 18:748–755.

84. Huang X, Warren JT, Gilbert LI: New players in the regulation of ecdysone biosynthesis. J Genet Genomics 2008, 35:1–10. [PubMed: 18222403]

85. Enya S, Ameku T, Igarashi F, Iga M, Kataoka H, Shinoda T, Niwa R: A Halloween gene noppera-bo encodes a glutathione S-transferase essential for ecdysteroid biosynthesis via regulating the behaviour of cholesterol in Drosophila. Sci Rep 2014, 4:6586. [PubMed: 25300303]

86. Zeng J, Kamiyama T, Niwa R, King-Jones K: The Drosophila CCR4-NOT complex is required for cholesterol homeostasis and steroid hormone synthesis. Dev Biol 2018, 443:10–18. [PubMed: 30149007]

87*. Pan X, Neufeld TP, O'Connor MB: A Tissue- and Temporal-Specific Autophagic Switch Controls Drosophila Pre-metamorphic Nutritional Checkpoints. Curr Biol 2019, 29:2840–2851 e2844. [PubMed: 31422886] This study demonstrates that autophagy in the PG cells regulates ecdysteroid biosynthesis by mediating cholesterol trafficking. Intriguingly, the autophagy process in the PG is induced by starvation in a stage-dependent way, which mediates the determination of the critical weight nutritional checkpoint.

88*. Texada MJ, Malita A, Christensen CF, Dall KB, Faergeman NJ, Nagy S, Halberg KA, Rewitz K: Autophagy-Mediated Cholesterol Trafficking Controls Steroid Production. Dev Cell 2019, 48:659–671 e654. [PubMed: 30799225] This study also reports on the role of autophagy in cholesterol trafficking and ecdysteroid synthesis in the PG. It shows that autophagy is regulated by the Hippo pathway in response to nutrition, and a basal level of autophagy occurs even under non-starvation condition which regulates ecdysteroid synthesis and body size of the animal.

89. Feyereisen R: A specific binding protein for the moulting hormone ecdysterone in locust haemolymph. Experientia 1977, 33:1111–1113. [PubMed: 891832]

90. Yamanaka N, Marques G, O'Connor MB: Vesicle-Mediated Steroid Hormone Secretion in Drosophila melanogaster. Cell 2015, 163:907–919. [PubMed: 26544939]

91**. Okamoto N, Viswanatha R, Bittar R, Li Z, Haga-Yamanaka S, Perrimon N, Yamanaka N: A Membrane Transporter Is Required for Steroid Hormone Uptake in Drosophila. Dev Cell 2018, 47:294–305 e297. [PubMed: 30293839] This study reports the identification of an ecdysone importer, EcI, which mediates the uptake of ecdysone in peripheral tissues. this helps cement the idea that steroid hormones do not simply pass through membranes by diffusion.

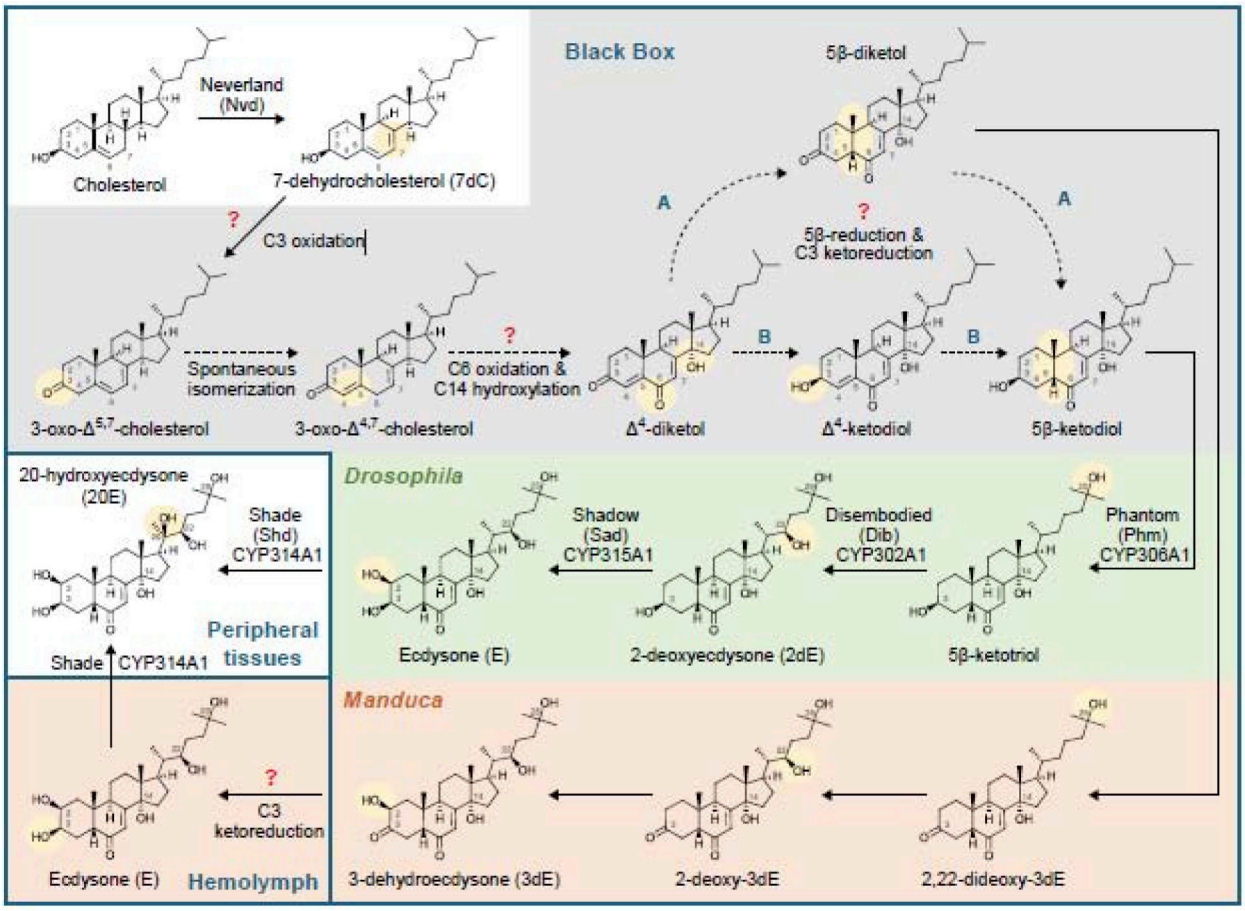

Figure 1.

The ecdysteroid biosynthetic pathway (see text for details). Light yellow circles mark the modification sites in each reaction. The Black Box reactions are marked by light grey background. Red question marks indicate the catalyzing enzymes are unknown. “A” and “B” presents the two alternative ways in which Δ^4-diketol is transformed into 5β-ketodiol. The post-Black Box reactions in *Drosophila* and *Manduca* are marked by light green and light orange background, respectively.

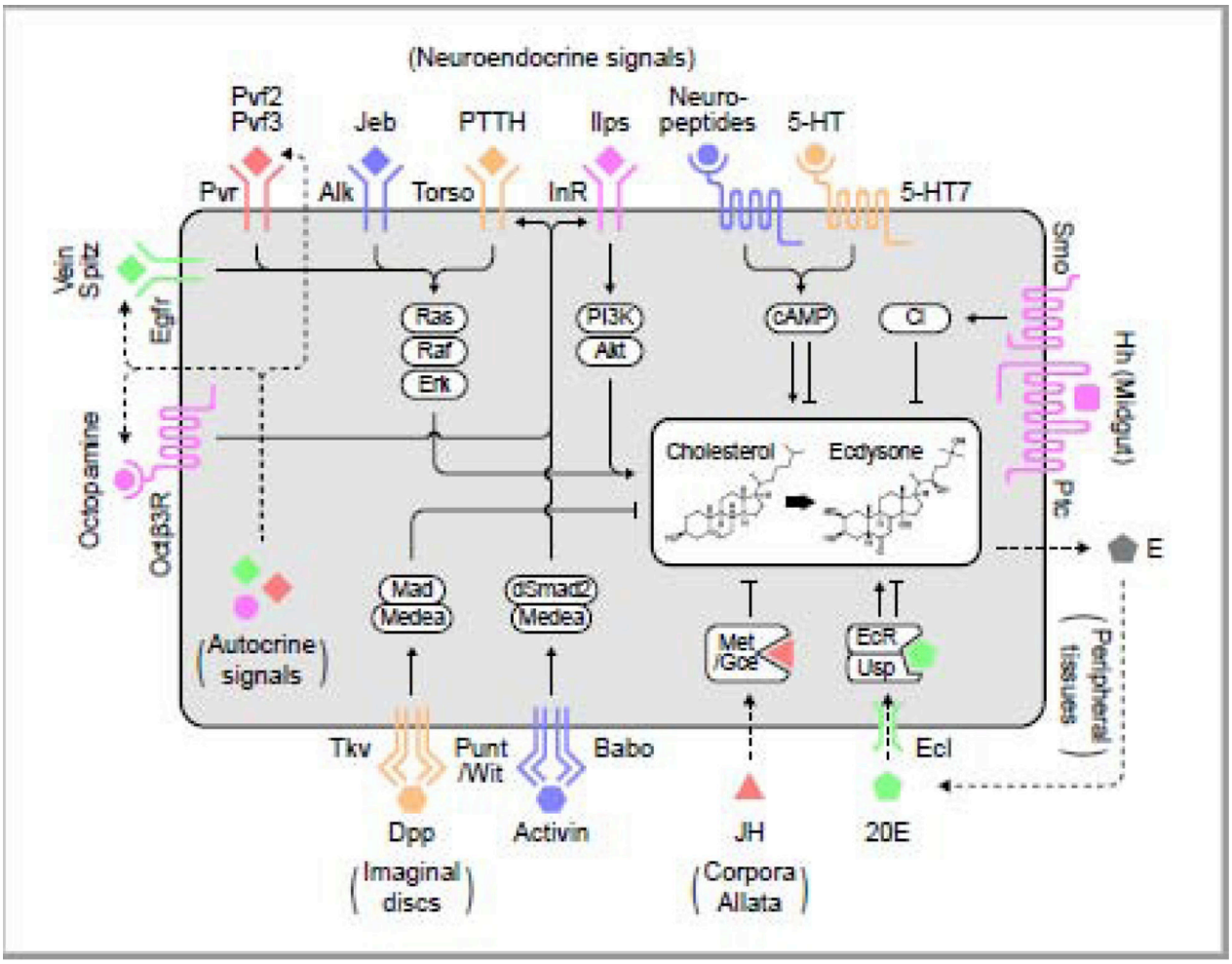

Figure 2.
Extracellular signals regulating ecdysteroid synthesis in the PG (see text for details).

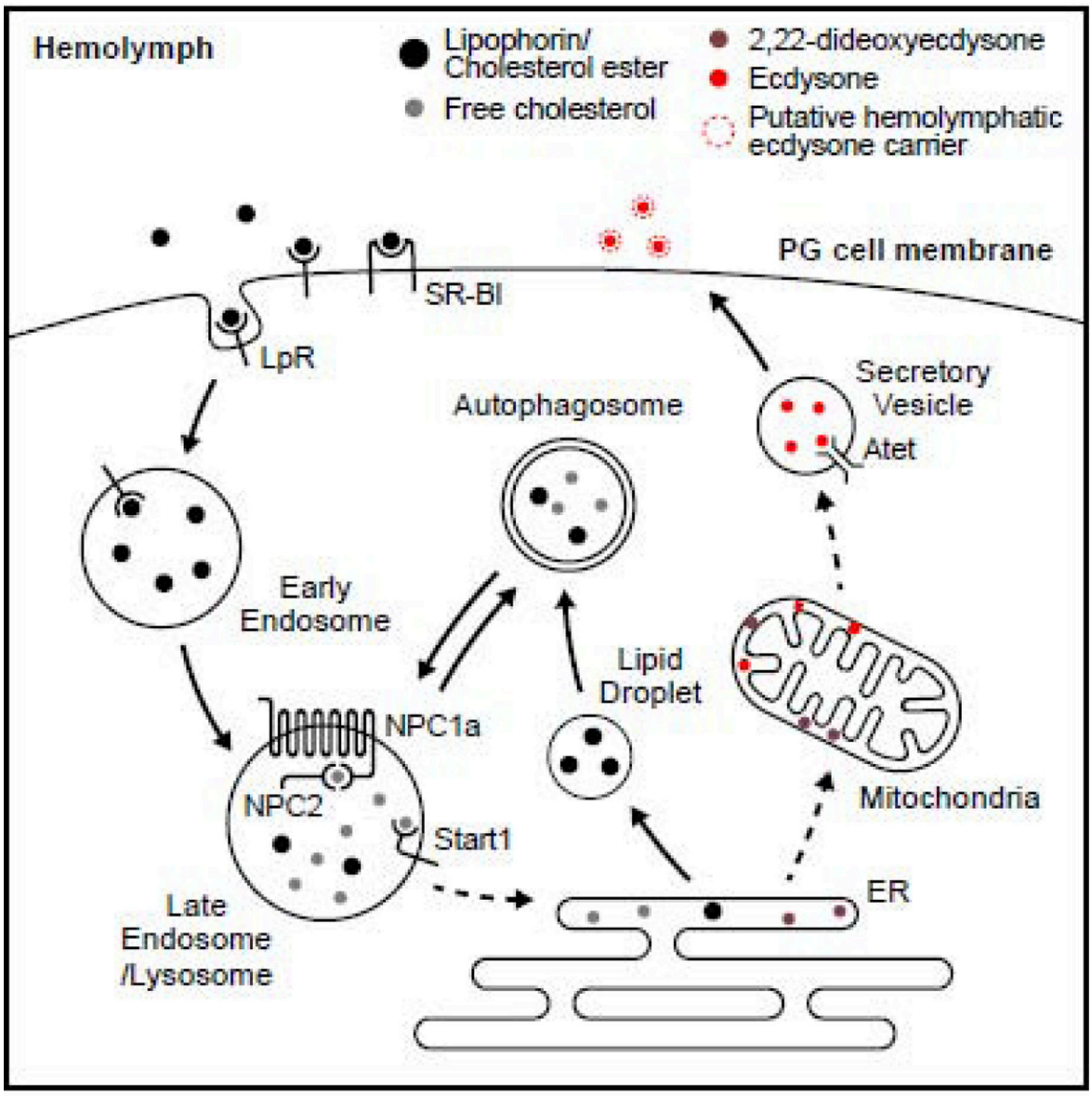

Figure 3.

Steroid trafficking during ecdysteroidogenesis in PG cells (see text for details). Dashed line indicates that the steroid trafficking process between the two cellular compartments are not fully understood.