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REVIEW

Cortical development and asymmetric cell divisions

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Abstract **The development of the mammalian neocortex involves rounds of symmetric and asymmetric cell division of neural progenitors to fulfill needs of both self-renewal of progenitors and production of differentiated progenies such as neurons and glia. The machinery for asymmetric cell division is evolutionarily conserved and extensively used in organogenesis and homeostasis of adult tissues. Here we summarize recent progress regarding cellular characteristics of different types of neural progenitors in mammals, highlighting how asymmetric cell division is utilized during cortical development.**

Keywords asymmetric cell division, radial glial cells, centrosome, spindle orientation

Introduction

The mammalian neocortex is the most complex region of the nervous system, responsible for performing the most sophisticated cognitive and perceptual functions such as sensory perception, generation of motor commands, spatial reasoning, conscious thought and language. Lying in the outer layer of the cerebral hemisphere and containing six layers, the adult neocortex comprises a plethora of projection neurons, interneurons and glial cells. Projection neurons are the main functional units, expressing excitatory neurotransmitters, with their long axons projecting into the subcortical or contralateral cortex of the brain. Most interneurons are inhibitory and form local circuitry with projection neurons to fine-tune neural activities. Glial cells are non-neuronal cells that maintain homeostasis, form myelin, and provide support and protection for neurons in the brain. The production of these neural cells during embryogenesis and postnatal development involves precisely coordinated proliferation, differentiation, and migration of progenitors and fate-committed cells. For example, projection neurons are generated from progenitor cells lying close to the cerebral ventricles following an inside-out pattern: early-born neurons reside in deep layers; late-born neurons migrate radially, passing progenitor cells and early-born neurons, to reside in superficial layers. Glia cells are produced succeeding to neurons, and cortical interneurons are generated in the ventral part of the telencephalon and migrate tangentially to the cortex. Neural progenitor cells (NPCs) divide symmetrically to expand their numbers in the earliest stage of cortical development. During the peak phase of neurogenesis (neuronal production), most NPCs divide asymmetrically to generate differentiated progenies and maintain certain numbers of progenitors simultaneously (Götz and Huttner, 2005; Lui et al., 2011).

Asymmetric cell division is a process by which a cell divides to generate two daughter cells that are already different at birth. Studies using *Caenorhabditis elegans* and *Drosphila melanogaster* have shown that a complex molecular machinery makes asymmetric cell division possible by polarizing cells and subsequently enabling cell-fate determinants to be differentially segregated into two daughter cells, thereby allowing them to adopt distinct fates (Fig. 1). The machinery for asymmetric cell divisions is evolutionarily conserved and widely used during development and tissue homeostasis in vertebrates, especially in cortical development (Knoblich, 2008). Here we review recent findings regarding cellular types of neural progenitors based on their location, morphology and division patterns during cortical development, with emphasis on how the molecular machinery for asymmetric cell division is utilized.

Cellular types of neural progenitor cells during cortical development

It takes weeks in rodents and months in primates to generate a

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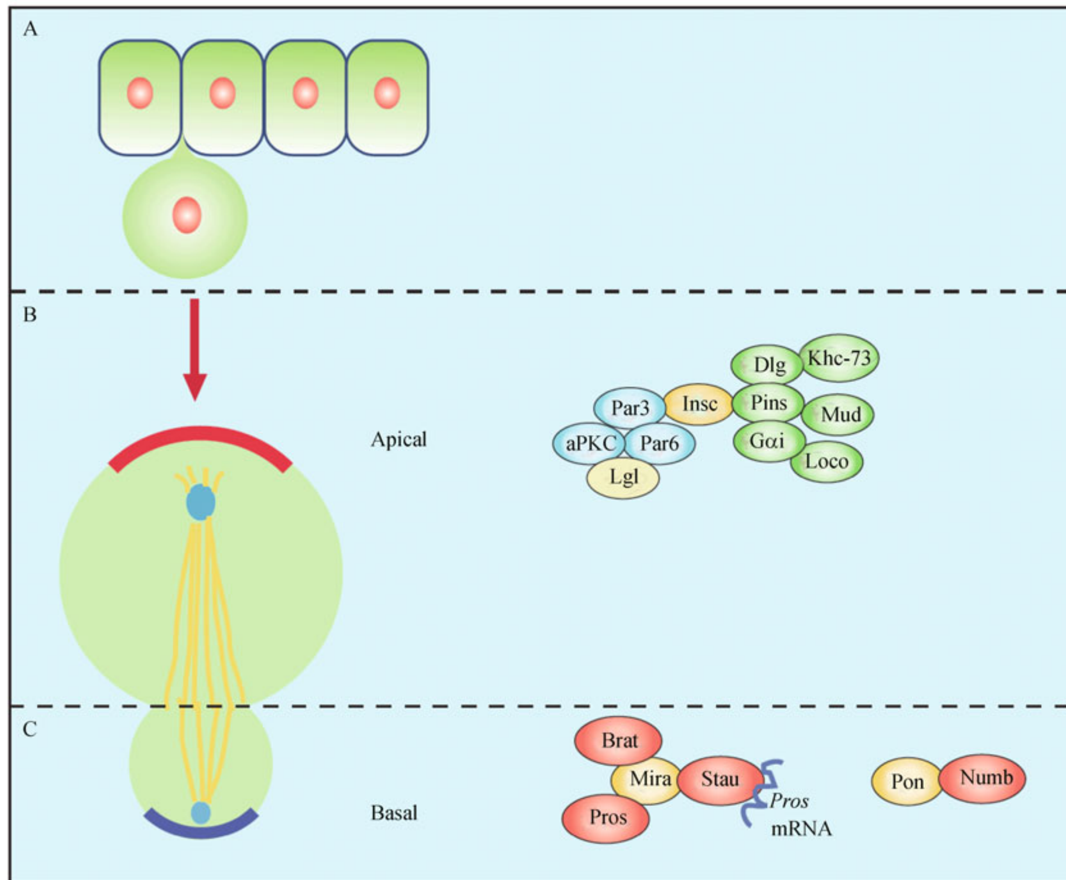

Figure 1 Asymmetric cell division of *Drosophila* neuroblasts. (A) Neuroblasts are neural progenitor cells that delaminate from the ventral neuroectoderm during embryogenesis of *Drosophila melanogaster*. (B) Neuroblast asymmetric divisions are controlled by two apically localized protein complexes linked by Inscuteable. The first protein complex is showed in blue and comprises Bazooka/Par3, Par6 and aPKC, with aPKC acting though the tumor suppressor Lethal giant larvae (Lgl) to exclude the basally localized proteins from the apical cortex. The second apical protein complex includes Partner of Inscuteable (Pins), Gαi and Locomotion defects (Loco). The Gαi/Pins/Loco complex mediates mitotic spindle orientation to ensure the cleavage plane is orthogonal to the apico-basal polarity axis. Pins also associates with the NuMa-related protein, Mushroom body defective (Mud), Disc Large (Dlg), and the astral microtubule plus end protein Khc-73 to ensure proper cortical polarity and spindle alignment. The apical daughter cell will remain as a neuroblast. (C) Cell-fate determinants like Brat, Prospero (Pros), Staufen (Stau) and Numb are localized to the basal cortex of the mitotic neuroblast, where they promote the daughter cell to differentiate into a ganglion mother cell (GMC).

highly organized layered structure during cortical neurogenesis and gliogenesis, with distinct types of neural progenitor cells in different locations involved. Polarized NPCs appear in the early stages and can be regarded as "founder" cells, whereas later-born multipolar NPCs are responsible for production of most projection neurons (Table 1).

Neuroepithelial (NE) cells

Before neurogenesis begins, the neural plate or neural tube is comprised of a single layer of neuroepithial cells, which form the neuroepithelium. The neuroepithelium seems multi-layered (pseudostratified), because the nuclei of neuroepithelial cells migrate up and down the apical–basal axis during the cell cycle (interkinetic nuclear migration), with their nuclei undergoing mitosis at the apical surface of the ventricular zone and migrating basally for the S phase part of cell cycle. NE cells show typical epithelial features and are highly polarized along their apical-basal axis (Huttner and Brand, 1997).

Radial glia (RG) cells

At approximately the time when cortical neurogenesis begins, around E10 in mouse, NE cells begin to acquire features related to glial cells, and are transformed into radial glia (RG) cells. RG cells express glial markers such as the astrocyte specific glutamate transporter (GLAST), the Ca^{2+} binding protein S100β, vimentin and brain-lipid binding protein (BLBP), in addition to Nestin, an intermediate filament protein, and transcription factor Pax6. Due to generation of neurons, the neuroepithelium becomes multi-layered, with RG cells residing in the layer that lines the ventricle, which is referred to as the ventricular zone (VZ). Whereas in

Table 1 Comparison of the properties of cortical neural progenitors

Properties	NE cells	RG cells	SNPs	oRG cells	Basal progenitors
Nuclear location	NE	VZ	VZ	oSVZ	SVZ
Apico-basal polarity	Present	Present	Present	Present	Absent
Apical contact	Present	Present	Present	Absent	Absent
Tight junctions	Present	Present (downregulated)	Unknown	Absent	Absent
Adherens junctions	Present	Present	Present	Absent	Absent
Nuclear movement	IKNM (NE)	IKNM (VZ)	Unknown	Basal soma movement	Absent
Basal lamina contacts	Present	Present	Absent	Present	Absent
Nestin expression	Present	Present	Present	Present	Absent
Astroglial markers	Absent	Present	Absent	Present	Absent
Pax6 expression	Present	Present	Present	Present	Absent
Tbr2 expression	Absent	Absent	Absent	Absent	Present
Division patterns	Symmetric (proliferative)	Sym & asym	Asymmetric	Sym & asym	Symmetric (terminal)

NE: neuroepithelium; RG: radial glial; SNPs: short neural progenitors; oRG: outer radial glial; VZ: ventricular zone; SVZ: subventricular zone; IKNM: interkinetic nuclear migration; Sym: symmetric; Asym: asymmetric

neuroepithelial cells the nuclei migrate through the entire length of the cytoplasm, the interkinetic nuclear migration of RG cells is restricted in the VZ. RG cells maintain polarized along their aplical-basal axis. Two thin radial fibers extend apically and basally from each RG cell body, touching the ventricular surface and pial surface respectively, and serve as scaffolds for radial neuronal migration. The apical processes (endfeet) have a specialized apical membrane domain that is separated by the adherens junctions from the basolateral domain and is in contact with the basement membrane (Anthony et al., 2004; Götz and Huttner, 2005). Live imaging experiments have shown that during the peak of neurogenesis, the majority of RG cells divide asymmetrically to self-renew and give rise to either neurons or basal progenitor cells (Fig. 2A).

Short neural precursors (SNP)

SNPs also reside in the VZ and divide at the ventricular surface similarly to RG cells. Although SNPs have apical

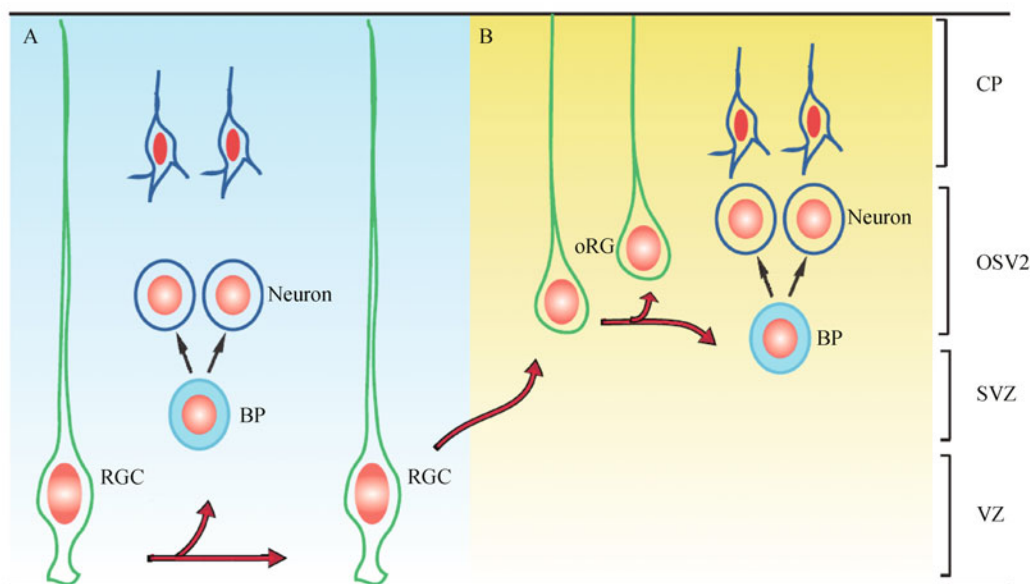

Figure 2 Asymmetric cell division of radial glial (RG) cells and outer radial glial (oRG) cells in cortical neurogenesis. (A) RG cells (RGC) have thin processes touching both the ventricular lumen and the pial surface of the developing brain, with their nuclei residing in the ventricular zone of the neuroepithelium. During indirect neurogenesis, a RG cell (RGC) divides asymmetrically to self-renew and produce a basal progenitor cell (BP), which then divides symmetrically into two postmitotic neurons that migrate radially to form the cortical plate (CP). BP cells are not polarized and reside in the subventricular zone (SVZ). BP cells can also divide symmetrically a few more rounds to expand themselves before terminal neurogenic divisions (not shown). (B) Outer radial glial (oRG) cells can be derived from asymmetric divisions of RG cells but lack apical processes. The somas of oRG cells localize in the outer subventricular zone (oSVZ) and express most RG cell markers. Similar to RG cells, most oRG cells divide asymmetrically during the peak phase of cortical neurogenesis.

processes touching the ventricle surface, their short basal processes are confined in the SVZ region. SNPs can be marked by their preferential expression of the tubulin α-1 promoter but not RG cell-specific GLAST and BLBP promoters (Gal et al., 2006; Stancik et al., 2010). It is not clear whether SNPs belong to a distinct progenitor cell type or are early intermediate progenitors that have not yet lost ventricular contacts.

Outer radial glia (oRG) cells

Recent studies have identified a new category of neural progenitors in primates and rodents, outer radial glia (oRG) cells. oRG cells are derived from RG cells and localize basally to the subventricular zone, the outer subventrucular zone (oSVZ). Unlike bipolar RG cells, oRG cells are unipolar, with basal fibers ascending toward the pial surface without apical fibers that descend toward the ventricle. Live imaging of oRG cell divisions in cultured slices of fetal neocortex shows that the cell soma of oRG cells rapidly ascends along the radial fiber during the hour preceding cell division, a process termed mitotic somal translocation. However, other than their nuclear location and physical appearance, oRG cells behave quite similar to RG cells, expressing radial glial markers, such as Nestin, Pax6, BLBP and GLAST, and dividing asymmetrically during the peak of neurogenesis (Fietz et al., 2010; Hansen et al., 2010; Lui et al., 2011; Wang et al., 2011).

Basal progenitor cells

A group of non-polarized progenitor cells appears in the subventricular zone (SVZ) at the advent of cortical neurogenesis. They originate from RG and oRG cells and their nuclei are localized basally to those of the RG cells. Without expressing markers for RGs and oRGs, they can be specifically labeled with T-box transcription factor Tbr2. Unlike RGs and oRGs, they only divide symmetrically either to two basal progenitors or two differentiated cells without features of interkinetic nuclear migration (Englund et al., 2005).

The proportion of oRG cells and basal progenitor cells is much greater in primates than that in rodents; this difference is believed to be responsible for the expansion of the cortical volume and surface to meet needs for more complicated neurological activities (Fig. 2B) (Fietz and Huttner, 2011; Lui et al., 2011).

Asymmetric cell divisions in *Drosophila* neurogenesis and in mammalian cortical development

Studies of the development of the nervous system of the fruit fly *Drosophila melanogaster* have provided the detailed molecular mechanisms of asymmetric cell fate determination. The majority of the molecular machinery and cell-fate determinants have mammalian counterparts that also play crucial roles in cortical development (Table 2) (Knoblich, 2008; Zhong and Chia, 2008). In this section, we will review the similarities and discrepancies in how asymmetric cell division is utilized in the neural progenitors of *Drosophila* and mammals, with emphasis on mammalian cortical neurogenesis.

Drosophila neural progenitors in the embryonic brain, called neuroblasts, undergo repeated asymmetric divisions, with each division producing a larger progeny that remains as a neuroblast and a smaller ganglion daughter cell (GMC). GMCs then divide terminally to generate two postmitotic neurons or glia. The differential fate of the neuroblast daughter cells is due to the asymmetric segregation of cell-fate determinants, such as Numb, Prospero and Brat, to the GMCs. This occurs because an apical-basal polarity is established by two protein complexes, the Par complex (Par3/Par6/aPKC), and a protein cassette related to hetero-trimeric G protein signaling (Gai/Pins/Loco). The two complexes are tethered by Inscuteable (Insc), which can bind both Par3 and Pins. Pins can also associate with the NuMa-related protein, Mud (Mushroom body defective), which is essential for proper spindle orientation, as well as Dlg (Discs Large) and the astral microbubule plus end protein Khc-73, to induce apical polarity (Fig. 1) (Schober et al., 1999; Betschinger and Knoblich, 2004).

The Par protein complex

In *Drosophila* embryonic neuroblasts, the polarity is aligned along the apico-basal axis and is initially defined by the Par3/Par6/aPKC complex that is already apically localized in the neuroepithelia from which neuroblasts delaminate. Then Insc and the G protein cassette are recruited to the apical cortex. The Par-portein complex provides positional cues for several processes that occur during asymmetric cell division in *Drosophila*, and directs asymmetric segregation of cell-fate determinants to the opposite, basal cortex by phosphorylating Lgl (Lethal giant larva). The G protein cassette is essential for orienting the mitotic spindle along the apico-basal axis (Schaefer et al., 2001). In mutants for any of the three proteins in the Par complex, the others are delocalized, cell fate determinants are uniformly distributed, and mitotic spindles orient randomly (Wodarz et al., 1999; Wodarz et al., 2000; Petronczki and Knoblich, 2001).

Mouse Par3 is concentrated at junctions in the contacts of the lateral membrane domains formed by ventricular endfeet of neighboring RG cells, and is apical to the adherens junction structures where beta-catenin and N-cadherin are localized. During the peak of neurogenesis, Par3 is asymmetrically segregated in more than half of RG cells. Accordingly, the fractions of neural progenitor cells that divide symmetrically

Table 2 Components of asymmetrical cell division and their roles in *Drosophila* and mouse

	Drosophila		Mouse homologs	
Functional categories	Symbol/name	Roles in cell fate specification of neuroblasts	Symbol	Roles in cortical neurogenesis
Cell polarity establishment	Par3/ Bazooka	After delamination of the neuroblast from the neuroepithelium, Bazooka provides an asymmetric cue in the apical cytocortex that is required to anchor Inscuteable. Bazooka is also responsible for the maintenance of apical-basal polarity in epithelial tissues (Kuchinke et al., 1998; Schober et al., 1999).	mPar3	1) mPar3 is enriched at the lateral membrane domain in the ventricular endfeet of RG cells during interphase, whereas it becomes dispersed and shows asymmetric localization during mitosis. mPar3 acts through the Notch signaling pathway in generating the asymmetry in radial glial daughter cell fate specification (Bultje et al., 2009). 2) Specifies axon-dendrite polarity in hippocampal neurons (Shi et al., 2003).
	Par6	Sets up apico-basal polarity. Loss of Par-6 leads to defects in the apical localization of Baz and Insc in neuroblasts and defects in the basal localization of Numb and Miranda, as well as the randomization of neuroblast spindle orientation; its loss also leads to defects in epithelial polarity (Petronczki and Knoblich, 2001).	Par6 (Pard6A) Pard6B Pard6G	1) Par6 specifies axon-dendrite polarity in hippocampal neurons (Shi et al., 2003). 2) Par6 Controls glial-guided neuronal migration (Solecki et al., 2004). 3) Par6 overexpression promotes the generation of RG cells *in vitro* and *in vivo* (Costa et al., 2008).
	aPKC	Sets up apico-basal polarity. aPKC mutation results in loss of apico-basal polarity, multilayering of epithelia, mislocalization of Insc, and abnormal spindle orientation in neuroblasts (Wodarz et al., 2000; Rolls et al., 2003).	aPKCλ & aPKCζ	aPKCλ is required for the maintenance of adherens junctions between endfeet of RG cells. In conditional aPKCλ knockout mice, adherens junctions are lost but neurons were produced at a normal rate (Imai et al., 2006).
	Lgl	Controls basal localization of cell-fate determinants(Ohshiro et al., 2000; Peng et al., 2000).	Lgl1	Maintains cell polarity of RG cells. Loss of Lgl1 in mice results in formation of neuroepithelial rosette-like structures. A large proportion of $Lgl1^{-/-}$ neural progenitor cells fail to exit the cell cycle and differentiate, but continue to proliferate and die by apoptosis. Dividing $Lgl1^{-/-}$ cells are unable to asymmetrically localize Numb (Klezovitch et al., 2004).
Mitotic spindle positioning	Insc	Inscuteable tethers together the Par and Pins complexes at the apical side. Loss of Insc results in misoriented mitotic spindles and randomized crescents of determinants. Ectopic expression of Insc in epithelial cells triggers a reorientation of the mitotic spindle into an apical-basal direction (Kraut et al., 1996).	mInsc	Orients mitotic spindles in retinal progenitors and RG cells: 1) In rat retinal explants, downregulation of mInsc inhibits vertical divisions and leads to proliferation and cell fate specification defects (Zigman et al., 2005). 2) Loss of mInsc results in defects of neurogenesis and depletion of basal progenitors. mInsc overexpression leads to expansion of basal progenitors (Postiglione et al., 2011).
	Pins	Pins and Inscuteable are dependent on each other for asymmetric localization in neuroblasts. In Pins mutants, neuroblasts showed defects in the orientation of their mitotic spindle and the basal asymmetric localization of Numb and Miranda (Schaefer et al., 2000; Yu et al., 2000).	LGN (Gpsm2)	Maintains planar divisions and RG cell fates.Knocking out LGN randomized the orientation of normally planar divisions of RG cells. The resultant loss of the apical membrane from daughter cells converted them into abnormally localized progenitors without affecting neuronal production rate (Konno et al., 2008).
			Ags3 (Gpsm1)	Receptor-independent activators of Gβγ signaling. Regulates spindle orientation and asymmetric cell fate of RG cells (Sanada and Tsai, 2005).
	Hetero-trimeric G proteins	The signaling mediated by the Gβ subunit of heterotrimeric G proteins determines asymmetric spindle formation. Lack of Gβ induces a large symmetric spindle and causes division into nearly equal-sized cells with normal segregation of the determinants (Fuse et al., 2003). Interfering with G protein function by Gαi overexpression or depletion of heterotrimeric G protein complexes causes defects in spindle orientation and asymmetric localization of determinants (Schaefer et al., 2001).	Hetero-trimeric G proteins	Interfering with Gβγ function in mouse neural progenitors causes a shift in spindle orientation from apical-basal divisions to planar divisions. This results in overproduction of neurons as a consequence of both daughter cells adopting the neuronal fate (Sanada and Tsai, 2005).

(*Continued*)

	Drosophila		Mouse homologs	
Functional categories	Symbol/name	Roles in cell fate specification of neuroblasts	Symbol	Roles in cortical neurogenesis
Cell fate determination	Miranda	Directs the basal cortical localization of multiple molecules, including Staufen and prospero RNA, in mitotic neuroblasts in an actin-dependent manner (Ikeshima-Kataoka et al., 1997; Shen et al., 1997).	N/A	N/A
	Numb	1) Specifies cell fates in the development of central and peripheral nervous system and myogenic lineage by antagonizing Notch signaling (Rhyu et al., 1994; Knoblich et al., 1995; Ruiz GÓmez and Bate, 1997). 2) Numb mutation results in overproliferation of neuroblasts and tumor formation in larval brain (Bowman et al., 2008).	Numb & Numbl	1) Numb and Numbl are redundant in asymmetric cell-fate specification of neural precursors. 2) Numb loss leads to depletion or hyperproliferation of neural progenitor cells in different genetic settings (Petersen et al., 2002; Li et al., 2003; Petersen et al., 2004). 3) Numb may be also required for maintenance of cadherin-based adhesion and polarity of RG cells (Rasin et al., 2007).
	Prospero	As a homeodomain-containing transcription factor, prospero represses genes required for self-renewal and activates genes for terminal differentiation. Loss of prospero causes neuroblast overproliferation (Doe et al., 1991; Choksi et al., 2006).	Prox1 & Prox2	1) Inhibit progenitor cell proliferation and promote horizontal cell genesis in the mouse retina (Dyer et al., 2003). 2) Prox1 mediates suppression of Notch1, thus relieves Notch's inhibition on neurogenesis and allows NPCs to exit the cell cycle and differentiate in chick and mouse spinal cords (Kaltezioti et al., 2010).
	Staufen	Mediates asymmetric localization and segregation of prospero RNA but not of Pros protein (Li et al., 1997; Broadus et al., 1998).	Stau1 & Stau2	Both are RNA binding proteins. Stau1 mediates mRNA decay including mRNA of ADP-ribosylation factor-1 (ARF1) (Kim et al., 2005; Gong and Maquat, 2011; Cho et al., 2012).
	Brat	Promotes neuronal differentiation and inhibits neuroblast self-renewal by inhibiting dMyc (Betschinger et al., 2006; Lee et al., 2006).	Trim32	Induces neuronal differentiation by inhibiting c-Myc and activating let-7 (Schwamborn et al., 2009).

significantly increased upon knockdown of or overexpression of Par3. However, the outcome in the daughter cell fate specification is quite different in opposite manipulations: while Par3 knockdown leads to an increase in neuron production, ectopic expression of Par3 promotes RG cell fate. The authors further suggested that Par3 promotes RG cell fates by positively regulating Notch signaling in the developing neocortex (Bultje et al., 2009). In summary, mouse Par3 shows polarized distribution in RG cells, and it may regulate asymmetric cell fates as a cell-fate determinant.

Rho-GTPase cdc42 has been implied in regulating the Par complex in apical polarity, junction formation, and asymmetric cell division in *Drosophila* and mammalian epithelium. Cappello et al. conditionally ablated cdc42 in cortical NPCs using Emx1-Cre. Although cdc42-deficient RG cells have normal cell cycle length, orientation of cell division, and basement membrane contact, the apical location of the Par complex and adherens junctions are gradually lost, leading to an increasing failure of apically directed interkinetic nuclear migration (IKNM). RG cells then undergo mitoses at basal positions and acquire the fate of basal progenitors. As expected, the neocortex became highly disorganized without a normal layered structure (Cappello et al., 2006).

Similarly, using Nestin-Cre to conditional knockout aPKCλ, one of two mammalian aPKC homologs, causes loss of adherens junctions, retraction of apical processes, and impaired IKNM, which results in disorganized neuroepithelial tissue architecture in VZ and SVZ. However, in contrast to cdc42 knockout mice, overall neurogenesis is not affected in aPKCλ conditional knockout embryos (Imai et al., 2006). The phenotypic discrepancies may be due to uncharacterized roles of cdc42 in RG cell-fate maintenance or cellular specificities of Cre lines used (please refer to detailed discussion below).

Spindle orientation and cell fate specifications

The polarity set up by the Par proteins has been shown to modulate the orientation of the mitotic spindle. In the *Drosophila* embryonic brain, the mitotic spindles of neuroblasts are aligned along the apico-basal axis by the Gαi/Pins/Loco complex, with Inscuteable (Insc) also involved. Mitotic spindles are important for the generation of asymmetry during mitosis in two ways: First, asymmetric position or shape of

the mitotic spindle can create daughter cells of different sizes. Second, the orientation of the mitotic spindle has to be coordinated with the asymmetric localization of cell fate determinants in order to ensure their asymmetric inheritance after mitosis. The orientation of mitotic spindle is randomized in *Insc* mutants (Kraut et al., 1996). Both Pins loss of function and overexpression of Gαi lead to mislocalization of Insc, aberrant spindle orientation and mislocalization of basal cell fate determinants (Schaefer et al., 2000; Yu et al., 2000). The role of G-proteins during asymmetric cell division is independent of extracellular signals. Instead, proteins containing GoLoco domains, such as Pins, mediate activation of the G proteins (Schaefer et al., 2001). The G proteins are required either to connect microtubules to the cell cortex or to activate molecular motors that pull on the microtubules.

In the past decade, studies have contradictory conclusions regarding whether spindle orientation of mitotic RG cells correlates with division pattern and cell fate choices. Initially, it had been suggested that vertical divisions, with mitotic spindle aligned along the apico-basal axis and a horizontal cleavage plane, are asymmetric; whereas horizontal (planar) divisions, with the spindle aligned along the ventricular surface, are symmetric (Chenn and McConnell, 1995). oRG cells, upon completing mitotic somal translocation, typically divide with a horizontal cleavage plane, with the more basal daughter cell always inheriting the basal fiber and remains as an oRG cell (Wang et al., 2011). It has been further suggested that a shift from horizontal to vertical divisions represents the transition from proliferation of RG cells to neuron production during peak phase of neurogenesis. Several studies appear to support this model. In one study, mouse inscuteable, mInsc, is found asymmetrically localized to the apical side of VZ progenitor cells and is involved in orienting the mitotic spindle in the rat retina. Knockdown of mInsc by RNAi inhibits vertical divisions and results in enhanced proliferation, consistent with a higher frequency of symmetric divisions generating two proliferating cells (Zigman et al., 2005). A recent study further showed that manipulation of mInsc expression in RG cells alters their mitotic spindle orientation, thus affecting the production of basal progenitors. Loss of mInsc results in abolishment of oblique and vertical mitotic spindles relative to ventricle surface and depletion of basal progenitors, while overexpression of mInsc has the opposite effect (Postiglione et al., 2011). Knockdown of Aspm, a gene encoding a protein localized in the mitotic spindle pole and mutated in hereditary microcephaly patients, results in more vertical divisions of RG cells (Fish et al., 2006). Similarly, compromise of heterotrimeric G protein signaling in cortical progenitors by interfering with Gβγ function or silencing AGS, a mammalian Pins homolog, leads to an increase in planar divisions of RG cells. But RG cells in this case increase differentiation rather than proliferation (Sanada and Tsai, 2005). A caveat in these studies is that the above-mentioned molecules may have dual roles in both spindle orientation and cell fate determination. Thus changes of cell fates and spindle orientation caused by mutations are parallel events rather than direct correlation.

In supporting this, a few other studies suggest spindle orientation and patterns of RG cells division can be decoupled. Noctor et al. performed elegant live imaging studies using cultured rat cortices and found that most divisions of RG cells are horizontal during the peak of neurogenesis. However, these cells divide asymmetrically to give rise to two different progenies (Noctor et al., 2004). A study by Konno and colleagues showed similar results. They found that knocking out LGN, another mammalian homolog of Pins, randomized the orientation of normally horizontal RG cell divisions and caused many RG cells to detach from the apical surface and become basal progenitor cells. However, loss of LGN had little effect on progenitor cell proliferation and neuron production (Konno et al., 2008). The same is true in the developing chick neuroepithelium (Morin et al., 2007). These findings suggest an alternative model that most RG cells have a relatively fixed spindle orientation along the ventricular surface and that correct spindle orientation is not for determining daughter cell fates but for proper organization of the neuroepithelium.

Centrosomes

As the microtubule-organizing center in eukaryotes, the centrosome is crucial for normal cell-cycle progression. All normal animal cells initially inherit one centrosome, comprising a pair of centrioles surrounded by amorphous pericentriolar material (PCM). The two centrioles differ in their birthdate, structure and function. During the G1 phase of each cell cycle, the centrosome replicates once in a semi-conservative manner, giving rise to two centrosomes: the "mother" centrosome inherits the early-born mother centriole while the "daughter" centrosome receives the late-born mother centriole (Meraldi and Nigg, 2002; Delattre and Gönczy, 2004). This intrinsic asymmetry in the centrosome has been showed to be crucial in proper spindle orientation of male germline stem cells and neuroblasts. Early in the cell cycle of *Drosophila* larval neuroblasts, the two centrosomes become unequal: one organizes an aster that remains near the apical cortex for most of the cell cycle, while the other loses PCM and microtubule-organizing activity, and locates near the basal cortex shortly before mitosis, recruiting PCM and organizing the second mitotic aster. Upon division, the apical centrosome remains in the stem cell, while the other goes into the differentiating daughter (Rebollo et al., 2007). A recent study by Wang et al. showed striking similarities in asymmetric cell divisions of RG cells during cortical neurogenesis. Using a photoconversion technique to differentiate "mother" centrosomes with the early-born centriole from the "daughter" centrosomes with the late-born centriole, they found more than two-thirds of centrosomes with the early-born centriole were located in the VZ and SVZ, while around two-thirds of centrosomes with the late-born centriole

were located in the intermediate zone and cortical plate. They further showed that removal of ninein, a mature centriole-specific protein, disrupts the asymmetric inheritance of the centrosome and causes premature depletion of RG cells from the VZ (Wang et al., 2009). Taken together, the preferential inheritance of the centrosome with the mature late-born mother centriole is required for maintaining RG cells in the developing neocortex.

Cell-fate determinants

In the *Drosophila* neuroblast lineage, asymmetric segregation of the cell-fate determinants Brat, Numb, and Prospero into GMCs assure distinct cell fates between the two daughter cells (Doe et al., 1991; Rhyu et al., 1994; Lee et al., 2006). Similarly, to construct the four-cell structure of the *Drosophila* external sensory organ, all three rounds of cell division are asymmetric, and Numb is exclusively distributed into one daughter cell in each division to differentiate two progenies by inhibiting the Notch signaling pathway. An interesting question is whether asymmetric segregation of cell-fate determinants in cortical RGs and oRGs is required for them to produce two distinct daughter cells after an asymmetric cell division.

Trim32 is the mouse ortholog of Brat. It is found asymmetrically concentrated in one of the two RG's daughter cells. Reminiscent of the scenario in *Drosophila*, Trim32 promotes neuronal differentiation in mouse: overexpression of Trim32 induces neuronal differentiation while Trim32 knockdown in RG cells causes both daughter cells to remain as progenitors. The authors further showed that Trim32 promotes differentiation by ubiquitinating c-Myc, a transcription factor involved in NPC self-renewal, and stabilizing let-7a, a microRNA that promotes neuronal differentiation (Schwamborn et al., 2009).

Numb has two mammalian homologs, m-Numb (Numb) and Numblike (Numbl). Mouse Numb is localized to the apical or apical-lateral side of RG cells, but Numb is excluded from the small apical cell membrane. The role of Numb proteins in cell-fate specification during cortical development is still controversial. Consistent with the findings in *Drosophila* and the idea that Numb may promote neuronal differentiation, conditional knockout of the Numb and Numbl genes in dorsal forebrain using Emx1-Cre results in hyperproliferation of neural progenitors, delayed cell cycle exit, and impaired neuronal differentiation. As a consequence, the neocortex in the double mutants in highly disorganized with disruption of adherens junctions at the apical (ventricle) surface and abnormal clustering of neurogenic foci in the cortex (Li et al., 2003; Rasin et al., 2007). However, using Nestin-Cre to ablate Numb in neural precursors in a Numbl mutant background results in depletion of neural progenitors and precocious productions of neurons, indicating a role for Numb in maintenance of NPCs (Petersen et al., 2002; Petersen et al., 2004). More specifically, ablation of Numb expression in RG cells during cortical neurogenesis using D6-Cre and hGFAP-Cre (unpublished data from Dr. Weimin ZHONG) also depletes neural progenitors dramatically, with thinning of cortex as a result of diminished neurogenesis, whereas the apical adhesions and polarity of RG cells remain intact. The drastic discrepancies in phenotypes from different Cre line-mediated gene ablation may reflect cell-type specificities among these lines. In vertebrates a major challenge in assigning gene function to a specific cell or cell-type, e.g. RG cells, is the difficulty in manipulating gene expression at the single cell-type level, which is essential to determining whether changes in cell behavior are a direct (intrinsic) or indirect (extrinsic) consequence of changes in gene function.

Conclusions and future directions

As discussed above, during cortical neurogenesis, RG cell polarity set up by conserved components of asymmetric cell division is essential to RG cell fate choice. However, when NPCs are cultured dispersed *in vitro* without extrinsic polarity cues, NPCs can still follow an intrinsic "clock" to undergo repeated asymmetric divisions, sequentially generating neurons and glia in their normal *in vivo* order: first preplate cells, including Cajal-Retzius neurons, then deep and finally superficial cortical neurons (Shen et al., 2006). Further studies will provide the molecular mechanisms for how polarity cues and intrinsic factors are integrated, and epigenetic evidence for how distinct cell fates are initiated and maintained. A molecular understanding of mammalian asymmetric cell division is not only relevant to basic development and stem-cell biology, but also has tremendous clinical importance for controlling stem cells therapeutically.

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