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REVIEW

Nutritional regulation of stem and progenitor cells in *Drosophila*

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ABSTRACT

Stem cells and their progenitors are maintained within a microenvironment, termed the niche, through local cell-cell communication. Systemic signals originating outside the niche also affect stem cell and progenitor behavior. This review summarizes studies that pertain to nutritional effects on stem and progenitor cell maintenance and proliferation in *Drosophila*. Multiple tissue types are discussed that utilize the insulin-related signaling pathway to convey nutritional information either directly to these progenitors or via other cell types within the niche. The concept of systemic control of these cell types is not limited to *Drosophila* and may be functional in vertebrate systems, including mammals.

KEY WORDS: *Drosophila*, Insulin, Dilp, Germline stem cell, Intestinal stem cell, Hematopoietic progenitor cell, Neural stem cell, Amino acid sensing, Nutritional control

Introduction

Stem cells possess the ability to self-renew and also to give rise to one or more differentiated cells that typically populate a single tissue type (Weissman, 2000; Scadden, 2006). The stem cell maturation process often includes progenitor cell types that retain many characteristics of their precursors (Weissman et al., 2001; Morrison and Spradling, 2008). Stem and progenitor cells occupy a specialized microenvironment, termed the niche, which emanates signals to control their proper maintenance, differentiation, proliferation and survival (Fuchs et al., 2004; Morrison and Spradling, 2008). Niche-mediated control of stem cells by their local environment has been extensively studied (Moore and Lemischka, 2006), and a number of local signals secreted by niches have been identified. These include bone morphogenetic proteins (BMPs), Wnts and Hedgehog (Hh), which act directly on the precursors and are necessary for their maintenance and their ability to proliferate (Li and Xie, 2005; Reya and Clevers, 2005; Scadden, 2006). In addition to niche-based local signals, those that arise from differentiating cells also maintain stem and progenitor cell populations (Hsu et al., 2011; Mondal et al., 2011).

The overall physiological status of an organism is also important in determining stem and progenitor cell fate. This aspect of control is important to ensure that these cells respond to rapid growth, injury and other environmental challenges in order to meet the needs of an organism. Accumulating evidence indicates that signals originating outside the niche are important for stem cell homeostasis (Conboy et al., 2005; Spiegel et al., 2008). A decade of research on *Drosophila* stem cells has provided novel insights into how the stem and progenitor cell pools are controlled and maintained in developing and adult tissues. Recent research has focused on nutrient-based regulation of stem and progenitor cell fate and how nutrient availability relates to metabolic regulation essential for the maintenance of tissue homeostasis. Given the conservation of tissue types and signals, it is reasonable to anticipate that stem cell populations in mammals would also share similar regulatory circuits in response to nutrient signals. Although this remains to be demonstrated, the systemic signal-mediated paradigms established in *Drosophila* are likely to be conceptually similar to stem cell signaling in mammalian systems (Ables and Drummond-Barbosa, 2011; Losick et al., 2011; Ables et al., 2012). In vertebrates, aged satellite cells are rejuvenated when exposed to an environment created from a younger animal (Conboy et al., 2005). Similarly, alteration of the systemic environment of the blood stem cell niche induces age-related processes that are dependent on insulin signaling (Mayack et al., 2010). Finally, it is well established that hematopoietic stem and progenitor cells interact dynamically with neurons and with the immune system (Spiegel et al., 2008). In the above examples from mammalian studies, the mechanisms by which stem and progenitor cells interact with specific systemic signals have not yet been elucidated, whereas in *Drosophila* the tools and technologies to allow such genetic dissection are readily available. This review focuses on the effect of nutrition on stem and progenitor cell development in different organ systems in *Drosophila*. In many cases, the pathways that sense nutritional input are also normally used for the development of various stem and progenitor classes.

Insulin signaling in *Drosophila*

The insulin-like growth factors (IGFs) and insulin in vertebrates, as well as insulin-like peptides in insects, help to coordinate nutritional status with systemic growth control (Hietakangas and Cohen, 2009; Johnson et al., 2013). The *Drosophila* insulin pathway is highly conserved and closely resembles the mammalian pathway in its physiological functions (Wu and Brown, 2006; Taguchi and White, 2008). Loss of *Drosophila* insulin-like peptides (Dilps; also known as Ilps) causes reduced growth, low triglyceride storage and high glucose/trehalose in blood circulation, similar to the symptoms presented by diabetic patients (Zhang et al., 2009). The *Drosophila* genome encodes eight Dilps. At least three of these (Dilp2, Dilp3 and Dilp5) are secreted from the insulin-producing cells (IPCs) in the brain, which are homologous to pancreatic beta cells in vertebrates. The IPC Dilps are regulated by signals that originate from the fat body, which is the liver/adipose tissue in the fly, in response to fat, sugar and amino acid levels (Colombani et al., 2003; Rajan and Perrimon, 2012). Dilps secreted from the brain IPCs bind to the Insulin receptor (InR; also known as Insulin-like receptor) in peripheral tissues (Fig. 1) (Ikeya et al., 2002; Rulifson et al., 2002) and transduction of this signal causes phosphatidylinositol 3-kinase (PI3K) activation and increase in phosphatidylinositol 3,4,5-trisphosphate (PIP3) levels. Other members of this canonical pathway include AKT (Akt1), PDK1 (Alessi et al., 1997) and the lipid phosphatase PTEN (Gao et al., 2000). One of the downstream

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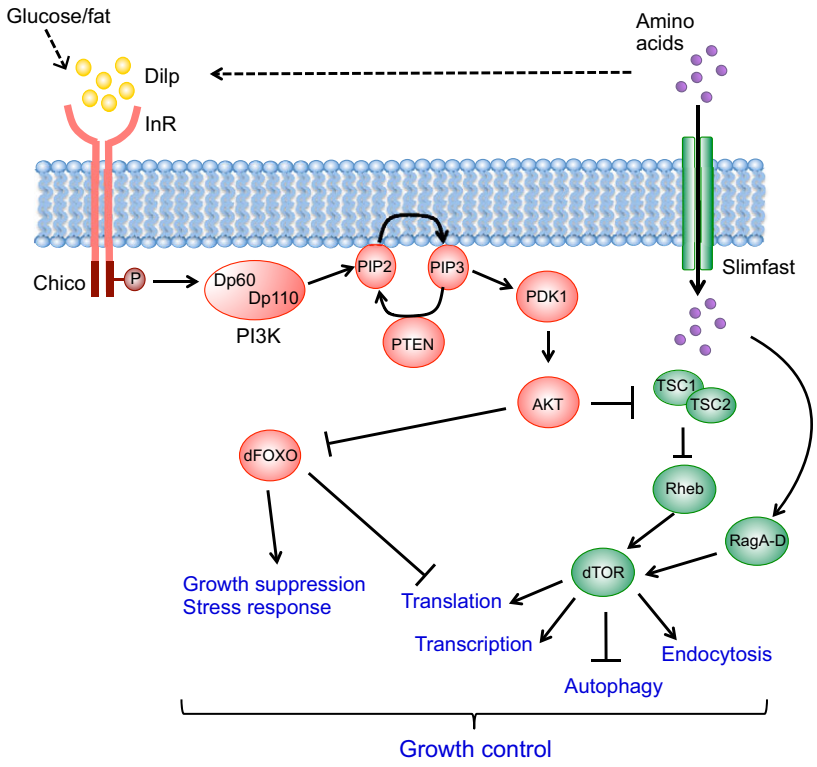

Fig. 1. The Insulin receptor pathway in *Drosophila*. The presence of sugars, fat and amino acids triggers the secretion of *Drosophila* insulin-like peptides (Dilps; insulin or IGF in mammals) from brain neuroendocrine cells. Dilp is recognized by Insulin receptor (InR; insulin receptor or IGFR in mammals) in the peripheral tissues and phosphorylates its substrate Chico (IRS1-4 in mammals). The downstream phosphorylation cascade includes the Dp60/Dp110 (Pi3K21B/Pi3K92E) complex (PI3K), PTEN, PDK1 and AKT, and controls the nuclear localization of dFOXO. In addition to the canonical InR/AKT/dFOXO pathway, AKT represses the TSC1/TSC2 complex, causing activation of Rheb and dTOR. Additionally, amino acids are imported into the cell by Slimfast (SLC7A family) and regulate dTOR via the RagA-D GTPase proteins. The modulation of the InR/dTOR pathways affects multiple cellular processes and thus links nutrient availability to growth control. The dashed arrows indicate indirect systemic controls of Dilp secretion through the fat body-derived Factor X or Upd2 (see also Fig. 6).

targets of AKT is the Forkhead box transcription factor *Drosophila* Foxo (dFOXO), which mediates growth control and age-related processes (Brunet et al., 1999; Puig and Tjian, 2005). AKT also inhibits the tumor suppressor proteins TSC1 and TSC2 (Gigas), which suppress a small GTPase called Rheb, an activator of *Drosophila* Tor (dTOR) (Potter et al., 2001; Saucedo et al., 2003; Zhang et al., 2003). Cells can also directly assess their nutritional status through the dTOR pathway in a process that is independent of Dilp/InR (Zhang et al., 2000). This is achieved by the direct sensing and transport of amino acids by the transporter Slimfast (Colombani et al., 2003) and the regulation of dTOR activity by amino acids that involves the Rag GTPases (Kim et al., 2008). The details of this activation process remain to be fully explored.

Female germline stem cells

Male and female germline stem cells (GSCs) are the best-understood and characterized stem cell systems in *Drosophila* (Fuller and Spradling, 2007). The simple morphology of GSCs and the availability of sophisticated genetic tools in *Drosophila* have expedited studies and provided novel insights into the developmental process of GSC specification and maintenance. The *Drosophila* ovary comprises several ovarioles, each of which consists of a series of egg chambers of increased maturity. Each ovariole contains a germarium, in which germline and somatic cells reside and the egg chamber is initially assembled. The GSC niche is located at the anterior tip of the germarium and consists of multiple somatic cells: terminal filament (TF) cells, cap cells (CCs) and a subset of escort cells (EsCs), all of which directly or indirectly contribute to GSC maintenance (Fig. 2A). Two or three GSCs are found in each germarium and form a direct connection with the CCs, from which they receive supportive signals (Lin et al., 1994). GSCs continuously self-renew by asymmetric division, in which the daughter cell that directly adheres to the CCs becomes another GSC, whereas the daughter that moves one cell away from the CCs gives rise to a cystoblast (CB). Signals secreted from the CCs are short range and cannot influence cells more than one cell diameter away. A CB is able to differentiate and divide in four rounds to form a 16-cell cyst (Xie and Spradling, 1998; Xie and Spradling, 2000; Song et al., 2004). In addition to a physical interaction with the CCs, the GSCs are encapsulated by EsCs that also play a role in GSC maintenance (Chen et al., 2011). The balance between self-renewal and differentiation is maintained by the microenvironment (Xie and Spradling, 2000) and improper niche function causes GSCs to either deplete (leading to infertility) or overproliferate, causing tumorous-like phenotypes (Xie and Spradling, 1998).

The reproductive system is highly sensitive to nutritional status (Renfree and Shaw, 2000). The *Drosophila* female germline exhibits delayed proliferation upon nutritional deprivation (Drummond-Barbosa and Spradling, 2001) and the number of GSCs is reduced (Hsu and Drummond-Barbosa, 2009). In response to changes in dietary protein concentration, egg production varies up to 60-fold in an insulin-dependent manner. Further analysis of the germline developmental stages showed that overall GSC maintenance is regulated by nutrient availability, abundance and quality (Hsu and Drummond-Barbosa, 2009). Nutritional regulation of *Drosophila* GSCs is mediated in part by Dilp expression, which in turn is essential to promote GSC proliferation. Ablation of the brain-derived Dilps results in reduced egg production and defects in yolk deposition (Ikeya et al., 2002), whereas loss of *chico*, which is the *Drosophila* ortholog of the mammalian insulin receptor substrate (IRS) genes (Fig. 1), results in infertility (Böhni et al., 1999). Loss of *InR* in the GSCs strongly affects GSC proliferation, cyst growth and yolk deposition. Similarly, ablation of Dilp-expressing neuroendocrine cells results in phenotypes similar to those seen upon loss of *InR* in the GSCs (LaFever and Drummond-Barbosa, 2005). Identical phenotypes are observed in the $chico^{1}$ mutant, and expression of a wild-type *chico* genomic construct is sufficient to rescue these phenotypes. However, the expression of a *chico* gene

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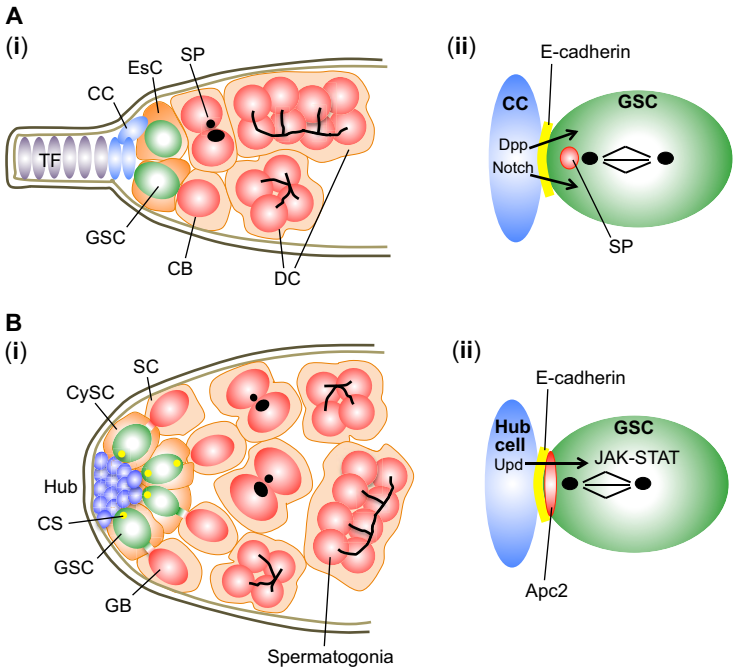

Fig. 2. Germline stem cells. (A) (i) The structure of the female germarium. (ii) Local maintenance and proliferation signals. CCs express Dpp and Notch, which maintain the stemness of GSCs; the GSCs are attached to the CCs via adherens junctions that are rich in E-cadherin (yellow). Notch is activated by Delta expressed on the TF cells. Spectrosomes (red) associated with one spindle pole (black) ensure asymmetric cell division. (B) (i) The structure of the developing male germline. (ii) Local maintenance and proliferation signals. Unpaired (Upd) originates from the hub cells and activates the JAK-STAT signaling pathway in the GSCs, suppressing their differentiation. Adherens junctions containing E-cadherin (yellow) in between the hub cell and the GSCs prevents GSC differentiation. Anaphase-promoting complex 2 (Apc2) colocalizes with E-cadherin to mediate the orientation of the mitotic GSC spindle. TF, terminal filament; CC, cap cell; GSC, germline stem cell; EsC, escort cell; CB, cystoblast; SP, spectrosome; DC, developing cyst cell; SC, somatic cyst cell; CySC, cyst stem cell; CS, centrosome; GB, gonialblast.

carrying a point mutation in PI3K binding sites does not rescue the phenotypes, indicating that PI3K mediates the effects of Dilp/InR in GSCs (Hsu et al., 2008). Moreover, $chico^{1}$;$dFOXO^{21}$/$dFOXO^{25}$ double mutants are able to rescue G2 delay in both InR^{E19}/InR^{339} and $chico^{1}$ mutants, implicating dFOXO downstream of InR in GSC proliferation. Although these studies have demonstrated that the insulin pathway specifically impinges upon the G2 phase via dFOXO, it is still possible that the GSCs employ alternative Dilp-independent mechanisms in controlling proliferation (Hsu et al., 2008). The second important target of the InR pathway, the dTOR protein, has a well-defined and conserved function in cell growth, survival and proliferation (Zhang et al., 2009). *dTOR* hypomorphic mutants exhibit a prolonged G2 phase, a phenotype that is not suppressed by *dFOXO* mutation, suggesting parallel and independent functions in the control of GSC proliferation (LaFever et al., 2010).

Other studies have focused on how systemic insulin integrates into various cellular functions. The miRNA pathway is known to regulate GSC self-renewal via a mechanism involving Dicer (Dcr-1) as the core enzyme (Jin and Xie, 2007). Ruohola-Baker and colleagues have found that the *Dcr-1*-deficient mutant rescues the starvation-induced slowing of GSC division, and that this recovery is mediated by the expression of Dacapo, which is similar to mammalian p21 (Cdkn1a) (Yu et al., 2009). Another study has shown that insulin regulates niche integrity by increasing Notch signaling in CCs as well as E-cadherin expression in the CC-GSC adherens junction. InR^{E19}/InR^{339} mutants eclose with fewer CCs than wild type, which indirectly affects the maintenance of GSCs. This phenotype is rescued by the expression of activated Notch in somatic cells of *InR* mutant germaria (Hsu and Drummond-Barbosa, 2009; Hsu and Drummond-Barbosa, 2011). In addition, CCs of the InR^{339} mutant significantly decrease their contact with the GSCs and express lower amounts of the E-cadherin that is important for maintaining the CC-GSC adherens junction. These results suggest that insulin controls GSC maintenance through Notch-mediated CC control and also by aiding CC-GSC adhesion with enhanced E-cadherin expression (Hsu and Drummond-Barbosa, 2009; Hsu and Drummond-Barbosa, 2011). Overall, it seems clear that the germline interprets insulin signaling in multiple cellular contexts to control GSC proliferation and maintenance. This can involve diverse mechanisms, including the miRNA machinery (Yu et al., 2009), Notch activity in CC proliferation, and E-cadherin expression in the GSC-CC contact (Hsu and Drummond-Barbosa, 2009; Hsu and Drummond-Barbosa, 2011), as well as the dFOXO and dTOR proteins. The complexity of this seemingly simple system should be kept in mind when interpreting results from the even more genetically complex mammalian niches.

Starvation is a stressful condition that causes a rapid reduction of insulin levels to protect the germline until favorable conditions return (McLeod et al., 2010). Physiological conditions such as aging and obesity gradually affect insulin levels (Hansen et al., 2013). Aging female flies significantly attenuate oogenesis due to a drop in GSC renewal, which is caused by a decrease in systemic insulin in addition to changes in cell-intrinsic factors (Xie and Spradling, 2000; Song et al., 2002; Pan et al., 2007; Hsu and Drummond-Barbosa, 2009). In mice and humans, obesity affects fertility in both males and females (Hartz et al., 1979) and it will be interesting to investigate the influence of such conditions on germ cell production.

Male GSCs

Adult *Drosophila* testes contain male germline cells and somatic support cells (Fuller, 1993). The apical tip of the testis has a compact, dome-shaped cluster of non-dividing stromal cells called the hub, which constitutes a signaling center involved in the maintenance of adjacent GSCs. Each GSC is flanked by two cyst stem cells (CySCs) that also contact the hub to produce squamous non-dividing somatic cyst cells (SCs), which ensure spermatogonial differentiation (Fig. 2B). Approximately six to nine GSCs surround and directly contact the hub cells as they initiate asymmetric divisions. A daughter cell displaced further away from the hub differentiates into a gonialblast, whereas the daughter directly connected to the hub remains a stem cell, similar to what is seen for the female GSC (de Cuevas and Matunis, 2011). Gonialblasts undergo four rounds of mitotic division with incomplete cytokinesis,

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generating 16 interconnected spermatogonia, which give rise to sperm (Fuller, 1998). The orientation of centrosomes, as determined by an E-cadherin and Centrosomin/Apc2 complex-dependent polarity cue, sets up daughter cell fate (Inaba et al., 2010). Additional mechanisms include the involvement of extracellular matrix components (Tanentzapf et al., 2007; Voog et al., 2008) and asymmetric distribution of newly synthesized histones (Tran et al., 2012). The cytokine Unpaired (Upd; also known as Outstretched) is secreted by the hub, triggering local JAK/STAT signaling essential for the maintenance of GSCs (Kiger et al., 2001; Tulina and Matunis, 2001).

Drosophila male germline cells also respond to nutritional status. Males raised on a diet lacking protein dramatically reduce the numbers of GSCs and CySCs due to slower proliferation. This phenotype is completely reversed by supplementing back the normal diet (McLeod et al., 2010). The mechanism by which the flies repopulate their stem cells could involve the process of dedifferentiation of early cyst cells to produce GSCs (Brawley and Matunis, 2004; Kai and Spradling, 2004; Cheng et al., 2008; McLeod et al., 2010).

InR responds to nutrient availability and mediates GSC/CySC maintenance in the male germline. *InR* mutants or the ablation of insulin-secreting neuroendocrine cells in wild-type flies results in rapid loss of GSCs and cyst cells similar to that seen during starvation (Ueishi et al., 2009; McLeod et al., 2010; Wang et al., 2011a). In addition, constitutive and simultaneous activation of insulin signaling in both the GSCs and the hub cells is necessary to ameliorate starvation-induced GSC loss. Thus, GSCs receive systemic insulin and autonomously coordinate nutritional information and stem cell behavior while, in addition, indirect GSC-related effects of insulin are mediated by the hub cells (McLeod et al., 2010). One of the known downstream mechanisms modified by systemic insulin is the control of the centrosome orientation checkpoint. Poor dietary conditions result in misorientation of the centrosome, which is reversed by activation of InR in the testis (Roth et al., 2012). Growth in nutrient-poor media also disrupts the expression and localization of Apc2, a cortical anchor of centrosomes in male GSCs, and mild overexpression of Apc2 is sufficient to recover centrosome misorientation under poor growth media conditions (Roth et al., 2012).

Hematopoietic progenitors

No self-renewing population of hematopoietic stem cells has been directly observed in *Drosophila*, although clonal analysis has suggested a short window in development when they might exist (Minakhina and Steward, 2010). The larval hematopoietic organ in *Drosophila* is the lymph gland, and it is here that blood cells develop and hematopoietic progenitors reside (Lanot et al., 2001; Holz et al., 2003; Jung et al., 2005; Minakhina and Steward, 2010). Several zones that house cells with different functions or at different stages of development have been identified within the primary lobe of the lymph gland (Jung et al., 2005) (Fig. 3). A zone called the posterior signaling center (PSC) contains cells that function as hematopoietic niche cells. These cells are positioned adjacent to the progenitors, which are contained within the medullary zone (MZ). The hematopoietic progenitors in the MZ are maintained primarily by Hh as well as other signals that emanate from the PSC (Krzemień et al., 2007; Mandal et al., 2007). The progenitors give rise to three different cell types, namely plasmatocytes, crystal cells and lamellocytes, and these differentiated cells occupy the cortical zone (CZ) at the outer most part of the lymph gland (Jung et al., 2005). In addition to signals

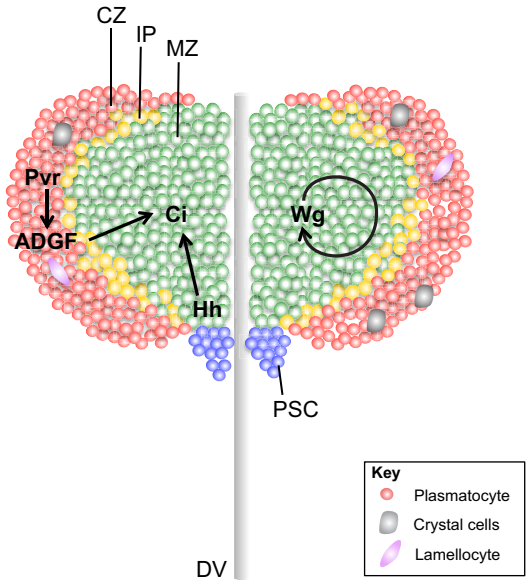

Fig. 3. The hematopoietic system. Hedgehog (Hh) originates from the PSC and is the primary signaling ligand involved in the maintenance of the hematopoietic progenitors through activation of Cubitus interruptus (Ci) in the MZ. Adenosine deaminase-related growth factor (ADGF) expressed downstream of PDGF- and VEGF-receptor related (Pvr) in the CZ cells functions as a retrograde signal also activating Ci. Progenitors are maintained by these signals as well as by Wingless (Wg), which is expressed in the MZ. PSC, posterior signaling center; MZ, medullary zone; CZ, cortical zone; IP, intermediate progenitor; DV, dorsal vessel.

secreted from the PSC, local factors such as Wingless (Wg) are expressed in the progenitors and autonomously regulate their maintenance and quiescence (Sinenko et al., 2009). Moreover, a recent study has found that a retrograde signal involving Adenosine deaminase-related growth factor (ADGF), which functions downstream of Pvr (PDGF- and VEGF-receptor related), is transmitted from the differentiating blood cells to the progenitors, providing a homeostatic balance between progenitor maintenance and differentiation (Mondal et al., 2011). The niche-derived Hh and the CZ-derived ADGF signals ultimately converge onto Cubitus interruptus (Ci), a transcription factor that is essential for progenitor maintenance.

Nutritional deprivation directly impinges on the maintenance of blood progenitors in the lymph gland and causes the expansion of mature blood cells (Benmimoun et al., 2012; Dragojlovic-Munther and Martinez-Agosto, 2012; Shim et al., 2012; Tokusumi et al., 2012). Twenty-four hour starvation of third instar larvae dramatically increases the differentiation of mature blood cells and concomitantly decreases blood progenitors (Benmimoun et al., 2012; Dragojlovic-Munther and Martinez-Agosto, 2012; Shim et al., 2012). Forty-eight hour starvation also generates a similar differentiation phenotype and additionally gives rise to a reduced PSC and lymph gland (Dragojlovic-Munther and Martinez-Agosto, 2012). Similar to the chronic inflammation observed in metabolic disorders (Wellen and Hotamisligil, 2005; Shoelson et al., 2006), starvation in larvae generates the hallmarks of inflammatory responses, including lamellocyte differentiation, the presence of which is normally restricted to the cellular immune response (Shim et al., 2012). These studies suggest that there is a conserved pathway linking metabolic disruption and blood cell differentiation in the *Drosophila* hematopoietic system.

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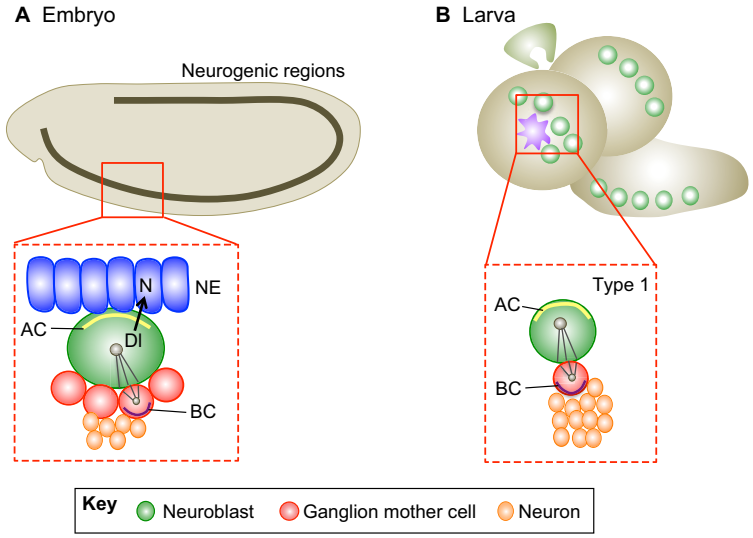

Fig. 4. Neural stem cells. (A) The structure of the embryonic neural stem cell niche. The neuroepithelium is composed of individual neurons arising in the ventrolateral region of the embryo. Neuroblasts are positioned adjacent to the neuroepithelium and express Delta (Dl), which represses neural fate in the neighboring epithelium by interacting with Notch (N). (B) A second wave of neurogenesis occurs in the first instar larva. The neuroblasts receive a Dilp signal from neighboring glial cells that causes them to enter the cell cycle. The inset shows the asymmetric division of a type 1 neuroblast giving rise to ganglion mother cells, which will further produce larval neurons. NE, neuroepithelium; AC, apical complex; BC, basal complex.

The systemic insulin level is sensed by InR both in the PSC and in the MZ cells and is required for the maintenance of the progenitor population (Benmimoun et al., 2012). Lack of InR in the PSC causes a reduction in the PSC cell number and overactivation results in an increase in the size of the niche (Tokusumi et al., 2012). Changes in PSC size indirectly affect progenitor cell fate, as loss of insulin signaling suppresses the expression of Hh, which is normally secreted from the PSC and maintains the undifferentiated state of the progenitors (Mandal et al., 2007; Tokusumi et al., 2012). Thus, insulin is sensed by the PSC and controls niche size, which in turn influences the maintenance signals, thereby indirectly controlling cell differentiation (Benmimoun et al., 2012; Dragojlovic-Munther and Martinez-Agosto, 2012; Tokusumi et al., 2012). In addition, the progenitors also directly sense insulin levels and can maintain their fate and proliferation in a cell-autonomous manner. Hyperactivation of insulin signaling in the progenitors by expression of activated PI3K ($PI3K^{CAAX}$) induces extensive differentiation of mature blood cells accompanied by a decrease in the pool of progenitors (Benmimoun et al., 2012). Conversely, knocking down insulin signaling in the progenitors gives rise to precocious differentiation with a significant reduction in the size of the lymph gland (Benmimoun et al., 2012; Shim et al., 2012), indicating that physiological levels of insulin signaling is required for their maintenance. These results are supported by the substantial enrichment of downstream effectors such as pAKT in the progenitors (Dragojlovic-Munther and Martinez-Agosto, 2012; Shim et al., 2012). Furthermore, hyperactivation of insulin signaling using *Tsc1* or *tsc2* mutants leads to a large increase in differentiation, along with a significant increase in the formation of lamellocytes, similar to the phenotype seen during starvation. These studies again imply that an adequate level of insulin signaling is crucial for lymph gland homeostasis and for the balance between progenitor maintenance and differentiation. Shim et al. (Shim et al., 2012) have shown that the expression of Wg, which is required for maintaining the progenitors in a cell-autonomous manner, is severely decreased upon loss of insulin signaling and that overexpression of Wg recovers progenitors lost upon starvation.

This intricate balance of blood cells is additionally maintained in a Dilp-independent manner by amino acids sensed by the progenitors themselves. Loss of *slimfast* in the progenitor population induces precocious differentiation of blood cells, suggesting that maintenance of the blood progenitors requires the direct sensing of amino acid levels (Shim et al., 2012). *dTOR* activation is also crucial for the maintenance of the precursors, and has also been implicated both in the InR pathway and in the direct sensing of amino acids (Shim et al., 2012). Dragojlovic-Munther and Martinez-Agosto have shown that activation of dTOR using *tsc2* RNAi in blood progenitors significantly expands the size of the lymph gland and increases reactive oxygen species (ROS) in the progenitors (Dragojlovic-Munther and Martinez-Agosto, 2012). Scavenging the higher ROS in the progenitors is enough to rescue the overproliferation phenotype, suggesting a possible connection between insulin signaling and ROS production (Owusu-Ansah and Banerjee, 2009; Dragojlovic-Munther and Martinez-Agosto, 2012). Overall, these studies suggest that systemic nutrient and insulin signals add to the local lymph gland-based signals to achieve blood progenitor maintenance (Benmimoun et al., 2012; Dragojlovic-Munther and Martinez-Agosto, 2012; Shim et al., 2012; Tokusumi et al., 2012).

Neural stem cells

Drosophila neurons are derived from the proliferation, self-renewal and differentiation of neural stem cells termed neuroblasts (NBs). Early NBs delaminate from the neuroepithelium in the ventrolateral region of the embryo. They undergo self-renewal by means of an asymmetric division that gives rise to a large daughter NB and a smaller ganglion mother cell, which is committed to producing a neuron and a glial cell upon division and differentiation (Fig. 4). Embryonic NB divisions give rise to all of the neurons in the larval central nervous system (CNS) and only 10% of the adult CNS. Following a cell cycle arrest, the embryonic NBs re-enter mitosis in the late first instar larva to generate neurons for the rest of the adult CNS in a second wave of neurogenesis (Artavanis-Tsakonas and Simpson, 1991; Skeath and Carroll, 1991; Egger et al., 2008; Reichert, 2011; Homem and Knoblich, 2012). Prior to NB formation, the neuroectoderm is maintained by Notch signaling, which inhibits the formation of NBs (Campos-Ortega, 1993; Campos-Ortega, 1995; Artavanis-Tsakonas and Muskavitch, 2010; Wang et al., 2011b). The formation of NBs from neuroepithelial cells is controlled by the *achaete*/*scute* complex of genes. In the embryo, these genes are expressed in clusters determined by the early patterning genes (Skeath and Carroll, 1994). Following a period of quiescence, the NBs of the central brain are reawakened

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and give rise to neurons of the larval central brain. The brain also contains a group of NBs termed type II NBs (Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008), as well as a separate pool of NBs that arise in the optic lobe from an independent primordium (Egger et al., 2007; Egger et al., 2010; Spindler and Hartenstein, 2010; Homem and Knoblich, 2012). As with the other stem cell systems described above, cell-intrinsic mechanisms influencing neural stem cell behavior in the brain work coordinately with systemic factors that influence stem cell populations (Ables and Drummond-Barbosa, 2011).

In a seminal discovery in 1998, Britton and Edgar showed that quiescent imaginal NBs are maintained in a proliferative state when they are co-cultured with the fat body, indicating the presence of a factor derived from the fat body that regulates the stem cells (Britton and Edgar, 1998). Although this signal is still unknown, the mechanism by which it functions is becoming clearer with recent reports (Chell and Brand, 2010; Sousa-Nunes et al., 2011). Chell and Brand investigated the nutrition-dependent behavior of NBs in the larval stages as they re-enter proliferation after a quiescent phase. Both loss- and gain-of-function genetic evidence implicates PI3K-mediated insulin signaling in NB reactivation from quiescence and subsequent cell division (Chell and Brand, 2010). NBs of larvae grown on an amino acid-deprived diet fail to show cellular growth or proliferation, indicating the requirement of a feeding trigger for NB reactivation. Regulation of this process appears to be mediated by Dilps, as glial-specific expression of Dilps induces the reactivation of NBs even in the absence of an amino acid diet. The expression of Dilp2 and Dilp6 is significantly reduced in the absence of amino acids in the diet, and homozygous *Dilp2*,*3*,*5*,*6* mutants display no NB reactivation. Furthermore, disruption of glial signals by Dilps blocks the proliferation and growth of NBs. Collectively, these findings establish the systemic regulation of neural stem cells by Dilps expressed in the glia. Sousa-Nunes et al. (Sousa-Nunes et al., 2011) also observed a delay in NB reactivation in Dilp as well as in *InR* mutants, supporting the observation of Chell and Brand (Chell and Brand, 2010). Additionally, Sousa-Nunes et al. assessed the *in vivo* requirement of the fat body-derived signal in NB reactivation. In an elegant genetic study, these authors demonstrated that an amino acid-derived nutritional signal activates the dTOR pathway in the fat body, causing it to secrete a soluble factor (X) that in turn causes the release of Dilps from glial cells and the IPCs. The glial-derived Dilp2/6 activates PI3K and dTOR signaling in NBs, causing them to exit quiescence and enter the cell cycle. Thus, blocking the dTOR signal in either the fat body or the NB reduces the number of NBs exiting quiescence, whereas NB-specific activation of the dTOR pathway is sufficient to trigger precocious exit from quiescence, even in the absence of a fat body-derived signal (Sousa-Nunes et al., 2011). Recent demonstration of Upd2 as a signaling molecule originating from the fat body that drives insulin secretion from the brain leads one to speculate that Upd2 could possibly play the role of this effector (Rajan and Perrimon, 2012). However, Upd2 secretion is triggered by fat or sugar intake and therefore the identity of the amino acid-triggered fat body signal remains to be established.

Following reactivation, as the NB enters a proliferative state during late larval stages, a growth-sparing program within the NB protects the brain from starvation. This is a phenomenon that is not found in any other cell type (Cheng et al., 2011). Lack of nutrient availability causes activation of the Anaplastic lymphoma receptor tyrosine kinase (ALK) by its ligand Jelly belly (Jeb), a secreted LDL repeat protein. This process maintains growth of the NB via the PI3K pathway, independent of InR and Slimfast. Activated ALK suppresses the function of both Slimfast and InR and thus enables late NB lineages to be less sensitive to amino acid and Dilp levels. Consequently, these later NBs can divide regardless of the nutritional status, and nutrient availability and Dilp levels are only critical in the reactivation process of early NBs (Cheng et al., 2011). In the early larval visual system, nutrients regulate the neural progenitor pool using InR/dTOR signaling (Lanet et al., 2013). However, this population later becomes dependent not on nutrition but on Ecdysone, which causes a switch from symmetric to asymmetric cell divisions in a Notch/Delta-dependent manner (Lanet et al., 2013). These strategies allow the brain to maintain neuronal diversity regardless of the nutritional status. This is in contrast to the other stem cell systems, which are critically and continuously dependent on nutritional status.

Intestinal stem cells

The *Drosophila* intestine comprises foregut, midgut and hindgut (Fig. 5A), which correspond to the esophagus/stomach, small intestine and large intestine/colon of the mammalian digestive system, respectively (Singh et al., 2011). The foregut and hindgut are of ectodermal origin, whereas the midgut derives from the endoderm. *Drosophila* intestinal stem cells (ISCs) are found in all three parts of the gut and are respectively termed gastric stem cells, midgut stem cells and hindgut stem cells based on their location. Gastric stem cells are found at the junction of foregut and midgut. They self-renew and give rise to progenitors in both the foregut (which differentiate into crop cells) and the anterior midgut (which differentiate into midgut cells) (Singh et al., 2011). The hindgut ISCs reside in a narrow segment between the midgut and hindgut boundary and are marked by high levels of JAK/STAT signaling (Takashima et al., 2008; Takashima and Hartenstein, 2012). The anterior pylorus region of the hindgut is also reported to contain a Wg-positive population of cells that only divides in response to serious tissue injury (Fox and Spradling, 2009). The midgut contains a pseudostratified epithelium made up of large polyploid enterocytes

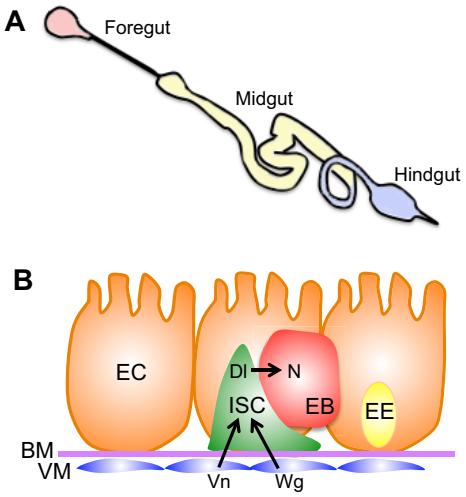

Fig. 5. Intestinal stem cell. (A) The three distinct segments of the *Drosophila* intestine: foregut, midgut and hindgut. (B) The midgut stem cells and their derivatives. Notch signaling promotes asymmetric division of intestinal stem cells (ISCs) to form enteroblasts (EBs). ISCs express the Notch ligand Delta (Dl), which activates Notch (N) in the EBs. Wingless (Wg) and Vein (Vn) are secreted from the visceral muscle and promote ISC maintenance and proliferation. BM, basement membrane; VM, visceral muscle; EE, enteroendocrine cell; EC, enterocyte.

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(ECs), diploid enteroendocrine (EE) cells and midgut stem cells (Fig. 5B). In the posterior midgut, only the stem cells proliferate (Casali and Batlle, 2009). The midgut ISCs divide to regenerate the stem cell pool and/or become enteroblasts (EBs) (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). The EBs then differentiate to an EC or an EE fate. Midgut stem cells are the most extensively studied stem cells among the three types of gut stem cells and are generically referred to as ISCs below.

Several signaling pathways that operate within the gut regulate the ISCs. Notch signaling promotes asymmetric division (Ohlstein and Spradling, 2007), whereas Wg, EGF, JAK/STAT and Hippo signaling are all implicated in ISC proliferation and maintenance (Karpowicz et al., 2010; Lin et al., 2010; Shaw et al., 2010; Xu et al., 2011). In addition to these local interactions, recent research has uncovered the impact of extrinsic factors such as nutrition on ISC behavior (McLeod et al., 2010; Choi et al., 2011; O'Brien et al., 2011).

Nutritional regulation of ISCs has again been shown to involve the insulin signaling pathway. McLeod et al. showed that fruit flies raised under protein starvation for 15 days significantly decreased ISC and EB numbers when compared with flies on a protein-rich diet (McLeod et al., 2010). Refeeding increased their gut size, and the number of ISCs and EBs reached levels comparable to those of control animals. Using a phosphorylated histone H3 marking assay, the authors found that the decrease in ISCs during starvation was due to a lack of proliferation upon protein starvation (McLeod et al., 2010). Bilder and colleagues (O'Brien et al., 2011) investigated the increase in midgut stem cell number in freshly eclosed flies that were either fed normally or fasted for the first 4 days following eclosion. The fasted flies failed to increase their stem cell number, as compared with a large increase in their fed counterparts. The cell population that increased upon feeding was due to both symmetric and asymmetric divisions in the gut (O'Brien et al., 2011).

The mechanism linking nutrition and ISC proliferation involves the insulin signaling pathway: Dilp2 and Dilp5 are upregulated in neurosecretory cells of 4-day-old fed flies, but not in freshly eclosed animals. However, on day 1, a more proximal signal, Dilp3, is upregulated in the midgut visceral muscle (VM), which acts as a niche for the ISCs (O'Brien et al., 2011). Dilp3 is upregulated in a food-independent manner up to day 1 after eclosion but becomes food dependent in the following days. The loss of Dilp3 upon starvation is reversible upon refeeding, and VM-specific knockdown or overexpression of Dilp3 causes a reduction or increase in ISC numbers, respectively. These results are further supported by the genetic manipulation of other members of the InR/PI3K pathway. (O'Brien et al., 2011). Parallel to the nutritional signal, Notch activates dTOR through the inhibition of TSC2 in the EB, leading to endoreplication and EC differentiation (Kapuria et al., 2012). In addition, chemical injury causes systemic insulin to induce ISC proliferation, enabling tissue repair (Amcheslavsky et al., 2009). Ohlstein and colleagues showed that nutrient deprivation results in the production of growth-delayed ECs and prolonged contact between ISCs and their daughter cells (Choi et al., 2011). They found that, in *Drosophila* adults fed a protein-poor diet, the number of stem cell-derived cells per clone increased but reached a lower plateau and at a slower rate than in well-fed fly guts, suggesting that nutrition affects the proliferation of ISCs. However, no decrease in the number of clones in the posterior midgut was seen in this study (Choi et al., 2011). Instead, a severe slowdown of the EC reduplication was observed when flies were fed a protein-poor diet (Choi et al., 2011). The discrepancy regarding the changes in ISC numbers between the studies could arise from differences in the way that the stem and progenitor populations have been marked. Choi et al. used a direct marker, Delta, for counting stem cells and this is likely to provide more accurate estimates of stem cell number. However, variations in feeding conditions, genetic backgrounds and the time points at which the tissue was analyzed could also contribute to some of the differences observed. Choi et al. (Choi et al., 2011) emphasize that the influence of nutrition on ISC proliferation is both direct and indirect: wild-type ISCs adjacent to *InR* mutant clones displayed significantly reduced proliferation, indicating a non-autonomous role for the insulin pathway in ISC proliferation, whereas an autonomous role is evident from the simultaneous removal of InR from ISCs and EBs that resulted in a

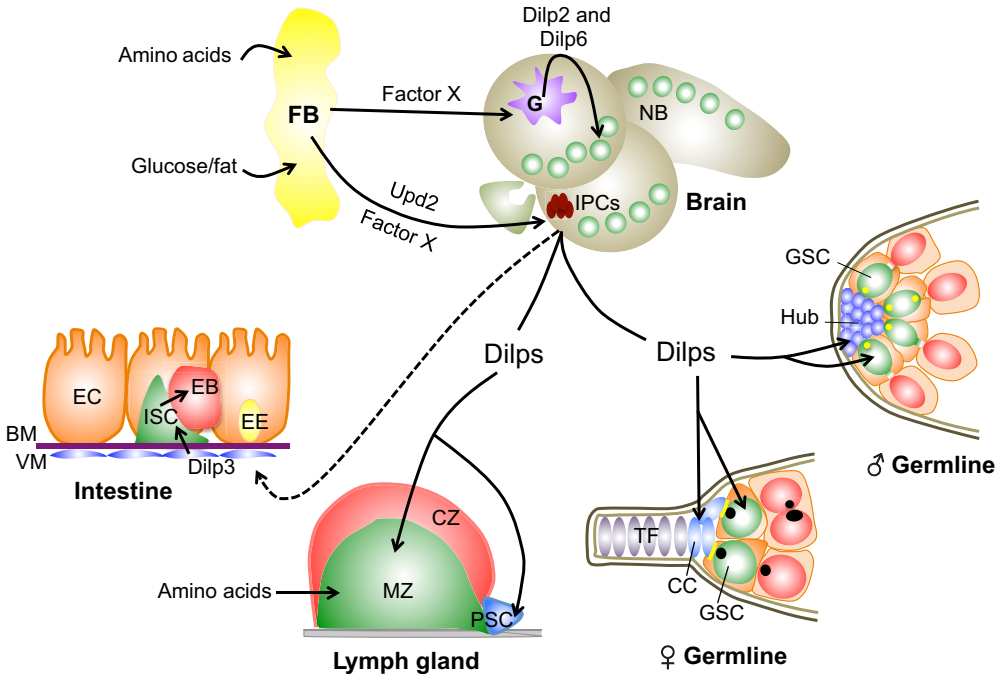

Fig. 6. Nutritional regulation of *Drosophila* stem/progenitor cell systems. Nutrients (amino acids and fat/glucose) are sensed by the fat body, which relays the signals to the insulin-producing cells (IPCs) in the brain or to glial cells in the case of neuroblast development. Fat and glucose trigger Upd2 and amino acids trigger unknown factors (X) to stimulate the secretion of Dilps. Various Dilps circulate throughout the body and regulate stem and progenitor cells as well as their niche in the lymph gland and the male and female germline. The dashed arrow pointing to ISCs is based on the findings of O'Brien et al. (O'Brien et al., 2011). Stem cells/progenitors are in green and the niche in blue. FB, fat body; G, glial cells; NB, neuroblast.

further block of proliferation. An interesting mechanism proposed by these studies is that the nutritional information is interpreted at the level of the strength of cell adhesion via DE-cadherin between the ISCs and their daughters. Thus, the prolonged contact between an ISC and an EB upon protein deprivation also causes an increase in E-cadherin levels, and knockdown of E-cadherin is sufficient to overcome the proliferation defects (Choi et al., 2011).

Conclusions

Research into the effect of nutrition on various stem and progenitor cell compartments in *Drosophila* has advanced our understanding of how different organs communicate with the external environment and with each other. In this review, we specifically focus on how nutrition and insulin regulate different types of stem and progenitor cells in *Drosophila* (Fig. 6). Although all stem cells utilize the same nutritional information via insulin, they process the signal in a manner distinctive to their niche. In the female germline, Dilps from the neuroendocrine cells maintain the niche integrity that indirectly affects GSC behavior. In addition, Dilps directly impact GSC proliferation and maintenance through multiple levels of control. The male germline interprets insulin signaling through the GSCs themselves as well as via the hub cells, similar to that in the female germline. In the male, one of the cellular mechanisms involved in this response is the control of centrosome orientation, which attenuates GSC proliferation under poor nutrient conditions. Blood progenitors in the lymph gland sense insulin both directly and indirectly via the PSC to maintain progenitor status and to control proliferation and differentiation. Additionally, nutrients such as amino acids are sensed directly by the progenitors, adding an additional level of sensitivity to the system. Despite the differences in tissue types and the lack of an identified blood stem cell population, the germline and the blood share several common features in terms of nutritional control. Both systems utilize brain IPC-derived Dilps, primarily Dilp2. In both tissues, systemic Dilp is perceived both by the niche cells and also directly by the stem/progenitor cells. Finally, in both cases, nutrition promotes the proliferation and maintenance of the stem/progenitor population.

Intestinal tissues react to poor diet and low insulin by reducing the proliferation of ISCs. This mechanism involves VM-derived Dilp3. This system is similar to that of the NBs in requiring local Dilp secreted from cells belonging to the niche. However, it remains possible that ISCs might also require additional Dilps derived systemically. Finally, unlike the other stem cells, NBs are controlled by Dilps from a different origin, namely the glial cells, where their levels are modified by nutritional status. Glial Dilps initiate the re-entry of NBs into a proliferative phase and promote their growth. Paradoxically, although systemic Dilps are produced in the brain, once secreted by the IPCs they do not have access to the NBs of the brain. Amongst the different stem/progenitor systems in flies, brain NBs are the most protected against nutritional effects and, following an early critical period, the later NBs are spared from the stress generated by limited periods of starvation.

Taken together, accumulating evidence supports the role of systemic factors, including nutrient availability and insulin levels, in regulating multiple stem cell compartments in *Drosophila*. This concept is conserved in vertebrates, although the specific nuances of how systemic signals integrate with local factors remain to be resolved. This area of research has potential for identifying therapeutic approaches to address stem cell interactions and defects in the context of external stimuli such as food, odor, infectious agents, stress and injury.

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Competing interests

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