

Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system

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The type and number of cell divisions of neuronal progenitors determine the number of neurons generated during the development of the vertebrate central nervous system. Over the past several years, there has been substantial progress in characterizing the various kinds of neuronal progenitors and the types of symmetric and asymmetric divisions they undergo. The understanding of the cell-biological basis of symmetric versus asymmetric progenitor cell division has been consolidated, and the molecular machinery controlling these divisions is beginning to be unravelled. Other recent advances include comparative studies of brain development in rodents and primates, as well as the identification of gene mutations in humans that affect the balance between the various types of cell division of neuronal progenitors.

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Introduction

The generation of neurons and glial cells during the development of the vertebrate nervous system involves symmetric and asymmetric divisions of various types of progenitor cells. Due to space limitation, we shall confine this review to the divisions of progenitors that, directly or indirectly, generate the neurons of the central nervous system (CNS) of vertebrates, notably mammals. Various classes of progenitor cells have been shown to generate CNS neurons (as will be discussed below): neuroepithelial cells, radial-glia cells and basal progenitors (also referred to as intermediate progenitors). Symmetric and asymmetric divisions of these progenitors can be defined either by the fate of the daughter cells or by the distribution, to the daughter cells during mitosis and cytokinesis, of subcellular structures and molecules that may affect cell fate. With respect to daughter cell fate, progenitor cell divisions can be classified into four principal types: sym-

metric proliferative (e.g. neuroepithelial cell → neuroepithelial cell + neuroepithelial cell); symmetric differentiative (e.g. basal progenitor → neuron + neuron); asymmetric mono-differentiative or self-renewing (e.g., neuroepithelial cell → neuroepithelial cell + neuron); and asymmetric bi-differentiative (e.g. neuroepithelial cell → radial-glia cell + neuron). Here, we will discuss recent progress in understanding such symmetric and asymmetric progenitor divisions, defined by daughter cell fate, at the cell biological level; that is, in terms of the distribution of relevant subcellular structures and molecules to the daughter cells during mitosis and cytokinesis.

Progenitors involved in CNS neurogenesis

Neuroepithelial cells

Neuroepithelial cells are the primary neural progenitors from which all other CNS progenitors and — directly or indirectly — all CNS neurons derive. Prior to neurogenesis, the neural tube wall consists only of neuroepithelial cells, which form a single-cell-layered, pseudostratified epithelium. Neuroepithelial cells extend from the apical (ventricular) surface to the basal lamina and show the typical features of epithelial cells, notably an apical–basal polarity, which has been reviewed recently [1,2]. Neuroepithelial cells undergo mitosis at the apical surface of the neuroepithelium.

Radial-glia cells

Concomitant with the production of neurons, the neuroepithelium changes into a multi-cell-layered tissue, with the progenitor cell bodies remaining in the apical-most cell layer (the ventricular zone) and the adjacent cell layer (the subventricular zone, which is most developed in the telencephalon), and the neurons migrating to the more basal cell layers. In the course of this transition, neuroepithelial cells give rise to the cells that succeed them, the radial-glia cells, which are highly related to neuroepithelial cells but are nonetheless distinct in certain properties, notably the expression of astroglial markers [2,3]. Radial-glia cells extend from the apical surface of the ventricular zone through the neuronal layers to the basal lamina. They retain apical–basal polarity, undergo mitosis at (or very close to) the apical surface of the ventricular zone [2] and, over the past few years, have been recognized as a major neuronal progenitor population [4].

Basal progenitors and subventricular-zone progenitors

At the onset of neurogenesis, a distinct type of neuronal progenitor appears that undergoes mitosis at the basal side of the ventricular zone and, during later stages of

neurogenesis, in the subventricular zone; it is henceforward referred to as basal progenitor or subventricular-zone progenitor [5^{**}–7^{**}]. These cells have recently been recognized as another major neuronal progenitor population [5^{**},6^{**}]. They originate from mitoses of neuroepithelial and radial-glia cells at the apical surface of the ventricular zone and, concomitant with the migration of their nuclei to the basal side of the ventricular zone and to the subventricular zone, they retract their apical extension [7^{**}]. Basal and subventricular-zone progenitors differ from neuroepithelial and radial-glia cells in their gene expression profiles; for example, they specifically express the non-coding RNA *Svet1* [8] and the transcription factors *Tbr2* [9], *Cux1* and *Cux2* [10,11].

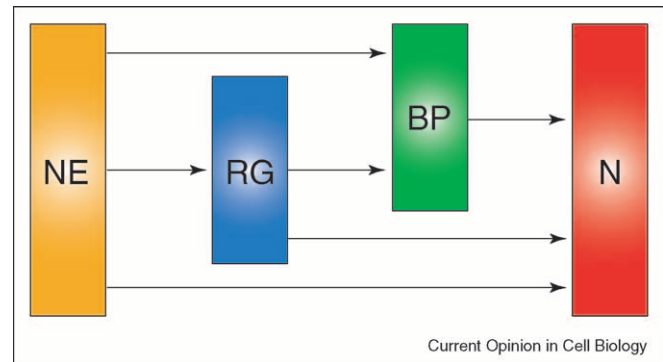
Outer subventricular-zone progenitors in primates

A primate-specific layer of progenitor cells called the outer subventricular zone has recently been described and shown to be a major site of production of cortical neurons in primates [12,13]. In contrast to basal and subventricular-zone progenitors in rodents, these cells in primates have a radial morphology [12,13], strongly suggesting that these are polarized cells. As little is known about the cellular organization of outer subventricular-zone progenitors at present, we shall concentrate in the subsequent discussion on the more extensively studied neuroepithelial and radial-glia cells and basal and subventricular-zone progenitors.

Types of progenitor cell division

Figure 1 summarizes the lineage relationships between neuroepithelial cells, radial-glia cells, basal and subventricular-zone progenitors and neurons that are known to

Figure 1



Lineage relationships between neuroepithelial cells (NE), radial-glia cells (RG), basal and subventricular-zone progenitors (BP) and neurons (N). The possible types of division of these progenitors are listed in Table 1. For details, see text.

exist, and Table 1 lists the various types of progenitor cell divisions that have been shown to, or hypothetically may, occur. These divisions fall into distinct groups depending on whether they result in an increase in progenitor cell number, the generation of a distinct type of progenitor, or the production of neurons.

Divisions that increase progenitor number

Both neuroepithelial and radial-glia cells increase in number by symmetric proliferative divisions (neuroepithelial cell → neuroepithelial cell + neuroepithelial cell; or radial-glia cell → radial-glia cell + radial-glia cell); for recent evidence of this by time-lapse imaging,

Table 1

The various types of progenitor cell divisions that have been shown to, or hypothetically may, occur.

Mother cell	Daughter cells				Type of division	Reported	Ref
	NE	RG	BP	N			
NE	2				Symmetric proliferative	Yes	[17,20]
NE	1	1			Asymmetric mono-differentiative	–	
NE		2			Symmetric differentiative	–	
NE	1		1		Asymmetric mono-differentiative	–	
NE			2		Symmetric differentiative	–	
NE	1			1	Asymmetric mono-differentiative	Yes	[6 ^{**} ,17]
NE				2	Symmetric differentiative	Yes	[20]
NE		1	1		Asymmetric bi-differentiative	Proposed	[2]
NE		1		1	Asymmetric bi-differentiative	Proposed	[2]
NE			1	1	Asymmetric bi-differentiative	–	
RG		2			Symmetric proliferative	Yes	[5 ^{**} ,7 ^{**}]
RG		1	1		Asymmetric mono-differentiative	Yes	[5 ^{**} ,7 ^{**}]
RG			2		Symmetric differentiative	–	
RG		1		1	Asymmetric mono-differentiative	Yes	[5 ^{**} ,7 ^{**} ,18,19]
RG				2	Symmetric differentiative	Yes	[7 ^{**}]
RG			1	1	Asymmetric bi-differentiative	–	
BP			2		Symmetric proliferative	Yes	[5 ^{**}]
BP			1	1	Asymmetric mono-differentiative	–	
BP				2	Symmetric differentiative	Yes	[5 ^{**} –7 ^{**}]

Only divisions along the 'forward' lineage (see Figure 1) are listed. BP, basal progenitor; N, neuron; NE, neuroepithelial cell; RG, radial-glia cell.

see [5^{••},7^{••}] (Table 1). Given that both types of progenitor extend across the entire apical–basal width of the neural tube wall, such divisions will increase the number of radial units [14]; that is, lead to lateral expansion of the neural tube wall.

An unresolved, key issue is whether basal and subventricular-zone progenitors also self-amplify by symmetric proliferative divisions (basal progenitor → basal progenitor + basal progenitor) before they undergo terminal neurogenic division (basal progenitor → neuron + neuron) (Table 1). As most, if not all, basal and subventricular-zone progenitors, at least in rodents, retract their apical extensions and consequently lose their radial morphology [7^{••}], their symmetric proliferative divisions could well lead to their accumulation in the radial (rather than lateral) dimension, thereby increasing the thickness of the subventricular zone. In this context, it will be important to determine whether the increase in the thickness of the subventricular zone during the evolution of mammalian brains reflects interspecies differences in the extent of a possible self-amplification of basal and subventricular-zone progenitors by symmetric proliferative divisions, rather than their increased production from neuroepithelial and radial-glia cells and/or their decreased consumption by symmetric neurogenic divisions.

The mammalian homologues of Numb, a key regulator of asymmetric division of *Drosophila* neuroblasts, have recently been shown to be important for the balance between symmetric proliferative, asymmetric neurogenic and symmetric neurogenic divisions of neuronal progenitors. Thus, loss of Numb and Numlike in the developing mouse forebrain results in an increase in neuronal progenitor cell number due to an increase in symmetric proliferative divisions (progenitor → progenitor + progenitor) and a reduction in asymmetric neurogenic (progenitor → progenitor + neuron) and symmetric neurogenic (progenitor → neuron + neuron) divisions [15]. It remains to be established which of the neuronal progenitor types (neuroepithelial cells, radial-glia cells, and/or basal/subventricular-zone progenitors) is increased in number. Similarly, forced expression of constitutively active Notch shifts the balance of radial-glia cell division from neurogenic to self-renewing/proliferative divisions [16].

Divisions that generate 'downstream' progenitors

With regard to radial-glia cells, little is known about the extent to which the transformation of neuroepithelial cells into these cells [3] occurs by asymmetric mono-differentiative division (neuroepithelial cell → neuroepithelial cell + radial-glia cell), symmetric differentiative division (neuroepithelial cell → radial-glia cell + radial-glia cell), or asymmetric bi-differentiative division (neuroepithelial cell → radial-glia cell + basal

progenitor; or neuroepithelial cell → radial-glia cell + neuron) (Table 1).

Regarding basal and subventricular-zone progenitors, time-lapse imaging has revealed that these cells arise from asymmetric mono-differentiative division of radial-glia cells (radial-glia cell → radial-glia cell + basal progenitor) [5^{••},7^{••}], but it is unclear whether they can also arise from symmetric differentiative division of the latter cells (radial-glia cell → basal progenitor + basal progenitor). As basal progenitors appear at the very onset of neurogenesis [6^{••}], that is prior to the appearance of radial-glia cells, they must also originate from neuroepithelial cells, but the underlying type of division remains to be elucidated (Table 1).

Divisions that generate neurons

Neurons are born by both asymmetric and symmetric divisions of various progenitor cells. With regard to asymmetric divisions, time-lapse imaging has shown that neurons originate from asymmetric mono-differentiative division of neuroepithelial cells (neuroepithelial cell → neuroepithelial cell + neuron) [6^{••},17] and radial-glia cells (radial-glia cell → radial-glia cell + neuron) [5^{••},7^{••},18,19]. Presumably, neurons also arise from bi-differentiative division of neuroepithelial cells (neuroepithelial cell → radial-glia cell + neuron) [2]. Other asymmetric divisions as a potential source of neurons remain to be investigated (radial-glia cell → basal progenitor + neuron) or appear unlikely (basal progenitor → basal progenitor + neuron) [5^{••}–7^{••}]. With regard to symmetric divisions, neurons have been shown, or are thought, to originate from such divisions of basal and subventricular-zone progenitors (basal progenitor → neuron + neuron) [5^{••}–7^{••}], radial-glia cells (radial-glia cell → neuron + neuron) [7^{••}] and, presumably, neuroepithelial cells (neuroepithelial cell → neuron + neuron) [20] (Table 1). Of course, symmetric neurogenic divisions of these progenitors (progenitor → neuron + neuron) may very well be asymmetric in terms of the neuronal subtype that arises. The observation [21] that pairs of neurons arising from single progenitors differ in the expression of certain transcription factors, such as Pax6 and Ngn2, is likely to reflect this at the molecular level.

Apical–basal cell polarity and symmetric versus asymmetric progenitor cell division Neuroepithelial and radial-glia cells

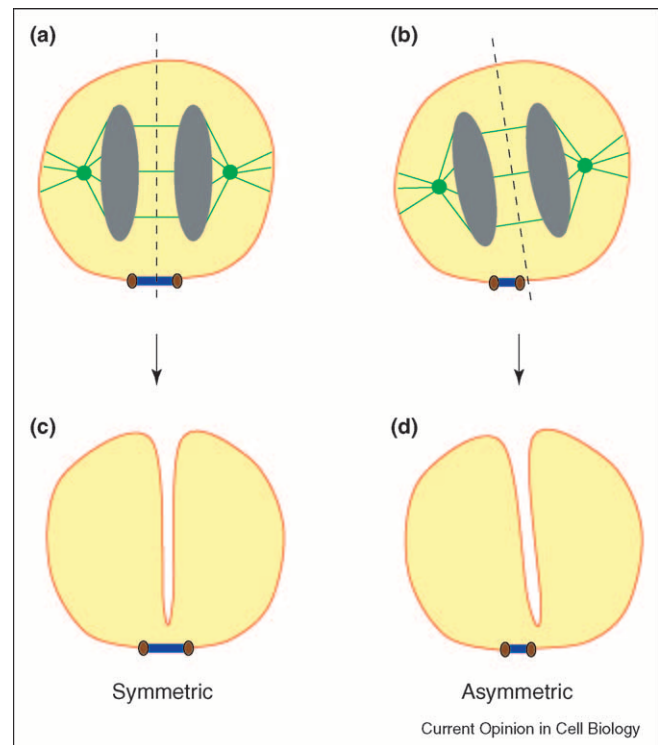
The apical–basal polarity of neuroepithelial cells and radial-glia cells is a prerequisite for the balance between symmetric and asymmetric divisions of these progenitors. Loss of the mammalian homologue of the *Drosophila* lethal giant larvae gene, *Lgl1*, results in disruption of neuroepithelial cell polarity and hyperproliferation of neuroepithelial and radial-glia cells in the developing mouse brain [22].

The apical–basal polarity of neuroepithelial and radial-glia cells also provides a cell-biological basis to explain their symmetric versus asymmetric division. Thus, a cleavage plane perpendicular to the luminal surface of the ventricular zone (vertical cleavage plane) has been proposed to result in symmetric divisions of neuroepithelial and radial-glia cells, because distinct apical and basal cell constituents would be distributed equally to the daughter cells, whereas a cleavage plane parallel to the luminal surface of the ventricular zone (horizontal cleavage plane) should result in asymmetric divisions of neuroepithelial and radial-glia cells because the apical constituents would be inherited by one daughter cell and the basal constituents by the other [23].

In the light of the scarcity of horizontal cleavage planes reported for many vertebrate neuroepithelial cells and radial-glia cells [24–27,28^{••}], an important change in the concept of the role of cell polarity in symmetric versus asymmetric division of mammalian neural progenitor cells originated from a consideration of the geometry of neuroepithelial and radial-glia cells [29]. As these polarized cells are very elongated, their apical plasma membrane and adjacent adherens junctions constitute only a minute fraction of the total plasma membrane (in fact, 1–2%; [28^{••}]). Hence, the orientation of the cleavage plane relative to the overall luminal surface of the ventricular zone is an insufficient criterion to predict whether key molecules with a polarized distribution along the apical–basal axis of neuroepithelial and radial-glia cells are passed on symmetrically or asymmetrically to the daughter cells. Indeed, cleavage planes perpendicular to the overall luminal surface of the ventricular zone (vertical cleavage planes) have been observed to result in either symmetric or asymmetric inheritance of the neuroepithelial or radial-glia cell apical plasma membrane, depending on whether this membrane is bisected (Figure 2a) or bypassed (Figure 2b), respectively, on cytokinesis [28^{••}].

Remarkably, using expression of the *Tis21* gene as a marker to specifically identify neurogenic divisions of neuroepithelial and radial-glia cells [6^{••},30], almost 90% of the divisions leading to an asymmetric inheritance of apical plasma membrane were found to be neurogenic, with >85% of the latter cells showing a vertical cleavage plane, whereas >80% of the divisions leading to a symmetric inheritance of apical plasma membrane were found to generate only progenitor cells [28^{••}]. In the case of the asymmetric neurogenic divisions of neuroepithelial and radial-glia cells, the daughter cell inheriting the apical plasma membrane is thought to remain a neuroepithelial or radial-glia cell, whereas the daughter cell not inheriting this membrane is thought to become the neuron [28^{••}]. (In this context, it has recently been claimed [31] that newborn neurons in the ventricular zone possess apical processes that reach the ventricle and form adherens junctions with adjacent cells. However, by confining

Figure 2



Symmetric versus asymmetric division of neuroepithelial and radial-glia cells with vertical cleavage plane orientation. (a,b) Orientation of the mitotic spindle. (a) Symmetric division – the orientation of the mitotic spindle is exactly perpendicular to the apical–basal axis, resulting in the cleavage plane bisecting the apical plasma membrane and adjacent junctional complexes. (b) Asymmetric division – the orientation of the mitotic spindle shows a small deviation from exactly perpendicular to the apical–basal axis, resulting in the cleavage plane bypassing the apical plasma membrane and adjacent junctional complexes. (c,d) Plasma membrane fusion on completion of cytokinesis. (c) Symmetric division – the membrane of the cleavage furrow fuses with the apical plasma membrane, resulting in equal inheritance of this membrane and adjacent junctional complexes by the daughter cells. (d) Asymmetric division – the membrane of the cleavage furrow fuses with the apical-most lateral plasma membrane, resulting in unequal inheritance of this membrane and adjacent junctional complexes (i.e. they are only inherited by one of the daughter cells). Blue, apical plasma membrane; brown, junctional complexes; red, basolateral plasma membrane; green dots, centrosomes; green lines, microtubules; grey, sister chromatids; dashed lines, cleavage plane. Note that the basal process maintained during M-phase [18,19] is omitted for clarity.

BrdU labeling to 2 hrs, Minaki *et al.* [31] fail to exclude the possibility that the mRNA of a ‘neuronal’ marker, *Neph3*, is actually expressed in dividing progenitor cells during G_1 — as is, for example, the mRNA for *Tis21* [30] — and hence the central conclusions of this study may be questioned.)

Thus, by providing a cell-biological explanation for how a vertical cleavage plane can result not only in symmetric, but also in asymmetric division of neuroepithelial and

radial-glia cells, the observations by Kosodo *et al.* [28**] resolve the seemingly contradictory observations (see also [32]) that neurons, at least during the early stage of mammalian neurogenesis, arise from asymmetric divisions of neuroepithelial and radial-glia cells [6**,19,23,33], but that the vast majority of neurogenic neuroepithelial and radial-glia cells show a vertical cleavage plane orientation [28**]. The concept proposed by Kosodo *et al.* [28**] may well hold true not only for mammalian neuroepithelial and radial-glia cells but also for those of lower vertebrates, given that vertical cleavage planes prevail during neurogenesis in the zebrafish neural tube in general, and specifically in progenitors dividing asymmetrically into a progenitor and a neuron [34,35].

In contrast to most of the studies determining the cleavage plane orientation in unmanipulated tissue fixed *in vivo*, in which as much as >90% of all mitotic neuroepithelial and radial-glia cells and >85% of the neurogenic neuroepithelial and radial-glia cells show a vertical orientation of the cleavage plane [24–27,28**], studies using time-lapse imaging in slice cultures have reported a significantly higher proportion of non-vertical cleavage planes (up to 50%) [17,23,36]. The reason for this discrepancy is presently unclear, but possible explanations include differences in the systems used (e.g., *in vivo* versus slice cultures [17], absence versus presence of serum, etc).

Neuroepithelial/radial-glia cell-derived apical membrane particles in the neural tube lumen

In mitotic neuroepithelial and radial-glia cells with a symmetric vertical cleavage plane (one that bisects the apical plasma membrane), the apical plasma membrane was found to constitute ~2% of the total cell membrane, whereas in cells with an asymmetric vertical cleavage plane (one that bypasses the apical plasma membrane) it was found to constitute only ~1% [28**]. How, then, do neuroepithelial and radial-glia cells reduce their apical plasma membrane? An unexpected possible explanation has arisen from the recent report [37*] that the luminal fluid of the embryonic neural tube contains a novel class of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133), referred to as ‘prominosomes’. Prominosomes exist in two size classes, ~600-nm particles and 50–80-nm particles, which presumably originate from specific structures of the apical plasma membrane of neuroepithelial and radial-glia cells. While the 600-nm prominosomes have so far only been observed in the neural tube fluid of the developing embryo, the 50–80-nm prominosomes also occur in various extracellular body fluids of adults, being distinct from the similarly sized exosomes [37*]. The functional significance of prominosomes remains to be elucidated, but a role in progenitor cell differentiation and/or intercellular communication are reasonable guesses.

Basal progenitors and subventricular-zone progenitors

Initially after their birth by divisions of neuroepithelial and radial-glia cells at the apical surface of the ventricular zone, basal and subventricular-zone progenitors in rodents possess a process extending towards the ventricular surface, but retract this process before M-phase [7**]. It will be important to determine whether the plasma membrane of this process contains, at least initially, apical membrane and the apical-most adherens junctions of the lateral membrane, or whether it consists only of lateral membrane. Given that the other daughter cell born along with the future basal or subventricular-zone progenitor at the apical surface of the ventricular zone is a neuroepithelial or radial-glia cell [7**] (which inherits apical membrane and adherens junctions), inheritance of apical membrane and adherens junctions by the newborn basal or subventricular-zone progenitor as well would imply that these distinct daughter cells arose from a cell-biologically symmetric division, whereas lack of such inheritance would imply an asymmetric division.

Whatever the answer, the retraction of the basal or subventricular-zone progenitor’s apical process prior to M-phase [7**] probably reflects down-regulation of its apical–basal polarity. Consistent with this view, rodent basal and subventricular-zone progenitors appear to undergo neurogenic divisions irrespective of the orientation of their cleavage plane, as two neuronal daughters have been observed to arise from progenitors with either vertical or horizontal cleavage planes [5**,6**].

Molecular machinery underlying symmetric versus asymmetric progenitor cell division

In light of the importance of cleavage plane orientation of polarized neuroepithelial and radial-glia cells for the fate of their daughter cells, the question arises of how this orientation is controlled. As the cleavage plane depends on the position of the mitotic spindle, the machinery governing mitotic spindle positioning in neuroepithelial and radial-glia cells becomes a central issue. Two sets of key players in this area have emerged recently: centrosomal proteins and heterotrimeric G-proteins.

Centrosomal proteins

A central component of the centrosome is Nde1 (mNudE) [38]. Nde1 mRNA is expressed in the ventricular zone, in particular at early developmental stages (E11) [38], when symmetric proliferative divisions of neuroepithelial cells prevail. Interestingly, ablation of Nde1 leads, first, to an increase in horizontal cleavage plane orientation of neuroepithelial and radial-glia cells at the expense of vertical cleavage plane orientation; second, to a progressive depletion of ventricular zone progenitors of the cerebral cortex that becomes particularly evident at later stages of neurogenesis; third, to an increase in early-born neurons and a reduction in

late-born neurons, and in consequence, to a smaller cerebral cortex [39^{••}].

Another gene whose loss-of-function phenotype is a smaller brain is *ASPM* (abnormal spindle-like microcephaly-associated) [40]. *Aspm* is expressed in the ventricular zone and, extrapolating from the role of the *Drosophila* orthologue *Asp* in mitotic microtubule organization at centrosomes [41], may well be involved in controlling the positioning of the mitotic spindle in neuroepithelial and radial-glia cells [40].

Thus, the consensus view emerges that centrosomal proteins such as *Aspm* and *Nde1*, which do not appear to be required for cell division as such, are of critical importance for the positioning of the mitotic spindle precisely perpendicular to the apical–basal axis of the neuroepithelial or radial-glia cell, a prerequisite for a symmetric proliferative division of these highly polarized cells [28^{••}]. The developmental regulation of *Aspm* and *Nde1* expression in the ventricular zone [38,40] is certainly consistent with these proteins being predominantly required in the subpopulation of neuroepithelial and radial-glia cells undergoing symmetric proliferative divisions. Given that the apical plasma membrane constitutes only a tiny fraction of the total plasma membrane of mitotic neuroepithelial and radial-glia cells [28^{••}], a reduction in the precision of spindle-pole positioning perpendicular to their apical–basal axis, resulting for example from a lack of centrosomal proteins such as *Aspm* and *Nde1*, will probably result, by default, in their asymmetric neurogenic division (with the cleavage plane bypassing the apical plasma membrane, Figure 2b) rather than their symmetric proliferative division (with the cleavage plane bisecting the apical plasma membrane, Figure 2a). This, in turn, would lead to premature switching from progenitor cell expansion to neurogenesis and, ultimately, to microcephaly. Consistent with this, other genes that cause primary microcephaly in humans when mutated also encode proteins (*CDK5RAP2* and *CENPJ*) expressed in the ventricular zone and localized to mitotic spindle poles [42].

Heterotrimeric G-proteins

Previous studies in *C. elegans* and *Drosophila* established a role for heterotrimeric G-proteins in the positioning of the mitotic spindle [43]. A recent study [44[•]] reports a similar role in the neuroepithelial and radial-glia cells of the mouse neocortex. Specifically, by manipulating the balance of heterotrimeric $G\alpha i3-\beta\gamma$ versus free $G\beta\gamma$ and by forcing free $G\beta\gamma$ heterodimers into an inactive complex with an inhibitor, it appears that when $G\beta\gamma$ heterodimers are free and thus able to interact with downstream effectors, as much as 50% of the cleavage planes of neuroepithelial and radial-glia cells show a non-vertical (i.e. horizontal or oblique) orientation. In contrast, when $G\beta\gamma$ subunits are in the heterotrimeric state or complexed to

an inhibitor (i.e. unable to interact with downstream effectors), 80–90% of the cleavage planes show a vertical orientation [44[•]]. $G\alpha i$ recruits a protein called LGN to the cell cortex, which in turn recruits the microtubule-binding protein NuMA [45]; $G\beta\gamma$ subunits too may interact with microtubules [44[•]]. Thus, in mammalian neuroepithelial and radial-glia cells, heterotrimeric G-protein subunits appear to be involved in directing the aster microtubules of the mitotic spindle to specific sites of the cell cortex. Consistent with this, $G\beta\gamma$ subunits form cortical domains [44[•]], and LGN is asymmetrically localized in mitotic neural progenitor cells [46]. However, the exact mechanism of spindle pole positioning, including the significance of the greater oscillation of spindle poles that precedes a subsequent horizontal (as opposed to vertical) cleavage plane orientation [36], remains to be elucidated.

A key issue in this context are the — as yet unidentified — integral constituents of the plasma membrane that contribute to the formation of the relevant spindle-pole-positioning cortical sites. It may be significant that $G\alpha i$, via its lipid anchors, partitions into cholesterol-based membrane microdomains called lipid rafts, whereas the free $G\beta\gamma$ heterodimer does not [47]. Perhaps cholesterol-based membrane microdomains have a role in the positioning of the mitotic spindle, maybe even in a differential manner between symmetric and asymmetric divisions.

Cytokinesis furrow and polarity of plasma membrane fusion

Both the asters and the midzone of the mitotic spindle determine the site of formation and direction of ingression of the cleavage furrow during cytokinesis [48[•]]. Interestingly, in mitotic neuroepithelial and radial-glia cells, the formation of a vertical cleavage furrow proceeds in a basal-to-apical direction [28^{••}]. Hence, given the small relative size of the apical plasma membrane [28^{••}], a symmetric distribution of this membrane and of the adjacent adherens junctions to the daughter cells will not only require an overall vertical orientation of the cleavage furrow (i.e. perpendicular to the mitotic spindle) but will also depend on whether the furrow plasma membrane eventually fuses with the apical (Figure 2c), rather than apical-most lateral (Figure 2d), plasma membrane on completion of cytokinesis. Little is known about the SNARE proteins mediating the final plasma membrane fusion during cytokinesis of neuroepithelial and radial-glia cells, or about their apical–basal polarity, but it has recently been hypothesized [2] that the symmetric versus asymmetric division of neuroepithelial and radial-glia cells — that is, the inheritance of critical apical constituents by both versus only one of the daughter cells — involves the control of basal–apical versus basal–lateral SNARE-mediated plasma membrane fusion, respectively. Consistent with this, the apical localization of the SNARE *Vamp7* (TI-VAMP) is profoundly

disrupted in *hvh* (hydrocephalus with hop gait) mice, which carry a mutation in the gene encoding α SNAP (a member of the membrane fusion machinery), and neuronal progenitors apparently switch prematurely from proliferative to neurogenic divisions [49^{*}], perhaps as a result of impaired basal–apical membrane fusion during cytokinesis.

Cell structures and key molecules with symmetric versus asymmetric inheritance

Cell structures and key molecules showing a polarized distribution in vertebrate neuroepithelial and radial-glia cells that are candidates to be inherited either equally or unequally and thereby to contribute to either symmetric or asymmetric daughter cell fate have recently been reviewed [1,2,32]. These include apical plasma membrane constituents, components of adherens junctions, and other players, notably Numb. Although in the case of the latter cell fate determinant the issue of symmetric versus asymmetric inheritance and daughter cell fate is unclear in the case of progenitor divisions with vertical cleavage planes [1,32], in the case of progenitor divisions with horizontal cleavage planes, the asymmetric inheritance of Numb appears to correlate with asymmetric daughter cell fate [1,17,32]. Below, we will concentrate on the basal process of neuroepithelial and radial-glia cells.

The basal process

An unexpected previous observation was that neuroepithelial and radial-glia cells undergoing mitosis at the ventricular (apical) surface of the ventricular zone retain, through M-phase, a thin process extending to the basal lamina [18]. Over recent years the interpretation of this finding has become broader than originally conceived, as the basal process, upon division of neuroepithelial and radial-glia cells, has been thought to be inherited by the neuron [18], the daughter neuroepithelial or radial-glia cell [50], or either [20], and both asymmetric [18,50] and symmetric [20] cell divisions (in terms of daughter cell fate) have been implicated. Irrespective of this, however, it is commonly thought that the basal process is inherited asymmetrically, in other words by only one of the daughter cells. With regard to symmetric neuroepithelial and radial-glia cell divisions, this would imply that such divisions are asymmetric in cell biological terms [1,20]. Given this lack of correlation between inheritance of cellular structure and daughter cell fate, it may be a worthwhile effort to re-address the issue of inheritance of the basal process by carrying out a detailed analysis of its structure and dynamics in M-phase neuroepithelial and radial-glia cells.

Planar polarity and asymmetric division of neuroepithelial and radial-glia cells

It was previously reported that during the development of the zebrafish retina, neuroepithelial cells, with respect to apical–basal polarity, show overwhelmingly vertical cleavage planes (i.e. planes parallel to the apical–basal axis of

the cells) [34,35,51], but, with respect to the two principal planar axes of the retina, change the orientation of the cleavage plane by 90° within the plane of the neuroepithelium, such that the daughter cells at early stages have predominantly central–peripheral positions and at later stages predominantly circumferential positions [51]. Although these observations would be consistent with planar polarity having a role in retinal progenitor divisions, a recent study reports that progenitor cleavage planes in the chick and rat retina are randomly oriented with respect to the planar axes, and concludes that planar polarity plays no significant role [52]. It remains to be investigated whether this discrepancy in observations reflects species differences or, as discussed above regarding the differences in cleavage plane orientation relative to the apical–basal axis, can be explained by the different experimental approaches used (e.g. live imaging versus analysis of fixed tissue).

In the developing mouse forebrain, compelling evidence for planar polarity and its role in asymmetric progenitor division has recently been provided [53^{*}]. Specifically, in mitotic neuroepithelial and radial-glia cells showing a vertical cleavage plane orientation, the EGF receptor is concentrated at the lateral cell cortex of one of the arising daughter cells, and the daughter cells arising from progenitors showing an unequal distribution of EGF receptor in M-phase have asymmetric (albeit glial, astrocyte/oligodendrocyte) fate, at least *in vitro* [53^{*}].

Symmetric versus asymmetric progenitor cell division and the role of cell cycle length

According to a recent model, referred to as the ‘cell cycle length hypothesis’ [54], an unequal inheritance of a cell fate determinant by the daughter cells upon progenitor cell division may or may not lead to asymmetric daughter cell fate, depending on the length of the cell cycle, in other words whether or not this cell fate determinant is allowed to function for a sufficient length of time (for review, see [2]). If the cell cycle is too short for the cell fate determinant to induce, for example, differentiation, even in the cell that inherited more of the determinant, both daughter cells will adopt a symmetric, undifferentiated fate. If the cell cycle is longer, such that the cell fate determinant is able to induce differentiation in the daughter cell that inherited more of it but not in the other daughter cell, the fates of the cells will hence be asymmetric. If the cell cycle is even longer, such that the cell fate determinant is able to induce differentiation in both daughter cells, then both will adopt a symmetric, differentiated fate [54].

In addition to the finding that lengthening the neuroepithelial cell cycle can alone be sufficient to trigger differentiation (i.e. neurogenesis) [54], three recent studies in mammalian embryos support the cell cycle length hypothesis. First, shortening the cell cycle, specifically G₁,

of mouse neuroepithelial or radial-glia cells by IGF-1 increases the probability of cell-cycle re-entry of their progeny; in other words, the balance between proliferative and neurogenic divisions of progenitors is shifted towards the former type of division [55]. Second, in the primate cortex, progenitors in the outer subventricular zone [12] of area 17 have a shorter G_1 phase, and their progeny have a greater probability of cell cycle re-entry, than do those of area 18, with cyclin E and p27 emerging as major positive and negative regulators, respectively, of cell cycle progression [13]. Third, for any given stage of neurogenesis and region of the developing mouse brain, progenitors undergoing neurogenic divisions have a longer cell cycle than those undergoing proliferative divisions [56].

Conclusions

Four major types of neuronal progenitors in the developing mammalian CNS have been recognized: neuroepithelial cells; radial-glia cells; basal and subventricular-zone progenitors; and the primate-specific outer subventricular-zone progenitors. These progenitors may undergo various types of division with regard to the resulting progeny. The issue of whether divisions are symmetric or asymmetric in terms of daughter cell fate is intimately linked to the polarized organization of the progenitor, the equal versus unequal inheritance of cell fate determinants by the daughter cells, and cell cycle length, as the latter factor determines the time determinants are allowed to act. The simplistic view that cleavage planes perpendicular to the apical–basal axis of neuroepithelial and radial-glia cells result in asymmetric division and cleavage planes parallel to this axis in symmetric division has been revised by the demonstration that the latter kind of cleavage plane orientation can lead to either symmetric or asymmetric division, depending on the equal or unequal inheritance of highly polarized cell constituents such as the apical plasma membrane. The molecular machinery controlling the positioning of the mitotic spindle and cleavage furrow is beginning to be unravelled, with centrosomal proteins and heterotrimeric G proteins emerging as key players. Besides further elucidating symmetric versus asymmetric divisions of neuronal progenitors at the level of molecular cell biology, the major challenges for future research will be to determine which aspects of the lineage from neuroepithelial cells to neurons are subject to change during the evolution of mammalian brains, how the changes are caused at the molecular level, and which mutations in the genome are responsible.

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