Focus Article

A niche for *Drosophila* neuroblasts?

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Stem cells, which can self-renew and give rise to differentiated daughters, are responsible for the generation of diverse cell types during development and the maintenance of tissue/organ homeostasis in adulthood. Thus, the precise regulation of stem-cell self-renewal and proliferative potential is a key aspect of development. The stem-cell niche confers such control by concentrating localized factors including signaling molecules which favor stem-cell self-renew and regulate stem-cell proliferation in line with developmental programs. In contrast, *Drosophila* neuroblasts (NBs), often referred to as neural stem cells/progenitors, can undergo asymmetric cell division to self-renew and produce differentiated daughters even in isolation (or in culture). Furthermore, these isolated NBs can also progress through an intrinsically regulated temporal series (of transcription factor expression) to generate diverse cell types *in vitro*. These data argue that NBs may depend only to a limited extent, if at all, on local environment (a niche) for their maintenance. On the other hand, there is increasing evidence which indicate that the interaction between NBs and their surrounding glia is critical for the control of NB proliferative potential and these glia, in conjunction with systemic regulation, perform the niche function to regulate NB behavior. Thus, these observations emphasize the importance of coordinated local microenvironment (niche activity) and systemic environment (global activity) on the regulation of NB behavior *in vivo*, and suggest NBs may conform to an alternative stem-cell/progenitor maintenance model.

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INTRODUCTION

In multicellular organisms, most tissue and organs arise from a fairly small pool of undifferentiated cells, referred to as stem cells or progenitors, which undergo multiply rounds of mitotic divisions to produce a large number of specialized cells with distinct functions. Theoretically, these stem cells or progenitors can divide indefinitely over the lifetime of the organism. In reality, these cells interact with their surrounding cells in such a way that their self-renew and proliferative capacity is regulated by these surrounding cells, which form a specialized microenvironment or the so-called niche. Such interactions are wide-ranging in different stem-cell systems and involve cell–cell interaction, cell–matrix interaction, as well as diffusible signaling molecules which activate or repress specific genetic programs within the stem cells in order to regulate their behavior.

The concept of niche in the maintenance of stem cells was first proposed by Schofield using hemopoietic stem cell as a model.[1] He hypothesized that the continued proliferation of the stem-cell population depends on its surrounding cells, which constitute a fixed position and a specialized microenvironment, a niche, to sustain long-term proliferative capacity. Removal of stem cells from their natively associated cells results in cellular maturation and differentiation. Despite his early scientific insight, the effects of niche on stem-cell behavior were only understood molecularly with the more recent studies of *Drosophila melanogaster* and *Caenorhabditis elegans* germline stem cells (GSC).[2–6] These studies based on invertebrate models thus provided some guidelines for the identification of stem-cell niches in other systems

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and the quest in understanding stem-cell niche is ongoing.

A COMMON FEATURE OF DEFINED NICHES

In adult *Drosophila*, several types of stem cells and their associated niches have been identified including male and female germline stem cells (GSCs), follicle stem cells, cyst stem cells, and intestinal stem cells.[7] In these systems, stem cells reside in close proximity to their surrounding cells which provide external signals for their long-term maintenance of stem-cell identity, although the nature of these external signals vary amongst the different systems.[8] In *C. elegans*, the distal tip cells (DTC) acts as the niche to ensure GSC self-renewal via GLP-1/Notch signaling.[9] Data from various mammalian stem-cell systems such as hematopoietic stem cells, satellite muscle cells, epithelial stem cells, and intestinal stem cells also unequivocally point to the existence of a niche constituting various signals emitting from those supporting cells in proximity to the stem cells, which are essential for their maintenance.[7,10] Although these niche systems do not have conserved architecture, nor do they utilize the same signaling components and molecular pathways, they have the same general design of having heterologous types of cells providing the necessary signals to maintain stem-cell identity and the self-renewing potential of stem cells is strongly influenced by the niche-associated (external) signals.

DROSOPHILA NEUROBLASTS

In contrast, *Drosophila* neuroblasts (NBs), the neural progenitors or often referred to as neural stem cells, appear to deviate from these stem-cell systems as the balance between self-renewal and differentiation is primarily intrinsically regulated instead of relying on extrinsic cues provided by the surrounding cells. There are two important aspects that underlie *Drosophila* neurogenesis during embryonic and larval stages: asymmetric cell division and temporal factor switching. The former involves unequal division of NB along its apicobasal axis (the apical cortex is arbitrarily defined by the localization of the Par protein complex) to produce a larger self-renewing apical daughter that retains NB fate and a smaller basal daughter known as ganglion mother cell (GMC), which divides terminally to generate two neurons and/or glial cells (Figure 1);[11,12] while the latter process is crucial in generating neuronal diversity as well as to terminate NB division according to an intrinsic program in coordination with the developmental clock (Figure 2).[13,14] As both the topics had been extensively discussed and reviewed,[12–17] this article will only focus on some important features pertaining to intrinsic regulations of NB division.

The majority of NBs undergo two proliferative stages separated by a transient quiescent stage; the first phase occurs mainly during stages 9–14 of embryogenesis to generate the larval nervous system, while the second phase takes place starting at early second instar larval stage and continues until early

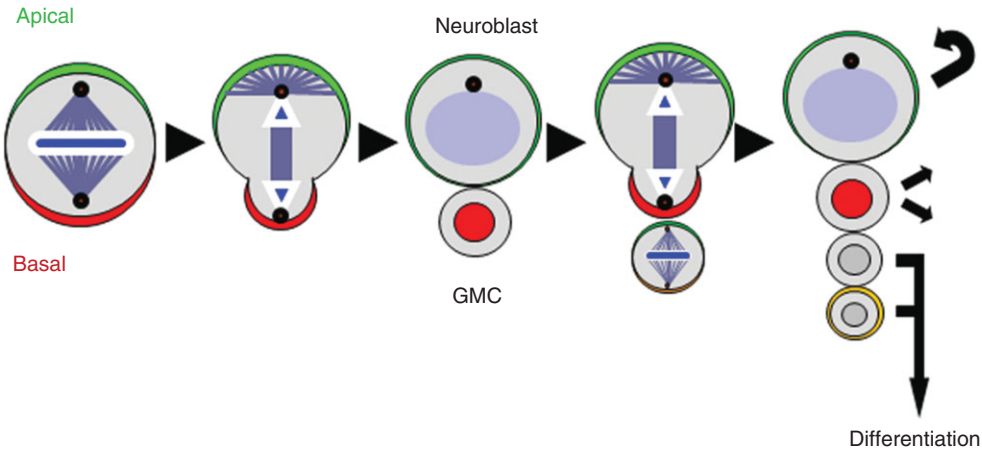

FIGURE 1 | Asymmetric division of neuroblasts (NBs). NBs undergo asymmetric cell divisions to produce a self-renewing neuroblast and a differentiating daughter cell (ganglion mother cell, GMC). The asymmetry of NB divisions is achieved through the establishment of a multi-protein complex at the apical cortex [including Inscuteable (Insc), Par6–Bazooka (Baz)–*Drosophila* atypical protein kinase C (DaPKC), and Partner of Insc (Pins)–G protein αi subunit (Gαi) signaling cassettes, in green], and the basal localization of neural cell fate determinants [e.g., Prospero (Pros), brain tumor (Brat), and Numb, in red] and the adaptor proteins Mira and Pon. The GMC then divides terminally to produce two ganglion cells which subsequently differentiate into neurons and/or glial cells.

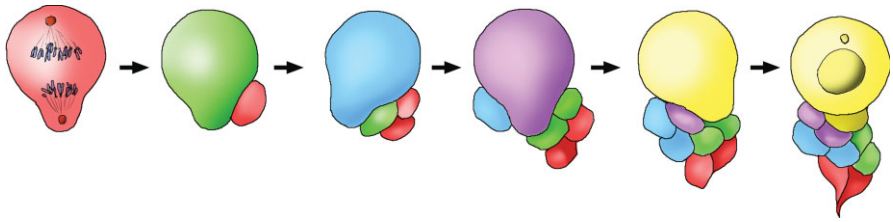

FIGURE 2 | Transcription factor switching in the embryonic neuroblast (NB). During embryonic neurogenesis, the NB expresses a series of transcription factors (temporal series) sequentially: Hunchback (Hb, red) → Kruppel (Kr, green) → POU homeodomain protein (Pdm, blue) → Castor (Cas, purple) → Grainyhead (Grh, yellow). The temporal transcription factor expressed in the NB is maintained in the ganglion mother cell (GMC) and the subsequent neuronal progeny that arise with the division of the GMC.

pupal stage to produce a functional adult nervous system.[18,19]

NBs are polarized cells with molecularly distinct apical and basal domains. The establishment of the apicobasal polarity in the NBs depends on the formation and maintenance of an apical complex molecular complex at its apical cortex starting from late interphase. This apical complex consists of inscuteable (Insc) and two conserved signaling cassettes: (1) the evolutionarily conserved Partition defective (Par) protein cassette comprising Bazooka (Baz), Par6, and *Drosophila* atypical protein kinase C (aPKC) and (2) partner of Insc (Pins), locomotion defective (Loco), and a subunit of the heterotrimeric G protein complex, G protein αi subunit (Gαi). The Par protein complex is essential for polarity regulation and segregation of the cell fate determinants to the basal cortex, whereas the Pins–Gαi cassette predominates in controlling the spindle orientation along the apical-basal axis of the NB.[20,21] The cell fate determinants which include Numb, prospero (Pros), and brain tumor (Brat) localize to the basal pole by binding to two coiled-coil adaptor proteins, partner of Numb (Pon, adaptor for Numb), and Miranda (Mira, adaptor for Pros and Brat). In addition, *pros* mRNA which is bound by its adaptor Staufen (Stau) is also segregated to the basal cortex. The interaction between microtubule associated proteins such as Disc-large (Dlg), kinesin heavy chain 73 (Khc-73), and mushroom body defect (Mud) with Pins on the apical cortex orients the mitotic spindle during metaphase such that the axis of NB division is orthogonal to apicobasal polarity. As a result, the cell fate determinants which repress NB fate are segregated exclusively into the daughter GMC upon completion of cytokinesis (Figure 1).

INTRINSIC REGULATION OF NB SELF-RENEWAL

Unlike stem cells which depend heavily on external signals for their self-renewal, there is thus far, no clear evidence showing that NBs require an extracellular signal for their maintenance and self-renewal. Indeed, isolated postembryonic NBs in culture retain the ability to divide asymmetrically to generate a self-renewing daughter NB, and a smaller daughter with neurogenic properties as evidence by Pros expression.[22] Moreover, the polarity markers such as Insc, Mira, and Numb are able to segregate asymmetrically into the daughter cells, despite the fact that their polarized distributions were delayed, being detectable only at late mitotic stage after the division plane was established.[22] Similarly, isolated embryonic NBs in the culture are capable of asymmetric division in the absence of extrinsic cue although there is a delay in the formation as well as concentration of Baz and aPKC crescents at the apical cortex.[23] Hence, in contrast to many other stem-cells systems like *Drosophila* male and female GSCs, or hematopoietic stem cells which rely on one or more extrinsic signals emanating from the niche to maintain their stemness, the ability of NBs to self-renew appears to be intrinsically regulated.

Further supporting evidence comes from the analysis of another intrinsically specified property of NBs: its ability to progress through a temporal series, which is exemplified by the sequential expression of a series of transcription factors in the NBs: Hunchback (Hb) → Kruppel (Kr) → POU homeodomain protein (Pdm1) → Castor (Cas) → Grainyhead (Grh) during embryonic neurogenesis.[24–26] This temporal series continues during larval development soon after NB reactivation with the expression of Cas → seven-up (Svp) and other unidentified factors, ultimately triggering cell-cycle exit in the NB upon the cessation of neurogenesis during early pupal stage.[27] The primary roles of the temporal series are to ensure that each NB will undergo specific rounds of divisions in a defined spatial and temporal context, and together with the asymmetric division machinery, to generate a stereotypic set of diverse cell fates within a largely invariant cell lineage. Experiments with isolated or dispersed NBs in culture support the notion that temporal identities of the NB are specified by intrinsic mechanisms.

The first evidence came from experiments, conducted by Furst and Mahowald in 1985, showing the intrinsic clock was unperturbed in disintegrated cultured NBs and these isolated NBs produced about the same number of progeny as they would *in vivo*.[28,29] Subsequent work from the same group revealed that the frequency of serotogernic and dopaminergic neurons produced by dispersed NBs in culture is remarkably similar to that found *in vivo*, suggesting that in the absence of their natively associated cells, these NBs can still generate the correct number of specific cell types.[30] In addition, *in vitro* NB lineages generated from single NBs in suspension contain subpopulation of neurons expressing Hb, Pdm1, Cas, and Grh with the temporal dynamics reflecting that seen *in vivo*.[25] However, these data were derived from cultured embryonic NBs and care must be taken when extrapolating to larval NBs *in vivo*.

IS THERE A NICHE FOR *DROSOPHILA NEUROBLAST?*

So, does a NB require a niche to maintain its stem-cell like characteristics? Strictly speaking, a stem cell has three defining characteristics: (1) the ability to self-renew; (2) the ability to allow multi-lineage differentiation from a single cell; and (3) the ability for *in vivo* functional reconstitution of a given tissue.[31] As discussed in previous sections, NBs are able to fulfill the first two criteria independent of other cell types. Yet, to assess the functional ability of the NBs to repopulate the entire neuronal lineages *in vivo*, it is essential to take into account the developmental context of the organism rather than to view the NB as an isolated entity. In particular, the embryonic and the larval NBs occupy architecturally distinct environments. Moreover, most NBs enter mitotic dormancy known as quiescence at the embryo to larval transition, a process regulated cooperatively by Hox genes and temporal transcription factors in conjunction with transcription cofactor Nab.[32] Here, we examine the requirements of NBs for a niche in light of the whole process of neurogenesis.

In the embryo, NBs are positioned adjacent to the neuroectoderm from which they are derived from with their apical poles abutting the basal surface of the epithelium (Figure 3(a)).[33] For successive rounds of division, NBs repeatedly orient themselves such that the GMCs are always budded off from the basal side to give rise to a tight neuronal cluster in the deeper layer of the embryo.[33] The correct alignment of the mitotic spindle is achieved via a 90° rotation from an anterior–posterior to apical–basal orientation during the first cell cycle of the NB soon after delaminating from

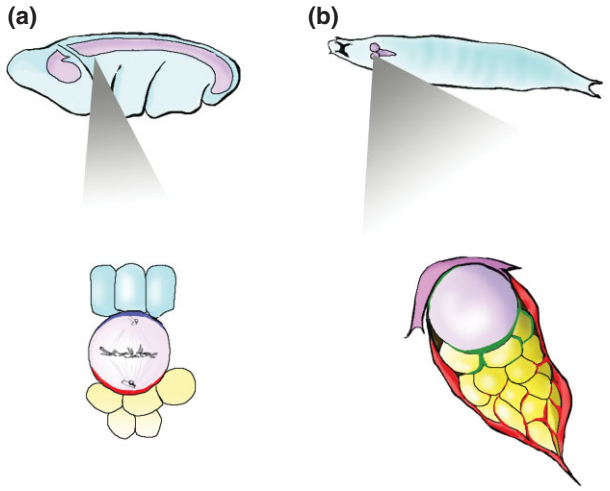

FIGURE 3 | Comparison between the local environments in which embryonic neuroblast (NB) and larval NB reside. (a) The embryonic NB (left panel, purple) delaminates from the ventr al neuroectodermal layer such that, during early divisions, its apical pole is always in contact with the epithelial cells (blue) which provide the signaling cue necessary for orientating the division axis of the NB. (b) In contrast, the larval NB (right panel, purple) arises from its quiescent form and divides to form a tight cluster of neuronal progeny (yellow). The proliferative control of the NB as well as the fasciculation and pathfinding of the neurites are regulated by the surface glia (pink) and cortex glia (red), respectively. DE-Cadherin is in green.

the neuroepithelium.[34] In contrast, spindle assembly during subsequent cell cycles of NBs rely on differential centrosome behavior such that the NB centrosome with higher microtubule-organizing center (MTOC) activity remains associated with the apical quadrant throughout the cell cycle, while the other centrosome moves the basal pole during prophase.[35] Hence, it has been speculated that the apical aster is instrumental in conveying the cortical polarity information from one cell cycle to another in an intrinsic manner.

However, it is also conceivable that the anchoring of the apical aster is a response toward extrinsic signal(s), as it has been shown that the timing and positioning of the Par protein complex, the orientation of the centrosome, and hence the alignment of the division axis depends on the site of epithelial–NB contact.[23] While the identity of the signal(s) being transmitted from the epithelial cells to the NBs remains elusive, absence of such extrinsic cue(s) in isolated NBs results in randomization of the division axis and dispersal of GMCs around the NB cortex.[23] Hence, the environment encompassing the epithelial cells provides functions (analogous to those provided by a niche) to organize the neuronal progeny in a correct spatial context.

Similar to the embryonic NBs, the larval NBs also have a generally unchanged axis of division such

that the neurons derived from a single NB form a column-like structure with the NB occupying the superficial end, followed by younger neurons, and with the older neurons situated deeper in the cortex (Figure 3(b)).[36] Intriguingly, the orientation of cortical polarity and the alignment of mitotic spindle are governed intrinsically through a memory function of the previous mitosis, independent of the surrounding tissue.[37] Using live imaging, it is apparent that polarity information can be passed from one cell division to the next through the interphase aster organized by the apical centrosome which remains fixed to the apical side of the NB.[37–39] This conclusion is substantiated by the fact that mutant NBs without or with unstable interphase aster such as *Sas-4*, *asl*, *pins,* and *polo*, as well as NBs subjected to microtubule depolymerization treatment have impaired orientation memory, leading to compromised GMCs spatial organization.[37]

It may be tempting to suggest that the cell-autonomous nature of cortical polarity orientation in larval NBs is due to the absence of an epithelial layer adjacent to the NB to provide the extrinsic cue. In actual fact, the larval brain has a more complex spatial–temporal organization, with the neuron cell bodies occupying the cortex in concentric layers while their axons are projected toward the core of the brain, forming the neuropile.[40] More interestingly, each NB lineage is encapsulated by processes of several cortex glial cells to form an enclosed chamber known as the trophospongium.[41] Presumably the dynamic growth and rearrangement of the glial processes during NB proliferation will compartmentalize neurons from one lineage to form a coherent bundle and to guide the trajectory of the cell body fiber tract.[42] Disruption of the trophospongium by over-expressing a dominant form of DE-cadherin (DE-Cad) in the cortex glia results in scattering of Cas-expressing neurons throughout the cortex, which would otherwise be restricted to the superficial layer with an intact trophospongium.[40] However, it is unclear whether this phenotype can be attributed exclusively to defective neuron-glial adhesion which would cause increased movement of neurons or whether there might be an alteration in neuronal fate as a consequence of environment change. Assuming that the latter scenario is true, it may indicate that the trophospongium provides a niche for NBs as correct specification of neuronal fate is a function of the NBs as neural progenitors. However, this possibility is subject to further investigation.

The proximal cell type which is important for the function of larval NBs as neuroprogenitors is the surface glia. These glia form a flat sheath structure on the surface of the brain, typically with their cell bodies located adjacent and above every NB, and underneath the surrounding basement membrane, forming the *Drosophila* CNS blood–brain barrier that separates neural elements from surrounding tissues and hemolymph.[43,44] Functionally, surface glial cells play an essential role in regulating the reactivation and proliferation of the NBs in the larval brain through some secreted molecules. The first evidence showing that surface glias are transducing signals which act directly and specifically on the larval NBs comes from the analysis of *anachronism* (*ana*) mutants. *ana* encodes a secreted glycoprotein which is expressed in surface glia. Mutations in *ana* leads to precocious development of NBs ahead of their normal developmental timing, ultimately causing morphogenetic anomalies in both the central brain and optic lobe.[45] Subsequent studies identified *terribly reduced optic lobes* (*trol*) as a downstream target of *ana*, and it functions to activate quiescence NBs in an *ana*-dependent manner.[46] In fact, *trol* encodes a *Drosophila* perlecan which is likely to stimulate NB proliferation by binding to growth factors like vascular endothelial growth factor and platelet derived growth factor (VEGF/PDGF), decapentaplegic (Dpp), wingless (Wg), and hedgehog (Hh).[47] In addition to its role in cortex glia in terms of trophospongium structure and function as discussed previously, DE-Cad is strongly expressed in the surface glia and is believed to control NB

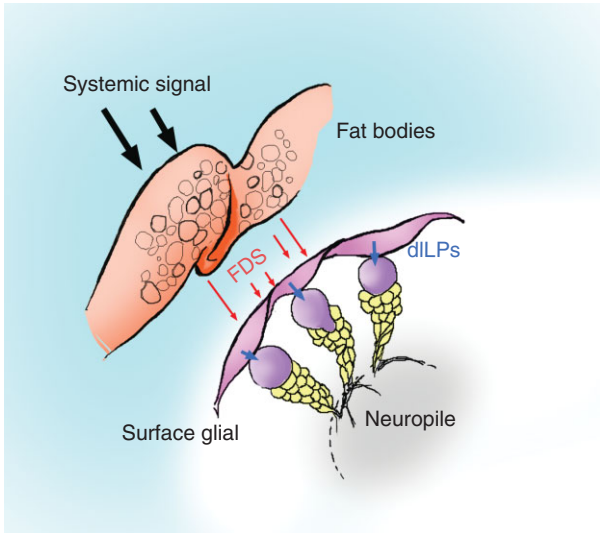

FIGURE 4 | The proliferation of the larval neuroblasts (NBs) are controlled by 'systemic' signals. The systemic changes of the organism during development signal the fat bodies (orange) to secret fat-body-derived signal (FDS) to the surface glial cells (pink) located superficial to the central brain. The signal is then relayed to the quiescent NBs (purple) located in close proximity to the glial cells in the form of insulin-like peptides (dILPs) such that NBs are reactivated and proliferate in line with systemic requirement.

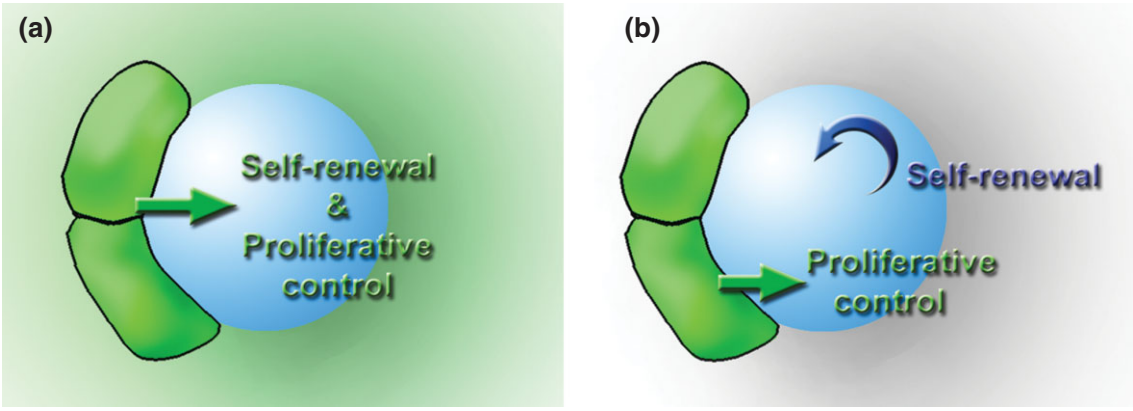

FIGURE 5 | Two modes of stem-cell maintenance. One class of stem cells (a), such as *Drosophila* germline stem cells, is maintained by the extrinsic signals emanating from the niche, without which these stem cells will be destined for their default mode of differentiation. In other stem-cell systems such as *Drosophila* neuroblast (NB) (b), the self-renewing capacity of the stem cell is intrinsically regulated, while other aspects of stemness such as growth and proliferation are partly controlled by the signaling event associated with their proximal cells.

proliferation via some unknown mechanism. Compromising DE-Cadherin function in these surface glial cells strongly impairs NB mitotic activity.[40]

Furthermore, two recent publications indicate that these surface glia provide local signals necessary for the reactivation of NBs during the late first instar larval stage.[44,48] Specifically, these surface glial cells act as a (local) source of insulin-like peptides (ILPs) in response to a fat-body-derived (systemic) signal (FDS) and these dILPs, in turn, likely bind to the insulin-like receptor (InR) on the NBs and activate the downstream phosphatidylinositol 3-kinase (PI3K) and target of rapamycin (TOR) signaling networks within the NBs (Figure 4).[44,48] Considering stem-cell proliferation control as a functional aspect of the niche, these data suggest that these surface glia perform a niche function in the control NB proliferation and, at the same time, respond to 'systemic' signals to ensure that proliferation of NBs is tightly regulated in line with the systemic state of the organism. However, as discussed in previous section, isolated NBs can self-renew (for instance, generate differentiated daughters and maintain temporal identity switching independent of these glia), suggesting that *in vivo*, these surface glia may act mainly to provide an interface for the efficient communication between NBs and the organism.

CONCLUSION

Niche is often referred to as a specialized local microenvironment where the stem cells reside and directly promotes the maintenance of the stem cells.[7] The strict requirement or nonrequirement of niche among different stem-cell systems suggests that there may be two distinct mechanisms for stem-cell maintenance. The first class of stem cells are preprogramed to differentiate by default, and this predisposition is suppressed by niche signals. *Drosophila* male and female GSCs, for instance, belong to this class as their maintenance requires localized external signals derived from the niche whose activity is to suppress the differentiation-promoting program.[8] While, *Drosophila* intestinal stem cells[7] and NBs may be similar to a second class of stem cells in which the primary inherent genetic program is self-renewal. In other words, differentiation is an induced process via inheritance of cell fate determinants (such as Notch signaling activation in the enteroblast, the differentiating daughter of ISC division and Pros, Brat, and Numb inheritance in GMCs) which trigger genetic programs to overwrite the default self-renewing mode. Under this classification, the decision of self-renewal and differentiation takes place in the stem cell for the first class of stem cells, but in the differentiating daughter cell for the second class of stem cells. Consistently, these two classes of stem cells have different dependence on their surrounding cells for their maintenance with stem cells in the first class depending heavily on niche-associated information, while maintenance of stem cells in the second class relies mainly on an intrinsic machinery but not external factors although their proliferative potential is subjected to external (local and systemic) regulation (Figure 5).

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