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Neural stem cells: balancing self-renewal with differentiation

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Stem cells are captivating because they have the potential to make multiple cell types yet maintain their undifferentiated state. Recent studies of *Drosophila* and mammalian neural stem cells have shed light on how stem cells regulate self-renewal versus differentiation and have revealed the proteins, processes and pathways that all converge to regulate neural progenitor self-renewal. If we can better understand how stem cells balance self-renewal versus differentiation, we will significantly advance our knowledge of embryogenesis, cancer biology and brain evolution, as well as the use of stem cells for therapeutic purposes.

Introduction

A defining feature of stem cells is their ability to continuously maintain a stem cell population (self-renew) while generating differentiated progeny. Thus, stem cells are faced with a uniquely difficult task: to avoid cell cycle exit and differentiation, and to avoid uncontrolled proliferation and tumor formation. How stem cells walk this developmental tightrope is an extremely interesting question that is of relevance to our understanding of the processes of cell differentiation and cancer, and of the developmental diseases that result from the premature loss of stem cell pools.

Here I review recent insights from studies of neural stem cells (NSCs) in *Drosophila* and mice. There are surprising similarities in the transcription factor profiles of NSCs in flies and mice, although many have not been functionally tested in both organisms. Both fly and mammalian NSCs have unique cellular contacts, but the role of these contacts (their 'niche') has only recently begun to be explored. Much more progress has been made on the role of cell polarity proteins in regulating self-renewal in *Drosophila* neuroblasts, and their conservation in mammalian cortical stem cells should lead to rapid progress in this system. Finally, I discuss the role of spindle orientation in regulating NSC self-renewal; recent identification of mutants that disrupt spindle orientation without affecting cell polarity in both flies and vertebrates now permits, for the first time, time-lapse imaging studies to correlate spindle orientation, cell polarity components and sibling cell fate. The goal of this review is to summarize recent research, to untangle conflicting results and to highlight areas for future exploration.

Neurogenesis in *Drosophila* and mammals

During *Drosophila* neurogenesis, neuroepithelial cells differentiate into neuroblasts (NBs), which divide to form a NB and a ganglion mother cell (GMC). GMCs are intermediate progenitors that have a limited mitotic potential and typically divide just once to generate a pair of postmitotic neurons (as summarized in Fig. 1A). Embryonic neuroepithelial cells are bi-potent cells that can form either NBs (stem-cell-like neural progenitors) or epidermis. This choice is determined by the level of proneural gene expression. High levels of the proneural genes *achaete*, *scute* or *lethal of scute* repress Notch activity and promote NB formation; low levels of proneural gene expression allow high Notch activity, which maintains neuroectodermal fate and ultimately leads to epidermal differentiation (Artavanis-Tsakonas et al., 1991). Thus, proneural genes promote neurogenesis (i.e. NB formation), whereas Notch signaling inhibits neurogenesis. In this review, I briefly discuss embryonic NBs and focus instead on the central brain NBs, where most is known about the mechanisms that regulate self-renewal.

Larval NBs, which have many attributes of self-renewing stem cells, lie in a specialized cellular niche; they are undifferentiated, do not express any known neuron- or glial-specific markers; are highly proliferative yet never form tumors; can undergo mitotic quiescence without differentiating; and, most importantly, can generate hundreds of neuronal progeny without losing their position, size, identity or mitotic potential. These features make larval NBs an ideal system in which to study the basic biology of stem cell self-renewal (see Box 1 for NB-based self-renewal assays). However, there is a potential limitation of larval NBs as a stem cell model: as they divide, they might gradually lose the ability to make early-born cell types within their lineage (termed a 'progressive restriction in competence'), similar to the situation for embryonic NBs (Isshiki et al., 2001; Pearson and Doe, 2003). If true, it would mean that the NB is not precisely self-renewing with every division. Nevertheless, mammalian NSCs of the cortex and retina also undergo progressive restriction (Desai and McConnell, 2000; Livesey and Cepko, 2001), and the study of *Drosophila* NBs might help us understand this process.

In the mammalian embryonic CNS, particularly in the ventral telencephalon during mid-neurogenesis and, to a lesser extent, in the dorsal telencephalon, neuroepithelial cells give rise to radial glia, which differentiate into basal progenitors that each form two postmitotic neurons (see Fig. 1B). Both radial glia and neuroectodermal cells can directly generate neurons (Gotz and Huttner, 2005), and both neuroepithelial cells and radial glia can self-renew while producing basal progenitors, neurons or glia. These self-renewing cell types share a similar epithelial morphology (they span the neuroepithelium), both express the intermediate filament Nestin and have an apically located mitotic spindle, and both can be distinguished by an array of molecular markers (Gotz and Huttner, 2005). By contrast, most basal progenitors lack self-renewal potential and typically generate two postmitotic neurons (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2001). They do not span the neuroepithelium and undergo mitosis in a basal region termed the subventricular zone (SVZ) (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2001; Noctor et al., 2004). Thus, only neuroectodermal and radial glial cells can self-renew, and as such are a focus of this review. [Excellent reviews have recently been published on neural progenitors of the mammalian spinal cord, retina, adult hippocampus and dentate gyrus (see Chapouton et al., 2007; Gould, 2007; Ninkovic and Gotz, 2007; Sutter et al., 2007).]

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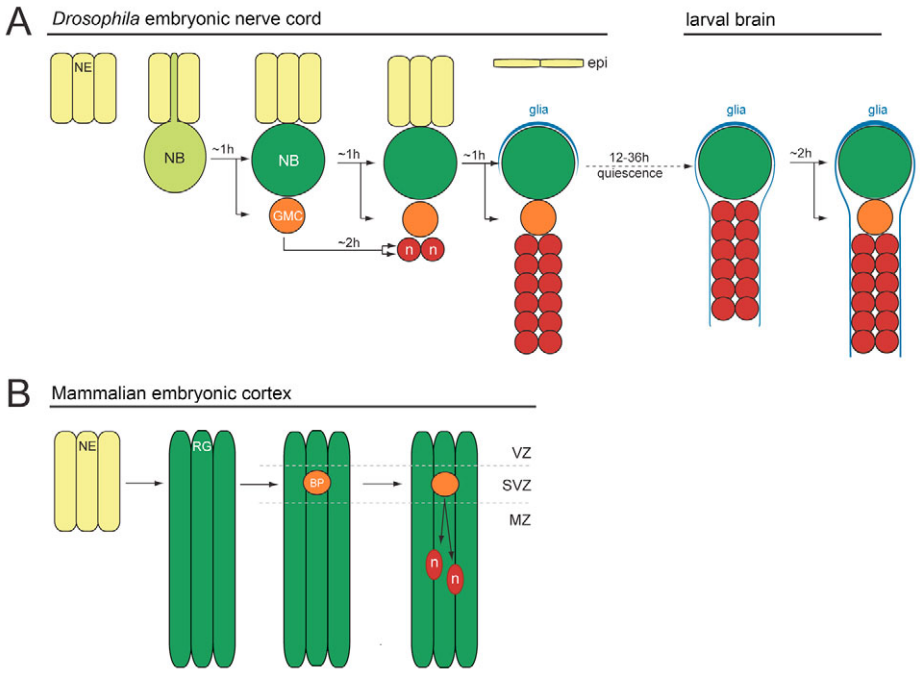

Fig. 1. Neural stem cell formation and neuronal differentiation. (**A**) *Drosophila* neurogenesis. Neuroectodermal cells (NE; yellow, the apical surface is uppermost) give rise to neuroblasts (NBs; green) by delamination, and each NB divides in a stem cell mode to bud off a chain of ganglion mother cells (GMCs; orange) from its basal surface. GMCs are intermediate precursors that typically generate two postmitotic neurons (n; red). Larval NBs are closely associated with glia (blue). Thoracic and brain NBs become mitotically quiescent in late embryos and resume proliferation during larval stages. Approximate cell cycle times or quiescence times are given in hours (h). epi, embryonic epidermis. (**B**) Mammalian embryonic cortical neurogenesis. Initially the cortex has only neuroepithelial cells (NE; yellow, the apical surface is uppermost), which mature into radial glia (RG; green). Radial glia and neuroectodermal cells generate basal progenitors (BP; orange), which are intermediate progenitors that generate a limited number of neurons (n; red). NE and RG can also generate neurons. VZ, ventricular zone, adjacent to the lumen; SVZ, subventricular zone; MZ, marginal zone.

The neural stem cell niche

Both *Drosophila* NBs and vertebrate NSCs lie in a unique cellular microenvironment compared with their differentiating progeny. Here I discuss the evidence for the role of niche-derived cues in regulating stem cell proliferation and self-renewal.

The *Drosophila* NSC niche

Larval NBs contact cortex glial cells on their apical and lateral sides (Dumstrei et al., 2003), while the basal side forms E-cadherin-rich contacts with new-born GMCs (Fig. 2A). Larval glia secrete the Anachronism (Ana) protein, which keeps NBs quiescent during early larval stages (Ebens et al., 1993). The possibility that glial-derived signals also promote larval NB proliferation is supported by the glial-specific expression of a dominant-negative E-cadherin protein, which results in fewer proliferating NBs (Dumstrei et al., 2003). Although the cellular basis for this phenotype is unknown, it is consistent with the loss of a glial-neuroblast contact and failure to transmit a glial-derived proliferation cue. Alternatively, non-specific effects, such as loss of the glial brain/hemolymph barrier, could generate this phenotype.

Is there any evidence that the Transforming growth factor β (TGFβ), Activin, Notch, Wnt, Hedgehog (Hh) or Fibroblast growth factor (FGF) signaling pathways have a role in maintaining *Drosophila* NB self-renewal or proliferation, as described below for mammalian NSCs? Recent work suggests that Activin, Hh and FGF promote NB proliferation, whereas Notch signaling promotes NB self-renewal; the role of the Wnt pathway has not yet been addressed. Activin and the redundant Activin-related Daw ligands are expressed in larval brain glia. NBs that lack the Activin receptor contain fewer cells per clone but maintain the NB (Zhu et al., 2008). Thus, Activin signaling regulates NB proliferation or neuronal survival, but not NB self-renewal. Similarly, decreased expression of the FGF receptor (Branchless), Hh, or the Hh- and FGF-binding protein Perlecan (Trol – FlyBase), reduces the number of proliferating NBs. Adding exogenous human FGF2 or increasing Cyclin E levels after this phenotype has become apparent rescues and returns to normal the number of proliferating NBs (Park et al., 2003), indicating that the affected NBs were mitotically quiescent rather than dead or differentiated. Thus, the mitogens FGF and Hh are necessary for maintaining NB proliferation but not for NB self-renewal or survival.

Finally, there is evidence that Notch signaling regulates NB self-renewal. Notch signaling is robust in larval NBs, based on the strong, specific expression of a Notch reporter gene (Almeida and Bray, 2005). Reducing Notch activity decreases central brain NB numbers (Wang et al., 2007), but has no effect on thoracic NB numbers (Almeida and Bray, 2005); conversely, increasing Notch activity by expressing a constitutively active Notch intracellular domain or by removing the Notch inhibitor Numb increases brain NB numbers (Lee et al., 2006a; Wang, H. et al., 2006). The identity and cellular source of the Notch ligand have not been determined, but this pathway is clearly implicated in supporting NB self-renewal, similar to its role in mammals (see below).

The mammalian NSC niche

Neuroepithelial and radial glial cells have a columnar epithelial morphology. Their apical process is exposed to the ventricular fluid, their basal (pial) process contacts the extracellular matrix (ECM), and they have lateral contacts with each other, including at the region

Box 1. Neural stem cell self-renewal assays in flies and mice

Neural stem cell (NSC) self-renewal assays in *Drosophila* include scoring the total number of NBs in the larval brain. Normally, ~200 central brain NBs exist per larval brain; mutants with increased NB self-renewal are expected to have increased NB numbers. Conversely, mutants in which NB self-renewal has failed should have fewer NBs.

Confirmation of a protein's role in NSC self-renewal in *Drosophila* is typically achieved by generating genetically marked single NB mutant clones. In wild-type flies, marked clones consist of a single NB and a family of marked GMC/neuronal progeny. If the mutated gene normally promotes differentiation, a homozygous mutant clone will generate multiple NBs. If the mutated gene normally promotes NB self-renewal, NBs will often be lost from the clone.

In mammals, self-renewal assays include a similar clonal analysis following the viral delivery of a marker gene (such as GFP) co-expressed with a gene-overexpression construct or a gene-knockdown construct [such as a short-hairpin RNA (shRNA)]. The in vitro 'neurosphere assay' is also widely used (Breunig et al., 2007), in which single cells derived from dissociated cortical tissue are cultured in vitro to determine the percentage that can generate a multi-lineage primary clone that contains single cells competent to generate a secondary multi-lineage neurosphere (reviewed by Jensen and Parmar, 2006).

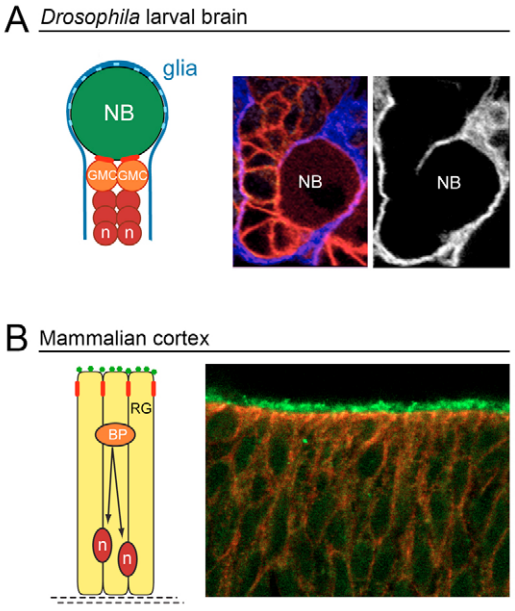

Fig. 2. Neural stem cell niche. (**A**) *Drosophila* larval neuroblast (NB) niche. (Left) Schematic showing NB/glial contact (light blue bars) and cadherin-rich NB/ganglion mother cell (GMC) contacts (red bars). (Right) Confocal image showing glial membrane staining relative to neuroblast and GMC membranes. Glial membrane was detected with repo-gal4 UAS-cd8:GFP (blue, or as a single channel in the right-hand image). NB, GMC and neuronal membranes were labeled with Scribbled (red). Image courtesy of Jason Q. Boone (University of Oregon). (**B**) Mammalian neuroepithelia/radial glia niche. (Left) Neuroepithelial/radial glia cells contact the ventricular fluid at their apical domain (green dots), neighboring cells via cadherin-rich adherens junctions (AJs; red bars), and the basal lamina at their basal domain (dashed lines). Basal progenitors (BP; orange) and neurons (n; red) lack access to apical and basal cues. (Right) E10 mouse neuroepithelial cells showing cadherin enrichment at the subapical AJs (red) and prominin 1 localization to the apical domain (green) that contacts the ventricular fluid [modified and reproduced with permission from Kosodo et al. (Kosodo et al., 2004)].

of subapical adherens junctions (Fig. 2B). Thus, cues from apical, basal or lateral directions could modulate neuroepithelial/radial glial self-renewal. Here I focus on the well-characterized roles of the Wnt, Notch and sonic hedgehog (Shh) pathways in regulating mammalian NSC self-renewal. Evidence for the role of the JAK/STAT, FGF, TGFβ and Toll-related pathways in regulating NSC proliferation and possibly self-renewal is summarized elsewhere (Rolls et al., 2007; Shi et al., 2008).

The canonical Wnt pathway promotes neuroepithelial/radial glial identity. The reduction of Wnt ligand levels or the removal of the canonical pathway component β-catenin results in fewer neuroepithelial/radial glial stem cells and in precocious neuronal differentiation (Machon et al., 2003; Zechner et al., 2003). By contrast, increased Wnt signaling expands the stem cell pool (Chenn and Walsh, 2002; Machon et al., 2007; Viti et al., 2003; Woodhead et al., 2006; Zechner et al., 2003). Wnt signaling also promotes NSC self-renewal during postnatal neurogenesis (Machon et al., 2007; Machon et al., 2003; Wexler et al., 2008; Zhou et al., 2004), where it can also promote the proliferation of committed neuronal progenitors (Lie et al., 2005). Wnt signaling directly activates cyclin D and the NSC factors Sox2 and Rest (RE1-silencing transcription factor) (Megason and McMahon, 2002; Nishihara et al., 2003; Takemoto et al., 2006), which may contribute to NSC maintenance. Later in cortical development, Wnt signaling is a potent inducer of neuronal differentiation, in part by activating the proneural gene neurogenin 1 (*Ngn1*; *Neurog1*) (Hirabayashi et al., 2004; Israsena et al., 2004; Muroyama et al., 2004; Viti et al., 2003). The difference in early versus late Wnt function is highlighted by the observation that the expression of stabilized β-catenin at embryonic day (E) 10 promotes neuroepithelial proliferation and self-renewal (Chenn and Walsh, 2002), whereas at E14 it promotes neuronal differentiation (Hirabayashi and Gotoh, 2005). It has been proposed that Wnt alone stimulates neuronal differentiation, whereas Wnt plus the mitogen Fgf2 inhibits neural differentiation (Israsena et al., 2004; Viti et al., 2003), although evidence against this model has also been presented (Hirabayashi et al., 2004). Thus, further study is needed to identify the context-dependent factors that switch Wnt signaling between promoting and inhibiting NSC self-renewal.

Notch signaling components are expressed in embryonic neuroepithelial/radial glial stem cells, as well as in adult NSCs (Mizutani et al., 2007; Stump et al., 2002). Mutations in the genes encoding Dll1 (a Notch ligand), Notch1 (a Notch receptor), RBPJk (Rbpj – Mouse Genome Informatics; a Notch transcriptional effector), Hes1, Hes3 or Hes5 (RBPJk-induced transcription factors) all lead to the depletion of radial glia stem cells and to precocious neuronal differentiation in the mouse embryo (de la Pompa et al., 1997; Handler et al., 2000; Hatakeyama et al., 2004; Mizutani et al., 2007; Yoshimatsu et al., 2006), and to NSC loss in the adult (Gaiano et al., 2000). Conversely, misexpression of Hes1, Hes3 or of activated Notch in the embryonic cortex blocks neuronal differentiation (Chambers et al., 2001; Ishibashi et al., 1994). Radial glia stem cells from *Dll1*, *Notch1*, *Rbpj*, *Hes1* and *Hes5* mouse mutants all have a reduced neurosphere-forming ability (see Box 1), indicating that they have a reduced ability to self-renew (Hitoshi et al., 2002; Ohtsuka et al., 2001; Yoon et al., 2004). Furthermore, radial glial cells that express a Notch-induced GFP reporter can be sorted by flow cytometry into Notch-high (GFP+) and Notch-low

(GFP–) populations; the Notch-high cells are more potent at generating primary and secondary neurospheres, and can be transplanted in vivo to generate all three neural lineages – neurons, astrocytes and oligodendrocytes (Mizutani et al., 2007). Thus, Notch signaling correlates with, and is required for, the maintenance of embryonic and postnatal NSCs.

The Shh pathway includes the Shh ligand, the transmembrane smoothened (Smo) protein, and the nuclear effectors Gli2/3, as well as many other proteins. Shh is expressed in the embryonic neuroepithelium (Lai et al., 2003), and in regions of adult neurogenesis – the hippocampus and dentate gyrus (Ahn and Joyner, 2005; Machold et al., 2003; Palma et al., 2005). When conditional Smo mutants are used to block Hh signaling in the postnatal hippocampus and dentate gyrus, these tissues produce fewer primary neurospheres when cultured in vitro (Machold et al., 2003). However, this effect could be due to a reduced stem cell population prior to explant; the ability to form multi-lineage secondary neurospheres was not assayed, which would have tested for stem cell self-renewal during neurosphere passage (see Box 1). Subsequent experiments showed that E18.5 cortical tissue from *Gli2* or *Gli3* mutant mice were deficient in both primary and secondary neurosphere formation, providing evidence that Shh promotes stem cell self-renewal (Palma and Ruiz i Altaba, 2004). Furthermore, there is compelling evidence that Shh both promotes proliferation and inhibits differentiation in postnatal cerebellar granule cell precursors (Argenti et al., 2005; Wechsler-Reya and Scott, 1999). Finally, it has recently been proposed that Shh might promote the transition of stem cells to more-rapidly dividing, committed progenitors (Agathocleous et al., 2007), rather than maintaining stem cell identity per se. Thus, the role of Shh in promoting NSC self-renewal needs further investigation.

Integrins are a family of cell-surface adhesion and signaling proteins that bind ECM proteins, such as laminin. β1-integrin (Itgβ1) is enriched at regions that contain embryonic and adult NSCs, and at the periphery of neurospheres where NSCs reside (Campos et al., 2004). When forebrain tissue from postnatal day 1 mutant mice that carry floxed *Itgb1* alleles is depleted of β1-integrin over a 10-day period, nestin^{+} stem cells from this tissue show a reduced neurosphere-forming ability and increased cell death (Leone et al., 2005), indicating that integrin signaling might also promote NSC survival.

Overall, findings to date show that in both mammals and flies, Notch signaling promotes NSC self-renewal. Wnt and Shh pathways might also regulate NSC self-renewal in mammals, but this role has yet to be tested in *Drosophila*. Less, however, is known about the cellular nature of the niche. In the mammalian cortex, it is not clear whether self-renewal cues come from ventricular fluid, the basal ECM, the neuroepithelial/radial glial cells themselves, or none of the above. In *Drosophila*, existing data suggest that glial cells are required for larval NB proliferation, but whether they serve as a local NB niche needs to be directly tested by glial ablation experiments.

Nuclear control of self-renewal

The recent identification of transcription factors (TFs) that are sufficient to reprogram human differentiated cells into cells that resemble embryonic stem (ES) cells (Takahashi et al., 2007; Yu et al., 2007) indicates that there also might be TFs or chromatin factors that specify the identity of tissue-specific stem cells. Numerous TFs are also known to be expressed in NSCs (see Table 1). In this section, I discuss TF/chromatin factor expression and function in NSCs.

Transcriptional regulation and NSC self-renewal in *Drosophila*

Genes transiently expressed in newly formed NBs include the proneural genes *achaete*, *scute* and *lethal of scute*. These encode basic helix-loop-helix (bHLH) TFs that promote the transition of a neuroectodermal cell to a NB, and thus are responsible for triggering NB delamination and NB-specific gene expression (epithelial genes off, NB-specific genes on). This is partly accomplished by the transient suppression of Notch signaling, as Notch signaling is necessary and sufficient to maintain neuroectodermal cell fate (reviewed by Artavanis-Tsakonas et al., 1991). Several other TFs are expressed in subsets of neuroectoderm and delaminating NBs, where they collaborate with the proneural proteins to promote NB formation. These include the SoxB group genes *SoxN* and *Dichaete*, which encode high mobility group (HMG) transcriptional activators (Cremazy et al., 2000; Nambu and Nambu, 1996; Russell et al., 1996). The first function of the proneural and SoxB genes is to induce neurogenesis within the ventral ectoderm, which otherwise would produce only epidermis.

A second class of NB TFs are permanently expressed in NBs but are not maintained in their GMC/neuronal progeny. These TFs are the best candidates for promoting NB self-renewal, and include the zinc-finger protein Worniu, the bHLH proteins Deadpan and Asense, and the SoxB family member SoxN (Ashraf and Ip, 2001; Bier et al., 1992; Brand et al., 1993; Cai et al., 2001; Cremazy et al., 2000). Surprisingly, very little is known about the function of these genes in regulating NB self-renewal. *deadpan* and *asense* single mutants have only mild post-embryonic CNS defects (Bier et al., 1992; Brand et al., 1993), although Deadpan can repress expression of the cell cycle inhibitor *dacapo* (Wallace et al., 2000), consistent with a role in promoting NB proliferation. Similarly, *worniu* mutants have mild defects in larval CNS axial shortening (Ashraf et al., 2004), and the Sox gene mutants have reduced embryonic NB numbers, but this is probably due to a failure in NB formation not self-renewal (Cremazy et al., 2000; Nambu and Nambu, 1996; Russell et al., 1996; Zhao et al., 2007). It is tempting to speculate that the Sox TFs act in NBs to prevent neuronal differentiation initiated by the proneural genes, similar to the proposed role of SoxB1 family TFs in vertebrates (see below). However, the function of Sox TFs in self-renewal has not yet been tested.

The flip side of NB self-renewal is neuronal differentiation. NBs rapidly lose the expression of the proneural genes, so what might promote neuronal differentiation in their lineage? The divergent homeodomain TF Prospero is crucial for initiating neuronal differentiation. *prospero* is transcribed and translated in all NBs, but is exported from the nucleus (Demidenko et al., 2001); the mRNA and protein are segregated into the GMC during NB asymmetric cell division (Broadus et al., 1998; Knoblich et al., 1995; Spana and Doe, 1995), where the protein enters the nucleus to repress cell cycle genes and activate neural differentiation genes (Choksi et al., 2006; Li and Vaessin, 2000). When *prospero* mutant clones are induced in single larval NBs, many GMCs fail to differentiate and instead form NB tumors (Bello et al., 2006; Betschinger et al., 2006; Choksi et al., 2006; Lee et al., 2006c).

What about chromatin remodeling genes? In mammals, the Polycomb group chromatin remodeling factor Bmi1 is required for postnatal NSC renewal (Molofsky et al., 2005; Molofsky et al., 2003), raising the possibility that *Drosophila* NBs might also require Polycomb for self-renewal. A recent paper tests this hypothesis by generating mutant clones null for several Polycomb group genes within single larval NBs (Bello et al., 2007). All Polycomb group mutant clones had fewer neurons and lacked the NB, consistent with

Table 1. Regulators of gene expression involved in neural stem cell self-renewal

Drosophila				Mammals			
Protein†	Expression‡	Function§	References	Protein†	Expression‡	Function§	References
SoxB	NE, NB	↑SR	(Cremazy et al., 2000; Nambu and Nambu, 1996; Russell et al., 1996)	SoxB1	NE, RG	↑SR	(Bylund et al., 2003; Ferri et al., 2004; Graham et al., 2003; Wang, T. W. et al., 2006)
E(spl)mγ	NB	?	(Almeida and Bray, 2005)	Hes1,3,5	NE, RG	↑SR	(Hatakeyama et al., 2004; Ishibashi et al., 1994)
Deadpan	NB	?	(Bier et al., 1992)	Hes related*			
Worniu	NB	?	(Ashraf and Ip, 2001)	Slug/Snail related*			
Asense	NB	?	(Brand et al., 1993)	Mash3 related*			
Musashi	NB	?	(Nakamura et al., 1994)	Musashi	NE, RG	?	(Kaneko et al., 2000; Siddall et al., 2006)
–	–	–		Rest	NE, RG	↑SR	(Ballas and Mandel, 2005)
Proneural	NE, NB	↑DIFF	(Skeath and Carroll, 1994)	Proneural	NE, N	↑DIFF	(Guillemot, 2007)
Prospero	GMC	↑DIFF	(Bello et al., 2006; Choksi et al., 2006; Knoblich et al., 1995; Lee et al., 2006c; Spana and Doe, 1995)	Prox1	BP, N	↑DIFF	(Dyer, 2003; Lavado and Oliver, 2007; Torii et al., 1999)
Brain tumor	GMC	↑DIFF	(Bello et al., 2006; Lee et al., 2006c; Betschinger et al., 2006)	Trim3	?	?	
p53	?	?		p53	N	↑DIFF	(Meletis et al., 2006)

†Protein orthologs or homologs are shown on the same line; –, gene ortholog has not been identified; *, groups of related proteins.
‡Expression in *Drosophila* neuroectoderm (NE), neuroblasts (NB), ganglion mother cells (GMC), neurons (N); or in mammalian telencephalon neuroepithelium (NE), radial glia (RG), basal progenitors (BP), neurons (N).
§Function in promoting self-renewal (↑SR) or in promoting differentiation (↑DIFF); ?, functional studies have not been reported.

a failure in NB self-renewal. However, the co-expression of the cell death inhibitor p35 rescued NB survival and normal clone size. Thus, the Polycomb group proteins are required to maintain NB survival, but are dispensable for larval NB self-renewal (Bello et al., 2007).

In conclusion, proneural genes promote NB expression of Worniu, Deadpan, Asense and Prospero. The first three TFs are good candidates for maintaining NB self-renewal, whereas Prospero is asymmetrically localized into the GMC where it promotes neuronal differentiation. This is an elegant mechanism for ensuring NB homeostasis while producing a constant stream of neurons.

Transcriptional regulation and NSC self-renewal in mammals

As in *Drosophila*, the bHLH proneural proteins Mash1 (Ascl1), Ngn1 and Ngn2 (Neurog2) are expressed in mammals in partially overlapping populations of neuroepithelial cells, where they are required for the acquisition of NSC properties. Subsequently, they are maintained in newly differentiating neurons, where they induce neuronal differentiation (Guillemot, 2007). How do neuroepithelial cells and radial glia express these proneural genes without differentiating? This is the role of the SoxB1 family members (Sox1, 2, 3). The SoxB1 proteins are expressed in embryonic and adult NSCs, as well as in a few postmitotic neurons (Graham et al., 2003; Wang, T. W. et al., 2006). A reduction in SoxB1 levels leads to precocious neural differentiation and to the depletion of the progenitor pool, whereas misexpression of SoxB1 family members can block neuronal differentiation and maintain the progenitor population (Bylund et al., 2003; Ferri et al., 2004; Graham et al., 2003), although without maintaining proliferation (Bylund et al., 2003). SoxB1 TFs antagonize the neuronal differentiation that is induced by the proneural proteins Mash1 and the Ngns (Bertrand et al., 2002; Bylund et al., 2003; Ge et al., 2006), and proneural proteins can directly bind and inhibit SoxB1 protein function. Thus, the balance of SoxB1 and proneural activity determines the tempo of neurogenesis. How this balance is regulated over time is unknown. One additional factor that promotes NSC self-renewal is the Rest transcriptional repressor, which is expressed in NSCs and in most non-neuronal cells, where it induces a repressive chromatin state that blocks the expression of neuronal differentiation genes (Ballas et al., 2005). Neurons express a small modulatory double-stranded (ds) RNA that induces differentiation by blocking Rest activity at the protein level (not the RNA level, surprisingly) (Kuwabara et al., 2004). Lastly, the RNA-binding protein musashi is expressed in both germline and NSCs (Kaneko et al., 2000; Siddall et al., 2006); it promotes germline stem cell self-renewal (Siddall et al., 2006), but its function in NSC self-renewal is yet to be determined.

An important stem cell attribute is the ability to proliferate. Maintenance of postnatal NSC proliferation is partly regulated by the Polycomb group transcriptional repressor Bmi1. Loss of Bmi1 results in an increase of the cell cycle inhibitor p16Ink4a (Cdkn2a) and in postnatal stem cell depletion, without affecting embryonic NSCs (Molofsky et al., 2005; Molofsky et al., 2003). One important negative regulator of proliferation might be Prox1, which is related to the *Drosophila* transcriptional repressor Prospero. Mash1 induces *Prox1* expression in newly differentiating neurons (Torii et al., 1999), and Prox1 inhibits proliferation in the mammalian retina (Dyer, 2003; Li and Vaessin, 2000), and might have a similar function in the cortex. Experimentally lengthening the cell cycle also increases progenitor differentiation (Calegari and Huttner, 2003). Thus, slowing or stopping the cell cycle can induce neuronal

differentiation, and prolonging cell cycle progression can prevent stem cell depletion, although quiescent stem cells clearly have a mechanism to prevent differentiation. How self-renewal and cell cycle pathways intersect will be an important and challenging area of future research.

In conclusion, data from flies and mice are consistent with a common model for neurogenesis, in which SoxB1 proteins confer progenitors (neuroepithelial cells in vertebrates, neuroectodermal cells and NBs in flies) with the potential to self-renew. Proneural proteins then induce progenitor delamination and neural differentiation, the latter being blocked by SoxB1 proteins. Finally, nuclear Prospero/Prox1 initiates cell cycle exit and neural differentiation. Several aspects of this model remain to be tested, including the role of the *Drosophila* SoxB proteins in antagonizing proneural activity and in promoting self-renewal, and the role of vertebrate Prox1 in promoting neuronal differentiation.

Cell polarity and self-renewal

Recent data suggest that cell polarity plays a key role in regulating self-renewal versus differentiation in both fly and mammalian NSCs, and that several of the proteins involved have evolutionarily conserved functions. But there are some surprising differences, and many proteins have only been tested in one animal to date.

Cell polarity and NSC self-renewal in *Drosophila*

Drosophila NBs divide asymmetrically to self-renew a NB while budding off a small, differentiating GMC. A growing number of proteins are known to be segregated into the NB or GMC during this asymmetric cell division. Proteins segregated into the NB include Bazooka (Baz/Par3), Cdc42, Par6, atypical protein kinase C (aPKC) (which may all form a single protein complex), Inscuteable (Insc), Partner of Inscuteable (Pins; Rapsynoid – FlyBase) and Gαi (G-iα65A – FlyBase) (which may form a distinct protein complex that links to Baz via Insc). Proteins partitioned into the GMC include the scaffolding protein Miranda and its cargo proteins Staufen, Prospero and Brain tumor (Brat), as well as Numb and Partner of Numb (Caussinus and Hirth, 2007; Gonzalez, 2007) (see Fig. 3A). The first protein identified to positively regulate NB self-renewal was aPKC. *aPKC* mutants have fewer NBs per larval brain lobe, and overexpression of a membrane-tethered aPKC in NBs dramatically increases brain NB numbers (Lee et al., 2006b). Similarly, *lethal (2) giant larvae* (*lgl*) mutants have ectopic cortical aPKC in NBs and a corresponding increase in brain NB numbers that can be fully suppressed in *lgl aPKC* double mutants (Lee et al., 2006b). Taken together, these data show that aPKC is sufficient to turn GMCs into NBs (ectopic NB self-renewal), but it is not absolutely required for NB self-renewal as *aPKC* mutants maintain a subset of their brain NBs. aPKC probably acts redundantly with a second pathway to promote NB self-renewal, most likely the Notch pathway, which is also sufficient but not necessary for NB self-renewal (see above). This model needs to be tested by assaying *Notch aPKC* double mutants for a complete loss of NB self-renewal.

How does aPKC promote self-renewal? One attractive model is that aPKC phosphorylates and inactivates neuronal differentiation factors – such as Lgl, Numb or the Miranda-Prospero-Brat complex – to keep these proteins out of the self-renewing NB. For example, aPKC is known to phosphorylate and inhibit the cortical localization of Numb (Smith et al., 2007) and Lgl (Betschinger et al., 2003), as well as to inhibit the cortical localization of Miranda by an unknown mechanism (Rolls et al., 2003). A more speculative model is that aPKC positively regulates cell cycle progression, and a speedy cell cycle promotes stem cell self-renewal. This model is inspired by

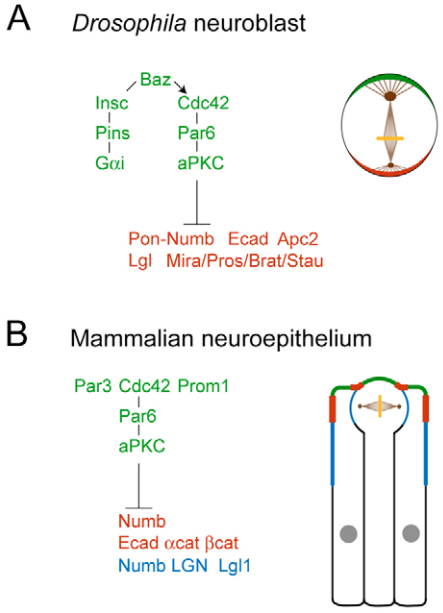

Fig. 3. Neural stem cell polarity. (**A**) *Drosophila* neuroblast cell polarity. (Left) Proteins that asymmetrically localize in a dividing NB. Green, apical proteins; red, basal proteins, including those associated with GMC contact site. Lines between proteins indicate physical interactions. Baz binds Par6 and aPKC (not shown). Arrow indicates that Baz is required for Cdc42 localization. T-bar indicates that aPKC excludes Lgl, Numb and Mira from the cortex, and Lgl excludes aPKC. (Right) Schematic of a dividing NB, showing the apical (green) and basal (red) cortical domains; spindle and centrosomes, brown; DNA, yellow. (**B**) Mammalian neuroepithelial (NE) cell polarity. (Left) Proteins that asymmetrically localize in a dividing NE cell. Green, apical proteins; red, AJ-enriched proteins; blue basolateral proteins. Lines between protein names indicate physical interactions. Par3 binds Par6 and aPKC (not shown). T-bar indicates that aPKC excludes Lgl and Numb from the cortex. (Right) Schematic of a dividing NE (center). Spindle and centrosomes, brown; DNA, yellow. Baz, Bazooka (fly Par3); Insc, Inscuteable; Pins, Partner of Insc; aPKC, Atypical protein kinase C; Lgl, Lethal giant larvae; Pon, Partner of Numb; Mira, Miranda; Pros, Prospero; Brat, Brain tumor; Stau, Staufen; Ecad, E-cadherin; Apc2, Adenomatosis polyposis coli 2; Prom1, prominin 1; αcat, α-catenin; βcat, β-catenin; LGN (Gpsm2), a homolog of Pins.

data showing that increasing cell cycle length triggers the differentiation of vertebrate neural progenitors (Calegari and Huttner, 2003). Consistent with this model, *aPKC* mutant NBs prematurely stop dividing (Rolls and Doe, 2004), although whether the NB becomes quiescent, dies or differentiates is unknown. It would be interesting to determine if the overexpression of aPKC can speed up the GMC cell cycle, and whether this is the cause of the extra NB phenotype; conversely, does lengthening the NB cell cycle induce precocious differentiation and reduced NB numbers?

A second protein required for NB self-renewal is Pins, a scaffolding protein that binds to Gαi, Insc and many other proteins (reviewed by Wodarz and Nathke, 2007). *pins* mutants initially show normal NB numbers in early larval development but have dramatically fewer NBs in late larval stages (Lee et al., 2006b). In addition, whereas wild-type NB clones always contain one NB and a family of GMC/neuronal progeny, *pins* mutant NB clones contain fewer total cells and often lack a NB (Lee et al., 2006b). *pins* mutants fail to localize aPKC to the apical cortex of larval NBs (Lee et al.,

2006b), which may contribute to the defect in self-renewal, but it is unknown whether forced expression of membrane-tethered aPKC can rescue the *pins* mutant phenotype. Surprisingly, *pins* mutant brain tissue can form tumors when transplanted into adult hosts (Caussinus and Gonzalez, 2005). The reason for this discrepancy is unknown, but a possible explanation is that transplanted cells are prone to genomic instability (Caussinus and Gonzalez, 2005), and any *pins* mutant cell that loses the *lgl* gene from the tip of chromosome 2 would generate *lgl pins* double-mutant cells that are known to form massive brain tumors (Lee et al., 2006b).

Proteins that negatively regulate NB self-renewal (i.e. that promote neuronal differentiation) usually segregate into the differentiating GMC during NB asymmetric cell division, and include the Miranda coiled-coil scaffolding protein, its cargo proteins Prospero and Brat, Lgl and Numb. Loss of any of these proteins transforms GMCs into NBs and produces a stem cell overgrowth phenotype (Bello et al., 2006; Betschinger et al., 2006; Choksi et al., 2006; Lee et al., 2006b; Lee et al., 2006c; Li and Vaessin, 2000; Wang, H. et al., 2006). Transplantation of larval brain tissue from these mutants into adult *Drosophila* hosts also leads to metastatic tumor formation (Caussinus and Gonzalez, 2005). Each of these proteins probably has a slightly different mode of action. Prospero is a transcriptional repressor that downregulates cell cycle genes (Choksi et al., 2006; Li and Vaessin, 2000), whereas Brat is a translational repressor that is required to restrain cell growth, in part by blocking *myc* (*dm* – FlyBase) translation (Betschinger et al., 2006), as well as having a poorly understood role in maintaining Prospero levels (Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006c). Numb is a multi-functional protein that antagonizes Notch signaling (Yoon and Gaiano, 2005), which is one mechanism it uses to inhibit NSC self-renewal in *Drosophila*. However, mammalian Numb also regulates Hh signaling and levels of the tumor suppressor p53 (Trp53) (see Box 2), which have yet to be tested for a role in *Drosophila* NB self-renewal.

Two regulators of cortical polarity also act as tumor suppressors in *Drosophila* larval brain development: the Polo and Aurora A (Aurora – FlyBase) kinases. Both are evolutionarily conserved centrosomal and cytoplasmic kinases that regulate cell cycle progression (Taylor and Peters, 2008). *polo* mutants have supernumerary larval NBs at the expense of neurons, both in homozygous mutant larval brains and in homozygous mutant single NB clones (Wang et al., 2007). This phenotype is partly due to the failure of *polo* mutants to phosphorylate Partner of Numb, and the corresponding loss of the basal localization of Numb. In addition, *polo* mutant NBs show uniform cortical aPKC. Reduced Numb and ectopic aPKC in GMCs would both favor the transformation of GMCs into NBs. *aurora A* mutants show a similar phenotype: ectopic aPKC localization and reduced basal Numb localization leading to an increase in NB numbers at the expense of neurons (Lee et al., 2006a; Wang, H. et al., 2006). Whether these two kinases act in a common pathway (e.g. Aurora A activating Polo, or vice versa) remains to be determined.

Rapid progress has been made in the last two years on the role of cortical polarity in regulating NB self-renewal versus differentiation, but many questions remain unsolved. How are apical and basal polarity proteins delivered and tethered to their respective membrane domains? What are the targets of aPKC and the Notch signaling pathway that promote NB self-renewal? Might it be sufficient to merely prevent exposure of the NB to the differentiation factors Prospero and Brat? Do aPKC and Notch act in the same or parallel pathways? Teasing out the relationship between cell cycle, cell polarity and self-renewal will be a key task for the next few years.

Box 2. The complexity of being Numb

In *Drosophila*, Numb is required to promote neuronal differentiation and to inhibit NB self-renewal (Lee et al., 2006c; Wang, H. et al., 2006). In mammals, the situation is more complex. The conditional deletion of Numb/Nbl early or late in neurogenesis (at E8.5 or E14, respectively) results in loss of neuroepithelial/radial glial progenitors (Petersen et al., 2002; Petersen et al., 2004). Conversely, the removal of Numb/Nbl with *Emx1*-Cre at E9.5 results in neural progenitor hyperproliferation, delayed cell cycle exit, and depletion of late-born neurons (Li et al., 2003). Most recently, the same Numb/Nbl *Emx1*-Cre conditional mutant was shown to have a loss of adherens junctions and defective apical/basal polarity owing to reduced E-cadherin-positive vesicle targeting to the junctional domain (Rasin et al., 2007). This might deplete NSC numbers, as seen with the early loss of apical/basal polarity following Par3 or Par6 depletion, but in fact the authors report no effect on progenitor maintenance or neuronal differentiation (Rasin et al., 2007). This 'Numb paradox' could be resolved by using the neurosphere stem cell self-renewal assay with Numb/Nbl mutant tissue, which, surprisingly, has never been reported. An even better experiment would be to perform clonal analysis of Numb/Nbl mutant cells in a wild-type background to determine whether the mutant cells leave the apical domain and differentiate, or remain in the apical domain and form progenitor tumors or rosettes.

A final complexity when studying Numb is to identify the relevant effector(s). Numb can block Notch signaling (Yoon and Gaiano, 2005), but it can also inhibit Shh signaling by promoting the ubiquitylation of Gli proteins (Di Marcotullio et al., 2006), and it can elevate levels of the p53 tumor suppressor by blocking its degradation (Colaluca et al., 2008). This latter function might be highly relevant to NSC self-renewal, as a reduction of p53 leads to increased NSC self-renewal at the expense of neuronal differentiation (Meletis et al., 2006; Piltti et al., 2006; Vanderluit et al., 2007). Thus, both Numb and p53 may be required for timely neuronal differentiation. This model has yet to be tested in *Drosophila*.

Cell polarity and NSC self-renewal in mammals

Neuroepithelial cells and radial glia both have an epithelial morphology and apical/basal cell polarity (Fig. 3B); by contrast, basal progenitors lack epithelial morphology and localization of apical/basal polarity markers has not yet been analyzed in these cells. Neuroepithelial cells localize the Par-complex proteins Par3 (Pard3), Par6 (Pard6α), aPKC (aPKCλ; Prkcι) and Cdc42 to the apical cortex early in mouse cortical neurogenesis when neuroepithelial/radial glial self-renewal is maximal, with levels declining at later stages concurrent with the loss of self-renewal potential (Cappello et al., 2006; Costa et al., 2008; Imai et al., 2006). Consistent with these findings, the reduction of Par3 or Cdc42 levels in neuroepithelial cells (at E9.5 using *Emx1*-Cre for Cdc42; at E10 using shRNA-expressing lentiviral vectors for Par3) leads to loss of $Pax6^+$ neuroepithelial/radial glial cells, smaller clone sizes, and to precocious neuronal differentiation (Cappello et al., 2006; Costa et al., 2008). Conversely, the overexpression of Par3 or Par6 results in larger clone sizes that contain additional $Pax6^+$ NSCs (Costa et al., 2008). The removal of one of the two aPKC isoforms (at E15.5 using nestin-Cre to remove aPKCλ) or of Cdc42 (at E14 using GFAP-Cre) from radial glial cells led to a similar but milder phenotype (Cappello et al., 2006; Imai et al., 2006). Thus, Cdc42 and the Par complex are apical proteins that are necessary and sufficient to maintain NSC identity in the embryonic cortex. These proteins have not yet been tested for a role in adult NSC self-renewal, in which apical/basal polarity is not as well defined.

Mice mutant for the adherens junction (AJ) component α-catenin lack AJs and have a faster neuroepithelial/radial glia cell cycle progression, which results in additional neuroepithelial/radial glia stem cells and neurons being formed, without a change in their ratio. This results in enlarged brains (Lien et al., 2006). Transcriptional profiling has shown that Hh-response genes are upregulated in α-catenin mutant brains; indeed, virtually all aspects of the α-catenin mutant phenotype can be suppressed by a Hh pathway inhibitor (Lien et al., 2006). Do AJs act via a contact-based inhibition of a proliferation mechanism that keeps Hh levels low? If so, then why is there no striking increase in stem cell proliferation following AJ disruption in *Cdc42* or Par-complex mutant mice? One possibility is that the Cdc42-Par complex is required for both junctional integrity and rapid cell cycle progression.

Another apical protein that promotes NSC self-renewal in the embryonic cortex is the AJ protein β-catenin. Forced expression of a stabilized β-catenin results in a large brain owing to increased numbers of proliferative progenitors and a corresponding decrease in differentiated neurons (Chenn and Walsh, 2002). Because β-catenin has a dual role, as a junctional protein and in canonical Wnt signaling, the phenotype could be due to increased Wnt signaling (which is linked to NSC self-renewal, see above) or to increased junctional stability, which might decrease the formation of basal progenitors (owing to a failure to dissolve apical junctions). It would be informative to distinguish these two pathways by specifically reducing Wnt signaling (e.g. in *Lef1/TCF1α* mutants) or AJs (e.g. in *Cdc42* mutants) to see which is required for the stabilized β-catenin phenotype.

If apical proteins promote NSC self-renewal, are basolateral proteins required for differentiation? The vertebrate Lgl1/2 (Llgl1/2 in mouse) proteins are located basolaterally in *Xenopus* and in mammalian epithelia, as is the related *Drosophila* Lgl protein (reviewed by Lien et al., 2006). *Drosophila lgl* mutants have increased NB numbers and decreased neuronal differentiation (Lee et al., 2006b); similarly, *Lgl1*-knockout mice have neuroepithelial cells with fewer AJs, increased proliferation, decreased neuronal differentiation, and a neural rosette morphology that resembles that of primitive neuroepithelial tumors (Klezovitch et al., 2004). The *Lgl1* mutant phenotype might be partly due to reduced Numb function, as Numb protein is delocalized in these mutants and expression of the Notch reporter Hes5 is increased (Klezovitch et al., 2004). Thus, the basolateral Lgl1 protein is required for Numb localization and neuronal differentiation, paralleling its function in the *Drosophila* CNS.

The role of the related Numb and numb-like proteins (henceforth referred to as Numb/Nbl) in mammalian neurogenesis is controversial (see Box 2). Recent microscopy studies clearly show that Numb localizes to AJs and to the basolateral membranes in embryonic neuroepithelial/radial glial cells and to the postnatal ependymal cells of the SVZ (Kuo et al., 2006; Rasin et al., 2007), consistent with previous reports of Numb having a basolateral localization in many animals, from fly to chick (see Rasin et al., 2007). Thus, Numb is an evolutionarily conserved basolateral protein that is excluded from the apical membrane domain. Identifying its precise role in NSC self-renewal, and the pathways that it regulates, await more-detailed future studies.

The kinase Akt (Akt1) and the phosphatase Pten have opposing functions in the Akt/Pten pathway (Narbonne and Roy, 2008), and have opposing NSC self-renewal phenotypes. Reduced Akt levels lead to loss of neuroepithelial/radial glia self-renewal in sequential neurosphere assays (Sinor and Lillien, 2004), whereas mice lacking Pten in the embryonic CNS have a larger brain, supernumerary stem cells, and shorter cell cycle times (Groszer et al., 2001). Compared with the wild type, *Pten* mutant mice generate neurospheres that can be maintained for longer in serial culture assays while maintaining their multi-lineage potential (Groszer et al., 2006). This indicates that *Pten* mutant stem cells have an increased self-renewal capability. Consistent with a role for wild-type PTEN in promoting neuronal differentiation, human *PTEN* mutations are associated with brain tumors and macrocephaly, and mouse *Pten* mutations with germline teratomas (reviewed by Stiles et al., 2004). In *Drosophila*, Pten co-localizes with the self-renewal-promoting factor aPKC (von Stein et al., 2005), so it is tempting to speculate that aPKC and Pten act antagonistically on common targets to regulate self-renewal.

Spindle orientation and self-renewal

Spindle orientation can impact stem cell self-renewal by positioning daughter cells relative to extrinsic or intrinsic self-renewal cues. It is thus important to monitor both extrinsic and intrinsic asymmetry relative to spindle orientation, to determine which correlates with self-renewal. For example, a change in spindle orientation relative to extrinsic landmarks might be meaningless if there is no change in the relationship of the spindle to functionally important intrinsic determinants. In the section below, I describe the progress, and limitations, in our understanding of spindle orientation relative to intrinsic and extrinsic cues and how it relates to NSC self-renewal.

Spindle orientation in *Drosophila* neuroblasts

Drosophila NBs invariably align their mitotic spindle along the apical/basal cell polarity axis (Fig. 4), resulting in the NB inheriting the apical proteins, while the differentiating GMC inherits the basally localized proteins (see Fig. 3). Spindle orientation is fixed at prophase, when one centrosome becomes anchored at the future apical cortex, while the other migrates throughout the cytoplasm before settling down at the basal cortex (Rebollo et al., 2007; Rusan and Peifer, 2007). By tightly linking spindle orientation with proven intrinsic determinants and with potential extrinsic cues, every NB division results in a self-renewed NB and a differentiating daughter cell. This precisely maintains brain NB numbers while constantly increasing neuron numbers.

Although it is commonly assumed that aligning the mitotic spindle with the intrinsic cortical polarity axis is essential for generating NB/GMC siblings, this has never been rigorously tested. For example, if the two spindle poles are functionally asymmetric, as suggested by recent studies (Rebollo et al., 2007; Rusan and Peifer, 2007), and this asymmetry helps specify NB versus GMC identity, then any spindle axis may reliably generate NB and GMC siblings, irrespective of spindle/cortical polarity alignment. A prerequisite for studying the role of spindle orientation in self-renewal is to identify mutations that alter spindle orientation without disrupting cortical polarity; this has only been shown for one, perhaps two, genes so far. One is *aurora A*, which encodes a centrosomal and cytoplasmic kinase. *aurora A* homozygous mutants assayed at an early larval stage, when some maternal Aurora A protein was still present, showed defects in spindle alignment relative to apical/basal cortical polarity, and a slight increase in brain NB numbers (Lee et al., 2006a; Wang, H. et al., 2006). However, neither study directly showed that the NBs with misaligned spindles always or ever produced two sibling NBs. Furthermore, NBs from older mutants had ectopic cortical aPKC and delocalized Numb proteins, raising the concern that the younger mutants might have mild defects in aPKC or Numb that cause the increase in NB

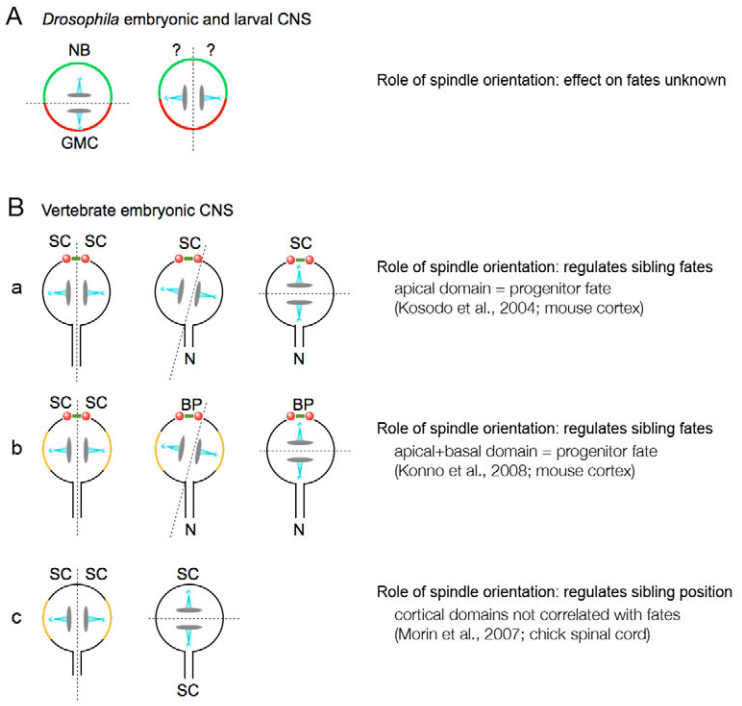

Fig. 4. Relationship between spindle orientation and sibling cell fate in neural stem cells. (**A**) *Drosophila* neuroblasts. Wild-type neuroblasts (left) invariably align their spindle along the apical/basal polarity axis, resulting in apical neuroblast (NB) and basal GMC cell fates. The cell fates that are acquired when the spindle is misoriented (e.g. in *aurora A* or *mud* mutants; right) have not been established. Spindle, blue lines; cleavage furrow plane, dotted line; apical domain, green; basal domain, red. (**B**) Vertebrate neuroepithelial cells. Conclusions from three different studies are shown. AJs, red balls; apical domain, green; basolateral domain, orange. SC, neuroepithelial cell; BP, basal progenitor; N, neuron. Apical is uppermost. (a) In Kosodo et al. (Kosodo et al., 2004), spindle orientation was concluded to regulate sibling cell fates in mouse embryonic neuroepithelial cells: if both siblings receive apical components (green), they both self-renew as progenitors (SCs; left); but if one cell lacks apical components, it differentiates into a neuron (N, middle and right). This type of asymmetric division can occur when the spindle is positioned in a near-planar orientation (middle) or in an apical/basal orientation (right). Thus, spindle orientation alone is insufficient to predict cell fate outcome. (b) Konno et al. (Konno et al., 2008) concluded that spindle orientation regulates sibling cell fates in mouse embryonic neuroepithelial cells: only siblings that inherit both apical and basal components will self-renew as progenitors (left); cells containing only apical domain become basal progenitors (BP; middle), whereas cells containing only the basal process become neurons (N; middle and right). (c) Morin et al. (Morin et al., 2007) concluded that spindle orientation does not affect sibling cell fates in the chick spinal cord. Normally, all divisions during early neurogenesis have a planar spindle orientation and form two progenitors (left). However, when the basolateral protein LGN (Gpsm2) was reduced by siRNA, the spindle could align with the apical/basal axis, yet both siblings still maintained progenitor identity by molecular marker expression (right), although the non-apical sibling was displaced out of the ventricular zone. Apical membrane and junction markers were not used in this study and thus are not shown.

number. Stronger evidence that spindle orientation defects can lead to expansion of the NB population comes from *mushroom body defective* (*mud*) mutants. Mud shares domain organization and limited sequence similarity with vertebrate NuMA (Numa1); both are primarily localized to the centrosome, and Mud can also be detected at the apical cortex during prophase (Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006), when spindle orientation is established (Rebollo et al., 2007; Rusan and Peifer, 2007; Siller and Doe, 2008). Similar to early *aurora A* mutants, *mud* mutants have normal metaphase cortical polarity but fail to align the mitotic spindle with the cortical polarity axis (see Fig. 4A), and have too many brain NBs (Bowman et al., 2006; Izumi et al., 2006). It is important to note that the NBs with misaligned spindles were not directly shown to produce two sibling NBs in these experiments. The best experiment would be to perform in vivo live imaging of mutant NBs that express vital spindle, polarity and cell fate markers. This would reveal whether spindle alignment defects always produce two NBs, whether they occasionally produce two GMCs, or whether spindle alignment is completely unrelated to the expansion in NB number in these mutants.

Spindle orientation in mammalian neural progenitors

The relationship between spindle orientation and cell fate has been studied in apical neuroepithelial cells and radial glia, but not in basal progenitors. Neuroepithelial cells have a small prominin 1^+ apical membrane domain that contacts the ventricular surface, as well as an adjacent ring of AJs and a long basal membrane domain that contacts the pial surface. In early studies of neuroepithelial cells, a horizontal mitotic spindle alignment (perpendicular to the apical/basal axis; planar cell division) was reported to result in both siblings maintaining neuroepithelial/radial glial identity; by contrast, vertical spindle alignment (along the apical/basal axis; apical/basal cell division) results in only the apical cell inheriting the apical membrane domain and remaining a progenitor, with the most-basal sibling taking a neuronal fate (Cayouette and Raff, 2003; Chenn and McConnell, 1995). More recently, it has been reported that planar divisions might actually be asymmetric apical/basal cell divisions because the tiny apical domain is partitioned into only one sibling (Kosodo et al., 2004) (see Fig. 4Ba). Furthermore, the long basal process may only be partitioned into one sibling in planar and apical/basal divisions (Das et al., 2003; Miyata et al., 2001; Miyata et al., 2004). This raises an extremely important point: what is the structure that is associated with neuroepithelial/radial glial self-renewal – the apical domain, the AJs, the basal process, or none of these? Two groups have reported that the apical cortical domain is a good predictor of neuroepithelial progenitor fate (Kosodo et al., 2004; Sanada and Tsai, 2005) (see Fig. 4Ba). By contrast, another group has shown that only cells that inherit both the apical domain and the basal process will remain as neuroepithelial progenitors (Konno et al., 2008) (Fig. 4Bb). Finally, another group reports that spindle orientation is unrelated to progenitor fate, but instead regulates daughter cell position (Morin et al., 2007) (Fig. 4Bc).

A large group of centrosomal proteins are required to maintain planar spindle orientation during the early phase of neuroepithelial expansion prior to E11.5 (Feng and Walsh, 2004; Fish et al., 2006; Konno et al., 2008; Morin et al., 2007; Xie et al., 2007). As predicted by the results of Konno et al., most of these mutants have a depleted apical neuroepithelial pool and have ectopic proliferating cells in more-basal regions of the CNS (Konno et al., 2008). At least some of these ectopic cells express neuroepithelial progenitor markers but

not basal progenitor markers (Feng and Walsh, 2004; Fish et al., 2006; Konno et al., 2008; Morin et al., 2007; Xie et al., 2007). Taken together, it appears that spindle orientation plays an important role in maintaining neuroepithelial/radial glial progenitors within the neuroepithelium, but the role of spindle orientation in regulating sibling cell fate remains an open question.

Conclusions

The last few years have seen phenomenal progress in our understanding of NSC self-renewal in *Drosophila* and mammals, based in part on new methods. Marked mutant clones (MARCM) technology has made it easier to generate single NB clones in *Drosophila* that lack a particular gene and to determine whether NB numbers increase or decrease in response to a specific gene mutation. In mammals, mosaic analysis with double markers (MADM) allows the simultaneous creation of a GFP-marked homozygous mutant clone and a RFP-marked wild-type clone (Zong et al., 2005), which permits comparison of stem cell numbers with and without the activity of a candidate self-renewal regulator. This technique is a valuable addition to existing self-renewal assays.

But despite rapid progress, important questions remain. Many evolutionarily conserved polarity proteins are known to regulate self-renewal, but the exact mechanisms by which they promote self-renewal or differentiation remain unknown. Similarly, recent studies in both flies and mice strongly suggest that the modulation of spindle orientation can alter stem cell pool size. However, time-lapse studies to determine spindle alignment relative to intrinsic polarity in a stem cell are still needed, so as to track the resulting sibling cell fates. Yet another key area for future research is the identification of TFs or other regulatory molecules that confer stem cell identity. The role of the cell cycle in regulating self-renewal versus differentiation is also an important area for future work. Why does lengthening the cell cycle trigger differentiation in mammalian neural progenitors? How do quiescent NBs in *Drosophila* or slowly dividing adult stem cells in mammals avoid differentiating?

What is clear is that there has never been a better time to study NSCs: molecular tools can be used to identify the stem cell transcriptome and proteome; genetic tools can be used to identify self-renewal mutants; and cellular tools allow unprecedented imaging of multiple proteins or organelles within stem cells in whole brains or brain slices.

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