Published in final edited form as:
Dev Biol. 2021 July 01; 475: 256–264. doi:10.1016/j.ydbio.2021.01.016.

Integrating animal development: how hormones and metabolism regulate developmental transitions and brain formation

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Abstract

Our current knowledge on how individual tissues or organs are formed during animal development is considerable. However, the development of each organ does not occur in isolation and thus their formation needs to be done in a coordinated manner. This coordination is regulated by hormones, systemic signals that instruct the simultaneous development of all organs and direct tissue specific developmental programs. In addition, multi- and individual-organ development requires the integration of the nutritional state of the animal, since this affects nutrient availability necessary for the progression of development and growth. Variations in the nutritional state of the animal are normal during development, as the sources and access to nutrients greatly differ depending on the animal stage. Furthermore, adversities of the external environment also exert major alterations in extrinsic nutritional conditions. Thus, both in normal and malnutrition circumstances, the animal needs to trigger metabolic changes to maintain energy homeostasis and sustain growth and development. This metabolic flexibility is mediated by hormones, that drive both developmental encoded metabolic transitions throughout development and adaptation responses according to the nutritional state of the animal. This review aims to provide a comprehensive summary of the current knowledge of how endocrine regulation coordinates multi-organ development by orchestrating metabolic transitions and how it integrates metabolic adaptation responses to starvation. We also focus on the particular case of brain development, as it is extremely sensitive to hormonally induced metabolic changes. Finally, we discuss how brain development is prioritized over the development of other organs, as its growth can be spared from nutrient deprivation.

Introduction

Hormonal signalling is extremely important to regulate animal development and growth in both physiological and adverse conditions. Hormones are soluble long-range signalling molecules produced by specialized endocrine organs or by sensing tissues, that serve as systemic messengers to coordinate and control organ growth and development (Droujinine and Perrimon, 2016). At their target tissues, hormones trigger tissue and stage specific responses that ultimately coordinate systemic growth and the development of all organs.

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During normal development, several hormones are produced at specific stages and their combined actions trigger developmental transitions, organ re-arrangements, spurts in growth or cell death (Berger and Dubrovsky, 2005; Dubrovsky, 2005; Flatt et al., 2005).

Under ideal conditions, animal development goes through distinct stages that have different growth rates and nutrient requirements. At the same time nutrient accessibility and animal behaviour also vary throughout development. As the animal's intrinsic status is highly dependent on external conditions, development must adapt to all these changes. This is especially true for the nutritional state of the animal, since variations in nutrient availability during development are normal. Egg development, for instance, depends exclusively on internal nutrient sources, whereas larval stages require both internal and external nutritional sources (Prasad et al., 2003). Hormones are essential to regulate the animal adaptation mechanisms to these developmentally encoded changes in nutritional requirements, serving as signals that connect the metabolic status of the animal to organ specific strategies (Chatterjee et al., 2014).

Similar mechanisms are applied to deal with extreme changes in environmental conditions that can cause a reduction or even absence of nutrients throughout periods of development. In these situations, the animal needs to trigger metabolic changes to re-route energy storage/consumption pathways to maintain energy homeostasis and sustain developmental progression and growth (Yasugi et al., 2017). Under these adverse conditions, hormones are again essential to trigger metabolic adaptation mechanisms to maintain homeostasis or alter the speed of development to ensure survival (Colombani et al., 2012; Garelli et al., 2012; Hoagland, 1957). Globally, hormonal signalling is thus essential to deal with overall changes in homeostasis or stresses such as deficient nutrition or injury (Colombani et al., 2012; Garelli et al., 2012; Hoagland, 1957).

Although hormones influence systemic metabolism, different developing organs and tissues can have specific responses to hormonal signals. (Talbot et al., 1993). Hormonal regulation of metabolism in a tissue specific manner is a conserved mechanism, having also been described to occur in humans (Moøller and Joørgensen, 2009; Sinha et al., 2014). However, it is still not well understood how tissue-specific metabolic responses influence organ formation and whole animal development. In this review, we focus in the development of *Drosophila melanogaster* to highlight recent studies where hormone induced metabolic transitions were studied, yielding new insights into understanding how development is shaped by hormonally regulated metabolic changes. Moreover, we also focus on the particular case of the brain as an example of an organ whose development is highly dependent on hormonally induced metabolic changes. This organ, contrary to the rest of the body, can bypass growth impairments induced by nutritional deprivations in certain developmental windows.

Hormonal regulation of *Drosophila* development and metamorphosis

Drosophila development goes through a series of stages which involve an impressive increase in cell size and number, as well as animal size and form. It comprises an embryo stage, three larval stages (L1, L2 and L3) and a pupal stage, after which

development ends and the adult animal ecloses (Figure 1A). Several hormones control *Drosophila* development,whose pathways interact with each other, as the neuropeptide prothoracicotropic hormone (PTTH), Insulin, the Juvenile Hormone (JH) and the steroid hormone ecdysone (in its active form 20-hydroxyecdysone, 20E).

Ecdysone is the master regulator of developmental transitions. It is produced by the prothoracic gland (PG), one of three glands that compose a larger endocrine organ called ring gland. Ecdysone increases its concentration in pulses throughout development (Garen et al., 1977; Warren et al., 2006) and in peripheral tissues it is modified into 20E. There, 20E binds to the ecdysone receptor complex, a heterodimer between ecdysone receptor (EcR) and Ultraspiracle, to control the transcription of ecdysone-responsive genes (Hu et al., 2003) in a tissue specific manner (Koyama et al., 2013; Talbot et al., 1993; Von Kalm et al., 1994). Each of the pulses of ecdysone triggers a different set of events and distinct developmental transitions. The balance between the concentration of ecdysone and JH, which is produced by the corpus allatum, another endocrine gland part of the ring gland (Berger and Dubrovsky, 2005), defines the type of developmental transition taking place (Jia et al., 2017; Liu et al., 2018; Mirth et al., 2014). During development, in the presence of high JH concentrations, ecdysone triggers larva-to-larva moults. However, in the L3 stage, a lower concentration of JH allows for the three small pulses of ecdysone to mark developmental milestones necessary to start metamorphosis (Liu et al., 2018). The first peak is particularly important as it signals the reach of critical weight (CW), the minimal body mass necessary for the animal to go through metamorphosis without developmental delays. CW achievement triggers the start of the terminal growth period, the period between CW attainment and the cessation of growth. This period has a largely fixed duration that cannot be extended even in the event of starvation. This means the absence of optimal growth conditions after CW no longer delay metamorphosis (Beadle et al., 1938; Hironaka et al., 2019; Mirth et al., 2005). The second peak in L3 promotes the segregation of glue genes that allow the animal to adhere to vertical surfaces once it forms a puparium. The third peak promotes the induction of wandering behaviour and the cessation of feeding that occurs previous to pupariation (Rewitz et al., 2013). Finally, in late L3 development, a high concentration pulse of ecdysone initiates puparium formation and the cessation of growth (Berreur and Porcheron, 1984; Yamanaka et al., 2013).

To connect developmental progression with systemic growth, ecdysone production is regulated in response to PTTH and Insulin. PTTH is produced from two prothoracicotropic neurons that innervate the PG, where it promotes the synthesis of ecdysone (Rewitz et al., 2009). A recent study identified a feedback mechanism where the peak of ecdysone that occurs at CW attainment in early L3 development upregulates *Ptth* expression. Consequently, this leads to PTTH release to the PG and more ecdysone synthesis, producing the next ecdysone peaks in L3 stage (Christensen et al., 2020).

The insulin signalling pathway (IIS)/insulin-like growth factor (IGF) and the target of rapamycin (TOR) signalling pathway are crucial players to ensure that systemic growth is adjusted to the nutritional conditions available to the organism (Clemmons, 1997). During larval development, insulin acts in the PG to promote the growth of this gland, which enables production of enough ecdysone to trigger the first small ecdysone peak of the L3

stage, signalling the achievement of CW (Koyama et al., 2014; Mirth et al., 2005). In response to the presence of dietary nutrients, Insulin Producing cells (IPCs), located in the brain, produce insulin-like peptides (dILPs). *Drosophila* has 8 dILPs described and, in target tissues, dILPs-1 through 7 bind to the insulin receptor (dInR) to repress growth inhibition and stress response pathways, allowing growth to occur (Lin and Smagghe, 2018). dILP-8 has a different mechanism of action, binding to relaxin receptor homolog Lgr3 in both the PG and brain to slow growth in response to injury (Garelli et al., 2012; Jaszczak et al., 2016).

Moreover, in response to local metabolites, mainly amino acids, the TOR pathway can be activated cell-autonomously in all tissues, in parallel to IIS (Colombani et al., 2003). This pathway promotes cellular growth through translational initiation, ribosome biogenesis, nutrient storage, endocytosis, and autophagy (Arsham and Neufeld, 2006). Furthermore, TOR is described to also have a role in CW achievement. During early L3 stage TOR signalling promotes the endoreplication of PG cells in early L3 development. This event triggers activation of the ecdysone synthesis pathway in an irreversible manner and, hence, production of the ecdysone CW peak (Ohhara et al., 2017; Zeng et al., 2020). Nonetheless, to integrate systemic and local cues, the TOR and IIS pathways are interconnected, sharing key regulators, like Protein Kinase B (also known as Akt), serine/threonine protein kinase and Forkhead Box class O (FoxO) (Grewal, 2009).

Thus, developmental progression results from the integration of several hormonal and signalling pathways. In the next chapter, we will highlight several studies showing how hormonal signalling can culminate in the alteration of metabolic strategies essential to drive developmental transitions.

Developmentally coordinated metabolic switches

During the different stages of development, the body relies on different nutrient sources to grow. Embryonic development is dependent on the nutrients deposited by the mother into the egg during its production, whereas during larval stages, growth depends on nutrients obtained from food sources. Then, pupal development is dependent on metabolites obtained from the remodelling of larval tissues or nutrients stored during larval development. This means that each stage of *Drosophila's* development has different energetic and metabolic requirements. From embryonic development to larva there are major differences in the source of metabolites, thus, this requires considerable changes in cellular metabolic strategies. Consistently, it was shown that, before larval development starts, a major systemic metabolic transition occurs in embryonic cells. Mid-way through embryo development, at 12–18 hr after egg laying, the orphan nuclear receptor Estrogen-Related Receptor (dERR) is activated by a still unknown mechanism or an unidentified ligand (Tennessen et al., 2011). dERR then promotes the expression of glycolytic and pentose phosphate pathway (PPP) enzymes, as well as Lactate dehydrogenase (ImpL3) (Figure 1B panel I). This expression turns ON the aerobic glycolysis metabolic program, a metabolic state normally associated with cell proliferation. Upon hatching, this transcriptional activation enables cells to utilize metabolites such as carbohydrates to support the rapid proliferation and major growth that occurs during larval development (Tennessen et al.,

2011). Before this glycolytic switch, cells present an increased expression of genes involved in lipid metabolism such as enzymes for b-oxidation, carnitine palmitoyltransferase and lipase 1, necessary for fatty acid break down. However, these genes are co-ordinately downregulated in late embryogenesis, in parallel with the onset of the glycolytic pathway induction. This again indicates that, in preparation for hatching, the embryo switches its metabolic strategy program from fatty acid break down to aerobic glycolysis (Tennessen et al., 2014). Therefore, dERR directs a coordinated metabolic switch that is of key importance to establish the metabolic requirements for larval development as it allows cellular metabolism to rely on nutrients from dietary intake and support the growth that occurs during larval development.

The ability to support growth during larval stages is a crucial feature, because the final adult body size is determined by the animal size at the end of larval development (Nijhout et al., 2014). During larval development, IIS stimulates high levels of lipogenesis (Church and Robertson, 1966; DiAngelo and Birnbaum, 2009), required to sustain the ~200-fold increase in body mass that occurs during this stage. The animal also stores metabolites later required as energy sources and building blocks to complete metamorphosis (Carvalho et al., 2012). Interestingly, a recent article demonstrated that the CW peak is important to promote storage over consumption of different metabolites, including Trehalose, to ensure the animal has sufficient nutrients to go through metamorphosis (Yamada et al., 2020). Before pupal development starts, the larva starts wandering, leaves its food source, and eventually forms a white pre pupae, a process called pupariation (Figure 1A). When wandering behaviour starts, the animal stops feeding and thus does not have access to additional nutrients. At the onset of wandering behaviour, another metabolic switch occurs, that alters larval lipid metabolism through upregulation of the transcriptional regulator complex composed by cyclin-dependent kinase 8 (CDK8) and its regulatory partner cyclin C (CycC) in nthe fat body, the equivalent to the adipose tissue of *Drosophila.* The CDK8/CycC complex is part of the larger Mediator complex, a general regulator of gene expression that connects specific transcription factors to the RNA Polymerase II complex (Soutourina, 2018). CDK8/CycC negatively regulates the lipogenesis pathway (Zhao et al., 2012), and is also required for proper activation of EcR-target genes (Xie et al., 2015) (Figure 1B panel II). Hence, this allows larval metabolism to shift from lipogenesis, a energy storage strategy, to energy consumption during metamorphosis. This was confirmed by the observation that, at this point, lipids stop accumulating for the first time during larval development (Carvalho et al., 2012).

At the end of the third larval stage, the high concentration peak of ecdysone that triggers pupariation is described to promote another metabolic transition. At this point, ecdysone signalling leads to the repression of dMyc expression in the fat body (Figure 1B panel II). This inhibition restricts ribosome biosynthesis and translation efficiency in fat body cells, which induces an arrest of the growth program, initiating the transition from larval to pupal development (Delanoue et al., 2010). Metamorphosis is the last developmental event of *Drosophila's* life cycle. During this period most larval tissues are histolysed and recycled to form building blocks and supply metabolites. This allows the proliferation and differentiation of imaginal discs, to form the adult body structures (Held, 2002; Thummel, 2001).

Systemically, the metabolic rates during metamorphosis follow a U-shaped curve, where energy consumption is high during the first 24 hr, declines in the mid-pupal stages and increases again in the last stages, before adult eclosion (Merkey et al., 2011). At the onset of pupariation, the high concentration peak of ecdysone induces the expression of Trehalose transporter genes and trehalase, the enzyme that catalyses the conversion of Trehalose into glucose (Figure 1B panel III). This triggers a feedforward mechanism by which ecdysone promotes the catabolism of Trehalose to produce the energy and building blocks to sustain development at this stage. This increase in Trehalose and glucose are also required for ecdysteroid biosynthesis necessary to promote pupation, the prepupa to pupal transition that occurs at 12 hr after puparium formation (APF) (Nishimura, 2020).

In mid-pupae development (starting at 48 hr APF) there is an upregulation of glycolysis, followed by an increase in the PPP at 72 hr after puparium formation (Figure 1B panel IV). Similar to the metabolic switch occurring during embryonic development, this upregulation seems to be under the control of dERR, as dERR conditional mutants show a delay in the expression of the genes involved in these pathways. Furthermore, dERR conditional mutant flies have reduced levels of citrate and ATP citrate lyase, both required for lipogenesis, indicating that dERR is also indirectly involved in the upregulation of lipogenesis during this stage (Beebe et al., 2020).

The adult that emerges from the pupal case is a motile and reproductively active animal. Adult animals must find food, escape predators, copulate and produce progeny, an energetically high demanding activity. This again, means that the metabolic requirements of the adult fly are very different from those of pupal development. At the onset of the adult stage, dHNf4, a nuclear receptor, regulates another metabolic transition. dHNf4 is a ligand-regulated transcription factor that promotes mitochondrial oxidative phosphorylation (OxPhos) by regulating nuclear and mitochondrial gene expression (Figure 1B panel V) (Barry and Thummel, 2016). This triggers a systemic transcriptional switch in newly emerged adults that supports mitochondrial function. The actions of dERR and dHNf4 are thought to be necessary to allow for a more efficient way to produce the energy required for proper adult function and motility. It remains to be explained, however, how the upregulation of glycolysis, PPP and lipogenesis pathways are necessary mid-way through pupal development. Possibly, this upregulation is necessary to store energy reserves to support the first hours of the newly eclosed adult animal, before it starts feeding (Aguila et al., 2007; Chiang, 1963). Alternatively, it could support the production of hydrocarbons required for the synthesis of pheromones and the waterproofing of the adult cuticle (Storelli et al., 2019). It is still not clear how these nuclear receptors may interact with hormonal signalling. Nonetheless, a recent study indicated that ecdysone represses dERR target genes involved in glycolysis in larval tissues and S2 cells, shedding a light on the possible interaction between these two pathways (Kovalenko et al., 2019).

Adaptation to starvation

When the optimal nutritional conditions are not met, development must adapt. Starvation is, therefore, a very interesting condition to study how hormonal signals and metabolic strategies are interconnected. In the event of nutrient deficits, multiple secreted factors,

which are sensors of different nutrient derived metabolites, converge in the regulation of IIS. Nutrient sensing tissues, such as the gut and the fat body, send signals to the brain in order to regulate dILP expression and secretion from the IPCs, informing the rest of the organs about the body's nutritional state. Under normal fed circumstances, the fat body, one of the major nutrient sensing tissues, is known to secrete several factors that induce animal growth through IIS (Figure 2). The human homolog of Leptin, Unpaired 2 (Upd2), is produced by the fat body in the presence of lipids and glucose from the diet. Upd2 activates Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signalling pathway in GABAergic neurons in the brain, supressing their inhibitory effect on the IPCs. This allows the secretion of dILPs to promote systemic growth and fat storage (Rajan and Perrimon, 2012). Moreover, in response to high nutritional levels of protein and glucose, the fat body produces Growth Blocking Peptides (GBPs), the peptide hormone CCHamide- 2 (CCHa2) and the peptide Stunted (Sun). In the IPCs, CCHa2 acts through its receptor CCHa2-R, promoting the expression of dILP-5 and the release of dILP-2, inducing growth (Ren et al., 2015; Sano et al., 2015). On the other hand, GBPs and epidermal growth factor (EGF)-like cytokines activate EGFR signalling in inhibitory neurons, relieving inhibition of the IPCs and, as consequence, promoting dILP secretion (Koyama and Mirth, 2016; Meschi et al., 2019). Sun binds to its receptor Methuselah in the IPCs, which is necessary for dILP-2 release and growth promotion (Delanoue et al., 2016). Interestingly, Sun is also part of the F1F0-ATP synthase complex present in the mitochondria (Cvejic et al., 2004; Kidd et al., 2005), suggesting that hormonal signalling can potentially regulate cellular metabolism directly.

In the event of starvation, animals enter a survival mode and can either promote the conversion of stored nutrients for utilization or repress growth pathways to slow down or even inhibit growth. In response to low levels of carbohydrates in the haemolymph, the adipokinetic hormone (AKH), the functional homolog to vertebrate glucagon, is released from the corpus cardiacum (CC), the third structure of the ring gland (Figure 2). In an attempt to sustain growth, AKH binds to and activates its receptor, AKHR, in the fat body and induces cyclic adenosine monophosphate/protein kinase A signalling. This stimulates the conversion of stored glycogen to trehalose and triglyceride to diglyceride, allowing the systemic maintenance of lipids and sugar levels (Gr et al., 2007). Trehalose, in turn, acts on the CC to stimulate the release of AKH, which then acts on the IPCs to induce the secretion of dILP-3 and promote larval growth (Kim and Neufeld, 2015). Another metabolic hormone, Eiger, the homologue of Tumour-necrosis factor alpha, was shown to mediate the response to protein starvation. Under protein shortage, Eiger is released from the fat body into the haemolymph and once it reaches the IPCs it binds to its receptor, Grindelwald. This leads to the activation of the Jun- N-terminal kinase pathway and to the inhibition of dILP-2 and dILP-5 expression, which culminates in growth inhibition (Figure 2) (Agrawal et al., 2016). Secreted decoy of InR (SDR) is also released to inhibit IIS in response to starvation, being described to bind to dILPs. It is produced by glial cells and binds to circulating dILP-3 in the haemolymph, negatively regulating systemic growth by antagonizing IIS (Figure 2) (Okamoto et al., 2013).

Starvation occurring during the terminal growth period, signals the release from the fat body of Imaginal morphogenesis protein-Late 2 (Imp-L2), the functional homolog of vertebrate

insulin-like growth factor-binding protein 7. Once released to the haemolymph, Imp-L2 acts in the IPCs to bind to and inactivate dILP-2, repressing growth (Honegger et al., 2008; Kwon et al., 2015; Lee et al., 2018). A recent study by Yamada et al. revealed a new role for Imp-L2. Independently of IIS, Imp-L2 inhibits trehalase after CW attainment, allowing for the maintenance of carbohydrate reserves even in the event of starvation, thus ensuring metamorphosis has metabolic conditions to occur (Yamada et al., 2020).

The inputs coming from these different hormones and metabolite sensing mechanisms can occur simultaneously and need to be integrated in the brain to relay a unique systemic response. However, it is not yet explored how these different hormones interact with each other and how this interaction influences the organism's response.

Hormone control of metabolism to direct cellular proliferation and fate – the particular case of brain development

The brain is one of the most important and complex organs in the body and its formation is particularly sensitive to the developmental and metabolic stages of the animal. During brain formation, neural progenitors proliferate in a stereotypical manner to produce different types of neurons and glia, critical for adult circuit formation. However, to form a correctly functioning nervous system, neurogenesis must be synchronized with developmental timing as well as to the hormonal and metabolic state of the organism (for a detailed review on neurogenesis see Homem and Knoblich, 2012 and Homem et al., 2015). Hence, understanding how systemic hormonal signals integrate with neural progenitor metabolism, fate and proliferation has become an interesting issue to uncover in the field. We will use *Drosophila* brain development as an exciting example where recent research showed how hormonal control and metabolic regulation influence cell fate and proliferation.

Regulation of brain development through IIS

Drosophila brain development is largely divided in two rounds of neurogenesis - an embryonic and a larval neural progenitor proliferation wave (Figure 3A). In the embryo, neural stem cells, the neuroblasts (NBs), start dividing to self-renew, i.e. form another NB, and generate differentiated neurons and glia. At the end of embryogenesis, most NBs are eliminated through apoptosis. However, in the central brain and thoracic ventral nerve cord, NBs suffer a cell cycle arrest, entering a quiescent state (G0) through an intricate transcriptional program (Champlin and Truman, 1998; White et al., 1994).

Upon larval hatching and feeding, the increase in circulating amino acids is perceived as a nutritional cue that triggers NB cell cycle re-entry, a process dependent on inter-organ signalling (Figure 3A). This nutritional cue is sensed by the fat body that, in turn, sends a secreted signal to stimulate the production and secretion of dILP-6 in a subset of glia that neighbours NBs (Chell and Brand, 2010). This fat-body derived signal is not yet identified, but some potential candidates include the previously mentioned Sun, GBP1/GBP2 and Upd2 factors (Delanoue et al., 2016; Koyama and Mirth, 2016; Rajan and Perrimon, 2012). In underlying dormant NBs, dILPs activate the InR and the downstream phosphatidylinositol 3-kinase (PI3K)/Akt pathway that, together with TOR signalling, trigger NB exit from

quiescence(Chell and Brand, 2010; Yuan Id et al., 2020) (Figure 3A). Although the precise metabolic alterations that take place upon NB reactivation are not fully elucidated, PI3K/Akt signalling is traditionally associated with an increase in anabolic metabolism and promotion of growth, whereas the TOR pathway constitutes a potent mediator of growth, as previously mentioned (Britton et al., 2002). Hence, PI3K/Akt and TOR pathways act in synergy in dormant NBs to drive metabolic changes, growth and ultimately cell cycle re-entry for timely NB reactivation (Chell and Brand, 2010; Sousa-Nunes et al., 2011). dILPs were also shown to be essential mediators of NB reactivation. In the larval ventral nerve cord, overexpression of dILP-6 or dILP-2 in glia, under nutrient restriction, is sufficient to trigger NB reactivation from quiescence (Chell and Brand (2010)). Consistently, dILP-2 overexpression in IPCs or in glia can rescue brain NB reactivation in a dILP-2 mutant background. Interestingly, this work also hinted that dILP-2 can regulate dILP-6 levels. Together, these studies suggest that, upon larval feeding, IPCs produce dILP-2, which possibly cross regulates the expression of other dILPs, such as the glia-specific synthesis and secretion of dILP-6 to promote NB reactivation (Chell and Brand, 2010; Yuan Id et al., 2020).

In sum, brain development in the early larva is tightly linked with the nutritional state of the organism. The reactivation of NB proliferation results from an interplay of hormonal signals from multiple tissues in the animal, both inside and outside of the brain. This event marks the start of the second round of neurogenesis.

Regulation of brain development through ecdysone signalling

Once NBs re-enter cell cycle in larval stages, the second wave of neurogenesis begins, continuing until early pupa stages, when NBs decommission and neurogenesis ends (Maurange et al., 2008; Homem et al., 2014). During this second wave, ecdysone signalling is a crucial coordinator of brain development. In this section, we will focus on how ecdysone can influence neural progenitor transcription and metabolism to promote major neuronal fate changes, as well as the end of neurogenesis.

Once NBs have generated their complete neural lineages, which for central brain and thoracic NBs mainly occurs during pupal development, NBs are decommissioned (Maurange et al., 2008; Yang et al., 2017). Interestingly, prior to their decommissioning, this set of pupal NBs ceases to grow after each division, progressively reducing their size and culminating with cell cycle exit (Figure 3A). Contrary to expected, reduction in NB size is not regulated by the nutritional status of the animal nor through IIS. NB decommissioning program requires ecdysone, which acts directly in NBs by binding to EcR in these cells (Homem et al., 2014). EcR acts along with the Mediator complex to promote several NB transcriptional changes affecting the expression levels of several metabolic enzymes during the larva-to-pupa transition. These transcriptional changes trigger a metabolic reprogramming in NBs, which change from a glycolytic metabolism towards a more OxPhos dependent metabolism, promoting NB cell size reduction and NB decommission (Homem et al., 2014) (Figure 3A). Interestingly, this metabolic switch in pupal NBs occurs at a stage when the animal is no longer feeding, so it remains unknown what is the origin of the metabolite sources, normally lipids and amino acids, necessary to fuel NB-OxPhos.

As the levels of anabolism in stem cells can be critical players in regulating fate and proliferation (reviewed in Folmes and Terzic, 2016), this metabolic switch is pivotal for NB decommission. Hence, ecdysone signalling is crucial to modulate NBs' metabolism and terminate neurogenesis.

Besides being a major regulator of neural progenitor fate and proliferation, ecdysone has also been described as a key player in regulating the temporal expression of transcription factors that modulate neuronal fate (Syed et al., 2017). Additionally, ecdysone is also involved in neuronal circuit assembly through the precise temporal expression of neuronal wiring genes (Jain et al., 2020). How temporal expression of transcription factors influence brain development and how ecdysone contributes to this process is reviewed elsewhere (see Doe, 2017) and will not be addressed here.

Brain development under nutrient restriction – the brain sparing phenomenon

The nutritional status of the animal is incredibly important to control the final size of adult organs. Nevertheless, the brain is mostly spared from nutrient deficiencies, as a slight change in its developmental pace is enough to trigger irreversible brain deficiencies (Cheng et al., 2011). After achieving CW in the L3 stage, the animal is bound to progress through development and if dietary nutrients are scarce/absent, the growth rate of the larval tissues slow down leading to the formation of smaller animals. However, the central nervous system continues to grow at an almost regular rate to ensure the brain is correctly formed and functional (Lanet et al., 2013) (Figure 3B).

This is called the brain sparing phenomenon, where NB proliferation becomes independent of animal nutrition during the last instar of larval development. Upon a reduction of dietary amino acids and, consequently, a reduction in dILPs, neural progenitors can maintain growth through the Anaplastic lymphoma kinase (Alk). This tyrosine kinase can activate the IIS in the absence of dILPs and bypass the amino acid sensing routes by regulating TOR signalling downstream effectors S6K and 4E-BP. This is possible as the Alk ligand, Jelly belly (Jeb), is constitutively expressed in NB neighbouring glial cells, independently of nutritional conditions (Cheng et al., 2011) (Figure 3B). Thus, Jeb/Alk activation constitutes a bypassing mechanism to re-direct NB metabolism toward proliferation in a nutrient/TOR/IIS-independent manner. Further details on how the brain develops under nutritional restriction are reviewed in Lanet and Maurange (2014).

Recently, another mechanism emerged as a regulator of brain sparing through Pathetic (Path), a broad specificity membrane transporter expressed in both NBs and glia (Feng et al., 2020). In NBs, *path* is a direct Notch target via Su(H) binding at an intronic enhancer, PathNRE, and is required to maintain NB proliferation rate. Nevertheless, Path expression in glial cells is required for brain sparing in nutrient restriction conditions. Although the mechanism remains to be elucidated, one of the hypotheses is that, in glial cells, Path can sense environmental amino acid levels and regulate the expression of Alk/Jeb accordingly. Additionally, considering that *pathNRE* is upregulated after NB reactivation, a process

dependent on nutrition, *path* can also be required in NBs to bypass or switch-off the activity of the TOR pathway in later stages of larval development (Feng et al., 2020).

Regardless of the mechanism by which Path induces NB starvation resistance, it seems that glial cells play a major role in regulating overall brain sparing. A transcriptome atlas of the *Drosophila* first instar brain, demonstrated that, under starvation, there are global changes in the glial transcriptional program, revealing metabolic changes prioritizing lipid catabolism (Brunet Avalos et al., 2019). Considering that glial cells are key players in the metabolic fuelling of brain cells (e.g glia secrete alanine and lactate to support neuronal mitochondrial metabolism (Volkenhoff et al., 2015)), glial metabolic rewiring can constitute an adaptive mechanism to sustain brain development under starvation in early larval stages. Maybe glia also contributes to brain sparing under starvation by providing metabolites to fuel NB metabolism, ensuring that NB proliferation remains constant in later larval stages.

Interestingly sparing mechanisms can also influence other neural cell types. A recent report describes a sparing phenomenon that influences dendrite arborization of neurons. Dendrite growth does not result from increases in cell number, but through membrane expansions. Under nutrient restriction, somatosensory neurons in the peripheral nervous system maintain low levels of FoxO compared to their neighbour epidermal cells which allows for the overgrowth of their dendrite arbors (Poe et al. (2020)). FoxO is a transcription factor activated under stress conditions, such as starvation, that supresses TOR signalling and induces autophagy. Hence, in starvation conditions, non-neural cells suffer a reduction in IIS/TOR signalling, that along with high levels of FoxO, significantly slows down cell growth. In contrast, TOR signalling in somatosensory neurons is not further suppressed by FoxO, therefore, these cells can bypass this growth suppression. This means these neurons possess their own intrinsic genetic program to prime brain sparing through the regulation of the TOR pathway.

Since periods of nutrient deprivation are not such rare events in the wild, animals have evolved fascinating mechanisms to maintain brain development. Interestingly, the beginning of brain sparing is triggered only after the hormonal-induced CW, a point by which blocking animal growth will not affect developmental timing. Since the brain cannot withstand a block/reduction in cell proliferation and growth, the nutritional sparing constitutes a bypassing mechanism of extreme importance for the correct brain formation and overall survival of the organism.

Conclusion

Over the years, we have witnessed a significant progress in the understanding of endocrine regulation of developmental transitions in animals. However, the connection between animal development and hormonally induced metabolic changes is less explored. The studies summarized here demonstrate that the integration of hormonal regulation throughout development is crucial to modulate both systemic and organ metabolic strategies. This is done according to the energetic requirements of each developmental stage, in both physiological and starvation conditions. At the organ-specific level, we focused our attention on brain development that highly depends on hormonal-mediated metabolic regulation.

Thus, it is vital that future metabolic studies consider development of the organism as a key factor, as it is clear it has a major influence on metabolic and proliferation strategies. In particular, it would be interesting to uncover how the multiple hormones in circulation may interact with each other to drive metabolic responses during development. Moreover, it would also be relevant to explore the role of hormones other than ecdysone and insulin, might have in metabolic regulation during brain development.

Acknowledgements

Work in C.F.H's laboratory is supported the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (H2020-ERC-2017-STG-GA 759853-StemCellHabitat); by Wellcome Trust and Howard Hughes Medical Institute (HHMI-208581/Z/17/Z-Metabolic Reg SC fate); and by EMBO Installation grant (H2020-EMBO-3311/2017/G2017). A.C.O. is funded by Fundação para a Ciência e Tecnologia (PD/BD/136895/2018)

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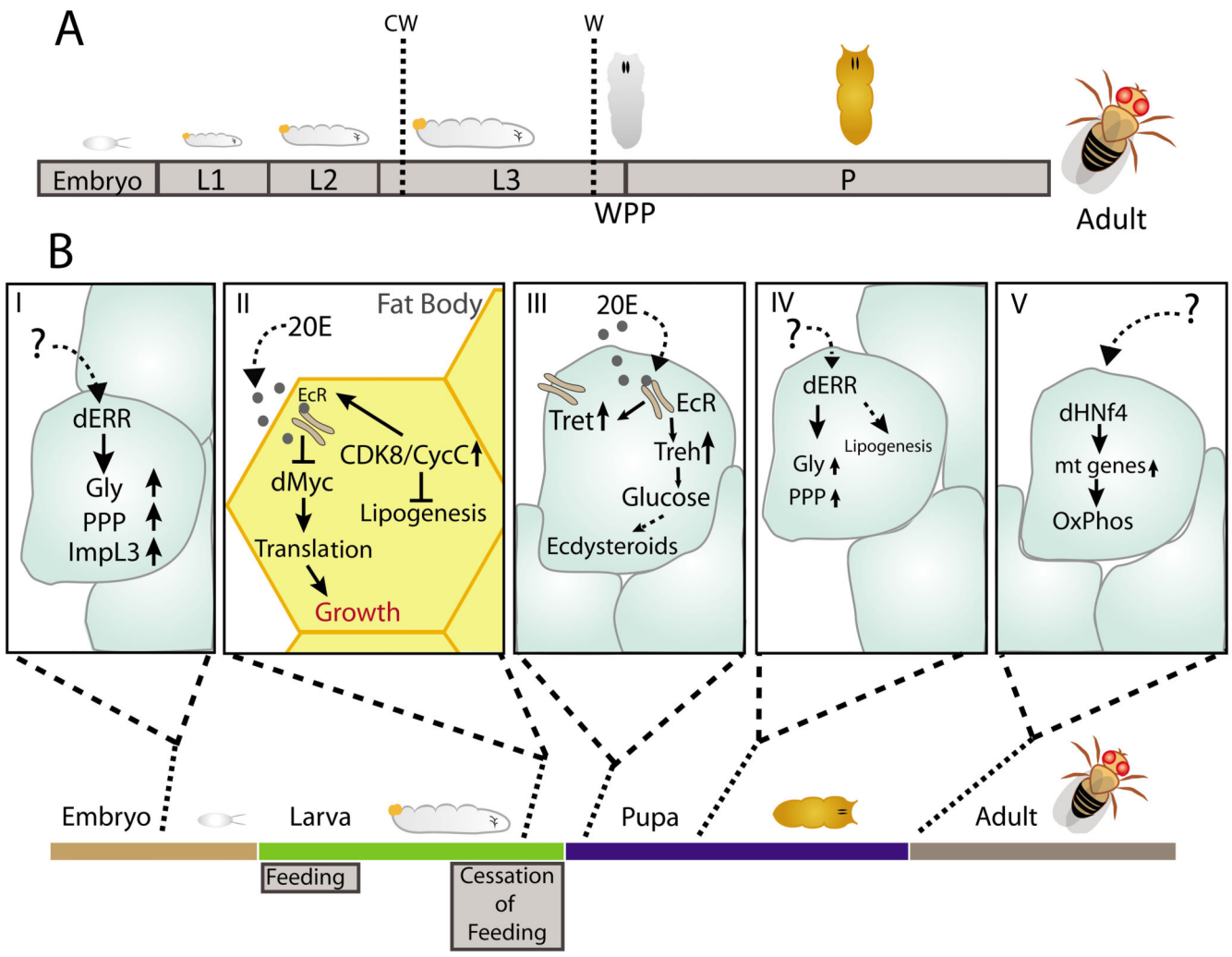

Figure 1. Developmental metabolic transitions regulated by hormonal signalling.

(A) Diagram of the *Drosophila melanogaster* life cycle: The developmental timing of *Drosophila* is highly influenced by temperature and other circumstances, as food availability. *Drosophila* life cycle starts with an embryonic phase that lasts 24 hr, followed by hatching of the first instar larva (L1) from the egg. 24 hr later L1 instars moult to a second larval instar (L2). The L2 instar lasts for another 24 hr and is followed by a second moult to the third larval instar (L3), the last larval stage. At the beginning of this stage, that spans 48 hr, larvae reach critical weight (CW), and in late L3 development larvae cease feeding and start wandering out of the food (W) in order to form a white pre pupa (WPP). The pupal stage (P) that follows lasts for 5 days after which an adult individual ecloses from the pupal case. The duration of development is dependent on extrinsic conditions like temperature, nutrient availability and injury. The average time of development from egg to adult at 25ºC and optimal conditions is 10 days. **(B)** Midway through embryonic development, the *Drosophila* Estrogen Related Receptor (dERR) promotes the upregulation of Glycolysis (Gly), Pentose Phosphate pathway (PPP) and Imaginal morphogenesis protein-Late 3 (ImpL3) to capacitate the embryonic cells to be able to metabolize nutrients acquired from food ingestion (Panel

I). At the end of larval development fat body cells upregulate the cyclin-dependent kinase 8/cyclin C complex (Cdk8/CycC) that acts to repress lipogenesis and activate EcR target genes. Ecdysone signalling at these stage acts to promote the cessation of growth by repressing translation efficiency through dMyc (Panel II). At the onset of pupariation, ecdysone promotes the expression of trehalase (Treh) and trehalose transporters (Tret) genes to catalyse the conversion of trehalose into glucose. Glucose can be used as an energy source or to form cholesterol (Panel III). During pupal development a new metabolic shift occurs to promote the expression of genes involved in Glycolysis, PPP and lipogenesis pathways which are induced by dERR (Panel IV). At the onset of the adult stage dHNF4 promotes Oxidative Phosphorylation (Oxphos) by regulating the expression of mitochondrial genes (Panel V).

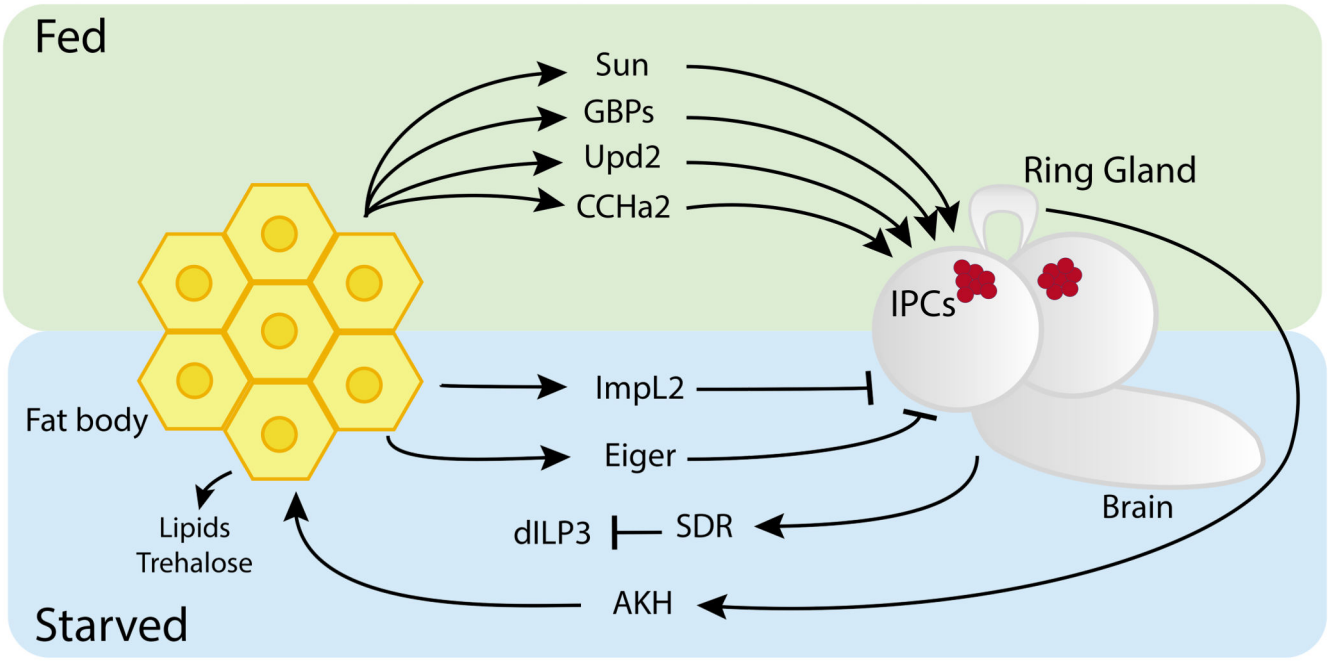

Figure 2. Hormonal pathways of nutrient sensing.

Schematic representation of nutrient sensing mechanisms. Under normal fed conditions (upper panel) the presence of dietary nutrients promotes the release from the fat body (yellow cells) of hormones like Stunted (Sun), Growth blocking peptides (GBPs), Unpaired 2 (Upd2) or CCHamide- 2 (CCHa2) that will act on the insulin producing cells in the brain (IPCs in red) to promote growth of tissues and developmental progression. In conditions of nutrient restriction (lower panel) the fat body releases Imaginal morphogenesis protein-Late 2 (ImpL2) or Eiger to act in the IPCs to halt the growth program. Glial cells in the brain release the Secreted Decoy of InR (SDR) that binds to and inactivates Insulin like peptide 3 (dILP3) in the haemolymph. To promote the utilization of stored metabolites the Ring Gland releases adipokinetic hormone (Akh) to act in the fat body and promote the release of lipids and trehalose to the haemolymph.

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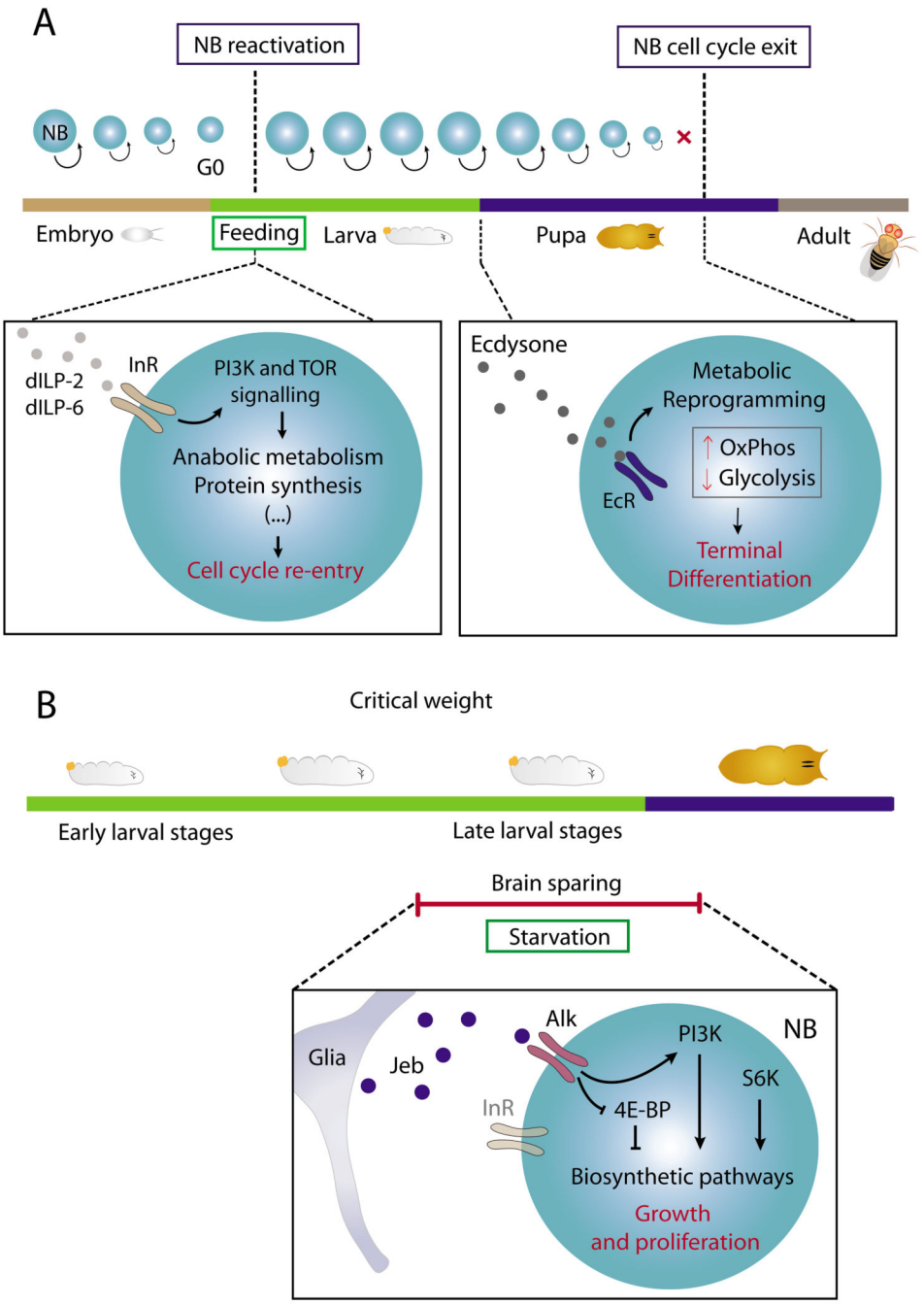

Figure 3. The influence of nutrition and hormonally induced metabolic changes in neurogenesis.

(A) Scheme depicting the timeline of neurogenesis along *Drosophila* development. Neuroblasts (NBs, blue circles) are first generated in the embryo and do not re-grow at each cell division. At late embryo stages, NBs become quiescent (G0). Upon animal feeding, in early larval stages, NBs re-enter cell cycle through the activation of biosynthetic and anabolic pathways in an insulin and nutrient dependent manner, initiating the post-embryonic wave of neurogenesis. Larval NBs re-grow upon each division and proliferate until early pupal stages. At the larva-to-pupa transition, the steroid hormone ecdysone

contributes to the reduction in NB growth and decommission through a metabolic switch from glycolysis towards an Oxidative Phosphorylation (OxPhos)-dependent metabolism. **(B)** Schematic representation of a timeline of *Drosophila* larval and early pupal stages. After reaching the critical weigh (CW) at mid-larval stages, if the animal suffers from starvation it slows down growth rate. Nevertheless, brain growth can be protected through a glia dependent Anaplastic lymphoma kinase (Alk)/Jelly belly (Jeb) mediated pathway. This signalling cascade induces biosynthetic pathways in NBs, independent of insulin receptor (InR) activation and nutritional conditions, promoting NB growth and proliferation. *Drosophila* insulin-like peptide (dILP); Ecdysone receptor (EcR); Phosphoinositide 3-kinase (PI3K); Target of rapamycin (TOR); Serine/threonine kinase (S6K); Translation initiation factor 4E-binding protein (4E-BP).

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