



Neuroendocrine Regulation of Reproductive Dormancy in the Fruit Fly *Drosophila melanogaster*: A Review of Juvenile Hormone-Dependent Regulation

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Animals can adjust their physiology, helping them survive and reproduce under a wide range of environmental conditions. One of the strategies to endure unfavorable environmental conditions such as low temperature and limited food supplies is dormancy. In some insect species, this may manifest as reproductive dormancy, which causes their reproductive organs to be severely depleted under conditions unsuitable for reproduction. Reproductive dormancy in insects is induced by a reduction in juvenile hormones synthesized in the *corpus allatum* (pl. *corpora allata*; CA) in response to winter-specific environmental cues, such as low temperatures and short-day length. In recent years, significant progress has been made in the study of dormancy-inducing conditions dependent on CA control mechanisms in *Drosophila melanogaster*. This review summarizes dormancy control mechanisms in *D. melanogaster* and discusses the implications for future studies of insect dormancy, particularly focusing on juvenile hormone-dependent regulation.

Keywords: reproductive dormancy, *corpus allatum* (*corpora allata*), juvenile hormone (JH), monoamine, insulin-like peptides (ILPs), ecdysone, ecdysis-triggering hormone, circadian clock

INTRODUCTION

In animals, neuroendocrine systems play a crucial role in facilitating adaptation to a wide variety of environments (Schmidt-Nielsen, 1997). In the temperate zone, winter is a challenging season for many animals because of the freezing temperatures and food shortages, which are sustained over several months. Hence, in winter, animals often suspend or slow down their normal physical functions, a process known as dormancy (Hand et al., 2016). To date, dormancy is recognized as an adaptive phenomenon in a wide variety of animal species (Hand et al., 2016).

Insects are one of the main animal groups that have been intensively used in dormancy studies. This is because control of insect dormancy could benefit many aspects of industry and agriculture. For example, since the silkworm *Bombyx mori* possesses an egg dormancy stage, a technology to flexibly regulate the induction and end of egg dormancy is useful to enable sericulture throughout the year (Yamashita and Yaginuma, 1991). In addition, pest dormancy studies are thought to

be important because understanding the seasonal distribution of pests is critical for generating predictive models to accurately determine when pests are destructive (Denlinger, 2008). Moreover, there have been attempts to select specific dormancy phenotypes in biocontrol agents, which cannot become dormant in a season when crop pest populations continue to grow (Lirakis and Magalhães, 2019). In this regard, it is noteworthy that a branch of the United Nations promoted a coordinated research project on dormancy management to enable mass-rearing and increase the efficacy of sterile insects and natural enemies from 2014 to 2019 (Food and Agriculture Organization of the United Nations, 2013).

Previous studies have revealed that the developmental stages of dormancy differ between insect species (Tauber et al., 1986; Danks, 1987, 2006; Schiesari and O'Connor, 2013). For example, as described above, *B. mori* enters dormancy only in the egg stage (Yamashita and Yaginuma, 1991). However, in other species, dormancy is not restricted to the egg but can occur at other stages, including the adult life. Adult dormancy results in many metabolic and behavioral changes, such as the slow-down of reproduction, decreased food ingestion, suppressed metabolism, increased stress resistance, and extended adult lifespan (Danks, 1987; Tatar and Yin, 2001; Hahn and Denlinger, 2011; Kubrak et al., 2014). Among the multiple changes, reproductive slow-down is thought to be a key event of adult dormancy, known as reproductive dormancy. Since adult insects allocate a considerable amount of energy to gametogenesis, reproductive dormancy allows insects to reduce their energy consumption and produce offspring again in the spring when winter is over. Reproductive dormancy is found in both male and female adults; In particular, females exhibit a drastic suppression of gametogenesis (oogenesis) by inhibiting vitellogenesis, accessory gland activity, and mating behavior (Saunders et al., 1989; Pener, 1992; Kubrak et al., 2016).

Previous studies have shown that reproductive dormancy and other types of dormancy are regulated by a complex interplay of multiple hormones and neurotransmitters in insects (Denlinger, 2002; Emerson et al., 2009a; Denlinger et al., 2012). In particular, several major hormones influence reproductive dormancy, including juvenile hormones (JHs), insulin-like peptides, and ecdysteroids (Denlinger, 2002; Emerson et al., 2009a; Denlinger et al., 2012). All of three classes of hormones are known to have a stimulatory effect on ovarian development, and a decrease in these signals in dormant individuals suppresses ovarian development (Denlinger, 2002; Emerson et al., 2009a; Denlinger et al., 2012; Uryu et al., 2015; Lenaerts et al., 2019; Santos et al., 2019; Swevers, 2019; Semaniuk et al., 2021). Among these three, JHs, the arthropod-specific sesquiterpenoid hormones, have been the most intensively studied in the long history of entomology to unravel their vital role in regulating reproductive dormancy in female adults. Since JHs are essential for promoting vitellogenesis in normal (non-dormancy-inducing) conditions, the reduction in JH levels is conversely required for suppressing vitellogenesis, leading to reproductive dormancy in females (Denlinger et al., 2012; Santos et al., 2019). This mechanism was well demonstrated in a study using the Colorado potato beetle *Leptinotarsa decemlineata*

(de Wilde and de Boer, 1961, 1969; Schooneveld et al., 1977; Kort, 1990), which has frequently been used for studies on reproductive dormancy. After hatching under short-day condition, the adults burrow into the soil and enter dormancy. When the endocrine organ known as the *corpus allatum* (*pl. corpora allata*; CA), responsible for biosynthesizing JHs, is dissected out from a non-dormant beetle and then transplanted to a dormant beetle, the dormant status is released from the beetle receiving the transplant. Such release of dormancy is also observed when JH is applied to a dormant beetle. In contrast, when the CA of a non-dormant beetle is surgically ablated, the ovarian development is inhibited even under long-day (non-dormancy-inducing) conditions. These results indicate that the reduced concentration of JH, which is biosynthesized in the CA, causes reproductive dormancy in *L. decemlineata* females. After this discovery, the importance of JH reduction to induce female reproductive dormancy has been confirmed in many insect species (Denlinger et al., 2012; Hand et al., 2016). Therefore, there must be essential, common mechanisms by which information about dormancy-inducing environmental cues, such as low temperature and short-day condition, are transmitted to the CA to control JH biosynthesis or release. However, the mechanisms at the molecular, cellular, and neuroendocrine levels have not yet been fully elucidated.

Among several insects used in laboratories, the fruit fly *Drosophila melanogaster* has contributed substantially to the discovery of various biological phenomena based on powerful genetic tools such as the GAL4-UAS system (Brand and Perrimon, 1993; Neckameyer and Argue, 2013). In the last decade, significant progress has been made in the study of reproductive dormancy in females of *D. melanogaster*. Specifically, there have been several recent studies to address how dormancy-inducing stimuli are transmitted to the CA to regulate reproductive dormancy through the reduction of JH titer. Importantly, a part of *D. melanogaster* research is beginning to contribute to understanding the molecular mechanisms of reproductive dormancy in other insects, as we describe below. This review summarizes current knowledge about the mechanisms of reproductive dormancy in *D. melanogaster* females and discusses the remaining questions of the *D. melanogaster* dormancy, particularly focusing on juvenile hormone-dependent regulation.

AN OVERVIEW OF *DROSOPHILA MELANOGASTER* DORMANCY

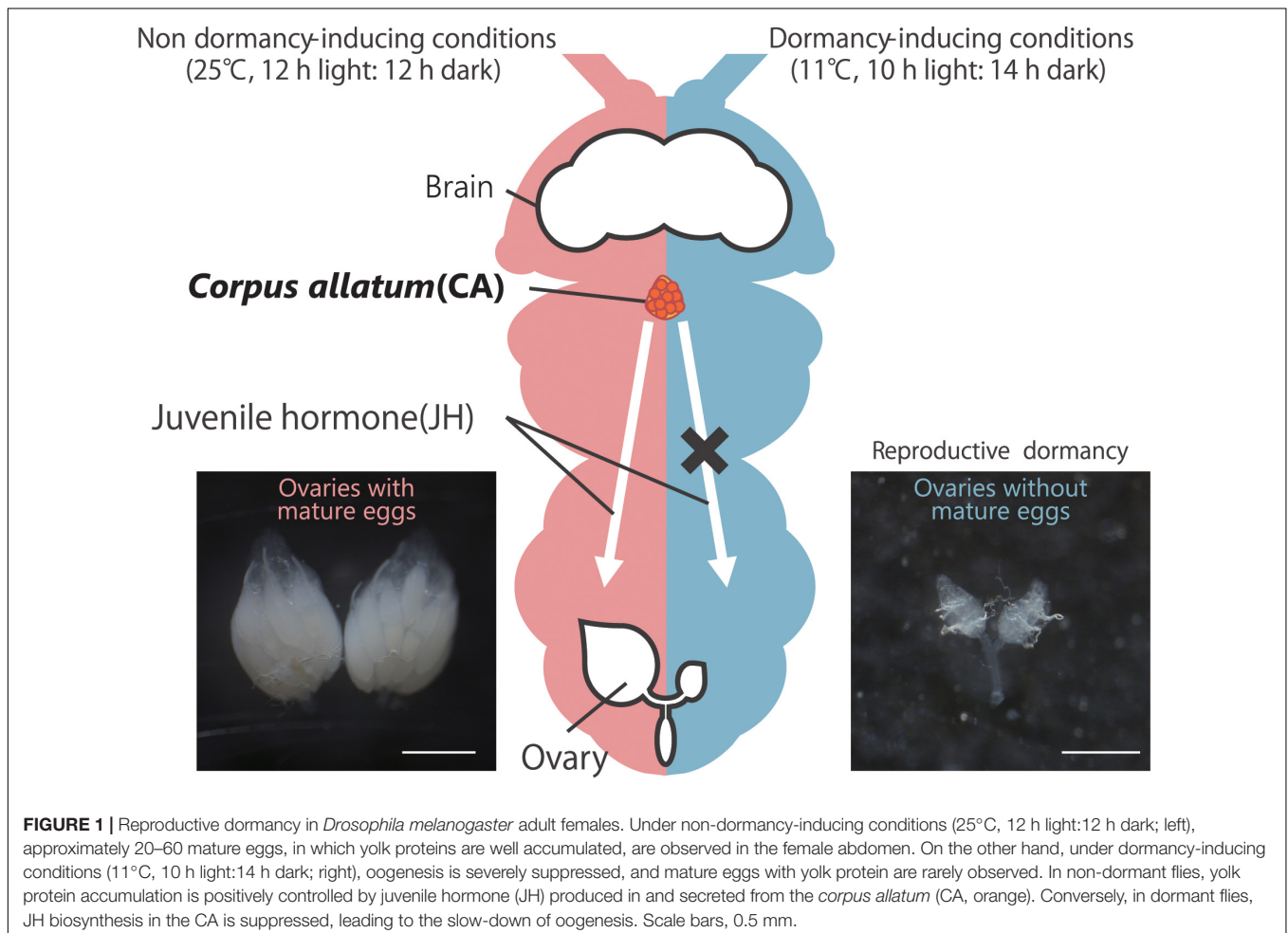
First, we would like to define our usage of the term “dormancy.” In general, dormancy can be broadly classified into two types, diapause and quiescence. Diapause is induced by seasonal cues that do not directly prevent development but foreshow the arrival of unfavorable conditions. On the other hand, quiescence is characterized by slowed metabolism and directly results from unfavorable environmental conditions such as low temperature (Košťál, 2006; Schiesari et al., 2011; Schiesari and O'Connor, 2013). There is still a debate about whether reproductive dormancy in *D. melanogaster* should be classified as diapause

or quiescence (Tatar et al., 2001; Košťál, 2006; Lirakis et al., 2018). However, it seems obvious that reproductive dormancy in *D. melanogaster* is influenced by both short-day conditions and low temperature (Schiesari et al., 2016; Zonato et al., 2017; **Figure 1**). This fact implies that reproductive dormancy in *D. melanogaster* has both quiescence and diapause properties. Therefore, most papers published in recent years simply use “dormancy” to describe the short-day and low temperature-induced changes in *D. melanogaster* adults (Kubrak et al., 2016; Liu et al., 2016; Andreatta et al., 2018; Lirakis et al., 2018; Ojima et al., 2018; Nagy et al., 2019; Abrieux et al., 2020). In this paper, as is customary, we will refer to the *D. melanogaster* phenotypes in terms of dormancy.

In laboratories, *D. melanogaster* dormancy is commonly induced by transferring virgin females into dormancy-inducing conditions shortly after (usually 2–6 h) eclosion under non-dormancy-inducing conditions. Such newly eclosed female flies are supposed to have previtellogenic or even few early vitellogenic egg chambers but not mature eggs. When the newly eclosed females are maintained in dormancy-inducing conditions, the flies continuously keep the immature ovaries even 2 or 3 weeks after eclosion (Zonato et al., 2017; Lirakis et al., 2018; **Figure 1**). However, since reproductive dormancy is a

“slow-down” and not a complete arrest, in ovarian development, the percentage of non-dormant flies increases about 4 weeks after hatching (Schiesari et al., 2016; Zonato et al., 2017; Lirakis et al., 2018). Meanwhile, rearing flies under conditions of temperature and day length that mimic winter can prolong the slow-down of oogenesis and maintain a higher reproductive dormancy rate than simple dormancy-inducing conditions even 3 months after eclosion (Zonato et al., 2017). It is still unclear why such environmental fluctuation is important to extend reproductive dormancy.

Under dormancy-inducing conditions in laboratories, *D. melanogaster* adults display some physiological changes, such as reduced metabolism (Saunders et al., 1989; Saunders and Gilbert, 1990; Tatar and Yin, 2001; Tatar et al., 2001; Kubrak et al., 2014; Anduaga et al., 2018). Previous studies have shown that absolute amounts of trehalose and glucose positively correlate with the dormancy rate (Kubrak et al., 2014; Anduaga et al., 2018). In addition, trehalose/glycogen and trehalose/glucose ratios also positively correlate with the dormancy rate (Watanabe et al., 2002; Kubrak et al., 2014; Anduaga et al., 2018). These observations raise a hypothesis that the biosynthesis of trehalose, an energy source and an anti-freezing material, is activated in dormant flies (Crowe, 2007; MacRae, 2010; Hahn and Denlinger,



2011). On the other hand, protein amount does not correlate with dormancy rate (Kubrak et al., 2014; Anduaga et al., 2018). Dormancy-associated physiological changes also include an increase in stress tolerance, an extended lifespan, and the activation of the innate immune system in both male and female fly adults (Saunders et al., 1989; Saunders and Gilbert, 1990; Tatar et al., 2001; Tatar and Yin, 2001; Kubrak et al., 2014; Kučerová et al., 2016; Anduaga et al., 2018). In addition, in *D. melanogaster* dormant females, the intestine is markedly shortened (Kubrak et al., 2014). Some of these physiological changes are also observed in other insects when they enter dormancy (Tatar and Yin, 2001; Denlinger, 2002; Hahn and Denlinger, 2007, 2011; Sim and Denlinger, 2013).

Along with the changes in metabolic state, suppression of vitellogenesis in oogenesis is another major aspect of dormancy (Figure 2); hence this process is called reproductive dormancy. Among the 14 distinct stages of *D. melanogaster* oogenesis (King, 1970; Bastock and St Johnston, 2008), vitellogenesis occurs from stage 8 (Bownes, 1994; Soller et al., 1997). Therefore, oocytes before and at stage 7 are defined as previtellogenic oocytes, while those after and at stage 8 are defined as vitellogenic oocytes. Most studies classify flies as dormant if their ovaries have only previtellogenic egg chambers, while flies with ovaries containing vitellogenic egg chambers are characterized as non-dormant (Saunders et al., 1989, 1990; Saunders, 1990; Saunders and Gilbert, 1990; Williams, 1993; Richard et al., 1998; Tatar et al., 2001; Schmidt and Conde, 2006; Williams et al., 2006; Schmidt and Paaby, 2008; Schmidt et al., 2008; Emerson et al., 2009b; Lee et al., 2011; Fabian et al., 2015; Schiesari et al., 2016; Zhao et al., 2016; Zonato et al., 2017; Andreatta et al., 2018; Ojima et al., 2018; Nagy et al., 2019; Figure 1). However, there is still an ongoing debate about whether this commonly used classification is truly valid, as discussed in several papers (Tatar et al., 2001; Lee et al., 2011; Lirakis et al., 2018; Erickson et al., 2020). These papers claim that oogenesis in dormancy-inducing conditions is not arrested at previtellogenic stages but rather at stage 9 corresponding to early vitellogenesis. In fact, stage 9 oocytes are largely different from stage 10 and later oocytes from the following perspectives. First, stage 10 oocytes massively grow and are drastically enlarged compared to stage 9 oocytes (He et al., 2011). Second, stage 9 oocytes possess a very small amount of yolk, but stage 10 oocytes accumulate it well (He et al., 2011). Third, stage 8 oocytes self-synthesize yolk proteins, while stage 10 oocytes incorporate extracellular yolk proteins synthesized in the fat body cells and released into the hemolymph (Brennan et al., 1982). Importantly, several studies have shown that dormant oocytes incorporate hemolymph yolk proteins in lesser amounts (Saunders, 1990; Saunders et al., 1990; Richard et al., 2001b). This issue is still being debated.

Previous laboratory studies have reported that, like other insects, low temperature and short-day condition influence the induction of dormancy in *D. melanogaster* adults (Saunders et al., 1989; Saunders and Gilbert, 1990; Allen, 2007; Figure 1). However, in contrast to the importance of short-day condition to induce dormancy in many other insect species, it is known that low temperature, but not short-day condition, is the crucial dormancy stimulus in *D. melanogaster* (Saunders and Gilbert,

1990; Zonato et al., 2017; Anduaga et al., 2018). In other words, *D. melanogaster* adults enter dormancy at temperatures less than 13°C even in long-day condition, but they never enter dormancy in short-day condition if the temperature is higher than 13°C. In *D. melanogaster*, the photoperiod appears to modulate reproductive dormancy within a 10–13°C range of permissiveness temperature (Saunders et al., 1989; Saunders and Gilbert, 1990; Schiesari et al., 2011). Curiously, a recent study has suggested that a very subtle difference in temperature, such as a 0.3°C difference, possibly has a significant impact on the induction of dormancy (Anduaga et al., 2018).

A concern about most laboratory experiments is that these previous data were obtained under artificial light on/off conditions, which is the unnatural constant light-constant dark photoperiodic cycle. However, a recent study has claimed that such artificial light on/off conditions does not reflect natural light/dark change and, thus, underestimate the effect of photoperiod on *D. melanogaster* reproductive dormancy (Nagy et al., 2018). This study revealed that a photoperiod-dependent reproductive dormancy is more clearly observed in *D. melanogaster* European strains when the animals are reared under gradual elevation and reduction of light that mimic the natural light/dark change (Nagy et al., 2018). In addition to low temperature and short-day condition, starvation is an additional factor that enhances dormancy rate (Lirakis et al., 2018; Ojima et al., 2018).

In the wild, it has been well described that there are geographic variations in reproductive dormancy in *D. melanogaster* (Williams et al., 2006; Tauber et al., 2007; Sandrelli et al., 2007; Schmidt and Paaby, 2008; Schmidt et al., 2008; Emerson et al., 2009b; Kolaczowski et al., 2011; Lee et al., 2011; Fabian et al., 2012; Bergland et al., 2014; Kapun et al., 2016; Zhao et al., 2016; Zonato et al., 2017; Lirakis et al., 2018; Betancourt et al., 2021; Machado et al., 2021). *D. melanogaster* originated in sub-Saharan Africa and subsequently expanded its habitat, possibly in association with humans and agriculture (Flatt, 2020). The flies have colonized temperate habitats in Eurasia and, more recently, North America and Australia. Genotypes from some populations enter reproductive dormancy readily in response to low temperature and short-day conditions, whereas others have low or zero dormancy propensity (Williams, 1993; Schmidt and Paaby, 2008; Emerson et al., 2009b; Fabian et al., 2015). Seasonal high-latitude populations show much greater dormancy inducibility than flies from subtropical/tropical low-latitude populations. In addition, geographic variations in reproductive dormancy in wild *D. melanogaster* are associated with single nucleotide polymorphisms in several loci, as we discuss later, suggesting that the traits of reproductive dormancy are genetically constrained.

THE ROLE OF JUVENILE HORMONES IN *DROSOPHILA MELANOGASTER* REPRODUCTIVE DORMANCY

As in other insect species, the induction of *D. melanogaster* reproductive dormancy is associated with reduced JH levels

(Figure 1). A previous study using an *in vitro* assay system has shown that the dissected CA from animals under reproductive dormancy-inducing conditions exhibits reduced JH biosynthesis (Saunders et al., 1990). In contrast, administration of the JH analog methoprene can break reproductive dormancy (Tatar and Yin, 2001). Phenotypes of reproductive dormancy are also observed in female flies in which JH biosynthetic enzymes are enhanced or suppressed. For example, overexpression of a gene encoding juvenile hormone acid *O*-methyltransferase (*Jhamt*) significantly reduces the percentage of dormant flies (Shinoda and Itoyama, 2003; Niwa et al., 2008; Noriega, 2014; Ojima et al., 2018). Conversely, the CA-specific transgenic double-strand RNA interference (RNAi) of *Jhamt* increases the percentage of dormant flies (Ojima et al., 2018). Taken together, these observations are consistent with the fact that JH accelerates oogenesis in most insects, including *D. melanogaster*, under non-dormancy-inducing conditions.

The role of JH in the regulation of *D. melanogaster* reproductive dormancy has also been investigated at the level of oogenesis. It is well known that JH is essential for facilitating vitellogenesis from the oocyte stage 8 (Bownes, 1994; Soller et al., 1997). More precisely, JH promotes yolk protein production, which occurs mainly in the fat body, and also acts on the ovarian follicle cells to promote vitellogenesis by enhancing yolk protein uptake to oocytes (Postlethwait and Weiser, 1973; Bownes, 1989; Soller et al., 1997, 1999; Richard et al., 2001a; Santos et al., 2019). The suppression of yolk protein production in the dormancy-inducing condition correlates well with reproductive dormancy (Saunders et al., 1990; Figure 2). Experimentally, application of the JH analog to dormant females increased yolk protein production and the number of vitellogenic oocytes as compared to an experimental control group (Saunders et al., 1990; Tatar et al., 2001). These data indicate that, in *D. melanogaster* and many other insects, reproductive dormancy is due to the suppression of vitellogenesis, at least in part, by JH titer reduction.

NEUROENDOCRINE MECHANISMS THAT DIRECTLY OR INDIRECTLY CONTROL JUVENILE HORMONE BIOSYNTHESIS IN THE CORPUS ALLATUM IN *DROSOPHILA MELANOGASTER*

To understand how JH biosynthesis in the CA is suppressed when the animals are placed in dormancy-inducing conditions, it is important to unravel the mechanisms transmitting environmental cues to the CA to influence the activity of JH biosynthesis. Although the mechanisms have not yet been fully elucidated, recent studies on *D. melanogaster* have collectively argued that several neuroendocrine factors are crucial for inducing JH-mediated reproductive dormancy. Hereafter we are describing the recent knowledge of neuroendocrine regulation of reproductive dormancy in *D. melanogaster*. As we described earlier, insect reproductive dormancy is regulated not only

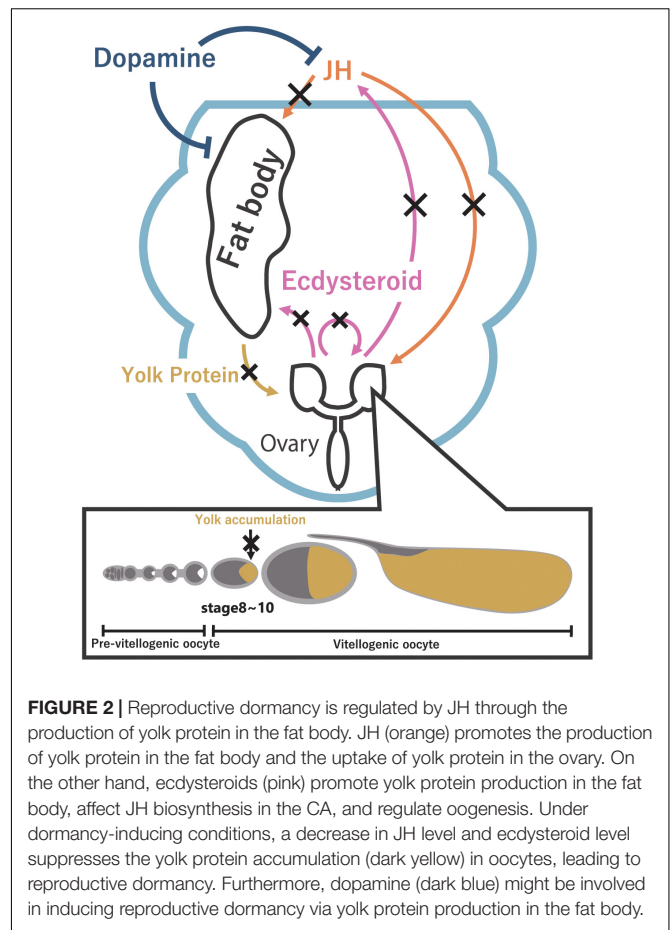
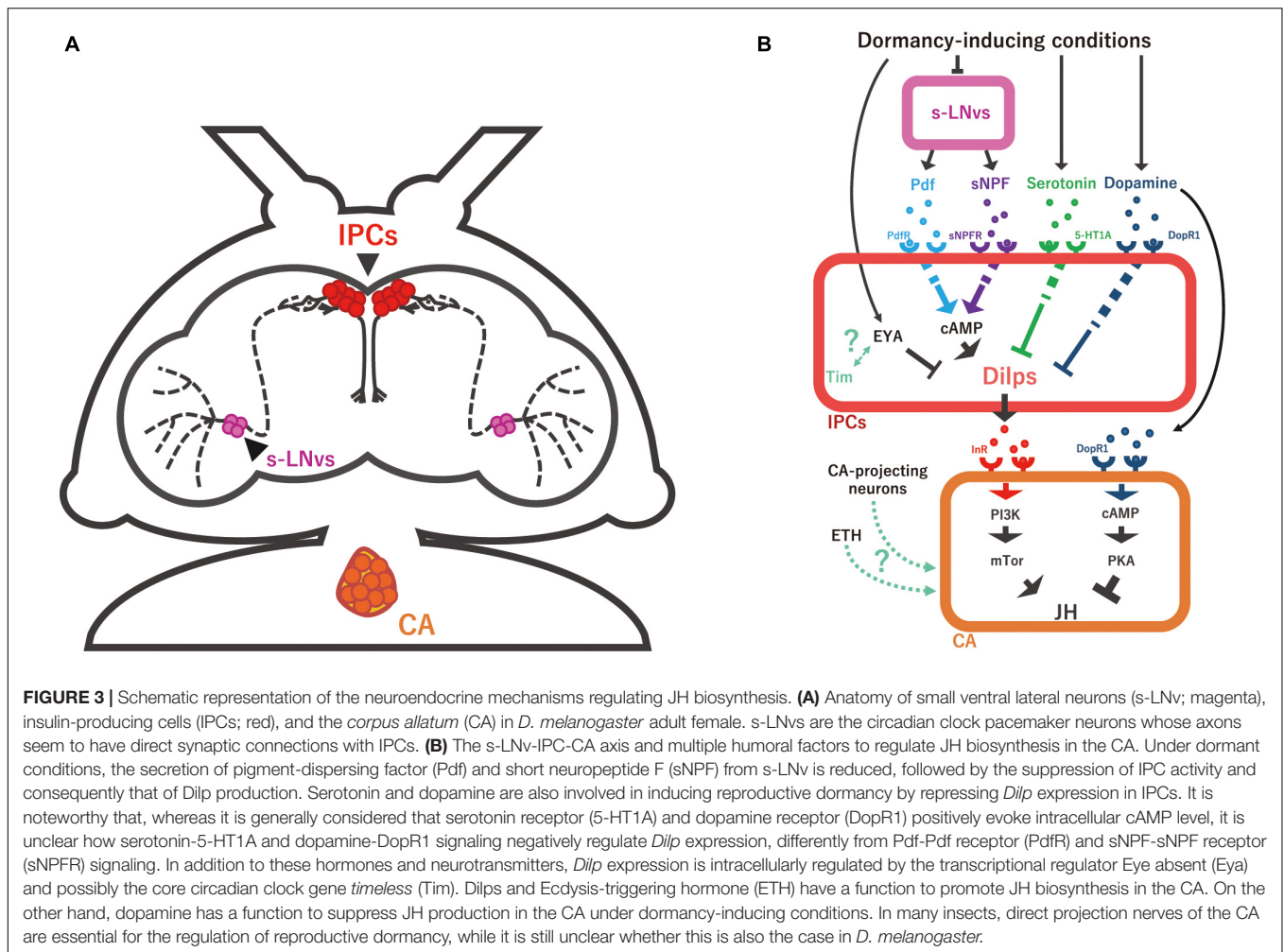


FIGURE 2 | Reproductive dormancy is regulated by JH through the production of yolk protein in the fat body. JH (orange) promotes the production of yolk protein in the fat body and the uptake of yolk protein in the ovary. On the other hand, ecdysteroids (pink) promote yolk protein production in the fat body, affect JH biosynthesis in the CA, and regulate oogenesis. Under dormancy-inducing conditions, a decrease in JH level and ecdysteroid level suppresses the yolk protein accumulation (dark yellow) in oocytes, leading to reproductive dormancy. Furthermore, dopamine (dark blue) might be involved in inducing reproductive dormancy via yolk protein production in the fat body.

by JHs but also many other hormones, including insulin-like peptides and ecdysteroids. Although it might be plausible to consider that insulin-like peptides and ecdysteroids have roles in reproductive dormancy independent of JHs, recent *D. melanogaster* studies have indicated that insulin-like peptides and ecdysteroids influence JH biosynthesis in the CA in multiple way. Therefore, in this review, instead of deliberately focusing on the whole picture of hormone interactions, we dare to focus on JHs and discuss the involvement of other hormones and neurotransmitters.

Mechanisms of Insulin Signaling-Mediated Reproductive Dormancy

Insulin signaling controls development, growth, and physiology by regulating nutrient metabolism and energy expenditure in animals. In *D. melanogaster*, insulin signaling has versatile functions, including reproductive dormancy (Sim and Denlinger, 2013; Kubrak et al., 2014; Liu et al., 2016; Schiesari et al., 2016; Ojima et al., 2018; Semaniuk et al., 2021). *Drosophila* insulin-like peptides (Dilps), orthologs of vertebrate insulin and insulin growth factors, are the endocrine factors activating insulin signaling in *D. melanogaster* (Semaniuk et al., 2021; Figures 3A,B). Their secretion is regulated by the activity of



insulin-producing cells (IPCs), which are neurons located in the pars intercerebralis (PI) of the brain. Dilps are then released from IPCs into the hemolymph (Semaniuk et al., 2021; **Figure 3A**). A previous study has demonstrated that, in flies reared at 11°C, short-day condition results in lower neuronal activity of IPCs than long-day condition (Ojima et al., 2018). Furthermore, when IPCs are artificially activated by overexpression of a transient receptor potential cation channel under dormancy-inducing conditions, the percentage of dormant flies decreases. Conversely, an artificial inhibition of IPCs by overexpressing an inward rectifier potassium channel increases the percentage of dormant flies (Venken et al., 2011; Schiesari et al., 2016; Ojima et al., 2018). These results suggest that IPCs negatively regulate reproductive dormancy. Phenotypes of reproductive dormancy are also observed in loss- and gain-of-function of *Dilps* and insulin receptor (*InR*). For example, overexpression of *Dilp2* and *Dilp5* in the IPCs suppresses the induction of dormancy (Schiesari et al., 2016). On the other hand, IPC-specific RNAi of *Dilp2*, *Dilp5* upregulates the induction of dormancy (Schiesari et al., 2016). Moreover, molecules downstream of Dilps and *InR* are also involved in reproductive dormancy. For example, Phosphatidylinositol-3 kinase (PI3K) influences

of the rate of reproductive dormancy in the North American population of *D. melanogaster* (Williams et al., 2006). In addition, FoxO, a well-known transcription factor downstream of Dilp signaling, also plays a crucial role in inducing reproductive dormancy, as revealed by an experiment using a FoxO-mediated gene expression reporter (Schiesari et al., 2016). Taken together, the production and secretion of Dilps are greatly suppressed in dormancy-inducing conditions, resulting in reduced insulin signaling, followed by the suppression of oogenesis (**Figure 3B**).

How does the reduction of Dilp signaling suppress oogenesis? One mechanism is that reduced Dilp signaling in the CA downregulates JH biosynthesis (**Figure 3B**). For example, overexpression of constitutively active *InR* or *PI3K* in the CA significantly decreases in flies with ovarian arrest in dormancy-inducing conditions (Ojima et al., 2018). Conversely, *InR* RNAi or dominant-negative *PI3K* or *mTOR*, another downstream element of insulin signaling, increases the proportion of flies with ovarian arrest in both normal and dormancy-inducing conditions (Ojima et al., 2018). On the other hand, besides the JH-mediated action of Dilps, there are other mechanisms by which Dilps directly act on ovarian cells to regulate oogenesis or indirectly influence oogenesis through the regulation of

ecdysteroid biosynthesis (Tu et al., 2002, 2005; LaFever and Drummond-Barbosa, 2005).

The role of insulin signaling in controlling developmental arrest has also been suggested in species other than insects, such as the nematode *Caenorhabditis elegans* and the annual killifish *Austrofundulus limnaeus*. In *C. elegans*, low food levels or other unfavorable environmental conditions lead to larval developmental arrest, known as dauer (Hu, 2007). Previous studies have identified and characterized genes mutated in various mutants exhibiting dysregulation of dauer arrest, called *daf* mutants. Some of the identified genes encode insulin-like peptides, insulin receptor, and signal transduction molecules downstream of insulin receptor (Golden and Riddle, 1982, 1984; Cornils et al., 2011; Sim and Denlinger, 2013). In the annual killifish, downregulation of insulin signaling appears essential for regulating embryonic diapause (Woll and Podrabsky, 2017). Considering that JHs are arthropod-specific and neither nematodes nor vertebrates biosynthesize JHs, it would be intriguing to examine whether and how insulin signaling interacts with other hormones and other bioactive molecules to regulate developmental arrest in these animals, such as dafachronic acids in *C. elegans* (Hu, 2007; Niwa and Niwa, 2014) and vitamin D3 in the annual killifish (Romney et al., 2018).

Mechanisms of Aminergic Signaling-Mediated Reproductive Dormancy

Previous studies on pupal dormancy in butterflies and moths have shown that hemolymph levels of aminergic neurotransmitters, such as dopamine and serotonin, are elevated during dormancy (Noguchi and Hayakawa, 1997; Isabel et al., 2001; Hsu et al., 2020; **Figure 3B**). Recent studies have revealed that serotonin and dopamine are also involved in regulating reproductive dormancy in *D. melanogaster* (Andreatta et al., 2018).

In *D. melanogaster*, the dopamine level in the whole adult body significantly increases under dormancy-inducing conditions (Andreatta et al., 2018). Furthermore, the percentage of flies with reproductive dormancy is reduced in mutants of DOPA decarboxylase (*Ddc*), which is involved in the biosynthesis of dopamine and serotonin (Sherald and Wright, 1974; Wright et al., 1976). On the other hand, activation of the dopaminergic neurons results in an increased proportion of flies with reproductive dormancy. These results indicate that dopamine, in contrast to Dilps, positively regulates reproductive dormancy. However, among the approximately 130 *bona fide* dopaminergic neurons in the *D. melanogaster* brain (Kasture et al., 2018; Karam et al., 2020), the dopaminergic neuron(s) important for inducing reproductive dormancy have not yet been identified.

A previous study has revealed that *Dop1R1*, the gene encoding one of the dopamine receptors, is expressed at the CA and the fat body (Gruntenko et al., 2012; **Figure 3B**). Knockdown of *Dop1R1* either in the CA or the fat body significantly reduces the percentage of dormant flies (Andreatta et al., 2018), suggesting that dopamine induces reproductive dormancy via both the CA and the fat body. It should be remembered

that the fat body is the main organ producing yolk protein, which is essential for vitellogenesis (Bownes, 1994). Therefore, these data raise the possibility that dopamine might inhibit yolk protein production in the fat body, which indirectly suppresses vitellogenesis in dormant flies (**Figure 2**). Besides the CA and the fat body, dopamine seems to act on IPCs as well. While it is unclear whether any of the dopamine receptors are expressed in IPCs, *Dop1R1* knockdown in IPCs also reduces the percentage of dormant flies (Andreatta et al., 2018). Therefore, Dilps production may be reduced when the IPCs receive dopamine (**Figure 3B**). Taken together, these data indicate that dopamine simultaneously acts on multiple tissues to regulate reproductive dormancy.

It is known that *Dop1R1* is coupled with the stimulatory trimeric G-protein, known as Gs, and utilizes intracellular cyclic AMP (cAMP) as a second messenger (Gotzes et al., 1994; Sugamori et al., 1995). A previous study has shown that the CA-specific forced expression of a dominant-negative form of protein kinase A (PKA), a cAMP-activating kinase (Francis and Corbin, 1994), represses reproductive dormancy in the dormancy-inducing condition (Andreatta et al., 2018). Moreover, the animals in this study exhibited expression changes in JH-responsive genes, which seemed to reflect the increased JH titer. These results are also consistent with the involvement of *Dop1R1* signaling in the CA in regulating reproductive dormancy (**Figure 3B**).

In addition to dopamine, serotonin also regulates reproductive dormancy in *D. melanogaster*. Artificial neuronal activation of serotonergic neurons by overexpressing a voltage-gated ion channel causes a significant increase in the percentage of dormant flies (Venken et al., 2011; Andreatta et al., 2018). Furthermore, one of the serotonin receptors, 5-HT1A, is expressed in IPCs and negatively regulates IPCs (Luo et al., 2012, 2014). Knockdown of 5-HT1A in IPCs greatly reduces the percentage of dormant flies. Consistent with this observation, the IPC-specific knockdown of 5-HT1A enhances the expression levels of *Dilp2* and *Dilp5*. These results indicate that serotonin acts on IPCs to promote dormancy via regulation of insulin signaling (**Figure 3B**).

Other bioactive monoamines include octopamine and γ -aminobutyric acid (GABA) (Nässel, 2018). It has been demonstrated that the activation of octopaminergic neurons suppresses reproductive dormancy, while GABAergic signaling does not consistently affect dormancy (Andreatta et al., 2018). Since the octopaminergic signal is known to influence the production and release of Dilps in non-dormancy-inducing conditions (Enell et al., 2010; Luo et al., 2014), a previous study has examined whether octopamine regulates reproductive dormancy by acting on IPCs. However, the silencing of the octopamine receptor in mushroom bodies (OAMB) in the IPCs does not affect the percentage of dormant flies (Andreatta et al., 2018). On the other hand, a previous study has suggested that octopamine suppresses JH degradation, leading to a rise in JH titer under heat stress (Gruntenko et al., 2000; Rauschenbach et al., 2001). Although this study does not focus on reproductive dormancy, this observation implies that activation of octopaminergic neurons may facilitate reproductive dormancy. However, it is still unclear which tissues receive

octopamine and whether octopamine-dependent control of reproductive dormancy requires JH signaling.

Mechanisms of Ecdysteroid Signaling-Mediated Reproductive Dormancy

The classical scheme of insect endocrinology describes how insect developmental transitions are regulated by three primary circulating factors: prothoracicotropic hormone, JHs, and ecdysteroids (Jindra et al., 2013; Truman, 2019). In *D. melanogaster*, besides JHs, ecdysteroids are also known to play an important role in regulating reproductive dormancy (Richard et al., 1998, 2001b). Ovaries of reproductively dormant flies exhibit lower ecdysteroid biosynthesis activity *in vitro* compared to those of non-dormant flies (Richard et al., 1998). On the other hand, the ovarian ecdysteroid level is elevated during the dormancy-breaking process (Richard et al., 1998). The elevation of the ovarian ecdysteroids occurs before mature eggs start to be produced in the dormancy-broken ovaries. Moreover, microinjection of 20-hydroxyecdysone (20E), the most active form of ecdysteroid, to reproductively dormant females evokes vitellogenesis (Richard et al., 1998, 2001b). Notably, vitellogenesis is severely suppressed in the ecdysone receptor (EcR) loss-of-function ovaries in non-dormant flies (Carney and Bender, 2000). Taken together, these results indicate that ecdysteroid biosynthesis in the ovary needs to be suppressed in the dormancy-inducing condition, while the elevation of the ovarian ecdysteroid level and the activation of 20E signaling are required for breaking reproductive dormancy (Figure 2).

Currently, however, the role of ecdysteroids in the regulation of reproductive dormancy in *D. melanogaster* has not been as well documented as that of the JHs. It is also unclear how the ovarian ecdysteroid levels are suppressed in the dormancy-inducing condition. Nevertheless, it has been hypothesized that three major pathways might be involved in the 20E-mediated regulation of reproductive dormancy. First, the downregulation of 20E signaling in the fat body seems to suppress yolk protein production in the dormancy-inducing condition, as 20E stimulates the fat body in non-dormant females, leading to the promotion of yolk protein production (Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980; Bownes, 1982; Handler and Maroy, 1989; Bownes et al., 1996; Richard et al., 2001a). Second, the downregulation of 20E signaling in the ovary might suppress many aspects of oogenesis in the dormancy-inducing condition. Previous studies have indicated that 20E has versatile roles in *D. melanogaster* oogenesis, including niche formation, germline stem cell proliferation and maintenance, cystocyte differentiation, and the developmental checkpoint at the stage 8–10 egg chambers (Belles and Piulachs, 2015; Uryu et al., 2015; Ameku and Niwa, 2016; Ameku et al., 2017; Swevers, 2019). Third, the downregulation of 20E signaling might suppress JH biosynthesis in the CA in the dormancy-inducing condition. In fact, very recent studies have just revealed the relationship between 20E signaling and JH biosynthesis, which is at least in part mediated by the peptide hormone ecdysis-triggering hormone (ETH). We provide more details in the next section.

Mechanisms of Ecdysis-Triggering Hormone-Mediated Reproductive Dormancy

ETH has been identified and characterized as the peptide hormone playing an essential role in regulating innate ecdysis behavior in the tobacco horn moth *Manduca sexta* and *D. melanogaster* (Žitòan et al., 1996; Kingan et al., 1997; Park et al., 1999). ETH is produced by Inka cells located close to tracheal pits in the thorax and abdomen and then secreted into the hemolymph. While early studies have shown that the released ETH is received in the brain, after which there is activation of a neuroendocrine mechanism to control ecdysis behavior, the recent studies using non-dormant *D. melanogaster* have also revealed that ETH has a function to promote JH biosynthesis in the CA, leading to ovarian development in females and mating behavior in males (Lee et al., 2017; Meiselman et al., 2017). In females, knockdown of *ETH* results in the reduction of JH levels and slow-down of ovarian development (Lee et al., 2017; Meiselman et al., 2017). In addition, live imaging experiments have shown that ETH is directly received by the ETH receptor on the CA to elevate intracellular Ca^{2+} level (Lee et al., 2017; Meiselman et al., 2017). Curiously, it is known that ETH production is promoted by 20E (Park et al., 1999). Therefore, it is plausible to expect that, in dormancy-inducing conditions, the downregulations of ecdysteroid biosynthesis and 20E signaling indirectly suppress JH biosynthesis via ETH, yet this fascinating possibility has not been experimentally tested in *D. melanogaster* so far (Figure 3B).

Interestingly, a recent study has shown that ecdysteroids regulate dormancy via ETH in the cabbage beetle *Colaphellus bowringi* (Guo et al., 2021). In this insect, reduction of ecdysteroid biosynthesis is required for suppressed oogenesis and accumulated lipid storage in the fat body in dormancy-inducing conditions. The reduction of ecdysteroid biosynthesis causes a decrease in ETH expression level, leading to a decrease in JH biosynthesis, which is essential for entering reproductive dormancy. The study on *C. bowringi* beetle nicely demonstrated that the ecdysteroid-ETH-JH relay is important to regulate reproductive dormancy. This study is an excellent example of how findings on the regulation of JH biosynthesis from analyses with *D. melanogaster* led to an understanding of the mechanisms of reproductive dormancy in other insects. Conversely, it would be intriguing to examine whether the ecdysteroid-ETH-JH-mediated mechanism found in *C. bowringi* also exists in *D. melanogaster*.

Is There a Direct Neuronal Regulation of Juvenile Hormone Biosynthesis in the Corpus Allatum in *Drosophila melanogaster*?

In some insects other than *D. melanogaster*, JH-mediated reproductive dormancy is regulated by not only humoral factors remotely acting on the CA but also by direct inputs from neurons projecting to the CA (de Wilde and de Boer, 1969; Shiga and Numata, 2000; Shimokawa et al., 2008). For example, in the

northern blowfly *P. terraenovae* and the bean bug *R. pedestris*, the neurons projecting to the CA (hereafter referred to as the CA-projecting neurons) are essential for inducing reproductive dormancy in dormancy-inducing conditions (Shiga et al., 2003; Shimokawa et al., 2008). In adults of the brown-winged green bug *Plautia stali*, the CA-projecting neurons co-produce several neuropeptides, such as Diuretic hormone 44, Insulin-like peptide 1, and *Plautia stali* myoinhibitory protein (Plast-MIP). In particular, Plast-MIP is considered to suppress JH biosynthesis in the CA directly (Matsumoto et al., 2017; Hasegawa et al., 2020; Hasebe and Shiga, 2021). In these insects, reproductive dormancy can be broken by surgically removing the CA-projecting neurons, confirming the importance of the CA-projecting neurons in the regulation of reproductive dormancy. However, in almost all cases, except *P. stali*, the neurotransmitter and/or neurohormone produced in the CA-projecting neurons has not been clarified. For a better understanding of the neuroendocrine mechanisms of JH biosynthesis in the CA, clarification and characterization of such neurotransmitters and/or neurohormones are crucial.

In *D. melanogaster* larvae, two types of neurons, designated CA-LP1 and CA-LP2 neurons, have been reported to directly innervate the CA (Siegmond and Korge, 2001). These neurons are involved in the epithelial movement of male genitalia by inhibiting JH biosynthesis during pupal development in non-dormancy-inducing condition (Ádám et al., 2003). It is possible that these neurons are also present in the adult stage and positively regulate reproductive dormancy via inhibiting JH biosynthesis. In addition, our recent study has reported new CA-projection neurons that produce the neuropeptide hugin in *D. melanogaster* adults, which is unexpected given that the CA-projecting hugin neurons may not have a significant impact on JH biosynthesis in the CA (Mizuno et al., 2021). Further studies would be needed to clarify the role of the CA-projecting neurons in reproductive dormancy in *D. melanogaster* (Figure 3B).

MECHANISMS OF DORMANCY CONTROL BY CIRCADIAN CLOCKS

In general, dormancy is significantly influenced by photoperiodism (Denlinger, 2002; Saunders, 2014). In *D. melanogaster*, although a low temperature is the main environmental cue to induce reproductive dormancy, photoperiodism additionally influences the dormancy entry; the short-day but not the long-day condition accelerates the entry of reproductive dormancy in *D. melanogaster* (Saunders et al., 1989; Saunders and Gilbert, 1990; Nagy et al., 2018). In a wide range of insects, photoperiodism and photoperiodism-associated dormancy are under the control of the circadian clock system (Bloch et al., 2013; Meuti and Denlinger, 2013; Meuti et al., 2015; Saunders, 2020). Indeed, photoperiodism significantly influences JH titers in *D. melanogaster* (Saunders, 1990) and other insects (Kramer, 1978; Khan et al., 1982; Meuti and Denlinger, 2013; Matsumoto et al., 2017), although molecular and cellular mechanisms of this phenomenon remain largely unclear. On the other hand, recent studies have shown that circadian clock neurons and circadian clock genes play an important role in

modulating the neuroendocrine system to control reproductive dormancy in *D. melanogaster*. Below, we mainly summarize the role of clock neurons and circadian clock genes in reproductive dormancy, and briefly mention a possible contribution of the clock machinery to JH biosynthesis in the CA.

The molecular machinery of the circadian clock has been extensively studied in *D. melanogaster*, where the circadian master clock comprises about 150 neurons located in the central brain (Helfrich-Förster, 2004). Among the identified circadian clock neurons, the small ventral lateral neurons (s-LNvs) act as central circadian pacemakers to regulate various daily changes in *D. melanogaster* physiology. Indeed, s-LNvs are involved in the dormancy-dependent regulation of neuronal activity of IPCs (Nagy et al., 2019). The axons of *D. melanogaster* s-LNvs project to the dorsal protocerebrum, where the cell bodies and dendrites of IPCs are located, implying that s-LNvs and IPCs might have direct synaptic contact with each other (Figure 3A).

The s-LNvs are known to produce and release two neuropeptides required for the normal functioning of the behavioral circadian rhythm, namely pigment-dispersing factor (Pdf) (Park et al., 2000) and short neuropeptide F (sNPF) (Johard et al., 2009). Overexpression of either Pdf or sNPF, specifically in s-LNvs, decreases the percentage of reproductively dormant flies in dormancy-inducing condition. Furthermore, inhibition of the sNPF receptor only in IPCs also increases the percentage of dormant flies in dormancy-inducing condition. Consistent with these results, administration of synthetic Pdf or sNPF ligand to cultured brains evokes an increase in intracellular cAMP, which acts as a second messenger for the Pdf receptor and sNPF receptor in IPCs (Nagy et al., 2019). According to these data, it is hypothesized that the change in day length during winter suppresses the production and secretion of Pdf and sNPF in s-LNvs, which might provide less stimulation for IPCs and consequently induce reproductive dormancy (Figure 3B). Therefore, the suppression of IPC neuronal activity might be due to reduced activation of the receptors for Pdf and sNPF (Ojima et al., 2018). In addition, it is very likely that Dilp-mediated regulation of JH biosynthesis in the CA would be under the control of Pdf- and sNPF-dependent regulation of the circadian rhythm; however, this hypothesis has not been closely examined.

It has been reported that Pdf-dependent regulation of reproductive dormancy has been observed in the blow fly *Protophormia terraenovae* (Shiga and Numata, 2009), the mosquito *Culex pipiens* (Meuti et al., 2015), and the bean bug *Riptortus pedestris* (Ikeno et al., 2014). These data imply that Pdf-producing clock neurons are involved in reproductive dormancy beyond Drosophilidae species.

Besides the regulation mediated by the central clock pacemaker neurons, the activity of IPCs is also regulated by transcription regulators expressed in IPCs. This mechanism has been revealed in a study on a gene called *eyes absent* (*eya*) (Abrieux et al., 2020). *Eya* encodes a highly conserved transcriptional coactivator and protein phosphatase that plays vital roles in multiple developmental processes in organisms ranging from *Drosophila* to humans. While the best-known function of *Eya* is to control eye formation, it is also known that *eya* is expressed in IPCs. Knockdown of *eya* in IPCs causes an

increase in the size of the ovary, implying that Eya negatively regulates oogenesis through IPCs. Interestingly, the expression of *eya* has a circadian rhythm (Abrieux et al., 2020). More precisely, the peak of Eya protein levels is delayed in the short-day condition in comparison to the long-day condition. In addition, the protein levels of Eya in IPCs are higher under the short-day condition than the long-day condition. In parallel, Eya protein level is also upregulated under low-temperature conditions. These observations raise the possibility that the upregulation of Eya protein in dormancy-inducing conditions is necessary for oogenesis suppression (Figure 3B).

In the same study (Abrieux et al., 2020), the authors have demonstrated that the *timeless* (*tim*) gene also functions in IPCs to regulate reproductive dormancy. The *tim* gene is notable for its role in the transcriptional-translational autoregulatory feedback loops of the circadian core clock system in *D. melanogaster* (Dunlap, 1999; Hardin, 2005). In the *tim* mutant, a higher rate of reproductive dormancy is observed in any daylight length condition compared to wild type, and the impact of the short-day condition is less effective to enhance reproductive dormancy (Tauber et al., 2007; Abrieux et al., 2020). Interestingly, Tim is involved in the regulation of the Eya protein level. For example, the circadian rhythm of Eya protein is abolished in the *tim* mutant under non-dormancy-inducing conditions (Abrieux et al., 2020). Furthermore, the increased level of Eya protein at low temperatures is thought to be mediated by Tim. The Tim-mediated upregulation of Eya seems to be regulated by a unique splice isoform generated only at low temperature (Anduaga et al., 2019; Foley et al., 2019), which physically interacts with EYA protein (Abrieux et al., 2020). Based on these findings, it is hypothesized that the low temperature-specific Tim splice isoform binds Eya to suppress IPC activity, while the nature of the cell types in which this physical interaction takes place has not been precisely elucidated. Taken together, IPCs activity seems to be regulated by the external and internal regulatory system of the circadian clock in *D. melanogaster*, both of which might be required for regulating reproductive dormancy by inhibiting JH biosynthesis in the CA (Figure 3B).

Lastly, we would like to mention that a part of the circadian clock-mediated mechanisms of photoperiodism in *D. melanogaster* might be conserved even in mammals. For example, *EYA3*, one of the mammalian orthologs of *eya*, is required for the photoperiodism-dependent expression change of the *thyroid-stimulating hormone* gene in the mouse and the sheep (Dardente et al., 2010; Masumoto et al., 2010; Hut, 2011; Wood and Loudon, 2014; Wood et al., 2015).

FUTURE PERSPECTIVES

Although the studies on *D. melanogaster* in the last decades have improved our understanding of JH-mediated regulation of reproductive dormancy, several issues remain to be clarified. Here, we list the five main possible research lines, in our opinion.

(1) Do hemolymph JH titers change during reproductive dormancy induction in *D. melanogaster* and how do they change? In fact, no previous study using *D. melanogaster* has made a

conclusive report about the temporal fluctuation of hemolymph JH titers. This information must be a solid foothold for *D. melanogaster* reproductive dormancy studies and, therefore, should be obtained by the *D. melanogaster* research community in the near future. Liquid chromatography-mass spectrometry-based methods to measure hemolymph JH titers have already been reported by several groups (Reiff et al., 2015; Lee et al., 2017; Meiselman et al., 2017; Ramirez et al., 2020; Zhang et al., 2021).

(2) How does the information regarding low temperature eventually get transmitted to the CA? It has been revealed that the antenna is a vital organ for detecting cold sensations (Gallio et al., 2011; Li and Gong, 2017; Alpert et al., 2020). Therefore, there must be a neuronal circuit from the antennal cold-sensitive sensilla to the CA; however, the nature of this circuit is largely unclear. At least, it is likely that the neuronal circuit may contain some dopaminergic neurons, based on the observations that dopamine suppresses JH biosynthesis and is elevated in dormancy-inducing conditions (Andreatta et al., 2018). On the other hand, it should be noted that low temperature suppresses overall biological activity, including feeding. Since a lower level of feeding results in the suppression of energy expenditure, the dormancy-inducing low temperature might negatively affect IPC activity, possibly leading to reproductive dormancy. Meanwhile, curiously and paradoxically, some studies have reported that IPCs are activated by cold-sensing neurons at 18°C rather than 25°C (Li and Gong, 2015; Zhang et al., 2019), whereas the function of cold-sensing neurons in dormancy-inducing conditions has not yet been investigated. Further studies will be needed to clarify how low temperatures influence IPC activity to induce reproductive dormancy.

(3) How does the reproductive dormancy status influence the physiology and remodeling in each tissue? It has been reported that reproductive dormancy suppresses oogenesis and is also associated with several changes in various organs of the body. For example, the intestine is markedly shortened, and the innate immune system is activated during reproductive dormancy in *D. melanogaster* (Kubrak et al., 2014); however, the mechanisms involved are still unknown.

(4) How is male reproductive dormancy regulated? It has been reported that low temperatures and short-day condition also induce male reproductive dormancy, such as the slow-down of spermatogenesis and degeneration of male accessory glands (Kubrak et al., 2016). However, the molecular and neuroendocrine mechanisms of male reproductive dormancy have not been investigated. It has not even been elucidated whether this process depends on JHs. In the future, it would be intriguing to examine whether and how the neuroendocrine regulatory system of female reproductive dormancy resembles that of male reproductive dormancy.

(5) How does the JH-mediated regulation of reproductive dormancy contribute to the evolutionary and ecological adaptation of wild *D. melanogaster*? To date, most studies on *D. melanogaster* reproductive dormancy have been conducted in laboratories. However, since reproductive dormancy is the adaptive response of organisms to the severe winter season, it must be important to investigate the reproductive dormancy of wild animals. As described earlier, geological differences of

genetic bias influence reproductive dormancy in *D. melanogaster*. Indeed, previous studies have shown that geographic variations in reproductive dormancy in wild *D. melanogaster* is associated with single nucleotide polymorphisms in the loci of *tim*, *InR*, *FoxO*, and *couch potato* (Williams et al., 2006; Sandrelli et al., 2007; Tauber et al., 2007; Schmidt et al., 2008; Paaby et al., 2010, 2014; Kolaczowski et al., 2011; Lee et al., 2011; Fabian et al., 2012; Bergland et al., 2014; Kapun et al., 2016; Zhao et al., 2016; Betancourt et al., 2021; Machado et al., 2021). In the case of *tim*, it is hypothesized that the allelic frequencies of two distinct isoforms, *ls-tim* and *s-tim* (Rosato et al., 1997), may have evolved to cope with winter when *D. melanogaster* expanded into Europe (Tauber et al., 2007). This hypothesis is based on the observation that flies expressing *ls-tim* have a higher dormancy rate than those with *s-tim* (Sandrelli et al., 2007; Tauber et al., 2007). It would be interesting to investigate whether such genetic variations and allelic frequencies among these geographically distinct *D. melanogaster* populations affect neuroendocrine mechanisms for reproductive dormancy.

(6) How does the JH-mediated regulation of reproductive dormancy contribute to the evolutionary and ecological adaptation of other Drosophilidae species? Other Drosophilidae species also enter adult dormancy in the winter, and many biological events during dormancy are essentially the same as we found in *D. melanogaster*, although the ease of dormancy varies among species (Carson and Stalker, 1948; Lumme et al., 1974; Schmidt and Conde, 2006; Yamada and Yamamoto, 2011; Salminen and Hoikkala, 2013; Zhai et al., 2016; Lirakis et al., 2018). Therefore, wild non-*melanogaster* Drosophilidae species are interesting research subjects for understanding the evolutionary significance of genetic and ecological variations in reproductive dormancy, which may influence flies' fitness in nature.

Well-developed and sophisticated genetic tools for *D. melanogaster* will shed light on the fundamental

mechanisms of JH-mediated reproductive dormancy, not only in *D. melanogaster*, but also in other insect species. As mentioned in the Introduction, studies on reproductive dormancy would increase our understanding of the seasonal distribution of pests (Denlinger, 2008). Notably, in recent years, several researchers have used the spotted wing Drosophilidae fly *D. suzukii* for studying reproductive dormancy (Toxopeus et al., 2016; Wallingford et al., 2016; Zhai et al., 2016, 2019). Since *D. suzukii* is a well-known agricultural pest, the motivation for these studies is to explore new pest control strategies to disrupt the mechanisms of reproductive dormancy. Therefore, future studies on reproductive dormancy in Drosophilidae species, including *D. melanogaster*, have the potential to contribute to the fields of basic animal physiology and applied agricultural biology in the future.

AUTHOR CONTRIBUTIONS

YK and RN wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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