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VIEWPOINT

Mammalian puberty: a fly perspective

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Mammalian puberty and *Drosophila* metamorphosis, despite their evolutionary distance, exhibit similar design principles and conservation of molecular components. In this Viewpoint, we review recent advances in this area and the similarities between both processes in terms of the signaling pathways and neuroendocrine circuits involved. We argue that the detection and uptake of peripheral fat by *Drosophila* prothoracic endocrine cells induces endomembrane remodeling and ribosomal maturation, leading to the acquisition of high biosynthetic and secretory capacity. The absence of this fat–neuroendocrine interorgan communication leads to giant, obese, non-pupating larvae. Importantly, human leptin is capable of signaling the pupariation process in *Drosophila*, and its expression prevents obesity and triggers maturation in mutants that do not pupate. This implies that insect metamorphosis can be used to address issues related to the biology of leptin and puberty.

Introduction

Puberty marks the transformation of the child into the adult. It constitutes a point of no return defined as the transitional period when sexual maturity is achieved along with important growth and behavioral changes [1,2]. When this change is initiated, upregulation of steroidogenesis leads to an irreversible juvenile-to-adult transition in humans. Insect metamorphosis is the developmental transition comparable to human

Abbreviations
5-HT, 5-hydroxytryptamine; 5-HT7, 5-hydroxytryptamine receptor 7; Alk, anaplastic lymphoma kinase; AMPK, AMP-activated protein kinase; apolpp, apolipophorin; AstA, allatostatin A; AstAR1, allatostatin receptor 1; Crz, corazonin; CrzR, corazonin receptor; Dpp, decapentaplegic; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Gce, germ cell–expressed; GnRH, gonadotropin-releasing hormone hypothalamic neurons; GRASP, green fluorescent protein reconstitution across synaptic partners; HPG, hypothalamic–pituitary–gonadal axis; IGF1, insulin-like growth factor I; IIS, insulin/insulin-like growth factor; Kiss1R, kisspeptin-1 receptor; KNDy, kisspeptin/neurokinin B/dynorphin neurons; Kr-h1, Krüppel homolog 1; Lgr3, leucine-rich repeat–containing G protein–coupled receptor 3; MAP, mitogen-activated protein; MC3R, melanocortin receptor 3; MC4R, melanocortin receptor 4; Met, methoprene-tolerant; mTOR, mammalian target of rapamycin; NPY/AgRP, neuropeptide Y and agouti-related peptide neurons; Octβ3R, β3-octopamine receptor; PDF, pigment-dispersing factor; PG, prothoracic gland; POMC/CART, pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript neurons; PTTH, prothoracicotropic hormone; $SE0_{PG}$, stomatogastric serotoninergic neurons; Sema1a, semaphorin1a; SIRT1, sirtuin 1; TGF-β, transforming growth factor-β; TkV, thickveins; tor, torso; TSC2, tumor suppressor tuberous sclerosis complex 2 and upd2: unpaired 2.

puberty, and despite more than 500 million years of evolutionary distance between insects and mammals, recent studies summarized in this Viewpoint have shown that the transition from juvenile to adult is well conserved in both and that it is governed by common molecular aspects ([3] and Fig. 1).

Endocrine control of mammalian puberty

In mammals, the reawakening of the hypothalamic–pituitary–gonadal (HPG) axis is what triggers pubertal maturation (Fig. 1). In response to incompletely defined nutrient, endocrine and environmental signals, neuroendocrine gonadotropin-releasing hormone (GnRH) hypothalamic neurons increase their firing properties [4,5], which stimulates the pituitary gland to release the gonadotropins luteinizing hormone and follicle-stimulating hormone. These hormones are then delivered via the circulatory system to promote the maturation of the gonads and the production of sex steroids, which signal back to the HPG axis.

GnRH neurons have the particularity of developing embryonically outside the brain, in the nasal placode, and they migrate to their final position in the hypothalamus before birth [6]. In addition, GnRH neurons during postnatal life recruit other populations—neuronal, glial (tanycytes and astrocytes), and endothelial—that connect with them to establish the 'GnRH neural network' [7–9]. This neural network fine-tunes GnRH production and secretion in time and space. Therefore, defects in the GnRH neurons themselves, or in the GnRH neural network, result in defective or delayed puberty [10]. Interestingly, the intercellular communication within the GnRH neural network is predominantly mediated by semaphorin signaling, which plays a fundamental role in the development of hypothalamic circuits but also in the control of GnRH release by circulating sex steroids [11]. Defective semaphorin signaling leads to hypogonadotropic

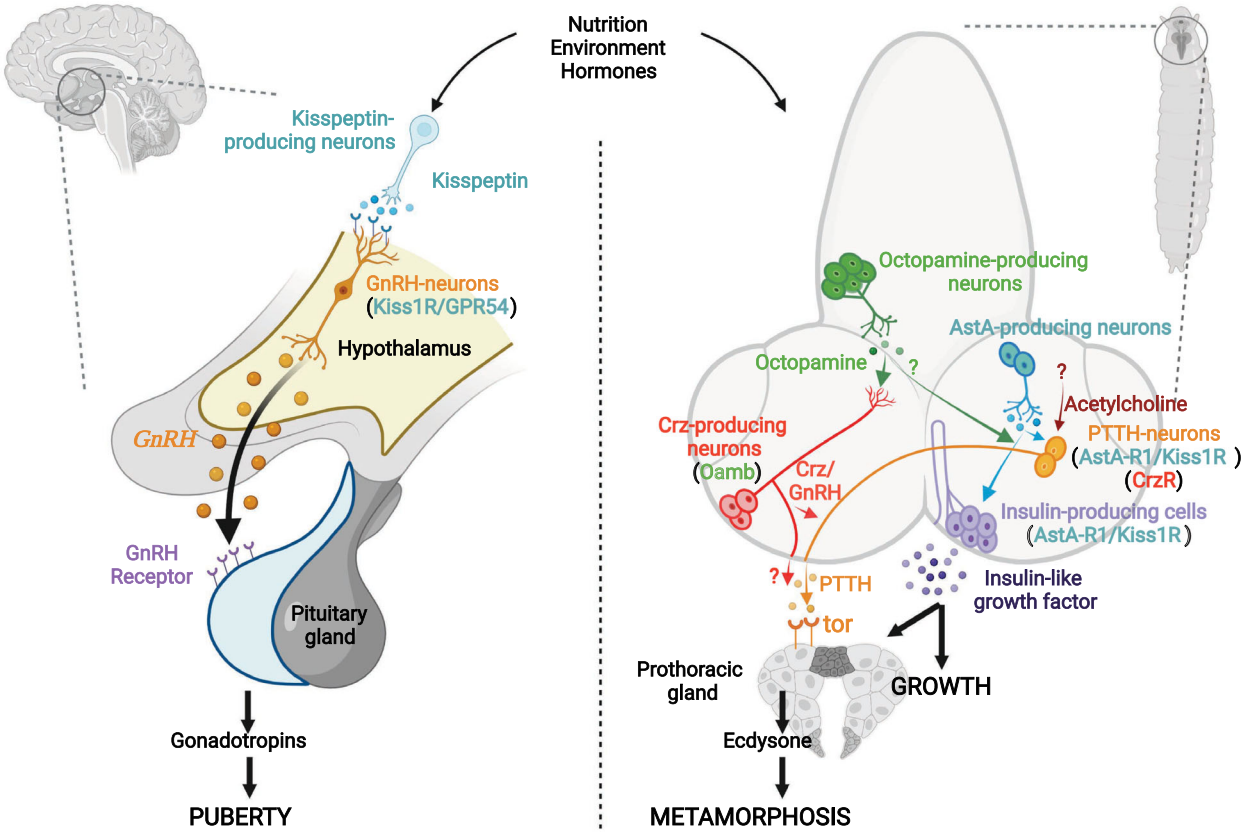

Fig. 1. Neuroendocrine circuits controlling puberty (left) and metamorphosis (right). Note that for simplicity, only one pathway in each *Drosophila* lobe is shown, although these neural circuits are duplicated in each lobe. We have also reversed the orientation of the *Drosophila* larval brain to facilitate comparison with the mammalian brain. AstA, allatostatin A; AstAR1, allatostatin A receptor 1; Crz, corazonin; CrzR, corazonin receptor; GnRH, gonadotropin-releasing hormone; Kiss1, kisspeptin 1; Kiss1R, kisspeptin 1 receptor; Oamb, octopamine receptor in mushroom bodies; PTTH, prothoracicotropic hormone; and tor, torso.

hypogonadism [12], defective neuroendocrine control of the adult ovarian cycle [13], and obesity [14].

The most potent upstream pubertal signal known to activate pulsatile firing of GnRH neurons is the neuropeptide kisspeptin, a product of the *Kiss1* gene, which activates the G protein–coupled membrane receptor Kiss1R (also known as GPR54) in GnRH neurons in the median eminence [15–18]. Mature kisspeptins in mammals are cleaved, and administration of decapeptide Kp10 (Kiss-10), the minimum active site, can elicit a robust increase in the circulating levels of GnRH [19]. Kisspeptin-positive neurons are widespread in the hypothalamus, mainly in the arcuate nucleus and in the rostral anteroventral periventricular area [20], with a third, less explored, population in the amygdala [21].

Kisspeptin neurons in the arcuate nucleus, named kisspeptin/neurokinin B/dynorphin (KNDy) neurons, also coexpress the tachykinin neurokinin B and the endogenous opioid peptide dynorphin [22,23]. Increased activity of the kisspeptin–Kiss1R signal in response to complex and ill-defined reciprocal positive and inhibitory signals mediated by KNDy neurons correlates with the GnRH pulse generator and the onset of puberty [24]. The kisspeptin neuron population in the anteroventral periventricular area is notably larger in female rodents and is involved in the preovulatory surge of gonadotropins [25]. Importantly, a lack of either kisspeptin or Kiss1R results in absent or delayed puberty onset in animals and hypogonadotropic hypogonadism in humans [26–28]. However, genetic ablation of kisspeptin or Kiss1R neurons using a diphtheria toxin fragment specifically in these cells leads to no change in the timing of the onset of puberty or attainment of fertility [29]. Interestingly, if kisspeptin neurons are ablated in adult mice, fertility is inhibited, suggesting that there is compensation during the formation of reproductive neural circuits that occurs early in development [30].

The onset of puberty is regulated by many permissive factors [31]. For example, in seasonal breeders the photoperiod signals the optimal time of year for the onset of puberty [32]. Among the different pubertal regulators, nutritional and metabolic cues have been shown to play a critical role in the central control of puberty, with numerous studies in rodents and humans indicating that a female's fat reserve must exceed a critical threshold to allow the onset of puberty [33] and thus signal the attainment of sufficient somatic growth to support pregnancy. In fact, the escalating prevalence of child obesity has been blamed for alterations of the age of onset of puberty [34], and malnutrition and intensive physical training can delay puberty [35,36].

In this regard, classical studies in mice [37] and humans [38,39] showed that a deficiency of leptin (a hormone secreted by fat cells) or its receptors (which signal the amount of energy stored in the body) leads to hyperphagia, early-onset obesity, and delayed or complete failure to initiate the pubertal transition. Despite these phenotypes and the fact that leptin modulates the expression of Kisspeptin in the hypothalamus [40], leptin receptors are low or null in Kisspeptin neurons and GnR neurons [20,41], which supports an indirect mode of action of leptin and other hormonal signals in the regulation of these neuronal populations via mechanisms that are poorly defined. For example, other populations that would play a fundamental role in these processes would be anorexigenic neurons expressing pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART neurons) and orexigenic neurons expressing neuropeptide Y and agouti-related peptide (NPY/AgRP neurons), which are activated in conditions of an excess and deficit of energy, respectively. NPY and AgRP have been shown to inhibit Kisspeptin neurons [42], whereas POMC neuropeptides (alpha-melanocyte stimulating hormone and CART) have been reported to modulate GnRH neurosecretory activity acting via melanocortin receptors (MC3R and MC4R) [43–45]. In a recent article, Lam et al. [46] reported that melanocortin signaling via MC3R also plays a fundamental role in the control of puberty in humans. Humans who carry loss-of-function mutations in MC3R showed delayed puberty accompanied by reductions in linear growth, lean mass and circulating levels of insulin-like growth factor I (IGF1), thus linking conserved nutritional cues to control of puberty [46]. Finally, an alternative to the canonical kisspeptin–HPG pathway involving *de novo* ceramide synthesis at the hypothalamic paraventricular nucleus and ovarian sympathetic innervation has recently been characterized as playing a fundamental role in obesity-induced precocious puberty in female rats [47].

While it is mainly lipid signaling molecules that seem to play a fundamental role in the onset of puberty, other master metabolic hypothalamic sensors, such as the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and sirtuin 1 (SIRT1) [48], have also been linked to metabolic/nutritional status and pubertal timing.

For instance, AMPK (a highly conserved serine/threonine kinase that senses glucose and energy status) and SIRT1 (a nicotinamide adenine dinucleotide [NAD+]-dependent deacetylase) are activated in conditions of negative energy balance, and both operate in kisspeptin-positive neurons in the arcuate

hypothalamus to delay pubertal timing independently of body weight [49,50]. At least for SIRT1, pubertal timing mechanistically involves epigenetic repression of the puberty-activating gene *Kiss1* [50]. In contrast, mTOR (a second serine/threonine kinase, which detects amino acid availability) is activated in the inverse pattern and has the opposite behavior to those of AMPK and SIRT1, at the time of puberty [51]. Mechanistically, in mouse embryonic fibroblasts, activated AMPK can inhibit mTORC1 by directly phosphorylating the tumor suppressor tuberous sclerosis complex 2 (TSC2) and the critical mTOR complex 1 (mTORC1)-binding subunit, raptor [52]; in addition, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme, has been shown to be a critical mediator of AMPK-driven SIRT1 activation [53]. Future research will determine the mode of action of these cellular sensors in Kiss1 cells or in other populations of the GnRH neural network in the control of puberty.

Integration of signals by PTTH neurons in the coordination of growth and metamorphosis

In the larval central brain of *Drosophila melanogaster*, a pair of prothoracicotropic hormone (PTTH)-releasing neurons located in each hemisphere has long been considered to be the primary promoting factor in the biosynthesis of the hormone ecdysone, the main steroid in insects. These neurons project and secrete PTTH, a neuropeptide initially identified in the silkworm *Bombyx mori* [54], into the prothoracic gland (PG), where they activate the Ras/Raf/ERK (extracellular-signal-regulated kinase) MAP (mitogen-activated protein) kinase pathway via its receptor tyrosine kinase, torso, to promote ecdysone production for regulating larval maturation [55]. *Ptth* gene transcription significantly increases 12 h before pupariation, and genetic ablation of PTTH neurons extends the third instar (L3) larval stage and delays the time to pupariation by 4 or 5 days, with a significant increase in adult body size [56]. A recent study in *Ptth*-null mutants showed only a modest delay of 1 day in the timing of metamorphosis [57], indicating that PTTH cannot be considered as the main promotor of ecdysone synthesis. The existence of one or more additional ecdysteroidogenic signals produced by the PTTH neurons could explain the difference in delay observed in response to PTTH loss compared with PTTH neuron ablation [58].

Recent studies have reported that the timing of PTTH secretion is controlled by different neuropeptides expressed in different larval cell populations (Fig. 1), including the *Drosophila* kisspeptin homolog allatostatin A (AstA) [59] and GnRH homolog corazonin [60], as well as the neurotransmitters acetylcholine and octopamine [61].

AstA is expressed in a pair of neurons located in the basolateral protocerebrum, and GRASP (green fluorescent protein [GFP] reconstitution across synaptic partners) analysis [62] detected a physical interaction between the axons of AstA neurons and the dendrites of PTTH neurons and insulin-producing cells [59]. AstA receptor 1 (AstAR1) is the insect homolog of mammalian Kiss1R, and its knockdown in PTTH neurons resulted in developmental delay and larger pupae—a phenotype similar to that of *Ptth*-null mutants [57,59].

A second neuropeptide, corazonin, is expressed in three pairs of neurons located in the dorsolateral and dorsomedial protocerebrum [63,64]. GRASP analysis on this cell population detected GFP expression in PTTH neurons and PG cells [60]. Interestingly, ultrastructural studies confirmed this communication and revealed bidirectional connectivity between corazonin and PTTH neurons [60]. In this case, knockdown of the corazonin receptor (CrzR), a member of the GnRH receptor superfamily on PTTH neurons, increased pupal body size without affecting pupariation timing [60].

Classical neurotransmitters that are widespread in the central larval nervous system are also involved in regulating the activity of PTTH neurons in *Drosophila*. In a recent report, Hao et al. [61] showed that among neurotransmitters, only acetylcholine and low doses of octopamine increased intracellular Ca^{2+} levels in PTTH neurons. Pharmacological treatment with cadmium chloride, a voltage-dependent Ca^{2+} channel antagonist, abolished octopamine-induced Ca^{2+} responses in the green fluorescent calcium sensor GCaMP6m (medium)-expressing PTTH cells [61], suggesting that low doses of octopamine might modulate the activity of PTTH cells via the G protein–coupled β3-octopamine receptors (Octβ3R). Octopamine-mediated signaling, likely occurring at the level of the subesophageal zone, has been shown to regulate corazonin neurons in systemic growth [60]. Genetic analysis performed only on acetylcholine receptors showed that depletion of the α1 and α3 subunits of nicotinic receptors, but not muscarinic receptors, reduced the Ca^{2+} responses of PTTH neurons to acetylcholine and also increased pupal body size without affecting pupariation timing [61]; this is a very similar phenotype to that obtained with the neuropeptide corazonin [60]. The lack of effect on pupariation time suggests that corazonin and acetylcholine neurons affect PTTH neurons to promote basal ecdysteroid biosynthesis, but not its peak. Monitoring of GCaMP6s (slow)

activity in PTTH neurons after corazonin neuron activation revealed a strong response only during the mid-L3 larval stage, but not later [60], which would support this hypothesis. In contrast, maximal AstA/AstAR1 activity has been observed toward the end of larval development [59], likely anticipating the rise in PTTH levels and the onset of metamorphosis.

Furthermore, extensive studies have shown that PTTH neurons are under the control of circadian rhythms in the blood-feeding hemipteran *Rhodnius prolixus* [65]. In *Drosophila*, pigment-dispersing factor (PDF)-producing clock neurons contact PTTH neurons, and PDF influences the transcriptional periodicity and attenuates the rise of *Ptth* transcription before pupariation [56] suggesting that circadian rhythms may control PTTH release in *Drosophila* as well. When larvae face tissue damage signals during their development, PTTH neurons and insulin-producing cells receive inputs from leucine-rich repeat–containing G protein–coupled receptor 3 (Lgr3)-positive neurons [66] to synchronize growth and maturation until the damage is resolved.

PG neurons as a neuroendocrine center in the coordination of growth and metamorphosis: a network of increasing complexity

As the major endocrine organ that dictates pupariation, the PG plays a central role in integrating multiple signals (Fig. 2) that inform the state of growth and maturation to ultimately control secretion from the organ and trigger the juvenile-to-adult transition. These cross talks include autocrine signals generated in the PG itself, which do not involve PTTH neuron activity [58,67,68], and brain-derived [64,69] and

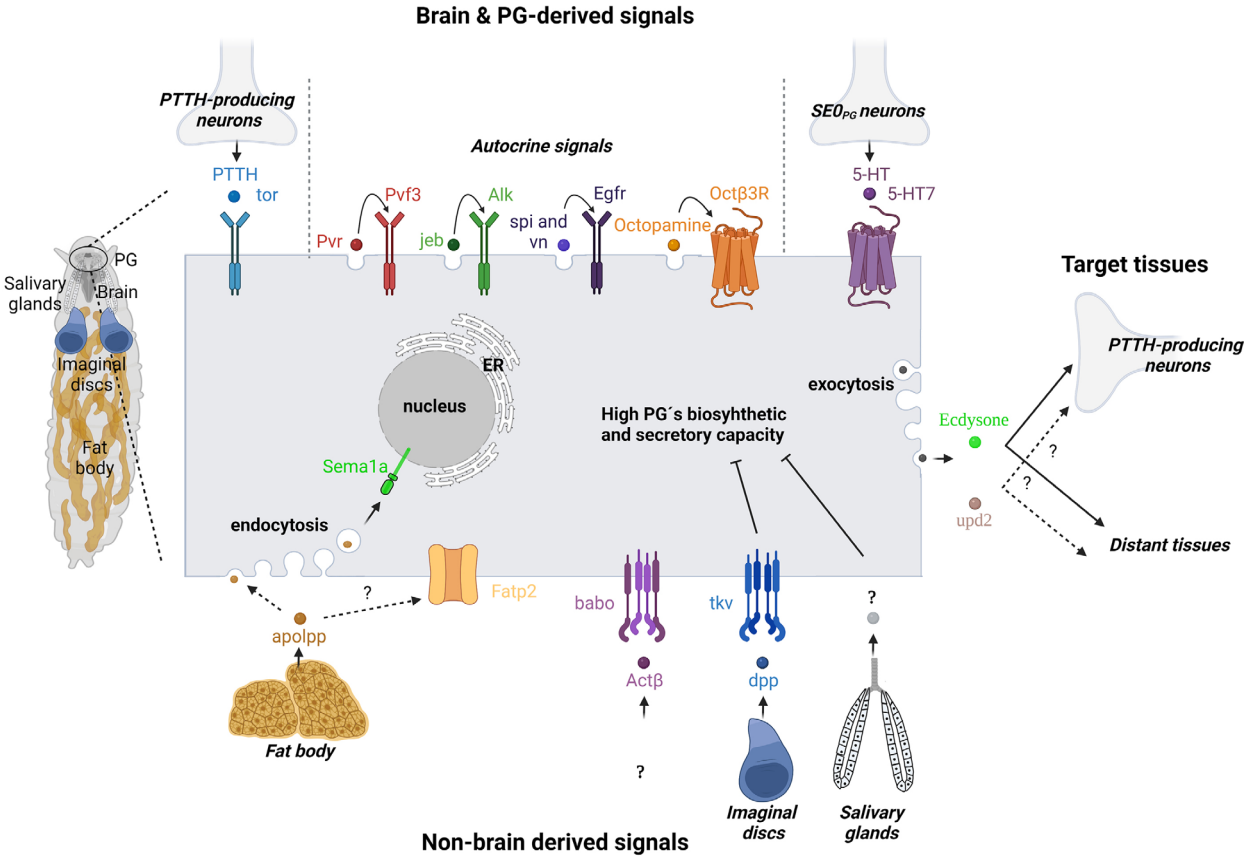

Fig. 2. Scheme of the integration of autocrine, brain- and non-brain-derived signals by the prothoracic gland for the coordination of growth and maturation in *Drosophila*. 5-HT, 5-hydroxytryptamine (serotonin); 5-HT7, 5-hydroxytryptamine (serotonin) receptor 7; Actβ, activin-β; Alk, anaplastic lymphoma kinase; apolpp, apolipophorin; babo, baboon; dpp, decapentaplegic; Ec, ecdysone; Egfr, epidermal growth factor receptor; ER, endoplasmic reticulum; Fatp2, fatty acid transport protein 2a; jeb, jelly belly; Octβ3R, octopamine β3 receptor; PTTH, prothoracicotropic hormone; Pvf3, PDGF- and VEGF-related factor 3; PG, prothoracic gland; Pvr, PDGF- and VEGF-receptor related; tkv, thickveins; Sema1a, semaphorin1a; spi, spitz; tor, torso; upd2, unpaired 2; and vn, vein.

non-brain-derived signals [70–73] from organs that convey nutritional status, environmental development and other unknown inputs that directly impact the PG.

Autocrine signals primarily include multiple receptor tyrosine kinases (anaplastic lymphoma kinase [Alk], PDGF- and VEGF-receptor related [Pvr], and epidermal growth factor receptor [EGFR]) that, together with PTTH/torso, seem to share the same signaling pathway to control pupation through Ras/ERK activation [58]. Why is there this redundancy of receptor tyrosine kinase signaling in the PG? Different explanations have been proposed: (a) to confer robustness and flexibility in response to changing developmental conditions, (b) to act synchronously or sequentially and achieve Ras/ERK activation that is strong enough to drive the massive PG secretion necessary for carrying out puparation or (c) to induce other unidentified downstream signaling pathways in addition to Ras/ERK signaling [58]. Although receptor tyrosine kinase activation is the predominant signaling in the PG, activation by monoaminergic autocrine signaling through the G protein–coupled octopamine-β3 receptor (Octβ3R) has also been reported to act upstream of insulin/insulin-like growth factor (IIS) ligand family and Ras/ERK signaling [68]. A second G protein–coupled receptor in the PG, 5-hydroxytryptamine receptor 7 (5-HT7), responds to food-related signals from a subset of stomatogastric serotoninergic ($SE0_{PG}$) neurons that directly innervate the PG [69]. In conditions of nutrient scarcity, the projections of $SE0_{PG}$ neurons are reduced [69], leading to decreased 5-HT signaling to PG cells and, as a result, reduced ecdysone release and delayed development, likely due to defective translation in those cells [74].

Multiple inputs from non-cerebral organs seem capable of influencing pupariation through diffusible signals reaching the PG (Fig. 2). Unlike the signaling of tissue damage mediated by the positive regulation of insulin-like peptide 8 [66,75], under normal physiological conditions, decapentaplegic (Dpp), a ligand of the transforming growth factor-β (TGF-β) signaling pathway, is released from the imaginal disks to the hemolymph and its signaling via the thickveins (Tkv) receptor also negatively regulates the production of ecdysone [72]. Activin, another TGF-β ligand, the source of which is unknown, functions in this process in the PG antagonistically to Dpp signaling [71]. Although the mode of integration of these pathways is not well defined, the hypothesis predicts that Dpp signaling must be reduced in the PG to allow pupariation to proceed. A recent study has shown that the salivary gland–derived peptide Sgsf is secreted into the hemolymph to regulate systemic growth via the IIS/target of rapamycin (TOR) signaling pathway without affecting metamorphic timing [73]. Interestingly, ablation of the salivary glands is necessary to control metamorphic timing, which also suggests the existence of one or more additional signals produced by salivary gland cells through mechanisms that are still unknown.

Despite this cross talk of multiple pathways converging on the PG, it remains to be clarified how these triggers of ecdysone biosynthesis affect cholesterol trafficking [76–78] and the transcription and chromatin remodeling of *Halloween* ecdysteroid biosynthetic genes [79,80] for timing ecdysone production in response to these stimulus-triggered signaling pathways. Although 20 ecdysteroidogenic transcription factors have been identified to date, we still do not fully understand their mechanism of action and whether it is direct or indirect, or their mode of interaction in the expression of *Halloween* genes. There is evidence that the transcription of *Halloween* genes is directly inhibited by epigenetic control, via the nuclear receptors methoprene-tolerant (Met) and germ cell–expressed (Gce) via Krüppel homolog 1 (Kr-h1) [81,82].

It is also unclear whether communication between the fat body, the larval fat and energetic reservoir, and the PG (either directly or indirectly via PTTH neurons) informs that adequate overall reserves of energy or specific nutrients/metabolites have been reached to meet the demands of subsequent reproduction and thus allow pupariation. If this were so, it would mean that despite the evolutionary distance, the process of metamorphosis is governed by principles more similar to puberty than we might think. We also mostly do not know what cellular processes occur at the level of the PG once the larva has passed the critical weight, leading to the production and secretion of ecdysone [83].

We have recently described that inhibiting lipid transport from the fat body via knockdown of *apolipophorin* (*apolpp*), or in the PG via knockdown of *Fatty acid transport protein 2* (*Fatp2*), *Semaphorin1a* (*Sema1a*) and leptin-like *unpaired 2* (*upd2*), precludes the transition through the critical weight without measurable impairment in the IIS signaling pathway and results in larvae that never left the food and continued growing and gaining weight until death [70]; these effects are analogous to those of leptin/LepR loss in patients and mice. Silencing of cationic amino acid and sugar transporters had no effect. These results support a critical role of lipid signaling, transport, and sensing in the events leading to sexual maturation commitment. Validating our conclusion, expression of the human leptin transgene in the PG rescued both the

obese and non-pupating phenotypes of *upd2*-mutant and *Sema1a*-mutant animals, suggesting that a fat sensor mechanism similar to the leptin system in mammals may act in the larval PG to coordinate body weight and growth for pupariation. Supporting the involvement of lipids in sexual maturation, a recent study has shown that enhancing cholesterol signaling in PG cells, a substrate for ecdysone production, leads to increased body growth and premature pupariation [84].

Interestingly, *Sema1a*^{i} PGs are distinctly different from PGs with knockdown in the IIS/TOR pathway: we did not detect any alteration in the size or endoreplication of the *Sema1a*^{i} PG cells, a feature previously linked to ecdysone synthesis [85]. Neither IIS/TOR nor PTTH/torso/Ras activation corrected larval arrest and obesity caused by *Sema1a* depletion in PG cells [70]. Our finding that TOR activation cannot rescue *Sema1a* deficiency in the PG suggests either that nutrient sensing by TOR is upstream of Sema1a or that Sema1a-mediated events may provide competence for responding to TOR. We favor this second possibility.

Analysis of the metabolic status of *Drosophila* larvae using an optimized NMR profiling assay and other commercial assays in dissected tissues and whole animals [86] showed elevated levels of sugar and lipids accumulated during the extension of feeding behavior [70]. Together with this metabolic analysis, we used super-resolution imaging of the PG to determine that the high secretory competence of the PG requires endocytosis, endoplasmic reticulum remodeling and ribosomal maturation for the acquisition of the high biosynthetic and secretory capacity of the PG cells; all these processes are Sema1a-dependent. Interestingly, we found that nanobody-based retention of upd2::GFP in the nuclei of PG cells led to a non-pupating and massively obese phenotype that was indistinguishable from the phenotype resulting from the knockdown of *upd2* in PG cells [70]. In the brain and peripheral organs, fat body cells and imaginal disks respond to upd2 and ecdysone to initiate nutrient-independent growth, differentiation, and maturation [70,87–89].

Conclusions

Recent studies of mammalian puberty and insect metamorphosis have shown that, despite their evolutionary distance and physiological differences, these critical events exhibit similar design principles and conservation of the molecular components involved. A variety of environmental and internal cues (e.g., nutrition, photoperiod, temperature, and tissue damage) appear capable of influencing sexual maturation through the activation of neuroendocrine organs, culminating in steroid production and secretion. Among these signals, the communication between peripheral body fat levels and endocrine organs seems crucial and allows the assessment of nutrient availability and the growth status of internal organs, ensuring that maturation starts at the right time. This is extremely concerning given the current rampant prevalence of childhood obesity and its possible relationship with the increasing incidence of early puberty [34], which affects growth and final body size, and which is associated with a number of adult morbidities [90–92].

Holistic investigation through the combined use of model organisms is certainly necessary to identify upstream signals (lipids, amino acids, etc.) and their sensors in the brain, as well as how these signals are integrated in the control of metamorphosis and puberty.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

JM, JG, and JC-V involved in writing—original draft; JM, JG, and JC-V contributed to review and editing; JM contributed to funding acquisition.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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