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Drosophila neuroblasts: a model for stem cell biology

Catarina C. F. Homem and Juergen A. Knoblich*

Summary

Drosophila neuroblasts, the stem cells of the developing fly brain, have emerged as a key model system for neural stem cell biology and have provided key insights into the mechanisms underlying asymmetric cell division and tumor formation. More recently, they have also been used to understand how neural progenitors can generate different neuronal subtypes over time, how their cell cycle entry and exit are coordinated with development, and how proliferation in the brain is spared from the growth restrictions that occur in other organs upon starvation. In this Primer, we describe the biology of *Drosophila* neuroblasts and highlight the most recent advances made using neuroblasts as a model system.

Key words: Asymmetric cell division, Neuroblast, Neurogenesis, Stem cells

Introduction

During brain development, neural stem cells proliferate in a spatially and temporally regulated fashion to generate the enormous number of diverse neurons that will drive the complex behavior of adult animals. How a limited number of neural stem cells generate all of the differentiated neurons and glial cells in the brain is still a largely unresolved issue. Typically, neurons arise from asymmetric divisions of a progenitor: one daughter cell retains self-renewal capacity, while the other is committed to neural differentiation. Different types of neurons are generated over time and this is facilitated by stereotyped transcriptional changes in the progenitor cell that follow a precise temporal order. Cell cycle entry and exit of neural stem cells are coordinated with developmental time to ensure that the right neurons are created at the right time and to prevent the formation of brain tumors. Furthermore, unlike other organs, the brain is spared from growth restrictions under starvation probably because a full complement of neurons is needed for the brain to function.

Surprisingly, all of these key features can be recapitulated in neuroblasts, the stem cells found in the developing brain of the simple invertebrate *Drosophila melanogaster*. The simplicity of *Drosophila* development and the sophisticated genetic tools available for studying *Drosophila* have allowed us to obtain insights into stem cell biology that would not be possible in a vertebrate model system. In this Primer (see Box, Development: the big picture), we summarize the exciting advances that have been made in this model system in recent years. We begin by describing the biology of *Drosophila* neuroblasts and discussing their relevance as a neural stem cell model. We then review how the study of stem cell proliferation and regulation has proven useful for understanding how stem cell tumors are originated, highlighting how tumor neuroblasts differ from wild-type neuroblasts and how they might bypass temporal and nutrient-sensing regulatory mechanisms. Finally, we discuss how the mechanisms that regulate neural stem cells in flies parallel those observed in vertebrates.

Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Dr Bohr Gasse 3-5, 1030 Vienna, Austria.

*Author for correspondence (juergen.knoblich@imba.oeaw.ac.at)

The development and cell biology of neuroblasts

Neuroblasts (NBs) are first formed during the embryonic stages (stages 9 to 11) of *Drosophila* development (Fig. 1A). NBs delaminate from a neuroepithelium located in the ventrolateral region of the embryo (Fig. 1B) and start dividing shortly afterwards to generate neurons and glia. Embryonic NBs are specified in a process called lateral inhibition in which Notch/Delta signaling refines the expression of proneural genes to individual cells (for reviews, see Artavanis-Tsakonas and Simpson, 1991; Skeath and Thor, 2003). NBs undergo repeated self-renewing asymmetric divisions (Reichert, 2011), giving rise to another NB and a smaller ganglion mother cell (GMC), which divides once to produce neurons and/or glial cells (Fig. 1B).

Embryonic NB divisions produce all the neurons that will form the larval central nervous system (CNS) but only 10% of the cells in the adult CNS (Prokop and Technau, 1991; Green et al., 1993). Most NBs in the abdominal regions of the embryo are eliminated through programmed cell death after completing their neuronal lineages (White et al., 1994). In the cephalic and thoracic regions, however, NBs arrest their cell cycle and exit from G1 into a G0-like quiescent state. Around 8-10 hours after larval hatching, during the late 1st instar stage, the NBs start exiting quiescence and re-enter mitosis. This second wave of neurogenesis (Fig. 1A) is responsible for the formation of 90% of the neurons in the adult CNS. Neurogenesis continues throughout larval stages into pupal stages, at which point the NBs exit from the cell cycle and disappear. (White and Kankel, 1978; Truman and Bate, 1988; Maurange et al., 2008).

The larval brain in particular has been used extensively to study how NB lineage progression is regulated. Unlike embryonic NBs, larval NBs re-grow to their original size after each cell division and are capable of dividing hundreds of times. Based on their position in the brain and their lineage characteristics, we can distinguish abdominal and thoracic NBs in the ventral nerve cord (VNC) and type I, type II, mushroom body and optic lobe NBs in the brain lobes (Fig. 1C). Whereas VNC and central brain NBs originate from embryonic NBs, optic lobe NBs arise only during larval stages (Egger et al., 2007). As excellent recent reviews have covered the special mode of neurogenesis in the optic lobes (Egger et al., 2010), we will focus on larval central brain type I and type II NBs.

Development: the big picture

This Primer is part of a series entitled 'Development: the big picture'. This series aims to highlight key developmental systems or processes that have been the subject of intense study because they have broad implications for other developmental, cell and molecular systems, or for disease and therapeutics. Keep an eye out for other articles in this series over the coming months!

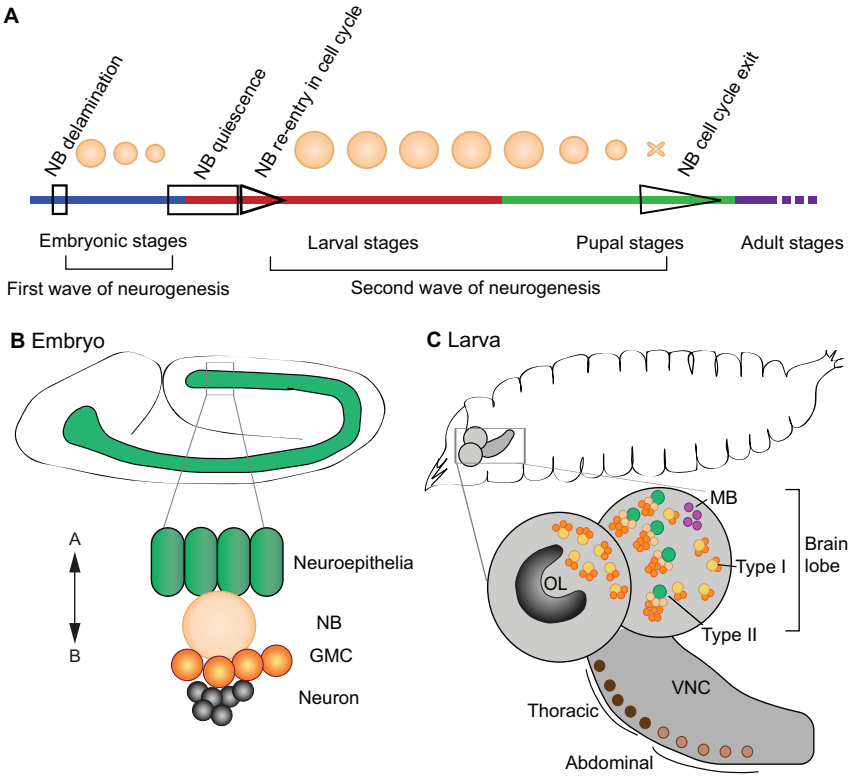

Fig. 1. Neurogenesis in *Drosophila* embryos and larvae. (**A**) Time line of the two waves of neurogenesis occurring during *Drosophila* development. Neuroblasts (NBs; beige) and their sizes are depicted throughout the timeline. NBs are generated during embryonic stages by delamination from the neuro-ectoderm. Embryonic NBs do not re-grow after each division. They become quiescent during late embryogenesis but re-enter the cell cycle to start a second wave of neurogenesis in larvae. Larval NBs re-grow after each cell division and therefore can divide more often. During the pupal stages, NBs disappear and this ends the second wave of neurogenesis. Different NBs exit the cell cycle at different time points; the cartoon depicts the cell-cycle exit mechanism described for thoracic NBs, which reduce their size until they undergo a size-wise symmetric division and differentiate. (**B**) A *Drosophila* embryo during neurogenesis. NBs (beige) delaminate from neuroectodermal cells (green). The polarity of the first embryonic NB division is inherited from the original epithelial cells. NBs divide to generate a ganglion mother cell (GMC; orange) that divides once more, giving rise to two neurons (gray). A, apical; B, basal. (**C**) A 3rd instar larva. The larval brain is shown in more detail, highlighting the main brain regions: ventral nerve cord (VNC) with its thoracic NBs (dark brown) and abdominal NBs (light brown); brain lobes with optic lobes (OL); central brain mushroom body NBs (MB; magenta); type I NBs (yellow); and type II NBs (green).

Type I NBs constitute the majority of central brain NBs and are located in both anterior and posterior sides of the brain. By contrast, type II NBs are located only in the posterior side of the brain and are characterized by a different lineage (Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008). Approximately 90 type I and eight type II NBs can be found in each brain lobe. Like the embryonic NBs, type I NBs bud off a GMC that divides once more to generate two differentiated cells (Fig. 2A). Type II NBs instead divide asymmetrically to generate a transit amplifying intermediate neural progenitor (INP), which undergoes a defined series of transcriptional changes to become a mature INP (Fig. 2B). Mature INPs are estimated to continue to divide asymmetrically between three to five times (Bello et al., 2008) and generate another mature INP and a GMC that divides once more to generate two differentiated neurons or glial cells (Fig. 2B). Therefore, INPs are a transit amplifying population that allows type II NBs to generate many more neurons than type I NBs. Together, type II NBs contribute ~5000 adult neural cells that form major neuropile substructures of the brain, such as the central complex (Izergina et al., 2009; Bayraktar et al., 2010).

Using neuroblasts to study stem cell biology

As highlighted above, the fly brain contains a variety of different neural stem cell lineages that can be used to study and characterize various specific aspects of stem cell biology. In recent years, studies of *Drosophila* neuroblasts have therefore provided us with key insights into some of the mechanisms that regulate stem cells.

Understanding asymmetric cell division

Asymmetric cell division is a process that generates two daughter cells that are specified to assume different cell fates shortly after mitosis. This use of the term ‘asymmetric cell division’ goes back to a ground-breaking review by Robert Horvitz and Ira Herskowitz (Horvitz and Herskowitz, 1992). As stem cells are defined by their ability to create both self-renewing and differentiating daughter cells, each stem cell division must be asymmetric in some sense. Self-renewing and differentiating daughter cells can be generated through purely stochastic means (Snippert et al., 2010; Simons and Clevers, 2011). Alternatively, extrinsic factors can act differently on the two daughter cells and direct them towards distinct fates. Finally, the unequal segregation of an intrinsic fate determinant can establish different fates in the sister cells.

Drosophila stem cells provide instructive examples for both the extrinsic and intrinsic mechanisms. In *Drosophila* germline stem cells (GSCs), for example, cells surrounding the stem cell niche supply self-renewal cues and/or release stem cell maintenance short-range signals that induce the polarity necessary for stem cell asymmetric divisions (Tulina and Matunis, 2001; Chen et al., 2011). By contrast, NB self-renewal does not require any extrinsic factors. Larval NB asymmetric divisions are not oriented with respect to an external axis, and NBs are still able to divide asymmetrically and self-renew in culture (Datta, 1999; Ceron et al., 2006; Rebollo et al., 2007). The regulation of asymmetric NB division has been studied extensively and most of the components involved in regulating this process have been identified (reviewed by Knoblich, 2008). Because many reviews have discussed this subject in detail, we will only briefly summarize it here.

The general mechanism for NB asymmetric cell division is conserved in all *Drosophila* NBs (Knoblich, 2008). NB asymmetric cell division involves four major steps: setting up of an axis of polarity; proper orientation of the mitotic spindle; asymmetric localization of cell fate determinants in the dividing NB; and differential segregation of cell fate determinants between the two daughter cells. In the embryo, when NBs delaminate, their apical-basal polarity is inherited from epithelial cells in the neuroectoderm. They retain apical localization of the Par complex

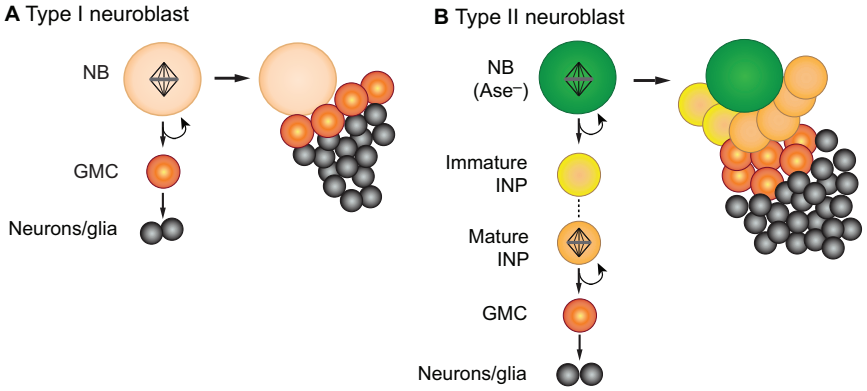

Fig. 2. Type I and type II neuroblasts and their mode of asymmetric cell division. (**A**) Type I neuroblasts (NBs) divide asymmetrically to self-renew and generate a ganglion mother cell (GMC, orange). GMCs divide once to generate neurons or glia (gray). (**B**) Type II NBs divide asymmetrically to self-renew and generate an immature intermediate precursor (INP; yellow). After a period of maturation, INPs start dividing asymmetrically to self-renew and to generate a GMC. The GMCs divide once into two differentiating neurons or glial cells (gray). Through INPs, type II lineages give rise to more neurons than do type I NBs.

(see Fig. 3, Par3/Bazooka, Par-6 and atypical PKC; aPKC) and undergo multiple rounds of asymmetric cell divisions along the apical-basal axis of the overlying epithelium (Yoshiura et al., 2011). Subsequent embryonic divisions (Rebollo et al., 2009) and divisions of larval NBs (Rebollo et al., 2007; Rusan and Peifer, 2007) are, by contrast, aligned relative to the axis of the previous division. For this, it is thought that the NB centrosome serves as a reference point for apical accumulation of the Par complex during interphase to establish an apicobasal axis of polarity within the cell (Rebollo et al., 2009). The mechanism by which cortical polarity is oriented relative to the centrosome is still unclear. Bazooka (one of the Par complex members) then links apical polarity to spindle orientation by anchoring Inscuteable (Insc), an adaptor protein, between the Par complex and the Gαi/Pins (Rap)/Mud complex (Schaefer et al., 2000; Yu et al., 2000; Schaefer et al., 2001). Through Mud, this complex binds astral microtubules and directs spindle orientation (Fig. 3) (see Table 1 for individual protein functions). The apical complexes also induce the asymmetric localization of the cell fate determinants Brain tumor (Brat), Prospero (Pros) and Numb to the opposite (basal) side of the cell, resulting in their segregation into the differentiating daughter cell following division. Asymmetric segregation of Numb, Pros and Brat is mediated by two adaptor proteins, Miranda (Mira) and Partner-of-Numb (Pon). Mira prevents Pros from regulating transcription in the NB by tethering it to the basal cortex during mitosis (Shen et al., 1997). Once segregated into the daughter GMC, however, Mira is degraded and Pros enters the nucleus to promote differentiation (Hirata et al., 1995; Ikeshima-Kataoka et al., 1997). Like Pros, Brat also binds to Mira and hence is co-segregated into the GMC during NB division. Pon assists in the asymmetric localization of Numb and its segregation into the GMC, although it is not strictly required for this to occur (Lu et al., 1998; Wang et al., 2007).

The mechanism by which the Par complex induces the asymmetric localization of cell fate determinants and adaptor proteins has been recently clarified. It was shown that aPKC directly phosphorylates Numb during mitosis, leading to its release from the apical NB cortex and its asymmetric distribution (Smith et al., 2007; Wirtz-Peitz et al., 2008). A similar mechanism has been demonstrated for Mira (Wirtz-Peitz et al., 2008; Atwood and Prehoda, 2009), indicating that polar phosphorylation is the underlying mechanism for asymmetric protein segregation during mitosis.

Once they are segregated into the GMC, the three cell fate determinants are thought to inhibit self-renewal and promote cell cycle exit and differentiation. Numb does this by promoting endocytosis of the Notch receptor, thereby inhibiting Notch in the differentiating daughter cell (reviewed by Schweisguth, 2004; Couturier et al., 2012). Pros is a transcription factor that inhibits cell cycle genes and activates pro-neural genes in the GMC (Choksi et al., 2006; Southall and Brand, 2009). Brat is a TRIM-NHL-domain protein that acts as a post-transcriptional regulator during embryogenesis (Arama et al., 2000; Sonoda and Wharton, 2001; Frank et al., 2002; Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006), but how it acts in NBs is not understood. Brat paralogs and orthologs can regulate microRNAs (Neumüller et al., 2008; Schwamborn et al., 2009), and, in the ovary, Brat functions as a differentiation factor, acting together with Pumilio to repress translation of differentiation genes (Harris et al., 2011).

In summary, these extensive studies have shown that asymmetric cell division is controlled intrinsically in NBs and that a combination of cortical determinants is segregated into one

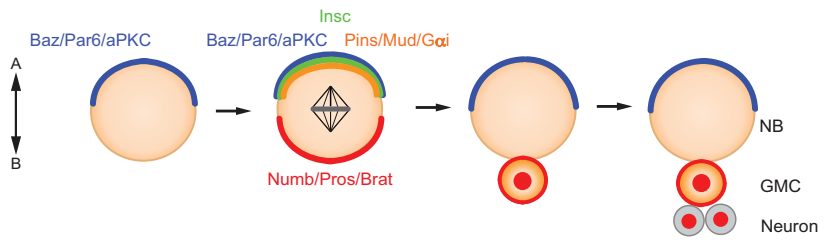

Fig. 3. Asymmetric cell division in neuroblasts. Neuroblasts (NBs) divide asymmetrically to self-renew and to generate a more differentiated daughter cell. The Par complex (Baz, Par6 and aPKC; blue line) localizes asymmetrically at the apical cortex of NBs. The Par complex recruits Inscuteable (Insc, green line), which in turn recruits the Pins/Mud/Gαi complex (orange line) to the apical cell cortex. Through Mud, these apical complexes orient the mitotic spindle with respect to the established apical-basal axis. Through a cascade of phosphorylation events, the apical Par complex directs the cell fate determinants Numb, Pros and Brat (red line) to the basal cell cortex. When the NB divides asymmetrically, these cell fate determinants are segregated to the GMC, where they promote differentiation rather than self-renewal. A, apical; B, basal.

Table 1. Regulators of neuroblast development and their mutant phenotypes

Drosophila gene/protein	Vertebrate orthologue(s)	Function	Defects associated with mutations in the nervous system	References
Asymmetric cell division				
Par complex: aPKC (Atypical Protein Kinase C), Bazooka/Par3, Par-6 (Partinioning defective 6)	PKCζ, PKCλ; Par3/Par-6	Establishes a polarity axis in NBs. Localizes and determines the apical side of the NB cell cortex. Inheritance by the undifferentiated NB after mitosis.	aPKC gain of function: NB-like cells and overproliferation Par complex loss of function: loss of apicobasal polarity; NBs prematurely enter cell cycle arrest	Reviewed by Knoblich, 2008
Insc (Inscuteable) and Gαi/Pins (Partner of Inscuteable)/ Mud (Mushroom Body Defective)	Mouse Insc, Gαi1-3, AGS3, LGN, NuMA	Insc: adaptor protein that links the Par complex to a second protein complex containing the proteins Gαi, Pins and Mud Gαi/Pins/Mud: apical complex; links apical cortex and astral microtubules to orient the mitotic spindle	Loss of function: misorientation of spindle during NB divisions	Reviewed by Knoblich, 2008
Lgl [Lethal (2) Giant Larvae]	Mgl	Lethal (2) giant larvae (Lgl) is a cytoskeletal protein that defines the basolateral domain and restricts the Par complex to the apical domain	Loss of function: overproliferation of NB-like cells	Reviewed by Knoblich, 2008
Numb	Numb, Numblike	Notch signaling inhibitor. Asymmetrically segregated to the basal daughter cell, where it lowers Notch levels and promotes cell differentiation.	Loss of function: overproliferation of NB-like cells	Wang et al., 2007
Pon (Partner of Numb)	–	Adaptor protein that facilitates the basal localization of Numb	Loss of function: overproliferation of NB-like cells	Wang et al., 2007
Brat (Brain Tumor)	Trim2, Trim3, Trim32	Translation inhibitor. Localizes basally in the dividing NBs. Is inherited by the basal differentiating daughter cell. Inhibits growth and self-renewal, and induces differentiation.	Loss of function: overproliferation of NB-like cells; reduction in number of differentiated cells	Bello et al., 2006; Betschinger et al., 2006
Mira (Miranda)	–	Adaptor protein that accumulates asymmetrically in the basal side of the dividing NB. Mira binds Brat and Pros, localizing these proteins to the basal cortex of NBs.	Loss of function: loss of Pros asymmetric localization in NBs; overproliferation of NB-like cells; reduction in the number of differentiated cells	Shen et al., 1997
Pros (Prospero)	Prox1	Homeodomain transcription factor. Represses expression of cell cycle genes and activates genes that specify cell fate and are required for terminal differentiation.	Gain of function: premature differentiation of NBs Loss of function: differentiating daughter cells revert back to Nb-like fate	Doe et al., 1991
Notch	Notch 1-4	Notch high levels are determinant of Nb fate	Gain of function: INPs revert back to NB-like cells Loss of function: loss of larval NBs	Bowman et al., 2008
Polo	Plk1 (Polo-like kinase1)	Cell cycle regulator, mitotic Ser/Thr protein kinase	Loss of function: overproliferation of NBs, and defective asymmetric localization of aPKC, Numb and Pon	Reichert, 2011
Aurora-A	Aurora	Cell cycle regulator, mitotic Ser/Thr protein kinase	Loss of function: overproliferation of NBs, and defective asymmetric localization of aPKC, Numb and Pon	Reichert, 2011
NB temporal identity				
Hb (Hunchback)	–	Member of the gap class of segmentation genes. Hb is a NB temporal transcription factor.	Gain of function: no switch to Kr^+ NBs; Pdm1/2 ectopic expression	For a review, see Kambadur et al., 1998
Kr (Kruppel)	Multiple (Klfs)	Member of the gap class of segmentation genes. Kr is a NB temporal transcription factor.	Loss of function: second identity NBs are not formed	Isshiki et al., 2001
Pdm1/2 (POU domain protein 1 and 2)	Pou family of transcription factors	Pou transcription factors 1 and 2 are involved in cell-identity decisions during CNS development. Pdm1 and Pdm2 are NB temporal transcription factors. Pdm1 regulates wing proliferation.	Loss of function: NB4-2 lineages do not generate RP2 neurons; premature embryonic quiescence of NB3-3 NBs	Yang et al., 1993; Tsuji et al., 2008
Cas (Castor)	Casz1	Cas is a NB temporal transcription factor	Loss of function: Pdm1 and Pdm2 are ectopically activated; embryo-larva quiescence is delayed	Kambadur et al., 1998; Tsuji et al., 2008
Svp (Seven up)	COUP-TFI and COUP-TFII	Steroid-hormone receptor gene. Required for the development of the embryonic CNS and specific photoreceptor cells of the eye.	Loss of function: embryonic NBs do not switch from Hb-Kr; larval thoracic NBs do not exit the cell cycle	Kanai et al., 2005; Maurange et al., 2008

Table 1. Continued on next page

Table 1. Continued

Drosophila gene/protein	Vertebrate orthologue(s)	Function	Defects associated with mutations in the nervous system	References
D (Dichaete)	Multiple Sox-domain containing genes	Sox protein and pair-rule segmentation gene. Dichaete is a transcription factor required for dorsal-ventral patterning of embryonic CNS and NB formation. Negative target of the Nb temporal transcription series.	Loss of function: CNS patterning defects	Nambu and Nambu, 1996; Buescher et al., 2002; Zhao et al., 2007; Maurange et al., 2008
Grh (Grainyhead)	Grainy head-like 1, 2 and 3	Transcription factor required for regulating NB mitotic activity during larval stages. Positive target of NB temporal transcription series. Grh, together with Abdominal A, end thoracic NB proliferation.	Grh maintains the self-renewal state of thoracic NBs Loss of function: thoracic NBa do not undergo apoptosis	Almeida and Bray, 2005; Cenci and Gould, 2005
Abd A (Abdominal A)	–	Hox protein expressed in a subset of postembryonic NBs. In a temporally restricted manner it regulates NB apoptosis.	Loss of function: thoracic Nb do not undergo apoptosis	White et al., 1994; Bello et al., 2003
Hox proteins: Antp (Antennapedia) and Abd A	Multiple vertebrate Hox genes	Regulate NB entry in quiescence at the end of embryonic stages	Loss of function of Antp or gain of function of Abd A: NBs do not enter quiescence at embryo-larva transition Loss of function Abd A: failure of larval abdominal NBs to apoptose	Bello et al., 2003; Tsuji et al., 2008

Genes regulating asymmetric cell division, temporal identity, NB growth and cell cycle entry or exit, and their mutant phenotypes.
NB, neuroblast.

daughter cell during mitosis to restrict self-renewal capacity following asymmetric division in NBs.

Insights into lineage progression

Mammalian stem cell lineages generally contain transit amplifying populations that allow even small stem cell populations to create large numbers of differentiating daughter cells. This raises several questions that cannot be addressed in the type I NBs. How, for example, is directionality encoded in the lineage to ensure that transit amplifying cells never revert back to stem cells? What is different in transit amplifying cells that makes self-renewal possible but only for a limited number of cycles? The recent discovery of type II NBs and their transit amplifying INPs has allowed these important questions to be addressed in the *Drosophila* brain.

Type II NBs are characterized by the lack of expression of the transcription factor Asense and this is why they have also been called PAN (posterior Asense negative) NBs (Bowman et al., 2008). Like type I NBs, they express the transcription factor Deadpan (Dpn) and divide asymmetrically by segregating Numb and Brat. Pros, however, is not present and this explains why type II lineages are more susceptible to tumor formation when asymmetric cell division is compromised (see also below). Upon division, type II NBs generate an immature INP, which is still Pros and Ase negative but also lacks Dpn, a marker and key regulator of all self-renewing *Drosophila* neural precursors (Wallace et al., 2000). Immature INPs do not divide but pass through a 4- to 6-hour period of maturation, after which they start re-expressing NB markers and are then called mature INPs (Bayraktar et al., 2010). They first turn on Ase followed by Dpn and Pros, and subsequently start dividing asymmetrically (Bayraktar et al., 2010). Like type I NBs, mature INPs give rise to a GMC, which generates two neurons in a symmetric division (Fig. 2B). The recent identification of several type II-specific lineage regulators has started to shed light on how this complex but stereotyped series of events is regulated.

Mature INPs, but not type I or type II NBs, express Earmuff (Erm), the *Drosophila* homolog of the Zn-finger transcription factors Fez and Fezl (Weng et al., 2010). In *erm* mutants, the maturation of INPs and even the first rounds of asymmetric INP division are normal. However, INPs eventually start reverting into type II NBs and this leads to an amplification of lineages and tumor-like overproliferation. As this de-differentiation can be suppressed by inhibiting Notch, Erm is thought to antagonize the Notch/Delta pathway in type II NB lineages. Erm may do this by inducing Pros expression in INPs, as Pros overexpression in INPs suppresses the *erm* phenotype and Erm overexpression induces accumulation of nuclear Pros in INPs, leading to their terminal differentiation. Therefore, Erm seems to stabilize the transit amplifying state by limiting proliferation of INPs and eventually promoting their terminal differentiation (Weng et al., 2010).

The Ets domain transcription factor Pointed seems to act further upstream in the type II lineage. Pointed is required for specifying the type II NB fate, presumably because one of its isoforms, PntP1, suppresses Ase expression in these cells (Zhu et al., 2011). However, Pointed must have other targets, as its ectopic expression can convert type I NBs into type II-like NBs and induce the formation of INPs, but this cannot be achieved by the sole knock down of Asense.

Another difference between type I and type II NBs relates to the Notch signaling pathway where Notch is known to regulate NB self-renewal (Wang et al., 2006). It is thought that the reduction of Notch activity induced by Numb in one of the two daughter cells is key for driving this daughter cell to differentiate. In addition, Notch enhances cellular re-growth after division in both type I and type II NBs (San-Juán and Baonza, 2011; Song and Lu, 2011). These Notch functions seem to be more important in type II lineages, as knock down of Notch in the brain reduces overall NB numbers (Wang et al., 2006), but causes a complete loss of all type II lineages (Bowman et al., 2008). Furthermore, overexpression of

the Notch targets Dpn or HLHmγ can transform INPs into NB-like cells, causing uncontrolled overproliferation and the formation of a tumor (San-Juán and Baonza, 2011; Zacharioudaki et al., 2012).

Although we have started to identify the first regulators of type II lineages, we are still a long way from a full understanding of lineage progression. A recent genome-wide RNAi screen (Neumüller et al., 2011) identified several additional type II-specific NB regulators. Among these are several regulators of chromatin states and their characterization might provide new insights into stem cell lineage control.

Defective neuroblast division: a model for tumor formation

Defects in NB asymmetric cell division can cause tumor formation in the *Drosophila* brain (Caussinus and Gonzalez, 2005). In fact, some of the key regulators of asymmetric cell division were first identified in genetic screens for tumor suppressor genes (Gateff and Schneiderman, 1974; Gateff, 1978; Gateff, 1994; De Lorenzo et al., 1999). It is thought that the inability of GMCs or INPs to undergo terminal differentiation results in their de-differentiation into additional NBs. These defects can also cause an exponential expansion of the stem cell pool and can ultimately lead to the formation of a tumor.

Tumor formation from stem cells is highly relevant for mammalian cancer research. It has long been hypothesized that defects in differentiation, rather than an impairment of proliferation control, can be the root cause of tumor formation (Harris, 2004). Moreover, tumors are heterogeneous and recent transplantation experiments have demonstrated that cells with stem cell properties are more potent in regenerating the entire tumor mass upon transplantation (Magee et al., 2012). In fact, tumors could even arise from an original defect in a small fraction of stem cells that would give rise to all the other cells present in the tumor (Reya et al., 2001). Although this hypothesis is still controversial, experiments in *Drosophila* can provide clear answers to questions regarding the role of stem cells in tumor formation and maintenance, which then may or may not apply to human cancers.

Several observations suggest that brain tumor formation in *Drosophila* involves more than just an amplification of NBs at the expense of neurons. First, several genes required for lineage cell fate commitment, but not for asymmetric cell division per se, can also cause NB tumors (Weng et al., 2010; Zhu et al., 2011) (see Table 1 for individual functions and phenotypes). Second, wild-type NBs exit proliferation during pupal stages, whereas tumors can survive into adulthood (Loop et al., 2004). Finally, tumors survive and continue to proliferate even after several cycles of transplantation into other adult hosts (Caussinus and Gonzalez, 2005). More likely, therefore, tumor formation involves the formation of a cell type that normally does not exist yet retains stem cell characteristics but no longer responds to proliferation control signals.

The detailed study of the tumor suppressor Brat has provided some information on how these unusual tumor cell types could form. In *brat* mutants, the type II NB still generates an INP that fails to commit to its fate and reverts back to a NB. The mutant immature INP first goes through a long delay in the cell cycle, leading to an initial underproliferation phenotype (Fig. 4). At some point, however, this cell cycle block is overcome and the cells start proliferating rapidly and indefinitely. Whether epigenetic modifications or the generation of DNA mutations are responsible for this tumor-initiating event is one of the most exciting issues in *Drosophila* tumor research. Furthermore, aneuploidy is observed upon tumor transplantation but has not been described in primary tumors (Caussinus and Gonzalez, 2005). Consistently, inducing

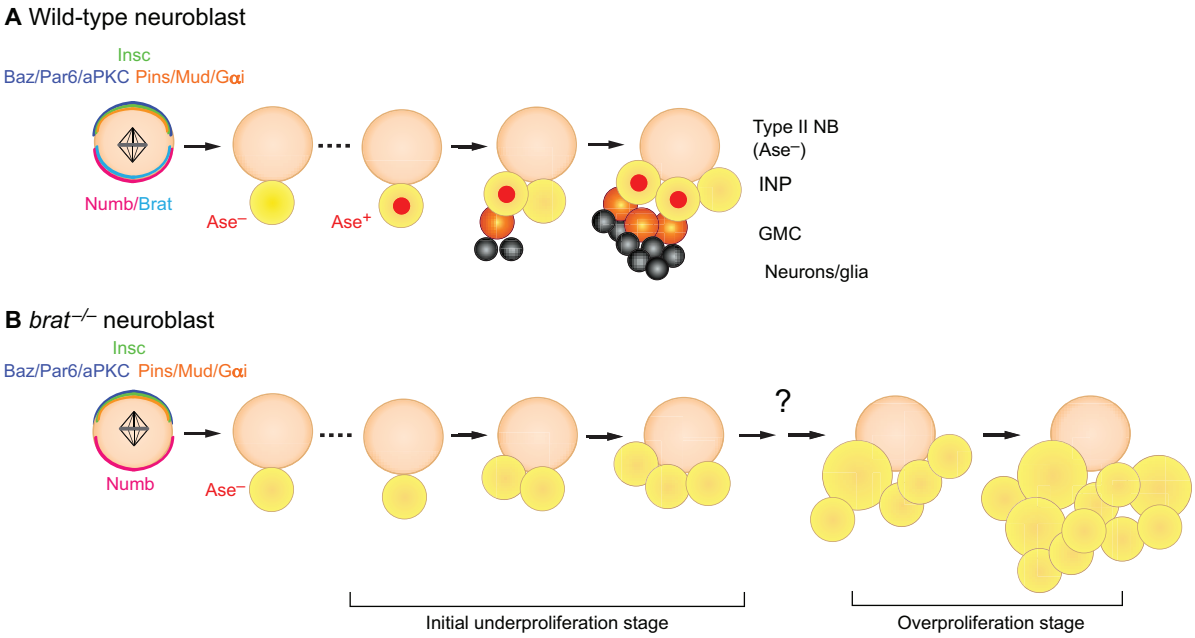

Fig. 4. Stages of tumorigenesis in the *Drosophila* brain. (**A**) Wild-type central brain type II neuroblasts (NBs) divide asymmetrically, segregating the cell fate determinants Numb (pink) and Brat (light blue) into the differentiating daughter cell. Type II NBs divide to self-renew and generate an immature, Asense-negative (Ase$^-$), intermediate progenitor (INP; yellow). After maturation, INPs start expressing the transcription factor Ase (red; Ase$^+$) and divide to generate ganglion mother cells (GMCs, orange). (**B**) In *brat* mutants, NBs divide asymmetrically and segregate the remaining cell fate determinant Numb (pink) into the immature INP. These mutant immature INPs do not mature or divide, explaining the initial underproliferation phase of tumor formation. This stage is followed by immature INP reversion to NB-like cells that start dividing indefinitely, causing a strong overproliferation defect.

genome instability in larval NBs does not result in the formation of transplantable tumors, suggesting that epigenetic modifications play a major role (Castellanos et al., 2008).

The observation that tumor NBs are different from normal NBs opens interesting avenues for potential tumor therapy. In fact, a recent study showed that NB tumors can be specifically suppressed. In NBs in which Notch is ectopically activated, neuronal differentiation does not proceed, leading to an increase in NB-like cells. Increased Notch signaling is accompanied by upregulation of elongation factor 4E (eIF4E) and Myc to promote cell growth. The overactivation of these growth pathways is specific for mutant NBs and inhibition of eIF4E suppresses and rescues the NB-like cell overproliferation phenotype without affecting wild-type NBs (Song and Lu, 2011). This effect can even be achieved by chemical inhibition of eIF4E using ribavirin, a substance that is in clinical trial for the treatment of acute myeloid leukemia (AML) in humans (Assouline et al., 2009).

Thus, *Drosophila* NBs allow us to study the early stages of tumor development that are much less accessible in vertebrate models. Although the evolutionary conservation of the resulting hypotheses needs to be tested in each case, the precise and reproducible answers that fruit flies provide may ultimately contribute to the development of early and effective treatments.

Insights into temporal identity

In *Drosophila* and vertebrates, the same neural progenitor can generate various neuronal subtypes over time. In NBs, this is mediated by a precise temporal cascade of transcriptional changes. In the embryonic stages of lineage development, NBs sequentially express a series of transcription factors: Hunchback (Hb), Seven-up (Svp), Kruppel (Kr), Pdm1/Pdm2 (Pdm) and Castor (Cas) (Fig. 5A). This series of transcription factors is not identical in all NBs and additional members can contribute to confer specific types of NBs with a temporal identity (Yang et al., 1997; Urbach and Technau, 2003; Karcavich, 2005). A network of feedback and feed-forward loops between the transcription factors controls their temporal changes in a cell-intrinsic manner (Grosskortenhaus et al., 2005). NB transcription factor expression is inherited by the daughter GMC and this, in turn, regulates the postmitotic expression pattern that specifies neuronal temporal cell fate (Kambadur et al., 1998; Isshiki et al., 2001; Novotny et al., 2002; Pearson and Doe, 2003; Kanai et al., 2005; Grosskortenhaus et al., 2006). However, what determines the switch between the different transcription factors is still unclear. A global timer that induces transitions simultaneously in all NBs has been excluded, as different NB types cycle at different developmental paces (Brody and Odenwald, 2000). Another hypothesis is that progression through this series is cell cycle dependent and happens after a specific number of NB divisions. In fact, the transition from Hb expression to Kr expression requires successful cell division; the switch does not happen when cell cycle arrest is induced and NBs are blocked as Hb-positive NBs (Isshiki et al., 2001; Grosskortenhaus et al., 2005). The subsequent transitions (Kr-Pdm-Cas), by contrast, are cell cycle independent and can occur even in isolated or G2-arrested NBs, supporting a mechanism that involves a neuroblast-intrinsic timer (Grosskortenhaus et al., 2005). Thus, the precise nature of the molecular mechanism that establishes the temporal identity of NBs is still unknown.

Another interesting issue is how the temporal identity of the daughter GMCs is determined by NB identity so that the right type of neuron is produced. Both Hb and Kr can regulate chromatin (Farkas et al., 2000), and could establish inheritable chromatin states such that only a subset of genes can be transcribed in GMCs and neurons. This hypothesis is supported by a recent study showing that Polycomb Repressor complexes (PRCs) restrict the window of competence for Kruppel NB identity (Touma et al., 2012). It is possible that an accumulation of chromatin changes progressively restricts the ability of NBs to generate specific neuronal subtypes.

It has recently been shown that the temporal transcription factor series progresses in postembryonic stages. VNC thoracic NBs express Cas at the end of the embryonic stages. When NB division

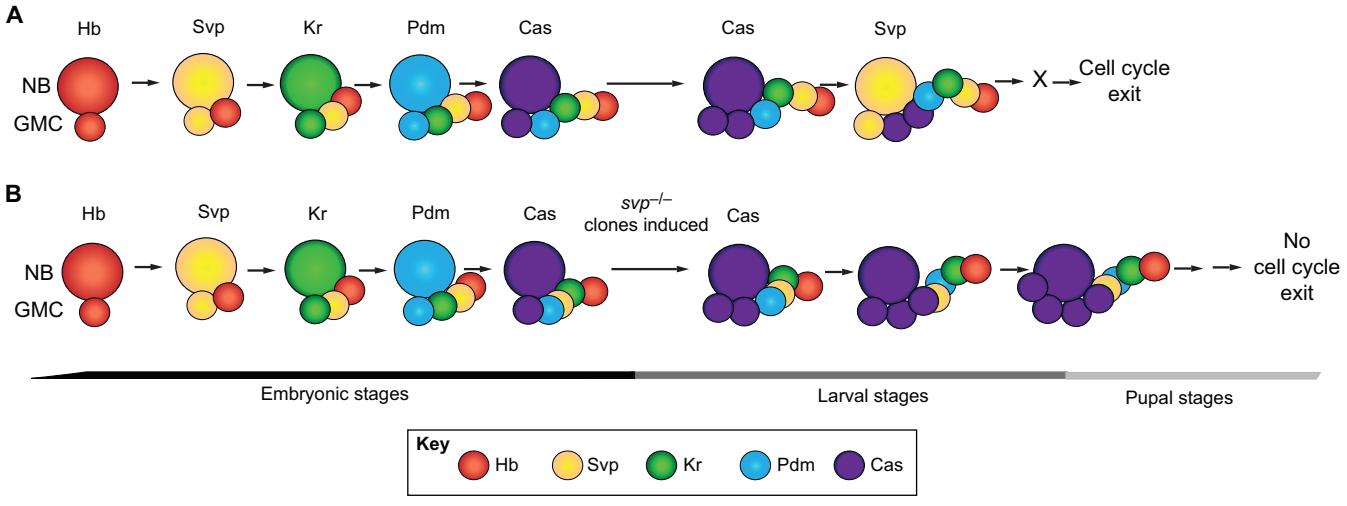

Fig. 5. The 'neuroblast clock': a series of transcription factors that regulate neuroblast temporal identity. (**A**) Embryonic neuroblasts (NBs) express a series of transcription factors that are inherited by the ganglion mother cell (GMC). They consecutively express Hunchback (Hb, red), Seven up (Svp, yellow), Kruppel (Kr, green), Pdm1/2 (Pdm, blue) and Castor (Cas, purple). At the end of the embryonic stages, Castor-positive NBs enter quiescence. When division resumes in the larval stages, ventral nerve cord thoracic NBs transition from Castor to Svp expression. Transcription factors expressed later in larval and pupal development have not yet been identified (X). (**B**) Larvae mutant for *svp* (*svp*$^{-/-}$ clones induced) do not switch off Castor expression and are stuck in a Castor-positive state. Such NBs do not exit the cell cycle at the appropriate time the during pupal stages.

restarts in larvae, NBs maintain Cas (Fig. 5A) and then switch to a second wave of Svp (Maurange et al., 2008). Thus, VNC thoracic NBs are capable of maintaining the expression of the last embryonic temporal transcription factor during embryo-larval quiescence and can then resume this series after exit from quiescence.

In larvae, the NB transcription factor series is paralleled by a neuronal identity series. Both Castor- and Svp-expressing NBs generate neurons positive for the transcription factor Chinmo. Later-born neurons express Broad Complex (Br-C) but the corresponding NB transcription factor is not yet identified. Thus, the NB clock must continue post Svp expression in larval NBs. As, however, only very few transcriptional states have been identified in larval NBs, it is likely that other mechanisms contribute to the vast number of different neurons generated during this stage. In addition, it is unclear whether the clock identified in the VNC also acts in the central brain. In addition, the existence of a transit amplifying population raises more questions. For example, does the INP inherit the identity of the NB? Are there two sets of temporal transcription factors series ticking at slightly different times in type II NBs and INPs?

Like other mammalian stem cells, NBs exit mitotic proliferation before adulthood (Fig. 1A; Fig. 5A). Interestingly, this happens at different times in distinct NB subtypes, suggesting that this is not a response to a global signal. Although central brain and VNC NBs cease dividing ~20-30 hours after pupa formation (APF), mushroom body NBs terminate division 85-90 hours APF (Ito and Hotta, 1992). Different mechanisms for cell cycle exit and NB death or differentiation have been identified for different NBs. Mushroom body NBs, which are the last ones to disappear, first decrease their proliferation in response to a decrease in insulin/PI3K signaling that occurs in the fasting pupal stages. This results in nuclear localization of the transcription factor Foxo (Forkhead box class O) followed by Reaper-dependent apoptosis and autophagy of these NBs (Siegrist et al., 2010). Abdominal VNC NBs undergo apoptosis in response to a pulse of the Hox gene *abdominal A* (White et al., 1994; Bello et al., 2003). Thoracic VNC NBs instead undergo a reduction in cell size until they are as small as a GMC. Pros then enters their nucleus and promotes terminal differentiation (Maurange et al., 2008). The correct number of progeny and timing for NB death/differentiation also seems to require the presence of the transcription factor Grainyhead (Grh). Grh is expressed in both embryonic and postembryonic NBs, and *grh* mutant NBs do not produce the correct number of progeny (Almeida and Bray, 2005; Cenci and Gould, 2005). Grh does not interfere directly with the temporal transcription factor series but rather is necessary to give NBs competence to respond to the downstream effectors of this series.

Although the precise mechanism that determines the timing of NB exit from proliferation is still unknown, there is some evidence that the temporal transcription factor series is involved. In *svp* mutants, in which the temporal series is interrupted, NBs do not exit the cell cycle and continue dividing until adult stages (Fig. 5B) (Maurange et al., 2008). This supports the notion that NBs have to be in a specific stage of the temporal series to be able to respond to the external signals that trigger cell cycle exit, death or differentiation.

Linking nutritional status to growth control

Although NB asymmetric division is regulated intrinsically, the rate of NB proliferation has to be coordinated with the developmental stages of the animal. Recent experiments have revealed the molecular mechanisms for this and have shown how NB proliferation is coordinated with nutritional status. In mammals, the rate of stem cell proliferation also varies in response to injury, hormonal signals and nutrition. Nutrition is a key regulator of tissue growth and the brain is particularly sensitive to changes in nutritional status. Diet affects neurogenesis in mice and dietary restriction has been shown to lead to increased neurogenesis (reviewed by Randhawa and Cohen, 2005). In humans, metabolic diseases such as diabetes have been shown to lead to cognitive impairment (reviewed by Szémán et al., 2012), which is hypothesized to be linked with reduced neurogenesis.

As discussed above, NBs enter quiescence at the end of embryogenesis (Fig. 1A; Fig. 6A). During the larval stages, food intake then activates the insulin receptor (InR) and target of rapamycin (TOR) pathways in dormant NBs (Chell and Brand, 2010; Sousa-Nunes et al., 2011), and triggers exit from quiescence, an increase in NB size and entry into the cell cycle. The insulin pathway is the main systemic sensor of nutrition. Downstream of insulin/InR, phosphatidylinositol 3-kinase (PI3K) and AKT are activated. At the cellular level, TOR senses cellular levels of amino acids and energy, and regulates the rate of growth by adjusting the cellular protein biosynthetic capacity (Russell et al., 2011). TOR can also be activated by PI3K/AKT, providing a convergence point between systemic and nutritional status (Fig. 6C).

In conditions of nutrient availability, increased concentrations of circulating amino acids activate growth of the fat body, which is an adipose hepatic-like tissue. Through Slimfast (Slif), an amino acid transporter, increased amounts of amino acids are detected and TOR is activated in the fat body (Fig. 6B). After TOR activation, the fat body releases a yet to be identified signal (termed the fat body derived signal; FDS) that activates the PI3K and TOR pathway in glial cells. These larval glial cells release insulin like peptides (ILPs) that act on quiescent NBs (for more information on glia functions, see Box 1). In this way, glial cells act as a niche for NBs, translating information regarding the nutritional status of the whole organism to NBs. The ILPs released from glial cells bind to the InR in NBs, leading to downstream activation of PI3K/AKT pathways. At the same time, circulating amino acids directly activate the TOR pathway in NBs. The InR and TOR pathways, in combination, stimulate translation and protein biosynthesis, and inhibit Foxo to stimulate growth and NB division (Fig. 6B,C) (for a review of growth regulation pathways, see Hietakangas and Cohen, 2009). Thus, the InR pathway acts at three distinct stages in the NB activation cascade.

Another level of NB regulation is provided by ecdysone, the major coordinator of all major metamorphic changes in *Drosophila*. Ecdysone is a steroid hormone that is secreted by the prothoracic gland. Its synthesis is tightly linked to the nutritional state of the animal and if the animal is undergrown and requires more time to reach a specific size, ecdysone synthesis is delayed (Layalle et al., 2008). It has recently been shown that a novel insulin like peptide ILP8 coordinates growth with developmental timing by regulating ecdysone biosynthesis (Colombani et al., 2012; Garelli et al., 2012). Ecdysone is also able to feed back on the insulin pathway by an unknown mechanism (Rusten et al., 2004; Colombani et al., 2005).

Although nutritional status affects most organs in the animal, the brain is normally spared from nutritional deprivation by an unclear mechanism. Nutritional restriction during human intrauterine growth, for example, results in small babies that have proportionally large heads (Gruenwald, 1963). In *Drosophila*, this brain-sparing phenomenon is surprisingly conserved, but is active only in late

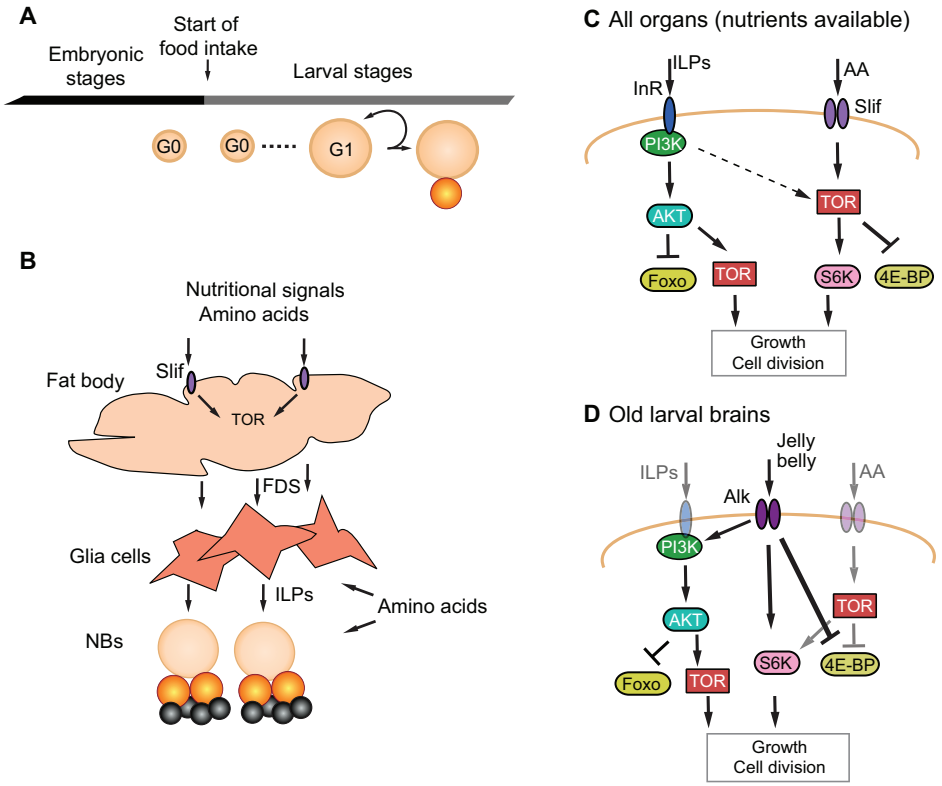

Fig. 6. Neuroblast growth control by nutritional signals. (**A**) Time line of reactivation of quiescent neuroblasts (NBs). When larvae hatch, NBs are in a G0-like quiescence state. Food intake in larval stages then induces NB growth, transition from G0 to G1, followed by cell division. (**B**) Larval feeding leads to an increase in insulin signaling and the concentration of circulating amino acids. Through the transporter Slimfast (Slif, purple), amino acids are detected by the fat body where the target of rapamycin (TOR) pathway is activated. TOR activation leads to production and secretion of an unknown fat body-derived signal (FDS). FDS activates the insulin pathway in glial cells, which in turn release *Drosophila* insulin-like peptides (ILPs), inducing NB growth and division. (**C**) Signaling pathways activated downstream of insulin and amino acids. ILPs bind the insulin receptor (InR, blue) and activate the PI3K/AKT pathway, which inhibits the growth inhibitor Foxo and activates TOR, leading to cell growth and division. Circulating amino acids are detected by Slif (purple), which also activates the TOR pathway. TOR activates S6K and inhibits 4E-BP, thus promoting protein translation, biosynthesis and ultimately cell growth and division. (**D**) The growth of older larval brains is independent of the nutritional status of the organism; NBs in older brains express the receptor Alk, which is activated by its ligand Jelly belly and directly modulates the TOR effectors S6K and 4E-BP, thus promoting cell growth and division. AA, amino acids.

larval stages. In young larval stages, the *Drosophila* CNS is very sensitive to the nutritional status of the organism. It depends on levels of insulin and amino acids for reactivating NB division after embryo-larva quiescence (Fig. 6C). It has long been known that starved flies grow smaller than normal. However, a recent study demonstrated that most fly organs grow at lower levels in poor nutrition conditions, whereas the brain continues to grow at a normal pace (Cheng et al., 2011). This brain sparing in *Drosophila* is mediated by anaplastic lymphoma kinase (Alk), a receptor tyrosine kinase that is strongly expressed in the developing CNS (Lorén et al., 2001). Alk is activated by its ligand Jelly belly, which is constitutively expressed by glial cells in a nutrient-independent manner (Cheng et al., 2011). In older brains, Alk kinase substitutes for InR and uncouples NBs from organismal growth control by inducing phosphorylation of the effector targets of TOR and the InR (Fig. 6C,D). In doing so, the InR and TOR pathways are bypassed, and the cell is no longer sensitive to the nutritional status of the whole animal. As this mechanism is obviously not as active in younger brains, where InR activity is crucial for NB exit from quiescence, it would be interesting to study how Alk is differentially regulated throughout larval development.

Parallels to mammalian neural stem cells

Although the mammalian brain is much larger than the *Drosophila* brain, many basic aspects of fly brain development are surprisingly conserved in mammals. As in flies, all neurons in the mouse cortex arise from asymmetric cell divisions of a small set of progenitor cells that generate several neuronal subtypes in a spatially and temporal controlled manner. Six individual layers can be identified in the adult mouse neocortex. Early during mouse brain development, at embryonic day 9 (E9.0), the cortex consists of neuroepithelial progenitors, which extend from the apical ventricular surface to the basal surface of the neural tube. Before these neuroepithelial cells divide, their nuclei undergo interkinetic nuclear migration and move apically to undergo mitosis at the apical-most position. Early divisions are symmetric and result in expansion of the progenitor pool. When neurogenesis starts, at around E11.0, neuroepithelial progenitors start expressing characteristic features of glial cells (Mori et al., 2005) and turn into the so-called radial glial (RG) cells. RG cells also extend apical and basal processes, and are restricted to the most apical area of the cortex: the ventricular zone (VZ) (Fig. 7A). They continue interkinetic nuclear migration and divide

Box 1. Glial cells in the *Drosophila* brain and their function

Glial cells are the second most abundant cell type in the nervous system. The Greek name 'glia' means 'glue', reflecting the supporting role that glial cells have in the brain. As in the mammalian brain, glia play multiple roles during *Drosophila* brain development, function and regeneration (Freeman and Doherty, 2006). They ensheathe neurons, provide axonal guidance and are necessary for axonal fasciculation and for neuronal survival (for a review, see Hartenstein et al., 1998; Hartenstein, 2011). In addition, through the secretion of molecules such as insulin like peptides (ILPs) or anachronism, glial cells regulate postembryonic neuroblast (NB) proliferation (Ebens et al., 1993; Sousa-Nunes et al., 2011). Glial cells are first formed in the *Drosophila* embryo. Lateral glia, the most abundant type of embryonic glia, are generated by glioblasts and neuro-glioblasts (reviewed by Hartenstein, 2011). Like NBs, glioblasts and neuro-glioblasts also delaminate from the embryonic neuroectoderm. Glioblasts generate only glial cells, whereas neuro-glioblasts are capable of generating both glia and neurons. The earliest event in the determination of lateral glial cell fate is the expression of glial cells missing (Gcm) (Hosoya et al., 1995; Jones et al., 1995). Gcm activates downstream transcription factors, such as reverse polarity (Repo) and Pointed, that are required for terminal glia differentiation (Klaes et al., 1994; Xiong et al., 1994). Simultaneously neuronal differentiation is repressed in glial cells by genes such as Tramtrack (Giesen et al., 1997), ensuring that these cells are committed to the glia fate. During larval stages, most glial cells divide, which increases their number, and a few neuro-glioblasts resume cell division to generate new glial cells. In contrast to NBs, glial cells continue to proliferate even in the adult brain, albeit at a low rate (reviewed by Hartenstein, 2011).

asymmetrically into one self-regenerating daughter cell and one cell that migrates into the more basally located cortical plate to differentiate into a neuron (Fig. 7A, in the process of direct neurogenesis). Alternatively, RG cells can generate one other RG and one intermediate progenitor cell (IPC) via a process known as indirect neurogenesis (Fig. 7B). IPCs reside in the cortical area between the VZ and intermediate zone (IZ), where they form the so-called subventricular zone (SVZ). They undergo at least one more symmetric division, which generates two terminally differentiating neurons. Indirect neurogenesis is thought to be the predominant mode of neurogenesis that occurs in the mouse cortex, at least during later stages, and resembles the mode of division used by *Drosophila* type I NBs.

Recent elegant live imaging studies have revealed another type of progenitor called an outer subventricular zone (OSVZ) progenitor or outer radial glial (oRG) cell (Fietz et al., 2010; Hansen et al., 2010; Wang et al., 2011b). These cells are located in the outer regions of the SVZ and arise from asymmetric divisions of RG cells. Although they lack a connection to the apical surface and no longer express apical plasma membrane markers, they contain a basal process, continue to express the RG markers Pax6 and Sox2, and continue to self-renew (Fig. 7C) (Fietz et al., 2010). In humans, most cortical neurons are actually thought to arise from oRG cells, whereas in the mouse they contribute only to a smaller fraction. These oRG lineages closely resemble type II lineages in *Drosophila*, and, therefore, both the type I and type II *Drosophila* NB lineages seem to be recapitulated in the mammalian brain.

Although most RG cell divisions occur parallel to the ventricular surface, some progenitors divide with an oblique orientation. As in *Drosophila* NBs, the polarity and spindle orientation machinery are conserved in the mammalian brain. Mammalian Par3, Par-6 (Pard6a – Mouse Genome Informatics) and aPKC are important for both apical-basal polarity and for spindle orientation (Suzuki and Ohno, 2006). Pins has two mammalian homologs, Ags3 and Lgn (G-protein regulator; Gpsm1 and Gpsm2 – Mouse Genome Informatics) (Yu et al., 2003; Sanada and Tsai, 2005). *Ags3*-null mice show no defects in brain morphology or function (Blumer et al., 2008). By contrast, knocking out *Lgn* randomizes the orientation of normally planar neuroepithelial divisions, consistent with it having a role in mitotic spindle orientation in the developing brain (Morin et al., 2007; Konno et al., 2008). The mammalian Mud homolog NuMA (a nuclear protein that associates with the mitotic apparatus; Numa1 – Mouse Genome Informatics) has a role in the establishment and maintenance of spindle poles (Sun and Schatten, 2006; Silk et al., 2009). Finally, the single vertebrate homolog of *Drosophila* Inscuteable is required and sufficient for inducing non-planar spindle orientation (Zigman et al., 2005; Konno et al., 2008; Postiglione et al., 2011). Thus, conserved molecular machinery regulates the orientation of progenitor divisions. How this influences cell fate in the daughter cells, however, is much less clear in vertebrates. Although the Notch pathway also plays an important role, its precise regulation in the mammalian brain may be very different from that occurring in flies (Pierfelice et al., 2011; Dong et al., 2012).

Unlike in *Drosophila*, in mammals, RG divisions can be asymmetric even when the mitotic spindle is parallel to the apical surface and the two daughter cells are of equal size (Siller and Doe, 2009). The asymmetry may result from unequal inheritance of the basal process (Konno et al., 2008), a structure that does not exist in flies. Nevertheless, the orientation of these divisions can influence the fate of the daughter cells. Upon deletion of mouse Insc, non-planar divisions are reduced and this results in a shift from indirect to direct neurogenesis. Overexpression of mouse Insc, by contrast, has the opposite effect and increases the number of IPCs (Postiglione et al., 2011). Strikingly, mouse Insc overexpression or expression of a dominant-negative version of Lgn also increases the number of oRG cells, indicating that non-planar divisions are more likely to generate the non-apical progenitor cells (Konno et al., 2008; Postiglione et al., 2011; Shitamukai et al., 2011; Wang et al., 2011a). As the number of oRG cells is dramatically increased in primates, this opens up exciting evolutionary perspectives for the spindle orientation field.

As in flies, a single progenitor generates different types of neurons throughout mammalian brain development. As this order can be recapitulated in cell culture (Gaspard et al., 2008), a transcription factor cascade similar to that observed in flies can well be envisaged. In fact, some evidence indicates that this cascade might function very similarly to the fly transcription factor cascade. Early progenitor cells in the ventricular zone produce deep layer neurons that express transcription factors, including SRY (sex determining region Y) box 5 (Sox5), Fez family zinc finger 2 (Fezf2) and chicken ovalbumin upstream promoter transcription factor-interacting protein 2 (Ctip2), which are required for their correct specification. Upper layer neurons, instead, are produced from progenitors in the subventricular zone and require SATB homeobox 2 (Satb2). The Fezf2/Ctip2 and Satb2 pathways appear to be mutually repressive, as Satb2 represses Ctip2 expression by recruiting histone deacetylases to the Ctip2 locus (Alcamo et al., 2008). Thus, Satb2 induces long-term epigenetic changes in chromatin configuration, which may enable cell fate decisions to be maintained during development. Conceptually, therefore, Stab2 and Ctip2 resemble *Drosophila* Chinmo and Br-C, which confer identity to postmitotic neurons. In addition, the Svp mouse

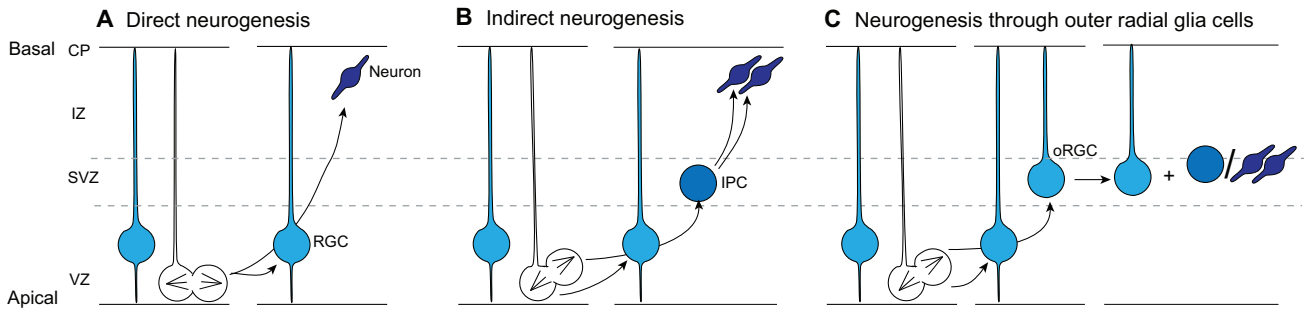

Fig. 7. Development of the mouse neocortex. (**A**) In direct neurogenesis, radial glia cells (RGCs, light blue) divide asymmetrically to self-renew and to generate a differentiated neuron (dark blue) that migrates to the basal cortical plate (CP). (**B**) RGCs can also divide obliquely to the ventricular surface to self-renew and generate an intermediate precursor cell (IPC) that resides in the subventricular zone (SVZ). IPCs then divide once more to produce two neurons. This mode of division is called indirect neurogenesis. (**C**) Neurogenesis can also occur through generation of outer radial glial cells (oRGCs). RGCs divide oblique to the ventricular surface and generate another RGC and one oRGC that localizes to the more basal region of the SVZ. oRGCs then divide to self-renew and generate an IPC or two neurons. IZ, intermediate zone; VZ, ventricular zone.

homologs COUP-TFI and COUP-TFII (chicken ovalbumin upstream promoter transcription factors I and II; Nr2f1 and Nr2f2 – Mouse Genome Informatics) have been shown to participate in the neuron/glia switch (Naka et al., 2008). COUP-TFI and COUP-TFII are transiently co-expressed in early neural progenitors in the ventricular zone of the early embryonic CNS before the switch to gliogenesis. The double knock down of COUP-TFI and COUP-TFII in embryonic stem cell-derived neural progenitors prolongs neurogenesis and the generation of early born neurons. These experiments suggest that COUP-TFI, COUP-TFII and Svp play evolutionarily related roles in neuronal temporal specification. Thus, specification of temporal identity may be conserved between flies and vertebrates, although the enormous number of cortical neurons present in vertebrates requires more complex regulatory mechanisms.

Thus, the basic processes that contribute to neurogenesis and neural progenitor regulation are conserved in a surprising manner. Conceptually, the relatively simple and clear insights obtained in *Drosophila* can help to uncover the more complex regulatory networks in vertebrates. Moving our knowledge from flies to mice, and ultimately to humans, certainly represents the greatest challenge in this field.

Conclusions

The analysis of *Drosophila* NBs has provided great insight into the mechanisms that allow cells to divide asymmetrically. More recently, tumorigenesis, growth control and temporal identity have been added to the biological processes that can be studied in this relatively simple model system. Among the greatest challenges in this field is the matter of how NBs become tumorigenic. Why do neural stem cells lose growth control when asymmetric cell division is impaired? What are the transcriptional circuits that allow a NB to undergo multiple rounds of self-renewal? How are those circuits modified in GMCs and INPs to allow progressive terminal differentiation? And why does this modification not happen in tumor mutants, such as *brat*? Do DNA mutations contribute, at the early stages or during metastasis?

The discovery of the transcriptional NB clock has raised another set of important questions. What regulates the precise temporal order of transcription factor expression? Much effort is being made to map the complete circuits for individual *Drosophila* behaviors. How is the information for generating those circuits encoded in the temporal identity program for neurons arising from individual NBs? In mice, functionally related neurons often arise from common lineages (Yu et al., 2009; Brown et al., 2011). Do similar relationships exist in flies and, if so, does a NB already contain information about the future behavioral role of the neurons it will give rise to?

The recent years have seen enormous technological advances in fly genetics. Transgenic RNAi allows almost any gene to be knocked out in a cell type-specific manner (Dietzl et al., 2007). This is now complemented by the creation of enhancer libraries that allow those RNAi lines to be expressed in almost any specific cell type (Pfeiffer et al., 2012). Hopefully, the speed at which results can now be obtained in flies will enhance their importance for stem cell and cancer biology even further. Given the enormous functional conservation in the developing brain, this is very likely to occur.

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Competing interests statement

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