Temporal regulation of the generation of neuronal diversity in Drosophila

Temporal regulation of the generation of neuronal diversity in *Drosophila*

Tetsuo Yasugi and Takashi Nishimura

Laboratory for Growth Control Signaling, RIKEN Center for Developmental Biology (CDB), 2-2-3 Minatojima-Minamimachi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan
Correspondence: t-yasugi@cdb.riken.jp, t-nishimura@cdb.riken.jp

Abstract

For the construction of complex neural networks, the generation of neurons and glia must be tightly regulated both spatially and temporally. One of the major issues in neural development is the generation of a large variety of neurons and glia over time from a relatively small number of neural stem cells. In *Drosophila*, neural stem cells, called neuroblasts (NBs), have been used as a useful model system to uncover the molecular and cellular machinery involved in the establishment of neural diversity. NBs divide asymmetrically and produce another self-renewing progenitor cell and a differentiating cell. NBs are subdivided into several types based on their location in the central nervous system. Each type of NB has specific features related to the timing of cell generation, cell cycle progression, temporal patterning for neuronal specification, and termination mechanism. In this review, we focus on the molecular mechanisms that regulate the proliferation of NBs and generate a large variety of neuronal and glia subtypes during development.

Key words:

central nervous system, *Drosophila*, neural stem cell, neuronal diversity, temporal regulation

Introduction

To create functional neural circuits, diverse types of neurons and glia must be generated in a spatially and temporally regulated manner (Greig *et al.*, 2013, Jessell, 2000, Kohwi & Doe, 2013, Livesey & Cepko, 2001). In the developing nervous system, multipotent neural stem cells proliferate and give rise to differentiating daughter cells. Extrinsic and intrinsic factors define the specification of neural stem cells and their descendants. These features are conserved between mammals and the fruit fly, *Drosophila melanogaster*. *Drosophila* neural stem cells, called neuroblasts (NBs), can divide asymmetrically to produce another self-renewing progenitor cell and a differentiating daughter cell. Although NBs essentially utilize the same molecular machinery for establishing asymmetric cell division, they can give rise to different numbers and different types of progeny. Over the past few decades, the molecular and cellular machineries that regulate NB proliferation have been intensively studied. In this review, we focus on the temporal control of NBs. We address the classification of NBs that are located in different positions within the developing central nervous system (CNS) and discuss different modes of proliferation. We also describe the mechanisms of NB generation, reactivation after quiescence, and termination. Additionally, we present birth order-dependent mechanisms that produce distinct types of neurons and glia over time.

Overview of neural development in *Drosophila*

There are two phases in neural development in *Drosophila*: the embryonic and larval/pupal stages. Embryonic NBs produce neurons of the larval CNS and a part of the adult CNS, whereas larval/pupal NBs produce neurons that will form approximately 90 % of the adult CNS (Truman & Bate, 1988). During embryonic stages, NBs delaminate from the neuroectoderm (Fig. 1A). Following the production of embryonic neurons, most embryonic NBs stop proliferating and become quiescent (Fig. 1C) (Ito & Hotta, 1992). The exceptions for this are four mushroom body NBs (MB NBs) and one lateral NB in each hemisphere that continue to proliferate during the transition from embryo to larva (Fig. 1C).

The larval CNS is morphologically divided into three parts: the medially located central brain; the optic lobe, which is located at the lateral side of the brain; and the ventral nerve cord (VNC). The VNC is further subdivided into thoracic and abdominal

regions. Larval NBs can be subdivided into several types according to their positions and mode of cell division (Fig. 1B). Furthermore, each type of NB shows a different pattern of proliferation (Fig. 1C). MB NBs, which are generated during the embryonic stage, continuously produce neurons until the late pupal stage (Ito & Hotta, 1992, Truman & Bate, 1988). NBs in the central brain and thoracic VNC stop proliferating at the early pupal stage, whereas abdominal VNC NBs die at the mid-third instar stage. Compared to NBs in the central brain or in the VNC, NBs in the optic lobe have the characteristic feature of producing asymmetrically dividing NBs during the larval stages. NBs in the optic lobe are generated from neuroepithelial cells (NE cells) during the larval stage and stop proliferating at the early pupal stage (see below).

NBs can be subdivided into three categories based on their method of cell division (Type 0, I, and II) (Fig. 1D). Type I NBs are the most common and divide to produce another Type I NB and a ganglion mother cell (GMC). The GMC divides again to generate two neurons or glia. Type II NBs are located in the posterior dorsal region of the larval brain and undergo asymmetric cell divisions to produce intermediate neural progenitors (INPs) (Bello *et al.*, 2008, Boone & Doe, 2008, Bowman *et al.*, 2008). INPs can self-renew and divide several times to generate GMCs and are therefore recognized as transit amplifying NBs. Recently, Type 0 NBs, which are located at the posterior tips of the outer proliferation center (tOPC) in the larval optic lobe, have been reported (Bertet *et al.*, 2014). Type 0 NBs divide and directly produce post-mitotic neurons. Although young tOPC NBs show a mode of Type 0 division, these NBs later divide to produce GMCs (Bertet *et al.*, 2014). Further analysis will clarify how many NBs undergo direct neurogenesis and whether it is a common feature of NBs to switch their mode of division during development.

Although there are several types of NBs that show different modes of proliferation and lineage specification, they essentially utilize the same molecular mechanism for establishing asymmetric cell division (Fig. 1E) (reviewed by Chia *et al.*, 2008, Gonzalez, 2007, Homem & Knoblich, 2012, Knoblich, 2008). During mitosis, the Par complex proteins Bazooka (Baz)-Par6-atypical protein kinase C (aPKC) localize to the apical cortex (Kuchinke *et al.*, 1998, Petronczki & Knoblich, 2001, Wodarz *et al.*, 2000). The Par complex directs three cell fate determinants to localize to the basal cortex. These cell fate determinants, which include the homeodomain transcription factor Prospero (Pros), the NHL domain protein Brain tumor (Brat), and the PTB

domain protein Numb, are selectively segregated into the GMC and induce neural differentiation (Bello *et al.*, 2006, Betschinger *et al.*, 2006, Doe *et al.*, 1991, Hirata *et al.*, 1995, Knoblich *et al.*, 1995, Lee *et al.*, 2006, Rhyu *et al.*, 1994). Brat and Pros require the adaptor protein Miranda, whereas Numb requires Partner of numb (Pon) for their basal localization. The Par complex interacts with the cytoskeleton adaptor protein Inscuteable (Insc) and recruits the Mushroom body defect (Mud)-Partner of inscuteable (Pins)-Gαi complex to the apical cortex. The Mud-Pins-Gαi complex regulates the orientation of the mitotic spindle and thereby ensures the asymmetric segregation of cell fate determinants into GMCs (Bowman *et al.*, 2006, Izumi *et al.*, 2006, Kraut & Campos-Ortega, 1996, Kraut *et al.*, 1996, Schaefer *et al.*, 2001, Schaefer *et al.*, 2000, Schober *et al.*, 1999, Siller *et al.*, 2006, Wodarz *et al.*, 1999). These asymmetrically localized proteins are segregated into either an apical daughter cell, which retains the stem cell fate, or a basal GMC, which undergoes differentiation.

Asymmetric cell division of neural progenitor cells is also observed in mammalian neurogenesis. After the expansion of neuroepithelial cells by symmetric cell division, neuroepithelial cells differentiate into radial glial cells, which divide asymmetrically to self-renew and to produce neurons or intermediate progenitor cells (Matsuzaki & Shitamukai, 2015). During asymmetric cell division of radial glial cells, Numb and Numb-like are asymmetrically distributed and regulate the behavior of daughter cells during mouse cortex development (Shen *et al.*, 2002, Zhong *et al.*, 1996). It is interesting to speculate that asymmetric protein localization during cell division of neural progenitors is fundamental for cell fate determination and the final number of neural cells.

Generation and temporal patterning of embryonic NBs

During embryonic stages, NBs delaminate from the neuroectoderm (Fig. 1A). Proneural genes of the *achaete-scute* complex (*AS-C*) including *achaete* (*ac*), *scute* (*sc*), and *lethal of scute* (*l'sc*) are expressed in proneural equivalence groups of neuroectodermal cells and give those cells the potential to differentiate into NBs (Cabrera *et al.*, 1987, Martin-Bermudo *et al.*, 1991, Skeath & Carroll, 1992). Within the 'proneural cluster' cells, single cells are selected to be NBs through the Delta (Dl)/Notch-dependent lateral inhibition mechanism, and other cells differentiate into epidermal cells (Artavanis-Tsakonas & Simpson, 1991, Hassan & Vaessin, 1996).

To achieve neuronal diversity, NBs produce different types of neurons in an invariant order. During embryonic development, NBs sequentially express at least four transcription factors: Hunchback (Hb), Krüppel (Kr), Pdm, and Castor (Cas) (Fig. 2) (Brody & Odenwald, 2000, Isshiki *et al.*, 2001, Kambadur *et al.*, 1998). These temporally expressed factors are inherited by the progeny of NBs and lead to their differentiation into different types of neurons and/or glia (Fig. 2) (Brody & Odenwald, 2000, Isshiki *et al.*, 2001, Kambadur *et al.*, 1998). Hb and Kr specify first-born and second-born cell fates, respectively (Isshiki *et al.*, 2001). *In vitro* experiments have shown that this Hb-Kr-Pdm-Cas progression occurs in a cell-intrinsic manner, where one temporal factor stimulates the expression of the next while also inhibiting the expression of previous factor (Fig. 2) (Grosskortenhaus *et al.*, 2005). First, Hb induces the expression of Kr. Next, Kr promotes the expression of Pdm, which in turn down-regulates Kr. Similarly, Pdm promotes Cas expression, which in turn inhibits Pdm expression. These 'positive feed-forward' and 'negative feed-back' networks ensure the temporal transitions between these transcription factors. In addition, another factor, Seven Up (Svp), is involved in the progression of the temporal window by terminating Hb expression (Fig. 2) (Kanai *et al.*, 2005). Svp is transiently expressed in Hb^+ NBs and subsequently switches off Hb expression to progress the NB into the next Kr^+ window (Kanai *et al.*, 2005). One remaining question is whether this temporal progression is coupled to the cell cycle. The transition from the Hb^+ window to the Kr^+ window requires cytokinesis, whereas the Kr-Pdm-Cas transition is cell cycle-independent (Grosskortenhaus *et al.*, 2005). Thus, the mechanism that coordinates the intrinsic molecular clock and the number of progeny remains unclear.

A similar form of temporal regulation is found in mammals during neural development. In the developing mouse retina, retinal progenitor cells (RPCs) sequentially produce specific retinal cell types at varying times during development. They first produce retinal ganglion cells, horizontal cells, and cone photoreceptor cells, followed by amacrine cells. At later stages, RPCs generate rod photoreceptor cells, bipolar cells, and Müller cells (Cepko, 2014). Ikzf1, a mouse ortholog of Hb, is expressed during early stages of development and defines early temporal competence (Elliott *et al.*, 2008). Casz1, a mouse homolog of Cas, is expressed in RPCs at mid/late stages (Mattar *et al.*, 2015). Casz1 suppresses the production of early-born retinal cell types and promotes mid- and late-born neural fates (Mattar *et al.*, 2015). Additionally,

Ikzf1 also acts as a temporal regulator for determining early neuronal fate in the cerebral cortex (Alsio *et al.*, 2013). Based on these similarities between *Drosophila* and mammals, it is interesting to speculate that sequential expression of transcription factors and birth order-dependent determination of neuronal identity are fundamental mechanisms to generate neuronal diversity.

Quiescence of embryonic NBs and re-entry into cell cycle during the larval period

Most embryonic NBs stop proliferating and undergo quiescence at the end of embryogenesis (Fig. 1C) (Ito & Hotta, 1992). Temporal factors and the mechanisms of asymmetric cell division determine the timing by which NBs enter quiescence. Pros is one of the basal components during asymmetric cell division, and it acts as a cell fate determinant (Fig. 1E) (Doe *et al.*, 1991). A high level of Pros expression induces cell differentiation, and the loss of Pros promotes the self-renewal of NBs (Bayraktar *et al.*, 2010, Cabernard & Doe, 2009, Choksi *et al.*, 2006). Pros shows a transient nuclear localization during the stage in which NBs exit the cell cycle. Transient nuclear Pros expression at low levels is responsible for inducing NB quiescence (Lai & Doe, 2014). Embryonic temporal factors also regulate the timing of quiescence induction. NB quiescence occurs precociously in *Pdm* mutants and is delayed in *cas* mutants; the nuclear localization of Pros is shifted earlier or later in these mutants, respectively (Lai & Doe, 2014, Tsuji *et al.*, 2008). The timing of quiescence ultimately affects the final number of neurons: *Pdm* mutants produce fewer neurons, and *cas* mutants produce more neurons than wild type (Tsuji *et al.*, 2008). These findings demonstrate that temporal factors regulate both neuronal cell fate and also the number of progeny.

Dormant NBs begin dividing again after hatching (Fig. 1C) (Truman & Bate, 1988). NB reactivation occurs in two steps: NBs first re-grow their size and then re-enter the cell cycle (Fig. 3A). Food intake and the cell-intrinsic activation of Insulin-like Receptor (InR)/PI3K/Target of Rapamaysin (TOR) signaling in NBs regulate the enlargement of NBs (Fig. 3B) (Britton & Edgar, 1998, Chell & Brand, 2010, Sousa-Nunes *et al.*, 2011). Glial-derived *Drosophila* insulin-like peptides (Dilps) activate InR/PI3K/TOR signaling in NBs to restore NB size. The secretion of Dilps in glia are regulated in part through gap junctions in the subperineurial glia, which cover the brain surface and act as the blood-brain barrier (BBB) in *Drosophila* (Speder & Brand, 2014). In addition, the fat body, which is the adipose tissue in *Drosophila*, plays a critical, non-

cell autonomous role in the reactivation of NBs (Britton & Edgar, 1998, Sousa-Nunes *et al.*, 2011). The fat body secretes a thus far unknown 'fat body derived signal (FDS)', which might act on glia or might act directly on NBs to cause them to regrow. The identification of the FDS and the analysis of the relationship between FDS and InR/PI3K/TOR signaling would further facilitate our understanding of this first step of reactivation.

After undergoing enlargement, NBs begin to proliferate (Fig. 3C). Anachronism (Ana) is a secreted glycoprotein that prevents premature re-entry into the cell cycle (Ebens *et al.*, 1993). Though it has been proposed that Ana expression in glia modulates the timing of reactivation, a recent finding showed that *miR-124* expression in NBs regulates NB proliferation by binding to the 3'UTR of Ana, suggesting the possibility that Ana is also required in NBs (Ebens *et al.*, 1993, Weng & Cohen, 2012). Terribly reduced optic lobes (Trol), which is a *Drosophila* Perlecan, promotes the G1-S phase transition in NBs either by inactivating Ana function or by acting downstream of Ana (Datta, 1995, Voigt *et al.*, 2002). In the developing brain, Trol is localized at the brain surface and modulates the Hedgehog (Hh) and FGF signaling pathways to promote NB reactivation (Park *et al.*, 2003). Hh and FGF operate in a mutual, positive feedback loop, and FGF is epistatic to Hh (Barrett *et al.*, 2008). The *Drosophila* steroid hormone ecdysone and the homeodomain protein Even skipped (Eve) are also involved in the G1-S phase transition step (Datta, 1999, Park *et al.*, 2001). It is possible that ecdysone regulates the temporal reactivation of NBs because ecdysone is the major endocrine hormone that regulates developmental timing (Yamanaka *et al.*, 2013). Interestingly, Eve function is not required in the CNS. Because ecdysone can rescue the *eve* mutant phenotype, Eve either acts upstream of ecdysone or modulates ecdysone. Because the *eve* mutation enhances the *trol* mutant phenotype, it is possible that Eve regulates the signaling activity of Hh and/or FGF. Another factor regulating NB reactivation is the lipoprotein Lipophorin (Lpp), which is localized in subperineurial glia and inside the brain. Lpp likely contributes to the proliferation of NBs by transporting GPI-linked proteins from the circulating hemolymph (insect blood) through the BBB and into the brain (Brankatschk & Eaton, 2010). These findings demonstrate the importance of glia surrounding the brain and NBs for the reactivation process. These glia may act as a niche to selectively transduce and integrate extrinsic signals coming from outside the brain to inhibit or promote cell growth and the proliferation of NBs.

Generation of optic lobe NBs during larval period

Visual information received by the retina is processed in the optic lobe. The adult optic lobe consists of four ganglia: the lamina, medulla, lobula, and lobula plate (Fig. 4A). The primordium of the optic lobe invaginates from the posterior procephalic region of the head epidermis during the embryonic stage (Green *et al.*, 1993). During the early larval stage, the NE sheet of the optic lobe primordium is divided into the outer proliferation center (OPC) and the inner proliferation center (IPC) (Fig. 4B and 4C) (Hofbauer & Campos-Ortega, 1990, White & Kankel, 1978). Cells in the OPC primarily produce lamina neurons and medulla neurons, whereas cells in the IPC differentiate into lobula distal cell neurons, lobula plate neurons, and lobula neurons. During OPC development, NE cells first proliferate through repetitive symmetric cell divisions. This NE expansion is assured by the nutrition supply and the activation of InR/TOR signaling in NE cells (Lanet *et al.*, 2013). Following the expansion of NE cells, the transition from NE cells to NBs occurs at the medial edge of the OPC, and this transition moves from the medial to the lateral OPC (Fig. 4D) (Egger *et al.*, 2007, Egger *et al.*, 2011, Yasugi *et al.*, 2008). The proneural wave, which is indicated by the transient expression of the proneural factor L'sc, sweeps across the NE sheet from the medial to the lateral region and determines the timing of the transition from NE cells to NBs (Yasugi *et al.*, 2008). Several signaling pathways are involved in the transition step, including EGFR, Fat/Hippo, JAK/STAT, and Notch. EGFR signaling is required for NE expansion, L'sc expression, NB differentiation, and the progression of the proneural wave (Morante *et al.*, 2013, Yasugi *et al.*, 2010). Fat/Hippo signaling regulates the proliferation of NE cells and also promotes the progression of the proneural wave by regulating the transduction of EGFR signaling (Kawamori *et al.*, 2011, Reddy *et al.*, 2010, Richter *et al.*, 2011). Unpaired, a ligand of JAK/STAT signaling, is expressed in the lateral-most NE sheet and activates the signal in the lateral side of the NE. The activation of JAK/STAT signaling prevents the precocious progression of the proneural wave (Ngo *et al.*, 2010, Wang *et al.*, 2011a, Yasugi *et al.*, 2008). Notch signaling is transiently activated at the transition zone between NE cells and NBs and inhibits precocious NE to NB transition (Ngo *et al.*, 2010, Orihara-Ono *et al.*, 2011, Reddy *et al.*, 2010, Wang *et al.*, 2011b, Weng *et al.*, 2012, Yasugi *et al.*, 2010). Ecdysone signaling also regulates the transition step by inhibiting Dl expression, although the precise

mechanism by which the systemic regulation assures this step-by-step progression remains unclear (Lanet *et al.*, 2013). The progression of the proneural wave must be tightly regulated because its precocious progression results in fewer medullar neurons (Yasugi *et al.*, 2008).

Compared to OPC NBs, less has been reported about NB development in the IPC. A recent paper showed an interesting feature of IPC differentiation (Fig. 4E) (Apitz & Salecker, 2015). IPC NBs are located at the distal surface of the optic lobe (d-IPC), and these NBs are differentiated from NE cells that are located at the more proximal region of the IPC (p-IPC). These d-IPC and p-IPC regions are connected by extensive cell streams. Progenitor cells emerge via an epithelial-mesenchymal transition (EMT)-like mechanism in the p-IPC region and migrate into the d-IPC region through the cell streams. In the d-IPC, progenitor cells differentiate into asymmetrically dividing NBs. L'sc is expressed in NE cells in the p-IPC and is required for the proper supply of IPC NBs.

Temporal regulation in larval NBs

Similar to embryonic NBs, larval NBs and INPs sequentially express transcription factors to generate neuronal diversity. In Type II lineages, INPs sequentially express at least three transcription factors (Fig. 5A) (Bayraktar & Doe, 2013). Newly differentiated INPs express the SOX-family protein Dichaete (D), whereas older INPs express the mammalian Pax6 homolog Eyeless (Ey). The CP2 family member Grainy head (Grh) is expressed in middle-aged INPs with some overlap with D or Ey. Like the temporal factors in embryonic NBs, there are feed-forward activation and feed-back repression mechanisms between the three factors. In young INPs, D induces Grh expression, whereas in middle-aged INPs, Grh shuts off D expression and promotes Ey expression. In old INPs, Ey terminates Grh expression. In addition to these interactions between temporal factors, the Prdm protein Hamlet (Ham) regulates temporal patterning by limiting the self-renewal capacity of INPs (Eroglu *et al.*, 2014). Ham is expressed in mature INPs and regulates both the transition from Grh^{+}/Ey^{+} INPs to Grh^{-}/Ey^{+} INPs and timely cell-cycle exit. Temporal factors also define post-mitotic cell types: young INPs produce Brain specific homothorax $(Bsh)^{+}$ neurons or D^{+} neurons, whereas old INPs produce Twin of eyeless $(Toy)^{+}$ neurons or $Repo^{+}$ glia. In addition to temporal patterning in INPs, Type II NBs express D and Cas, which is followed by the

expression of Svp (Bayraktar & Doe, 2013, Maurange *et al.*, 2008). Repo^{+} glial cells are produced during the early NB / old INP window, and Bsh^{+} neurons are produced from the late NB / young INP window (Fig. 5A). These findings suggest that the combination of the temporal status of NBs and INPs can expand neuronal diversity. Importantly, the down-regulation of Ey causes the anatomical failure of the brain and produces defects in negative geotaxis behavior, demonstrating a close relationship between brain structure and neural function (Bayraktar & Doe, 2013).

The adult medulla consists of more than 70 neuronal types with approximately 40,000 neurons. OPC NBs temporally and sequentially express several transcription factors to produce the neuronal diversity in the adult stage. OPC NBs can be subdivided into several regions, including main OPC NBs and tOPC NBs (Fig. 4B). In the main OPC, NBs sequentially express Homothorax (Hth), Klumpfuss (Klu), Ey, Sloppy paired (Slp), D, and Tailless (Tll) (Fig. 5B and 5C) (Li *et al.*, 2013, Suzuki *et al.*, 2013). Because the differentiation of NE cells to NBs progresses in a medial to lateral direction, young, Hth^{+} NBs are located laterally, and old, Tll^{+} NBs are located medially. The expression patterns of these temporal factors along with some overlaps in expression produces approximately 12 NB types. The tOPC is defined by Wingless (Wg) expression in the posterior region of the optic lobe, also referred as the glial precursor cell (GPC) area (Dearborn & Kunes, 2004, Perez & Steller, 1996). tOPC NBs use a similar but different set of genes from NBs in the main OPC as temporal factors (Fig. 5D) (Bertet *et al.*, 2014). Newly differentiated tOPC NBs sequentially express Distalless (Dll), Ey, Slp, and D. Dll^{+} NBs directly generate neurons expressing Dll, Spalt major (Salm) and Runt (Run). Ey^{+} NBs produce Svp-positive neurons. Slp^{+} NBs produce Toy^{+} neurons, and D^{+} NBs produce D^{+} and Toy^{+} neurons. Interestingly, Notch signaling induces cell death in Notch^{ON} cells from the Ey^{+} window and in Notch^{OFF} cells from the Slp^{+} and D^{+} windows. However, it is not yet clear how the Caspase-dependent cell death mechanism responds the Notch activity nor how the cellular status changes between Ey^{+} and Slp^{+} windows.

NBs in the d-IPC show an additional form of temporal competence where two phases of NBs generate different types of neurons (Fig. 5E and 5F) (Apitz & Salecker, 2015). Young NBs express D and Asense (Ase) and give rise to Toy^{+} distal cell neurons, which project neurites to the medulla and lobula or the medulla and lamina in the adult stage, whereas old NBs express Tll, Atonal (Ato), and Dachshund (Dac) and give rise to

Dac^+ lobula plate neurons (Apitz & Salecker, 2015). Similar feed-forward and feed-back regulatory mechanisms exist between these two stages, as D induces Tll expression, and Tll shuts down Tll expression. Thus, the serial expression of transcription factors in NBs and their mutual interactions are key mechanisms for producing the vast variety of neurons and glia found in the CNS. It is intriguing to ask whether cell cycle length and extrinsic factors also affect the progression of the temporal windows described above.

Temporal regulation in mushroom body neurons

MB NBs sequentially produce three types of neurons with distinct axonal projection patterns (Fig. 6A) (Lee *et al.*, 1999). From the embryonic to mid-third instar larval stages, MB NBs generate γ neurons, and between the mid-third instar stage and puparium formation, MB NBs generate α'/β' neurons. After puparium formation, MB NBs produce α/β neurons. Cell-extrinsic and intrinsic factors regulate the transitions of MB neurons. The BTB-zinc finger protein Chronologically inappropriate morphogenesis (Chinmo) is highly expressed in MB neurons during early stages, and its expression gradually decreases during development (Fig. 6A) (Zhu *et al.*, 2006). In *Chinmo* loss-of-function mutants, the number of late-born α/β neurons increases at the expense of early-born γ and α'/β' neurons, suggesting that the temporal gradients of Chinmo determine neuronal fate (Zhu *et al.*, 2006). Another BTB-zinc finger protein, Abrupt (Ab), is highly expressed in MB neurons during the larval stages, and its down-regulation during puparium formation is required for the switch from α'/β' to α/β neuronal generation (Fig. 6A) (Kucherenko *et al.*, 2012). Furthermore, ecdysone signaling acts as an extrinsic temporal regulator for the transition from α'/β' neurons to α/β neurons (Fig. 6A and 6B). Ecdysone induces the expression of *let-7* microRNA, which is required for the timely transition from α'/β' neurons to α/β neurons through the negative regulation of Chinmo and Ab expression (Fig. 6B) (Chawla & Sokol, 2012, Kucherenko *et al.*, 2012, Sokol *et al.*, 2008, Wu *et al.*, 2012). Contrary to these findings, the genetic ablation of prothoracicotropic hormone (PTTH)-producing cells, which results in the inhibition of ecdysone synthesis and thus elongates the larval period, does not shift the transition timing from α'/β' to α/β neuronal generation (Lin *et al.*, 2013). Further experiments will reveal the molecules that act upstream of Chinmo and regulate the γ to α'/β' transition and the precise mechanism of ecdysone signaling.

Developmental plasticity in response to nutrient conditions

Animal development and tissue growth are often influenced by environmental factors such as nutrition. It is challenging to understand how the intrinsic growth program adapts to the changes in the environment during development. This is also the case for the spacio-temporal production and diversification of the nervous system. Indeed, differences in nutritional conditions and in the timing of systemic growth affects the proliferation of a subset of NB types. One of the examples demonstrating neuronal plasticity in response to nutritional conditions is MB NBs. In this case, larvae that are cultivated in protein-starved conditions before being placed to the normal food produce excess γ neurons, from MB NBs suggesting that MB NBs are highly plastic to nutrient conditions (Lin *et al.*, 2013). In contrast, two antennal lobe lineages, anterodorsal projection neuron (adPN) lineage and lateral antennal lobe (lAL) lineage, do not show any differences in their final numbers of neurons nor in the neuronal cell types generated under protein starvation conditions (Lin *et al.*, 2013). In these lineages, both NB proliferation and temporal fate transitions are delayed under nutrient-restricted conditions. Similarly, nutritional restriction does not affect the diversity of the neuronal cell types produced by OPC NBs, although the total number of neurons is reduced due to the decrease of the overall number of OPC NBs (Lanet *et al.*, 2013). These studies reveal that both nutrient-dependent and independent growth programs exist during neural development. It will be interesting to uncover the mechanisms that underlie the differences between those NBs that show or do not show plasticity in response to nutritional restriction conditions and the timing of systemic growth.

Termination of NB proliferation

To produce the exact number of neurons and glia, the termination of NB proliferation must be precisely determined. As there are differences in the generation and proliferation modes of different types NBs, each type disappears at different time points of development via different mechanisms (Fig. 1C).

In the abdominal VNC during larval stages, there are only three NBs in each hemisegment. These NBs die approximately 72 hours (hr) after larva hatched (ALH) through an apoptosis-dependent manner (Fig. 1C and 7A) (Bello *et al.*, 2003, Cenci & Gould, 2005). At the end of embryogenesis, Cas-positive NBs induce Grh expression.

Following the quiescence stage, VNC NBs and Type I and Type II NBs in the central brain sequentially express Cas and Svp. These temporal factors (and unidentified temporal factors which are expressed after Svp) are required for the timely exit from the cell cycle. The Hox protein Abdominal-A (AbdA) is transiently expressed in abdominal VNC NBs at the larval stage, and this pulse of AbdA expression triggers apoptosis in abdominal NBs (Bello *et al.*, 2003). Grh maintains AbdA expression, and Grh and AbdA induce apoptosis in a parallel mechanism (Cenci & Gould, 2005).

Type I and Type II NBs in the central brain and thoracic VNC NBs use similar termination machinery, where they begin to shrink following puparium formation, and most NBs exit the cell cycle at approximately 24 hr after puparium formation (APF) (Fig. 1C and 7B) (Homem *et al.*, 2014, Ito & Hotta, 1992, Maurange *et al.*, 2008). As with abdominal VNC NBs, embryonic Cas induces Grh expression, and after hatching, Cas induces Hh expression in these NBs (Chai *et al.*, 2013). Hh signaling acts as an autocrine and/or paracrine factor to sustain Grh expression, which in turn prevents premature nuclear localization of Pros to exit the cell cycle (Maurange *et al.*, 2008). In addition to the regulation by temporal factors and Hh signaling, ecdysone and the mediator complex, which is a highly conserved transcriptional mechanism, cooperatively regulate NB shrinking and the termination of proliferation in Type I and Type II NBs in the central brain (Homem *et al.*, 2014). The mediator complex binds to Ecdysone receptor (EcR) and eventually up-regulates the level of oxidative phosphorylation (OxPhos), which results in an increase in the oxygen consumption rates of pupal NBs. This metabolic change inhibits cell growth and ultimately causes NBs to exit the cell cycle. It will be interesting to clarify the relationship between the Grh-dependent nuclear localization of Pros and the metabolic changes mediated by the mediator complex and ecdysone.

The termination of NBs in the optic lobe has not been extensively investigated. Old Tll^{+} NBs in the main OPC express Pros, implying that the accumulation of nuclear Pros induces cell cycle exit (Li *et al.*, 2013). It is also possible that the apoptosis-dependent mechanism regulates termination because the cell death occurs in both OPC and IPC regions during early pupal stages (Hofbauer & Campos-Ortega, 1990). In either case, it is plausible that NBs in the optic lobe disappear after a defined number of cell divisions and/or passage time following NB formation.

Compared to other NB subtypes, MB NBs persist for a longer time (Fig. 1C) (Ito

& Hotta, 1992, Truman & Bate, 1988). MB NBs begin shrinking approximately 72 hr APF and disappear by 96 hr APF (Siegrist *et al.*, 2010, Truman & Bate, 1988). The down-regulation of InR/PI3K signaling after 72 hr APF leads to the nuclear localization of the transcription factor Forkhead box class O (FoxO), and this accumulation of nuclear FoxO regulates the timing of caspase-dependent cell death (Fig. 7C) (Siegrist *et al.*, 2010). NBs can persist even to the adult stage and generate new neurons by eliminating both the FoxO and pro-apoptotic gene functions (Siegrist *et al.*, 2010). This implies that some NBs have the potential ability to survive and proliferate until the adult stage.

Although it has been thought that there is no neuronal production in the adult stage in *Drosophila* (Kato *et al.*, 2009, Siegrist *et al.*, 2010), a recent study has shown that there are cell divisions in the adult medulla cortex, those cell divisions produce neurons, and the cell division rate increases after injury (Fernandez-Hernandez *et al.*, 2013). This raises the possibility that some OPC NBs retain their ability to proliferate into the adult stage. In the mammalian brain, a small subset of slowly dividing neural stem cells are retained in adults primarily in the subgranular zone in the dentate gyrus and the subventricular zone of the lateral ventricles (Ming & Song, 2011). Newly generated neurons in the adult are thought to be required for functional plasticity and brain repair following injury (Deng *et al.*, 2010, Lin & Iacovitti, 2015). Additional studies of neurogenesis in the *Drosophila* adult brain could advance our understanding of neuronal plasticity and neurodegenerative disorders.

Conclusion

A considerable number of studies of *Drosophila* NBs have revealed molecular and cellular mechanisms of asymmetric cell division, lineage specification, and neuronal diversification during development. NBs produce a large variety of neurons and glia through the sequential expression of transcription factors. Still, much remains to be clarified about the establishment of these functional neuronal networks. One remaining question is how the specification of NB subtypes is determined. Three types of NBs exist in the larval central brain, Type I, Type II, and MB NBs, and each shows different proliferation patterns and generates different numbers of progeny. There are approximately 100 NBs in the central brain, and systematic clonal analysis has revealed that there are approximately 100 stereotyped neuronal lineages that show distinct

positioning of cell bodies and neuronal arborization patterns in the adult central brain (Ito *et al.*, 2013, Yu *et al.*, 2013). This raises the possibility that the identity of each NB is specified at the start of development, producing unique progeny. Another question is whether the transition between temporal transcription factors found in several types of NB lineages is coupled with cell cycle progression and is regulated by extrinsic factors. Lineage tracing techniques can be used to follow all neuronal types from a particular NB and will give insight into the temporal competence mechanism (Yu *et al.*, 2010). Additionally, it is interesting to speculate how the development of the CNS is plastic to environmental conditions and is related to the developmental timing of the whole body. It has been shown that the brain is actively protected relative to other tissues during nutrient-restricted conditions (Cheng *et al.*, 2011).

Neurogenesis in *Drosophila* and in mammals shares several key features in the generation of neuronal diversity. In both systems, the temporal regulation of neural stem cells defines the position and the connectivity of neurons, which provides the platform for brain function. Future analyses will deepen our understanding of the importance of timing in neural development.

Acknowledgements

We thank N. Okamoto and S. Yoshiura for their critical reading of the manuscript. T. N. is funded by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Alsio, J. M., Tarchini, B., Cayouette, M. & Livesey, F. J. 2013. Ikaros promotes early-born neuronal fates in the cerebral cortex. *Proc Natl Acad Sci U S A,* **110,** E716-725.

Apitz, H. & Salecker, I. 2015. A region-specific neurogenesis mode requires migratory progenitors in the Drosophila visual system. *Nat Neurosci,* **18,** 46-55.

Artavanis-Tsakonas, S. & Simpson, P. 1991. Choosing a cell fate: a view from the Notch locus. *Trends Genet,* **7,** 403-408.

Barrett, A. L., Krueger, S. & Datta, S. 2008. Branchless and Hedgehog operate in a positive feedback loop to regulate the initiation of neuroblast division in the Drosophila larval brain. *Dev Biol,* **317,** 234-245.

Bayraktar, O. A., Boone, J. Q., Drummond, M. L. & Doe, C. Q. 2010. Drosophila type II neuroblast lineages keep Prospero levels low to generate large clones that contribute to the adult brain central complex. *Neural Dev,* **5,** 26.

Bayraktar, O. A. & Doe, C. Q. 2013. Combinatorial temporal patterning in progenitors expands neural diversity. *Nature,* **498,** 449-455.

Bello, B., Reichert, H. & Hirth, F. 2006. The brain tumor gene negatively regulates neural progenitor cell proliferation in the larval central brain of Drosophila. *Development,* **133,** 2639-2648.

Bello, B. C., Hirth, F. & Gould, A. P. 2003. A pulse of the Drosophila Hox protein Abdominal-A schedules the end of neural proliferation via neuroblast apoptosis. *Neuron,* **37,** 209-219.

Bello, B. C., Izergina, N., Caussinus, E. & Reichert, H. 2008. Amplification of neural stem cell proliferation by intermediate progenitor cells in Drosophila brain development. *Neural Dev,* **3,** 5.

Bertet, C., Li, X., Erclik, T., Cavey, M., Wells, B. & Desplan, C. 2014. Temporal patterning of neuroblasts controls Notch-mediated cell survival through regulation of Hid or Reaper. *Cell,* **158,** 1173-1186.

Betschinger, J., Mechtler, K. & Knoblich, J. A. 2006. Asymmetric

segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. *Cell,* **124,** 1241-1253.

Boone, J. Q. & Doe, C. Q. 2008. Identification of Drosophila type II neuroblast lineages containing transit amplifying ganglion mother cells. *Dev Neurobiol,* **68,** 1185-1195.

Bowman, S. K., Neumuller, R. A., Novatchkova, M., Du, Q. & Knoblich, J. A. 2006. The Drosophila NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. *Dev Cell,* **10,** 731-742.

Bowman, S. K., Rolland, V., Betschinger, J., Kinsey, K. A., Emery, G. & Knoblich, J. A. 2008. The tumor suppressors Brat and Numb regulate transit-amplifying neuroblast lineages in Drosophila. *Dev Cell,* **14,** 535-546.

Brankatschk, M. & Eaton, S. 2010. Lipoprotein particles cross the blood-brain barrier in Drosophila. *J Neurosci,* **30,** 10441-10447.

Britton, J. S. & Edgar, B. A. 1998. Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development,* **125,** 2149-2158.

Brody, T. & Odenwald, W. F. 2000. Programmed transformations in neuroblast gene expression during Drosophila CNS lineage development. *Dev Biol,* **226,** 34-44.

Cabernard, C. & Doe, C. Q. 2009. Apical/basal spindle orientation is required for neuroblast homeostasis and neuronal differentiation in Drosophila. *Dev Cell,* **17,** 134-141.

Cabrera, C. V., Martinez-Arias, A. & Bate, M. 1987. The expression of three members of the achaete-scute gene complex correlates with neuroblast segregation in Drosophila. *Cell,* **50,** 425-433.

Cenci, C. & Gould, A. P. 2005. Drosophila Grainyhead specifies late programmes of neural proliferation by regulating the mitotic activity and Hox-dependent apoptosis of neuroblasts. *Development,* **132,** 3835-3845.

Cepko, C. 2014. Intrinsically different retinal progenitor cells produce specific types of progeny. *Nat Rev Neurosci,* **15,** 615-627.

Chai, P. C., Liu, Z., Chia, W. & Cai, Y. 2013. Hedgehog signaling acts with

the temporal cascade to promote neuroblast cell cycle exit. *PLoS Biol,* **11,** e1001494.

Chawla, G. & Sokol, N. S. 2012. Hormonal activation of let-7-C microRNAs via EcR is required for adult Drosophila melanogaster morphology and function. *Development,* **139,** 1788-1797.

Chell, J. M. & Brand, A. H. 2010. Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell,* **143,** 1161-1173.

Cheng, L. Y., Bailey, A. P., Leevers, S. J., Ragan, T. J., Driscoll, P. C. & Gould, A. P. 2011. Anaplastic lymphoma kinase spares organ growth during nutrient restriction in Drosophila. *Cell,* **146,** 435-447.

Chia, W., Somers, W. G. & Wang, H. 2008. Drosophila neuroblast asymmetric divisions: cell cycle regulators, asymmetric protein localization, and tumorigenesis. *J Cell Biol,* **180,** 267-272.

Choksi, S. P., Southall, T. D., Bossing, T. et al. 2006. Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. *Dev Cell,* **11,** 775-789.

Datta, S. 1995. Control of proliferation activation in quiescent neuroblasts of the Drosophila central nervous system. *Development,* **121,** 1173-1182.

Datta, S. 1999. Activation of neuroblast proliferation in explant culture of the Drosophila larval CNS. *Brain Res,* **818,** 77-83.

Dearborn, R., Jr. & Kunes, S. 2004. An axon scaffold induced by retinal axons directs glia to destinations in the Drosophila optic lobe. *Development,* **131,** 2291-2303.

Deng, W., Aimone, J. B. & Gage, F. H. 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci,* **11,** 339-350.

Doe, C. Q., Chu-Lagraff, Q., Wright, D. M. & Scott, M. P. 1991. The prospero gene specifies cell fates in the Drosophila central nervous system. *Cell,* **65,** 451-464.

Ebens, A. J., Garren, H., Cheyette, B. N. & Zipursky, S. L. 1993. The Drosophila anachronism locus: a glycoprotein secreted by glia inhibits neuroblast proliferation. *Cell,* **74,** 15-27.

Egger, B., Boone, J. Q., Stevens, N. R., Brand, A. H. & Doe, C. Q. 2007. Regulation of spindle orientation and neural stem cell fate in the Drosophila optic lobe. *Neural Dev,* **2,** 1.

Egger, B., Gold, K. S. & Brand, A. H. 2011. Regulating the balance between symmetric and asymmetric stem cell division in the developing brain. *Fly (Austin),* **5,** 237-241.

Elliott, J., Jolicoeur, C., Ramamurthy, V. & Cayouette, M. 2008. Ikaros confers early temporal competence to mouse retinal progenitor cells. *Neuron,* **60,** 26-39.

Eroglu, E., Burkard, T. R., Jiang, Y. et al. 2014. SWI/SNF complex prevents lineage reversion and induces temporal patterning in neural stem cells. *Cell,* **156,** 1259-1273.

Fernandez-Hernandez, I., Rhiner, C. & Moreno, E. 2013. Adult neurogenesis in Drosophila. *Cell Rep,* **3,** 1857-1865.

Gonzalez, C. 2007. Spindle orientation, asymmetric division and tumour suppression in Drosophila stem cells. *Nat Rev Genet,* **8,** 462-472.

Green, P., Hartenstein, A. Y. & Hartenstein, V. 1993. The embryonic development of the Drosophila visual system. *Cell Tissue Res,* **273,** 583-598.

Greig, L. C., Woodworth, M. B., Galazo, M. J., Padmanabhan, H. & Macklis, J. D. 2013. Molecular logic of neocortical projection neuron specification, development and diversity. *Nat Rev Neurosci,* **14,** 755-769.

Grosskortenhaus, R., Pearson, B. J., Marusich, A. & Doe, C. Q. 2005. Regulation of temporal identity transitions in Drosophila neuroblasts. *Dev Cell,* **8,** 193-202.

Hassan, B. & Vaessin, H. 1996. Regulatory interactions during early neurogenesis in Drosophila. *Dev Genet,* **18,** 18-27.

Hirata, J., Nakagoshi, H., Nabeshima, Y. & Matsuzaki, F. 1995. Asymmetric segregation of the homeodomain protein Prospero during Drosophila development. *Nature,* **377,** 627-630.

Hofbauer, A. & Campos-Ortega, J. A. (1990). Proliferation pattern and early differentiation of the optic lobes in Drosophila melanogaster. *Roux's Arch Dev*

Biol, **198**, 264-274.

Homem, C. C. & Knoblich, J. A. 2012. Drosophila neuroblasts: a model for stem cell biology. *Development,* **139,** 4297-4310.

Homem, C. C., Steinmann, V., Burkard, T. R., Jais, A., Esterbauer, H. & Knoblich, J. A. 2014. Ecdysone and mediator change energy metabolism to terminate proliferation in Drosophila neural stem cells. *Cell,* **158,** 874-888.

Isshiki, T., Pearson, B., Holbrook, S. & Doe, C. Q. 2001. Drosophila neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell,* **106,** 511-521.

Ito, K. & Hotta, Y. 1992. Proliferation pattern of postembryonic neuroblasts in the brain of Drosophila melanogaster. *Dev Biol,* **149,** 134-148.

Ito, M., Masuda, N., Shinomiya, K., Endo, K. & Ito, K. 2013. Systematic analysis of neural projections reveals clonal composition of the Drosophila brain. *Curr Biol,* **23,** 644-655.

Izumi, Y., Ohta, N., Hisata, K., Raabe, T. & Matsuzaki, F. 2006. Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. *Nat Cell Biol,* **8,** 586-593.

Jessell, T. M. 2000. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat Rev Genet,* **1,** 20-29.

Kambadur, R., Koizumi, K., Stivers, C., Nagle, J., Poole, S. J. & Odenwald, W. F. 1998. Regulation of POU genes by castor and hunchback establishes layered compartments in the Drosophila CNS. *Genes Dev,* **12,** 246-260.

Kanai, M. I., Okabe, M. & Hiromi, Y. 2005. seven-up Controls switching of transcription factors that specify temporal identities of Drosophila neuroblasts. *Dev Cell,* **8,** 203-213.

Kato, K., Awasaki, T. & Ito, K. 2009. Neuronal programmed cell death induces glial cell division in the adult Drosophila brain. *Development,* **136,** 51-59.

Kawamori, H., Tai, M., Sato, M., Yasugi, T. & Tabata, T. 2011. Fat/Hippo pathway regulates the progress of neural differentiation signaling in the Drosophila optic lobe. *Dev Growth Differ,* **53,** 653-667.

Knoblich, J. A. 2008. Mechanisms of asymmetric stem cell division. *Cell,* **132,** 583-597.

Knoblich, J. A., Jan, L. Y. & Jan, Y. N. 1995. Asymmetric segregation of Numb and Prospero during cell division. *Nature,* **377,** 624-627.

Kohwi, M. & Doe, C. Q. 2013. Temporal fate specification and neural progenitor competence during development. *Nat Rev Neurosci,* **14,** 823-838.

Kraut, R. & Campos-Ortega, J. A. 1996. inscuteable, a neural precursor gene of Drosophila, encodes a candidate for a cytoskeleton adaptor protein. *Dev Biol,* **174,** 65-81.

Kraut, R., Chia, W., Jan, L. Y., Jan, Y. N. & Knoblich, J. A. 1996. Role of inscuteable in orienting asymmetric cell divisions in Drosophila. *Nature,* **383,** 50-55.

Kucherenko, M. M., Barth, J., Fiala, A. & Shcherbata, H. R. 2012. Steroid-induced microRNA let-7 acts as a spatio-temporal code for neuronal cell fate in the developing Drosophila brain. *EMBO J,* **31,** 4511-4523.

Kuchinke, U., Grawe, F. & Knust, E. 1998. Control of spindle orientation in Drosophila by the Par-3-related PDZ-domain protein Bazooka. *Curr Biol,* **8,** 1357-1365.

Lai, S. L. & Doe, C. Q. 2014. Transient nuclear Prospero induces neural progenitor quiescence. *Elife,* **3**.

Lanet, E., Gould, A. P. & Maurange, C. 2013. Protection of neuronal diversity at the expense of neuronal numbers during nutrient restriction in the Drosophila visual system. *Cell Rep,* **3,** 587-594.

Lee, C. Y., Wilkinson, B. D., Siegrist, S. E., Wharton, R. P. & Doe, C. Q. 2006. Brat is a Miranda cargo protein that promotes neuronal differentiation and inhibits neuroblast self-renewal. *Dev Cell,* **10,** 441-449.

Lee, T., Lee, A. & Luo, L. 1999. Development of the Drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. *Development,* **126,** 4065-4076.

Li, X., Erclik, T., Bertet, C. et al. 2013. Temporal patterning of Drosophila medulla neuroblasts controls neural fates. *Nature,* **498,** 456-462.

Lin, R. & Iacovitti, L. 2015. Classic and novel stem cell niches in brain homeostasis and repair. *Brain Res*.

Lin, S., Marin, E. C., Yang, C. P. et al. 2013. Extremes of lineage plasticity in the Drosophila brain. *Curr Biol,* **23,** 1908-1913.

Livesey, F. J. & Cepko, C. L. 2001. Vertebrate neural cell-fate determination: lessons from the retina. *Nat Rev Neurosci,* **2,** 109-118.

Martin-Bermudo, M. D., Martinez, C., Rodriguez, A. & Jimenez, F. 1991. Distribution and function of the lethal of scute gene product during early neurogenesis in Drosophila. *Development,* **113,** 445-454.

Matsuzaki, F. & Shitamukai, A. 2015. Cell Division Modes and Cleavage Planes of Neural Progenitors during Mammalian Cortical Development. *Cold Spring Harb Perspect Biol,* **7**.

Mattar, P., Ericson, J., Blackshaw, S. & Cayouette, M. 2015. A conserved regulatory logic controls temporal identity in mouse neural progenitors. *Neuron,* **85,** 497-504.

Maurange, C., Cheng, L. & Gould, A. P. 2008. Temporal transcription factors and their targets schedule the end of neural proliferation in Drosophila. *Cell,* **133,** 891-902.

Ming, G. L. & Song, H. 2011. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron,* **70,** 687-702.

Morante, J., Vallejo, D. M., Desplan, C. & Dominguez, M. 2013. Conserved miR-8/miR-200 defines a glial niche that controls neuroepithelial expansion and neuroblast transition. *Dev Cell,* **27,** 174-187.

Ngo, K. T., Wang, J., Junker, M. et al. 2010. Concomitant requirement for Notch and Jak/Stat signaling during neuro-epithelial differentiation in the Drosophila optic lobe. *Dev Biol,* **346,** 284-295.

Orihara-Ono, M., Toriya, M., Nakao, K. & Okano, H. 2011. Downregulation of Notch mediates the seamless transition of individual Drosophila neuroepithelial progenitors into optic medullar neuroblasts during prolonged G1. *Dev Biol,* **351,** 163-175.

Park, Y., Fujioka, M., Kobayashi, M., Jaynes, J. B. & Datta, S. 2001. even skipped is required to produce a trans-acting signal for larval neuroblast proliferation that can be mimicked by ecdysone.

Development, **128,** 1899-1909.

Park, Y., Rangel, C., Reynolds, M. M. et al. 2003. Drosophila perlecan modulates FGF and hedgehog signals to activate neural stem cell division. *Dev Biol,* **253,** 247-257.

Perez, S. E. & Steller, H. 1996. Migration of glial cells into retinal axon target field in Drosophila melanogaster. *J Neurobiol,* **30,** 359-373.

Petronczki, M. & Knoblich, J. A. 2001. DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in Drosophila. *Nat Cell Biol,* **3,** 43-49.

Reddy, B. V., Rauskolb, C. & Irvine, K. D. 2010. Influence of fat-hippo and notch signaling on the proliferation and differentiation of Drosophila optic neuroepithelia. *Development,* **137,** 2397-2408.

Rhyu, M. S., Jan, L. Y. & Jan, Y. N. 1994. Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell,* **76,** 477-491.

Richter, C., Oktaba, K., Steinmann, J., Muller, J. & Knoblich, J. A. 2011. The tumour suppressor L(3)mbt inhibits neuroepithelial proliferation and acts on insulator elements. *Nat Cell Biol,* **13,** 1029-1039.

Schaefer, M., Petronczki, M., Dorner, D., Forte, M. & Knoblich, J. A. 2001. Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system. *Cell,* **107,** 183-194.

Schaefer, M., Shevchenko, A. & Knoblich, J. A. 2000. A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in Drosophila. *Curr Biol,* **10,** 353-362.

Schober, M., Schaefer, M. & Knoblich, J. A. 1999. Bazooka recruits Inscuteable to orient asymmetric cell divisions in Drosophila neuroblasts. *Nature,* **402,** 548-551.

Shen, Q., Zhong, W., Jan, Y. N. & Temple, S. 2002. Asymmetric Numb distribution is critical for asymmetric cell division of mouse cerebral cortical stem cells and neuroblasts. *Development,* **129,** 4843-4853.

Siegrist, S. E., Haque, N. S., Chen, C. H., Hay, B. A. & Hariharan, I. K. 2010. Inactivation of both Foxo and reaper promotes long-term adult neurogenesis in Drosophila. *Curr Biol,* **20,** 643-648.

Siller, K. H., Cabernard, C. & Doe, C. Q. 2006. The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. *Nat Cell Biol,* **8,** 594-600.

Skeath, J. B. & Carroll, S. B. 1992. Regulation of proneural gene expression and cell fate during neuroblast segregation in the Drosophila embryo. *Development,* **114,** 939-946.

Sokol, N. S., Xu, P., Jan, Y. N. & Ambros, V. 2008. Drosophila let-7 microRNA is required for remodeling of the neuromusculature during metamorphosis. *Genes Dev,* **22,** 1591-1596.

Sousa-Nunes, R., Yee, L. L. & Gould, A. P. 2011. Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in Drosophila. *Nature,* **471,** 508-512.

Speder, P. & Brand, A. H. 2014. Gap junction proteins in the blood-brain barrier control nutrient-dependent reactivation of Drosophila neural stem cells. *Dev Cell,* **30,** 309-321.

Suzuki, T., Kaido, M., Takayama, R. & Sato, M. 2013. A temporal mechanism that produces neuronal diversity in the Drosophila visual center. *Dev Biol,* **380,** 12-24.

Truman, J. W. & Bate, M. 1988. Spatial and temporal patterns of neurogenesis in the central nervous system of Drosophila melanogaster. *Dev Biol,* **125,** 145-157.

Tsuji, T., Hasegawa, E. & Isshiki, T. 2008. Neuroblast entry into quiescence is regulated intrinsically by the combined action of spatial Hox proteins and temporal identity factors. *Development,* **135,** 3859-3869.

Voigt, A., Pflanz, R., Schafer, U. & Jackle, H. 2002. Perlecan participates in proliferation activation of quiescent Drosophila neuroblasts. *Dev Dyn,* **224,** 403-412.

Wang, W., Li, Y., Zhou, L., Yue, H. & Luo, H. 2011a. Role of JAK/STAT signaling in neuroepithelial stem cell maintenance and proliferation in the Drosophila optic lobe. *Biochem Biophys Res Commun,* **410,** 714-720.

Wang, W., Liu, W., Wang, Y., Zhou, L., Tang, X. & Luo, H. 2011b. Notch signaling regulates neuroepithelial stem cell maintenance and

neuroblast formation in Drosophila optic lobe development. *Dev Biol,* **350,** 414-428.

Weng, M., Haenfler, J. M. & Lee, C. Y. 2012. Changes in Notch signaling coordinates maintenance and differentiation of the Drosophila larval optic lobe neuroepithelia. *Dev Neurobiol,* **72,** 1376-1390.

Weng, R. & Cohen, S. M. 2012. Drosophila miR-124 regulates neuroblast proliferation through its target anachronism. *Development,* **139,** 1427-1434.

White, K. & Kankel, D. R. 1978. Patterns of cell division and cell movement in the formation of the imaginal nervous system in Drosophila melanogaster. *Dev Biol,* **65,** 296-321.

Wodarz, A., Ramrath, A., Grimm, A. & Knust, E. 2000. Drosophila atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J Cell Biol,* **150,** 1361-1374.

Wodarz, A., Ramrath, A., Kuchinke, U. & Knust, E. 1999. Bazooka provides an apical cue for Inscuteable localization in Drosophila neuroblasts. *Nature,* **402,** 544-547.

Wu, Y. C., Chen, C. H., Mercer, A. & Sokol, N. S. 2012. Let-7-complex microRNAs regulate the temporal identity of Drosophila mushroom body neurons via chinmo. *Dev Cell,* **23,** 202-209.

Yamanaka, N., Rewitz, K. F. & O'connor, M. B. 2013. Ecdysone control of developmental transitions: lessons from Drosophila research. *Annu Rev Entomol,* **58,** 497-516.

Yasugi, T., Sugie, A., Umetsu, D. & Tabata, T. 2010. Coordinated sequential action of EGFR and Notch signaling pathways regulates proneural wave progression in the Drosophila optic lobe. *Development,* **137,** 3193-3203.

Yasugi, T., Umetsu, D., Murakami, S., Sato, M. & Tabata, T. 2008. Drosophila optic lobe neuroblasts triggered by a wave of proneural gene expression that is negatively regulated by JAK/STAT. *Development,* **135,** 1471-1480.

Yu, H. H., Awasaki, T., Schroeder, M. D. et al. 2013. Clonal development and organization of the adult Drosophila central brain. *Curr Biol,* **23,** 633-

643.

Yu, H. H., Kao, C. F., He, Y., Ding, P., Kao, J. C. & Lee, T. 2010. A complete developmental sequence of a Drosophila neuronal lineage as revealed by twin-spot MARCM. *PLoS Biol,* **8**.

Zhong, W., Feder, J. N., Jiang, M. M., Jan, L. Y. & Jan, Y. N. 1996. Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. *Neuron,* **17,** 43-53.

Zhu, S., Lin, S., Kao, C. F., Awasaki, T., Chiang, A. S. & Lee, T. 2006. Gradients of the Drosophila Chinmo BTB-zinc finger protein govern neuronal temporal identity. *Cell,* **127,** 409-422.

Figure Legends

Fig. 1. Classification and asymmetric cell division of NBs. (A) Embryonic NBs delaminate from the neuroectoderm (NE). NBs divide and generate GMCs on the basal side. GMCs divide again to produce two post-mitotic neurons. (B) Larval NBs can be subdivided into Type I, Type II, and MB NBs in the central brain (CB), OPC and IPC NBs in the optic lobe (OL), and thoracic and abdominal NBs in the ventral nerve cord (VNC). (C) Time course of proliferation of NBs. MB NBs continuously divide from the embryonic to late pupal stages. OPC and IPC NBs in the OL produce neurons and glia throughout the larval to early pupal stages. Type I and Type II NBs in the CB and VNC NBs are produced during embryogenesis. At the end of embryogenesis, they stop proliferating and become quiescent. These NBs begin dividing again after hatching. (D) Three different modes of NB cell division. All NB types divide asymmetrically to self-renew. Type 0 NBs directly produce post-mitotic neurons. Type I NBs generate GMCs, which divide again to differentiate into neurons and/or glia. Type II NBs divide asymmetrically and produce transit amplifying intermediate neural progenitors (INPs). INPs divide asymmetrically to self-renew, producing another INP and a GMC. GMCs divide again and produce neurons and/or glia. (E) During NB mitosis, the apically localized Par complex (aPKC/Baz/Par6) recruits the Pins/Gαi/Mud complex to the apical cortex through binding to Insc. The Par complex localizes cell fate determinants such as Pros, Brat, and Numb to the basal side, which are inherited by the basal progeny.

Fig. 2. Temporal patterning in embryonic NB lineages. Embryonic NBs sequentially express Hb, Kr, Pdm, and Cas as they age. The transient expression of Svp shuts off Hb expression. The ‘positive feed-forward’ and ‘negative feed-back’ regulatory mechanisms between the transcription factors are indicated.

Fig. 3. Reactivation of post-embryonic NBs. (A) NBs maintain a G0-like quiescent state just after hatching. NBs start growing again upon food intake and progress through the cell cycle. (B) Food intake activates InR/PI3K/TOR signaling in NBs, leading to their regrowth. The fat body (FB) secretes a thus far unknown ‘fat body derived signal (FDS)’ upon dietary amino acid intake. The FDS acts on glial cells and/or directly on NBs, leading to their reactivation. One possibility is that the FDS induces the expression of Dilps in glial cells, which activates InR/PI3K/TOR signaling in NBs. (C)

Extrinsic factors regulate the G1-S transition in NBs. Ana prevents precocious cell cycle re-entry. Hh and FGF show a mutual feedback loop to regulate the re-entry into the cell cycle, which is mediated by Trol. Trol acts either downstream of Ana or by inhibiting Ana function. Ecdysone stimulates the G1-S transition. The Eve function outside the brain controls the transition. Eve acts upstream of ecdysone or modulating ecdysone. Eve also genetically interacts with Trol.

Fig. 4. Optic lobe development. (A) The fly visual system at the adult stage. The OL consists of the lamina, medulla, lobula, and lobula plate. Light information received by the retina is processed through these optic lobe ganglia. (B, C) Lateral view (B) and horizontal section (C) of the larval OL; the OPC, NE, lamina, and IPC are shown. The OPC can be subdivided into the main OPC and the tOPC. (D) Horizontal section of the main OPC. The transition from NE cells to NBs occurs in the medial region of the NE sheet. The proneural wave, which is indicated by the transient expression of L'sc, sweeps in a medial to lateral direction. OPC NBs divide asymmetrically and produce differentiating cells towards the inner side of the brain. The dashed line divides the oldest NB lineage from younger lineages. (E) Horizontal section of the IPC. Progenitor cells in the p-IPC region migrate into the d-IPC region in streams that connect these areas. d-IPC NBs produce distal cells (dc) and lobula plate neurons (lopn).

Fig. 5. Temporal patterning of larval NB lineages. (A) INPs generated from Type II NBs serially express D, Grh, and Ey with some overlap. Ham is required for the transition from Grh^{+}/Ey^{+} INPs to Grh^{-}/Ey^{+} INPs. Old INPs from early NBs produce $Repo^{+}$ glia, whereas young INPs from late NBs produce Bsh^{+} neurons. (B, C) Main OPC NBs express Hth, Klu, Ey, Slp, D, and Tll in a continuous manner. Neurons derived from different NB competence windows express specific factors such as Bsh, Run, Drf, and Toy. Neurons in which Notch signaling is active express Ap, which further generates neuronal diversity. Tll^{+} NBs produce $Repo^{+}$ glia. The dashed line in (B) divides the oldest NB lineage from younger lineages. (D) tOPC NBs sequentially express Dll, Ey, Slp, and D. Dll^{+} NBs directly produce post-mitotic neurons that express Dll, Salm, and Run. Ey^{+} NBs produce Svp^{+} neurons. Slp^{+} NBs and D^{+} NBs produce Toy^{+} neurons. $Notch^{ON}$ cells from Ey^{+} NBs and $Notch^{OFF}$ cells from Slp^{+} NBs and D^{+} NBs undergo cell death. (E, F) Young IPC NBs express D and Ase, whereas old IPC NBs express Tll,

Ato, and Dac. Young IPC NBs produce Toy^+ distal cells (dc), whereas old IPC NBs generate Dac^+ lobula plate neurons (lopn).

Fig. 6. Temporal patterning in MB neurons. (A) MB NBs sequentially produce three distinct types of neurons (γ, α'/β', and α/β). Chinmo expression is high during the early stages and gradually decreases. At the time of puparium formation, ecdysone is induced. The expression of Ab decreases, and the expression of *let-7* increases during this time period. (B) The transition from α'/β' to α/β is regulated by ecdysone. Ecdysone induces the expression of *let-7*, which inhibits Chimno and Ab.

Fig. 7. Termination of NB proliferation. (A) Abdominal VNC NBs stop proliferating due to apoptosis at approximately 72 hr ALH. The temporal expression of AbdA during the larval stage induces apoptotic cell death. Cas expression during late embryogenesis induces Grh expression. Sustained Grh expression maintains AbdA expression, and Grh causes cell death independent of AbdA. Temporal factors such as Cas and Svp during larval stages might promote apoptosis through a parallel mechanism. (B) Type I and Type II NBs in the CB and thoracic VNC NBs exit the cell cycle at approximately 24 hr APF. Cas expression during late embryogenesis induces Grh, whereas larval Cas induces Hh. Hh signaling maintains Grh expression to promote the nuclear localization of Pros and cell cycle exit. After puparium formation, Mediator and ecdysone cooperatively change the energy metabolism in NBs. An increase in oxidative phosphorylation (OxPhos) levels reduces cell growth, which results in cell cycle exit. (C) MB NBs undergo apoptotic cell death at approximately 96 hr APF. The gradual decrease in InR/PI3K signaling induces caspase-dependent cell death.

Figure 1

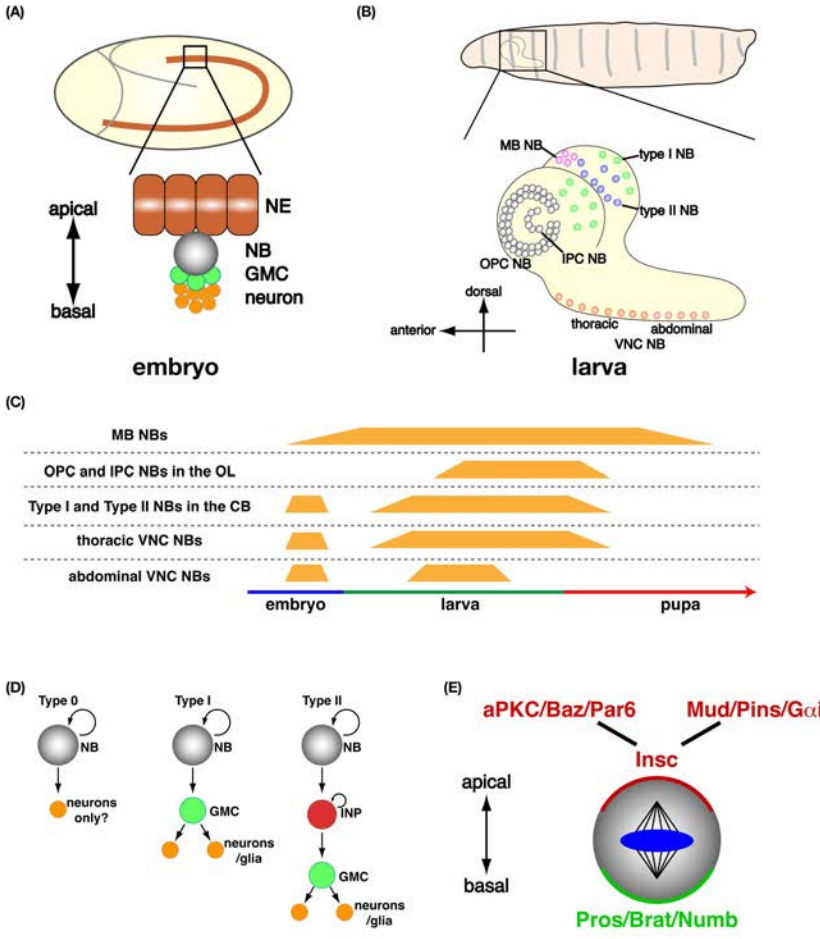

(A)
apical
basal
NE
NB
GMC
neuron
embryo
(B)
MB NB
type I NB
type II NB
OPC NB
IPC NB
dorsal
anterior
thoracic
abdominal
VNC NB
larva
(C)
MB NBs
OPC and IPC NBs in the OL
Type I and Type II NBs in the CB
thoracic VNC NBs
abdominal VNC NBs
embryo
larva
pupa
(D)
Type 0
NB
neurons only?
Type I
NB
GMC
neurons /glia
Type II
NB
INP
GMC
neurons /glia
(E)
aPKC/Baz/Par6
Mud/Pins/Gαi
Insc
apical
basal
Pros/Brat/Numb

Figure 2

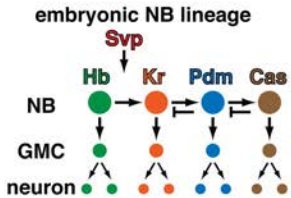
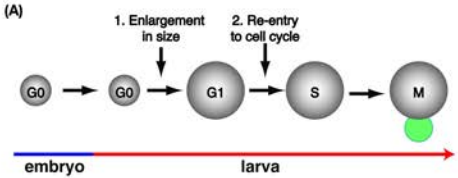
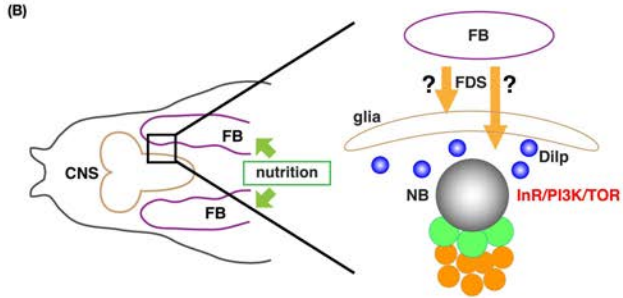


Figure 3


(A)
1. Enlargement in size
2. Re-entry to cell cycle
G0
G0
G1
S
M
embryo
larva


(B)
FB
? FDS ?
glia
CNS
FB
nutrition
FB
Dilp
NB
InR/PI3K/TOR

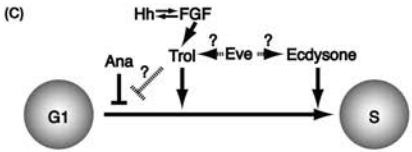

(C)
Hh⇌FGF
?
Ana
?
Trol
Eve
?
Ecdysone
G1
S

Figure 4

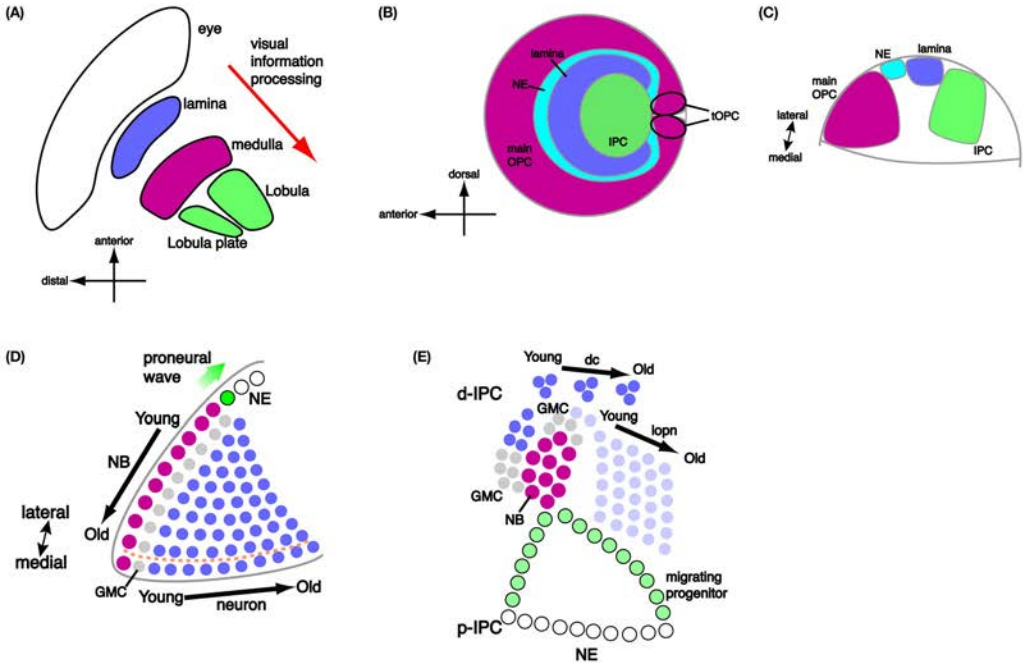

(A)
eye
visual
information
processing
lamina
medulla
Lobula
Lobula plate
anterior
distal
(B)
lamina
NE
tOPC
IPC
main
OPC
dorsal
anterior
(C)
NE
lamina
main
OPC
lateral
medial
IPC
(D)
proneural
wave
NE
Young
NB
lateral
medial
Old
GMC
Young
Old
neuron
(E)
Young
dc
Old
d-IPC
GMC
Young
lopn
Old
GMC
NB
migrating
progenitor
p-IPC
NE

Figure 5

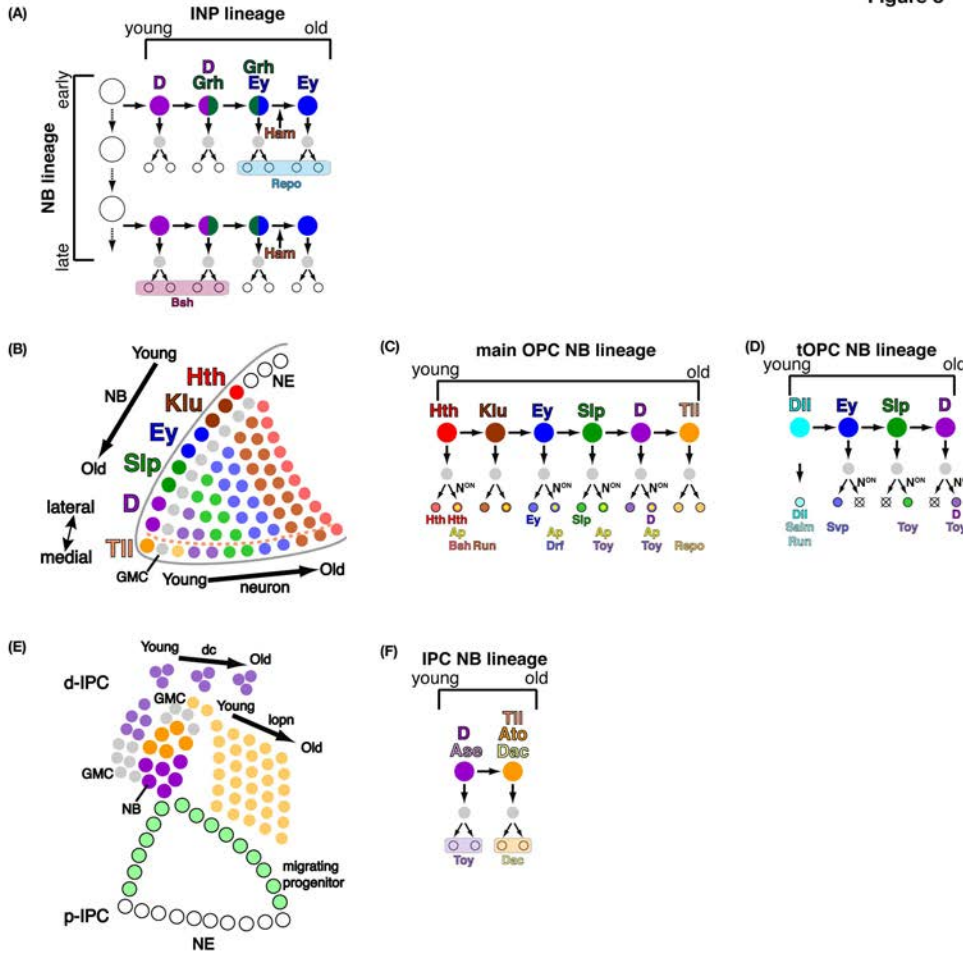
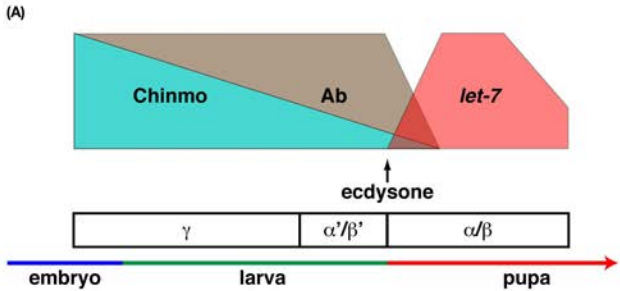

(A)
INP lineage
young
old
NB lineage
early
late
D
D Grh
Grh Ey
Ey
Ham
Repo
Ham
Bsh
(B)
Young
NB
Old
lateral
medial
Hth
Klu
Ey
Slp
D
Tll
NE
GMC
Young
Old
neuron
(C)
main OPC NB lineage
young
old
Hth
Klu
Ey
Slp
D
Tll
Hth Hth
Ap
Bsh Run
Ey
Ap
Drf
Slp
Ap
Toy
D
Ap
Toy
Repo
(D)
tOPC NB lineage
young
old
Dll
Ey
Slp
D
Dll
Salm
Run
Svp
Toy
D
Toy
(E)
Young
dc
Old
d-IPC
GMC
Young
lopn
Old
GMC
NB
migrating
progenitor
p-IPC
NE
(F)
IPC NB lineage
young
old
D
Ase
Tll
Ato
Dac
Toy
Dac

Figure 6


(A)
Chinmo
Ab
let-7
ecdysone
γ
α'/β'
α/β
embryo
larva
pupa

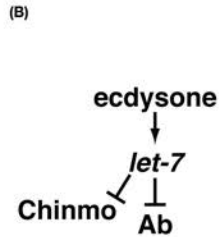
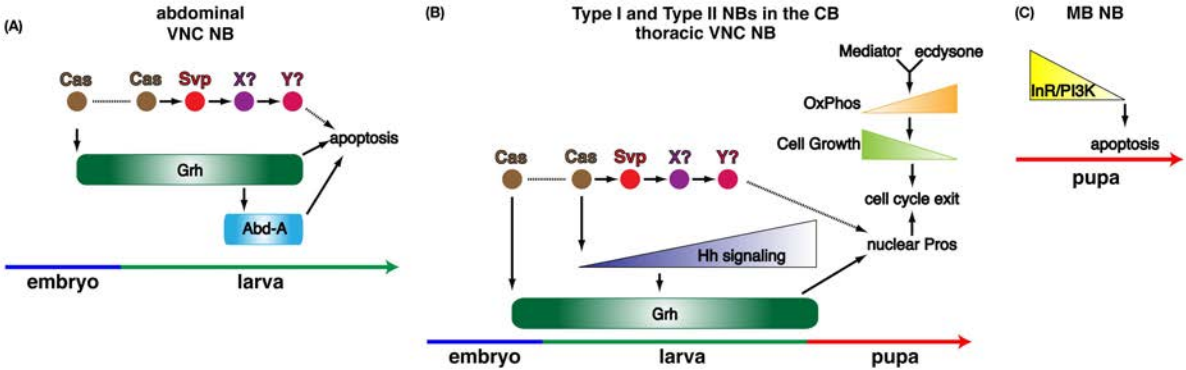

(B)
ecdysone
let-7
Chinmo
Ab

Figure 7


(A)
abdominal
VNC NB
Cas
Cas
Svp
X?
Y?
apoptosis
Grh
Abd-A
embryo
larva
(B)
Type I and Type II NBs in the CB
thoracic VNC NB
Mediator
ecdysone
OxPhos
Cell Growth
cell cycle exit
nuclear Pros
Cas
Cas
Svp
X?
Y?
Hh signaling
Grh
embryo
larva
pupa
(C)
MB NB
InR/PI3K
apoptosis
pupa