

Making bigger brains – the evolution of neural-progenitor-cell division

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Summary

Relative brain size differs markedly between species. This variation might ultimately result from differences in the cell biology of neural progenitors, which might underlie their different proliferative potential. On the basis of the cell-biological properties of neural progenitors of animals of varying brain size and complexity (namely, *Drosophila melanogaster*, rodents and primates), we hypothesize that the evolution of four related cell-biological features has contributed to increases in neuron number. Three of these features – the pseudostratification of the progenitor layer, the loss of (Inscuteable-mediated) mitotic-spindle rotation and the evolution of proteins (such as *Aspm*) that maintain the precision of symmetric progenitor division – affect the mode of cell division in the apically dividing progenitors of the ventricular zone. The fourth feature, however, concerns the evolution of

the basally dividing progenitors of the subventricular zone. In rodents, these basal (or intermediate) progenitors lack cell polarity, whereas in primates a subpopulation of radial, presumably polarized, progenitors has evolved (outer-subventricular-zone progenitors). These cells undergo basal mitoses and are thought to retain epithelial characteristics. We propose the epithelial-progenitor hypothesis, which argues that evolutionary changes that promote the maintenance of epithelial features in neural progenitors, including outer-subventricular-zone progenitors, have been instrumental in the expansion of the cerebral cortex in primates.

Key words: Brain size, Cerebral cortex, Neural stem and progenitor cells, Neurogenesis, Radial glia, Symmetric and asymmetric cell division

Introduction

One of the most fascinating topics in biology is the increase in relative brain size (brain size relative to body size) during vertebrate, and in particular mammalian, evolution. Notably, there is an approximate 15-fold increase in relative brain size from mouse to human (Table 1). Several hypotheses have been generated to explain the increases in relative brain size that have occurred during mammalian evolution, and these have generally agreed on two tenets: (1) the increase in brain size primarily results from an increase in total cell number (rather than cell size), and (2) the observed expansion of the cerebral cortex predominantly reflects an increase in surface area (lateral expansion) rather than cortical thickness (radial growth) (Fig. 1) (Caviness et al., 1995; Rakic, 1995; Rakic, 2007; Rockel et al., 1980). Cell number and the direction of cortical expansion and growth are determined by the number and mode of neural-progenitor-cell divisions (which can be either symmetric or asymmetric; Fig. 1) (see Box 1 for a glossary of key neurobiological terms). However, despite the wide acceptance of these tenets, the precise cell-biological mechanisms that underlie the evolutionary changes in the number and mode of neural-progenitor-cell divisions have yet to become the focus of discussion.

In this Commentary, we concentrate on the evolutionary changes that might have driven an increase in the number of neurons that are generated during development of the mammalian brain (Table 1) (rather than the number of postmitotic glial cells, which also contributes to increases in relative brain size). First, we describe certain basic cell-biological features of neural progenitor cells in animals that represent diverse levels of central nervous system

(CNS) size and complexity – *Drosophila melanogaster* as the invertebrate paradigm, rodents as the predominant mammalian model and primates as the phylogenetic group of ultimate interest. Because this Commentary focuses on evolution of the mammalian brain, we do not discuss zebrafish or chick, which have served as non-mammalian vertebrate models. On the basis of these data, we outline four related changes in the cell biology of neural progenitor cells that, we hypothesize, have been important for evolutionary increases in relative brain size in mammals. In particular, we focus on the processes that maintain symmetric cell division (thereby generating more neural progenitor cells), on the constraints that limit progenitor proliferation and on how neural progenitors have overcome these constraints in the process of mammalian encephalization.

Key features of neural progenitor cells

Neural stem cells and progenitor cells in *D. melanogaster*

In *D. melanogaster*, the development of the CNS involves three types of dividing cells – neuroectodermal cells, neuroblasts and ganglion mother cells – which occupy distinct layers within a stratified tissue in the embryo. The most-apical layer is the neuroectoderm, a sheet of cuboidal epithelial cells (Fig. 2A, bottom). In *D. melanogaster*, neuroblasts (neural stem and progenitor cells) lose their intercellular junctional connections and delaminate from, and subsequently divide basally to, the neuroectodermal epithelium (Fig. 2A, top). Neuroblast divisions are asymmetric, and generate another neuroblast and a smaller ganglion mother cell (not shown). In the most-basal layer, the ganglion mother

Table 1. Relative brain size in selected mammals

Species	Relative brain size (EQ)	Neurons in brain
Mouse	0.5	10 ⁷
Monkey	2.09	10 ⁹
Human	7.44	10 ¹¹

Relative brain size is given in terms of encephalization quotient (EQ) (Jerison, 1973); data are taken from Macphail (Macphail, 1982). The estimates of neuron number in the brain are taken from Braitenberg (Braitenberg, 2001).

cell undergoes a symmetric, consumptive division to generate either two neurons or two glial cells (Doe, 2008; Egger et al., 2008; Roegiers and Jan, 2004; Wang and Chia, 2005; Wodarz and Huttner, 2003).

Neuroectodermal cells and neuroblasts have distinct modes of cell division. This distinction is not due to differences in cell polarity, because both cell types are polarized along their apicobasal axes, but rather is caused by mitotic-spindle alignment with respect to the axis of polarity. In neuroectodermal cells, the spindle aligns parallel to the apical surface, which results in a cleavage plane that is parallel to the apicobasal axis (vertical cleavage plane, referred to in short as ‘vertical cleavage’) and in the symmetric distribution of polarized cell-fate determinants (Fig. 2A, bottom). By contrast, the spindle of neuroblasts rotates by 90° during metaphase, and ultimately aligns perpendicularly to the overlying epithelial (neuroectodermal) layer. Consequently, neuroblasts divide with a cleavage plane that is perpendicular to the axis of polarity (horizontal cleavage plane; referred to in short as ‘horizontal cleavage’), which leads to the asymmetric distribution of cell-fate determinants (Fig. 2A, top) (Doe, 2008; Egger et al., 2008; Knoblich, 2008; Roegiers and Jan, 2004; Wang and Chia, 2005; Wodarz and Huttner, 2003). *D. melanogaster* progenitors have thus served as a paradigm for the role of cell polarity in the control of symmetric versus asymmetric cell division.

Neural stem cells and progenitor cells in the rodent

During development of the rodent CNS, two principal classes of neural stem and progenitor cells can be distinguished on the basis of their location during mitosis, and these are referred to as apical and basal progenitors.

Rodent apical progenitors

Apical progenitor cells (APs) include neuroepithelial cells (the primary neural stem and progenitor cells), radial glia and short neural precursors (Fig. 2Bi) (Fishell and Kriegstein, 2003; Gal et al., 2006;

Götz and Huttner, 2005; Huttner and Kosodo, 2005; Kriegstein and Götz, 2003; Rakic, 2003b). Although they differ in certain molecular and cellular features, these cell types all share hallmarks of epithelial cells, including apicobasal polarity, apical junctional complexes and radial bipolar morphology (the presence of elongated apical and basal processes). In all three types of APs, the apical process contacts the ventricular lumen. By contrast, the basal processes of neuroepithelial cells and radial glia, but not those of short neural precursors, extend all the way to the basal lamina (Gal et al., 2006; Hartfuss et al., 2003; Noctor et al., 2002).

An important characteristic of APs is that they undergo interkinetic nuclear migration, in which the nucleus moves along the apicobasal axis of the cell in concert with the cell cycle (Götz and Huttner, 2005; Messier, 1978; Sauer, 1935; Takahashi et al., 1993). The resultant dispersion of AP nuclei is responsible for the pseudostratified appearance of the progenitor-cell layer that is known as the ventricular zone (VZ) (Boulder Committee, 1970). Importantly, although AP nuclei are located throughout the VZ, they migrate apically during the G2 phase of the cell cycle and undergo mitosis only when the nucleus is at the apical surface (Sauer, 1935) (Fig. 2Bi). AP divisions predominantly occur with a vertical or near-vertical cleavage plane (Estivill-Torrus et al., 2002; Fish et al., 2006; Heins et al., 2001; Huttner and Brand, 1997; Konno et al., 2008; Kosodo et al., 2004; Landrieu and Goffinet, 1979; Smart, 1972a; Smart, 1972b; Smart, 1973; Stricker et al., 2006). Self-renewing divisions of APs can be either symmetric (to generate two APs) or asymmetric (to generate one AP and one more-differentiated cell) (Fig. 2Bi) (Götz and Huttner, 2005; Huttner and Kosodo, 2005).

Rodent basal progenitors

Basal progenitors (BPs) constitute the second class of neural progenitors in rodents (Fig. 2Bii) and are also called intermediate, non-surface or subventricular zone (SVZ) progenitors (Götz and Huttner, 2005; Haubensak et al., 2004; Kriegstein et al., 2006; Miyata et al., 2004; Noctor et al., 2004; Pontious et al., 2008). BPs arise from an apical division, migrate basally and retract their apical process before mitosis. Importantly, BPs are found almost exclusively in the telencephalon and are only rarely observed in the hindbrain or spinal cord (Haubensak et al., 2004; Smart, 1972a; Smart, 1973). In the telencephalon, BPs are generated from the onset of neurogenesis and increase in number as neurogenesis proceeds, eventually accumulating to form a secondary proliferative layer, the SVZ, which lies basal to the VZ (Boulder Committee, 1970). BPs have a round, rather than a radial, morphology and are randomly distributed within the SVZ. Almost all BPs divide symmetrically (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Attardo et al., 2008; Noctor et al., 2008) (Fig. 2Bii, right). The vast majority (~90%) of these divisions

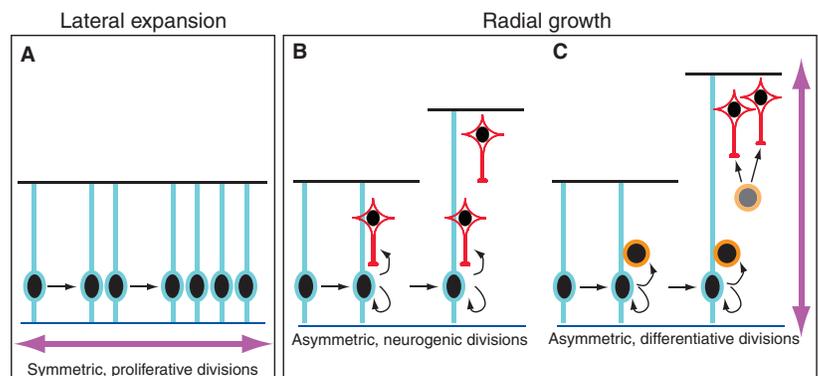


Fig. 1. The mode of neural-progenitor-cell division, and its effect on the direction of cortical expansion and growth (lateral expansion versus radial growth). (A) Lateral expansion (double-headed arrow), in which one AP generates two AP daughter cells, occurs as a result of the symmetric, proliferative division of APs (blue). (B,C) Radial growth (double-headed arrow) occurs as a result of either (B) asymmetric, neurogenic divisions of APs (blue), in which one AP generates a further AP as well as one neuron (red), or (C) asymmetric, differentiative divisions of APs (blue), in which one AP generates one AP and one BP (orange), which in turn generates two neurons (red) and is thereby consumed (dimmed orange).

Box 1. Glossary of key neurobiological terms**Interkinetic nuclear migration**

The apical-to-basal (G1 phase) and subsequent basal-to-apical (G2 phase) migration of the nucleus during the cell cycle of epithelial, notably neuroepithelial, cells.

Pseudostratified epithelium

An epithelium (such as the neuroepithelium) in which all cells extend from the basal lamina to the apical (luminal) surface to form a single-cell layer, but cell nuclei occupy various positions, which results in a stratified appearance.

Telencephalon

The most anterior, rostral part of the vertebrate brain.

Neural stem cell

A neural cell whose division results in self-renewal, with or without the generation of differentiated progeny (neural progenitors, neurons or glial cells).

Neural progenitor cell

A neural cell whose division generates an increased amount of differentiated progeny (an increased number of committed neural progenitors, neurons or glial cells).

generate two neurons, and the remaining ~10% generate two BPs (Noctor et al., 2004; Wu et al., 2005). In rodents, mitotic BPs lack apical-specific proteins, have no significant processes, and are, therefore, both non-epithelial and unpolarized (Attardo et al., 2008; Noctor et al., 2008) (Fig. 2Bii, right; compare Fig. 2Bii, left). The absence of apicobasal polarity is thought to restrict BPs to the symmetric mode of division and might explain why such divisions can occur with nearly random cleavage-plane orientation (Attardo et al., 2008), although symmetric BP divisions might have a higher incidence of horizontal cleavage-plane orientation at early developmental stages (Noctor et al., 2008; Stricker et al., 2006).

Neural stem cells and progenitor cells in primates

Histological descriptions of the developing primate brain suggest that the proliferative layers of the developing primate cortex exhibit features that are both similar to and distinct from the rodent cortex (Bystron et al., 2008; Kornack and Rakic, 1995; Molnar et al., 2006; Rakic, 2003a; Smart et al., 2002). The most striking difference is the dramatic increase in the thickness of the SVZ in the primate cortex relative to the rodent cortex. This expansion of the SVZ is characterized not only by an increase in the number of basally dividing progenitors, but also by their diversity; the SVZ of primates has been reported to contain two morphologically distinct cell layers (Smart et al., 2002). The first layer, which is called the inner SVZ (ISVZ), comprises cells that resemble rodent BPs, owing to their round nuclear shape and random organization (Fig. 2Cii). The second, and considerably thicker, cell layer, which is referred to as the outer SVZ (OSVZ) (Fig. 2Ciii), contains cells that have elongated, radially aligned nuclei, similar to the APs in the VZ (Fig. 2Ci). Other aspects of the cell biology of primate neural progenitors, including the orientation of the cleavage plane, the mode of cell division and the distribution of cell-fate determinants, are largely unknown.

Evolution of the cell biology of neural progenitors

What is the cell-biological basis of the lateral expansion of the mammalian neocortex, which ultimately explains the evolutionary

increase in the number of neurons that are generated during development (Table 1)? There are two principal – and not necessarily mutually exclusive – mechanisms by which neuron number (that is, the total number of neurogenic divisions of neural progenitors) could be increased. One is to increase the number of neural progenitors, which could be achieved by more rounds of symmetric, proliferative divisions of progenitors (in which one progenitor generates two progenitors, each of which subsequently generates a given number of neurons; Fig. 1A). The other mechanism is to increase the number of neurogenic divisions that occur per progenitor, which could be achieved by more rounds of asymmetric, neurogenic (or differentiative) divisions of each progenitor [in which one progenitor generates one progenitor and one neuron (or BP); Fig. 1B,C]. The considerations and hypotheses described below are based on the general concept that increasing the number of symmetric, proliferative divisions of neural progenitors will primarily lead to the lateral expansion of the cortex (Fig. 1A), whereas increasing the number of asymmetric, neurogenic or differentiative divisions per progenitor will predominantly result in increased radial growth, i.e. greater cortical thickness (Fig. 1B,C) (Rakic, 1995; Rakic, 2007).

Differences in the cell biology of neural progenitors among species might underlie differences in their proliferative potential, and thus might ultimately be responsible for differences in relative brain size. We hypothesize that the evolution of four related cell-biological features has contributed to increases in neural-progenitor number, and hence to lateral cortical expansion and to increases in relative brain size. Three of these – the pseudostratification of the VZ, the loss of spindle rotation and the precision of cleavage – concern APs, whereas the fourth, the basal location of progenitor-cell mitosis, concerns rodent BPs and primate OSVZ progenitors. We go on to suggest that the evolutionary changes that promote the maintenance of the epithelial nature of neural progenitor cells have been instrumental in the evolution of the mammalian, and in particular the primate, brain.

Pseudostratification and APs

During brain development in the *D. melanogaster* embryo, neural progenitors (the neuroblasts) undergo asymmetric, neurogenic divisions in a cell layer that is distinct from that of the neuroectodermal epithelial cells, which undergo symmetric, proliferative divisions. By contrast, the vertebrate brain develops from a pseudostratified epithelium (the neuroepithelium) (Rao and Jacobson, 2005), in which cells that undergo symmetric and asymmetric divisions occupy the same single-cell layer and are junctionally connected. Two features of the pseudostratified neuroepithelium of vertebrates are particularly important for increasing the number and proliferative potential of neural progenitors compared with invertebrates: the elongation of APs and the fact that APs undergo apical mitosis.

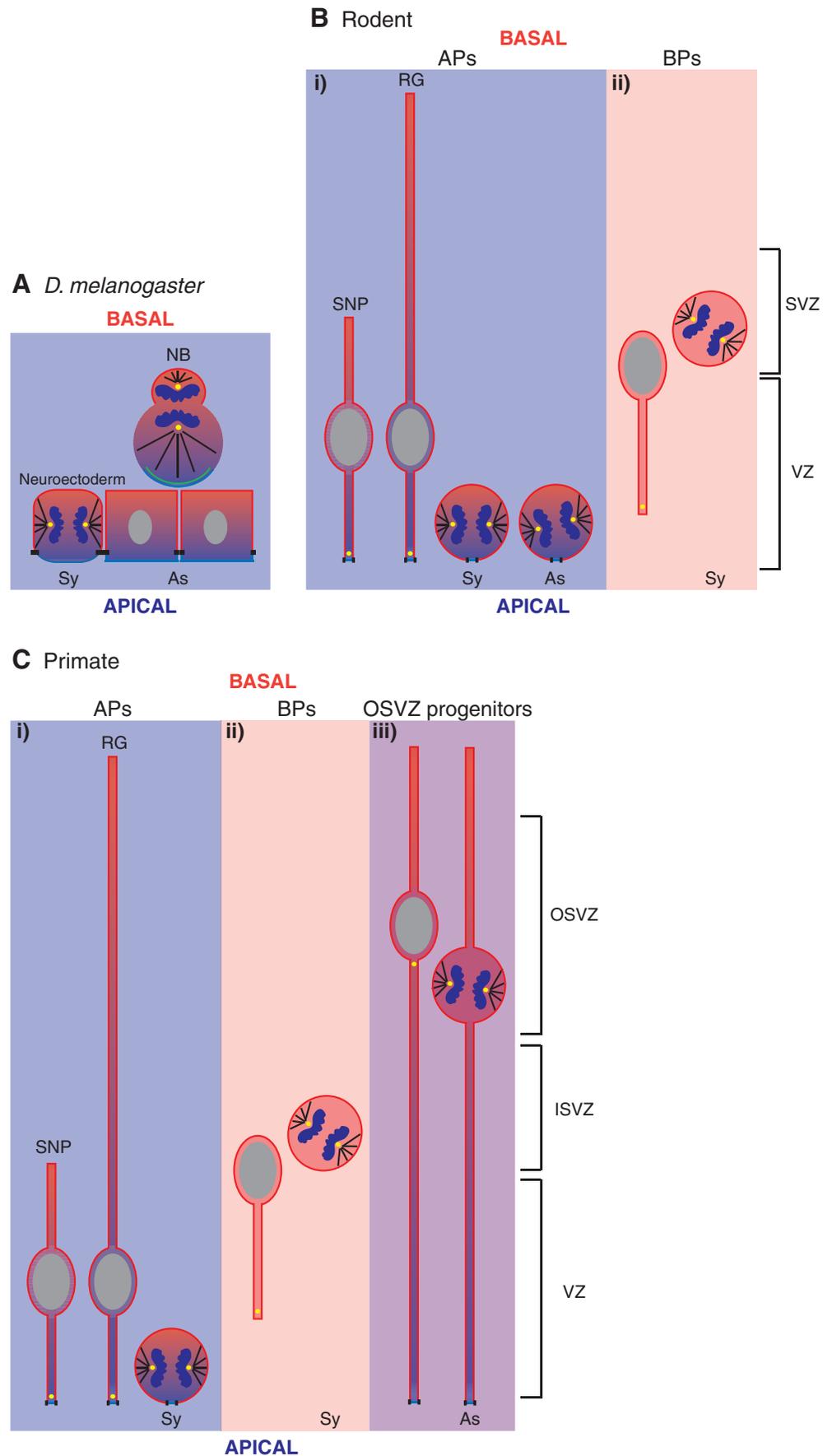
Elongation of APs

Comparative vertebrate neuroanatomy has shown that, during evolution, the external (pial) surface area of the cortex has increased disproportionately to the area of the apical (ventricular) membrane (Rakic and Lombroso, 1998; Smart and McSherry, 1986a; Smart and McSherry, 1986b); likewise, the number of progenitors per unit of apical area has increased in line with increasing relative brain size. This has been made possible because the APs of the vertebrate neuroepithelium are apicobasally elongated (Fig. 3Aii). Elongation is intimately linked to neural-plate formation and neural-tube

Fig. 2. Key features of the cell biology of neural stem and progenitor cells in (A) *D. melanogaster*, (B) rodents and (C) primates. The apical plasma membrane and corresponding cortical domains are represented by blue lines, and the basolateral plasma membrane and corresponding cortical domains are represented by red lines. Interphase nuclei are shown in grey and representative sister chromatids are in dark blue. Black rectangles represent junctional complexes and yellow dots indicate centrosomes or mitotic-spindle poles. For clarity, only the astral microtubules of the mitotic spindle are depicted (black lines).

(A) *D. melanogaster* neuroectodermal cells (bottom) divide with a cleavage plane that is parallel to their apicobasal axis (vertical cleavage), which results in symmetric (Sy) division. In neuroblasts (NB, top), Insc (green line) directs a 90° rotation of the mitotic spindle, aligning it along the apicobasal axis and thus generating a cleavage plane that is perpendicular to this axis (horizontal cleavage), which results in asymmetric (As) division.

(B) In rodents, APs of the VZ exhibit apicobasal polarity (apical junctional complexes and apically located centrosomes in interphase). APs include neuroepithelial cells (not shown) and, after the onset of neurogenesis, short neural precursors (SNPs) and radial glia (RG) cells, the basal processes of which terminate at the basal side of the VZ and at the basal lamina, respectively, in interphase (two left-hand cells). APs divide at the apical surface with a vertical or nearly vertical cleavage plane that can result in either symmetric (Sy) or asymmetric (As) division (two right-hand cells; M-phase basal processes are not shown for clarity). (Bii) By contrast, BPs of the SVZ are known to have an apical process in interphase (left-hand cell), which is retracted before mitosis. Mitotic BPs (right-hand cell) are unpolarized, lack adherens junctions and divide in a basal location. BP-cell divisions are symmetric, with a random cleavage-plane orientation. (C) In primates, APs (Ci) and BPs (Cii) that are similar to those in rodents are also present, and BPs constitute the ISVZ. In addition, a novel neural progenitor that undergoes mitosis in a basal location, the OSVZ progenitor (Ciii), has evolved. Most of the cell-biological features of this progenitor are unknown. However, we hypothesize that interphase (left-hand cell) and mitotic (right-hand cell) OSVZ progenitors maintain epithelial characteristics, including radial processes and apical junctional complexes. Other features might distinguish OSVZ progenitors from APs, such as a perinuclear location of the centrosome in interphase (left-hand cell). Additionally, we hypothesize that symmetric cell divisions of polarized neural progenitors predominantly occur in APs (Ci) of the VZ (right-hand cell), whereas OSVZ progenitors (Ciii) might be restricted to asymmetric, differentiative divisions (right-hand cell).



closure, both of which require that neuroepithelial cells constrict apically and elongate basally (Rao and Jacobson, 2005). The elongation of progenitors is a prerequisite for pseudostratification, in which the nuclei of adjacent progenitors stack on top of each other to allow more progenitors to be accommodated per unit of apical area (Fig. 3Bii). In this way, apical space can be reserved for mitosis, because interphase nuclei are localized basally. This, in turn, has implications for the distribution of apical cell constituents during cytokinesis, as is discussed below.

In addition to its impact on AP number, the elongation of APs promotes the switch from symmetric to asymmetric cell division. For AP division to be symmetric and proliferative, it appears to be necessary that both daughter cells inherit the apical plasma membrane and adherens junctions (Costa et al., 2008; Feng and Walsh, 2004; Fish et al., 2006; Huttner and Brand, 1997; Konno et al., 2008; Kosodo et al., 2004). However, as APs proliferate and become more elongated, the size of their apical domain shrinks (Fig. 3A,B); consequently, symmetric division in elongated APs can tolerate only a slight deviation of the cleavage plane from the vertical, apicobasal axis; beyond this deviation, division is asymmetric (Fig. 3Cii) (Götz and Huttner, 2005; Huttner and Brand, 1997; Huttner and Kosodo, 2005; Kosodo et al., 2004). In fact, bypassing (rather than bisecting) the apical plasma membrane and adjacent adherens junctions has evolved as the prevalent mode of switching from symmetric to asymmetric division in mammalian cortical APs (Konno et al., 2008; Kosodo et al., 2004).

Mitosis at the apical surface

The defining feature of vertebrate APs is that mitosis occurs apically. Not only is this an intrinsic feature of pseudostratification (which, as described above, increases the proliferative potential of APs by reserving the apical space for mitosis), it also has significant cell-biological implications. Specifically, another hallmark of these epithelial cells is that the primary cilium is located at the apical plasma membrane (Dubreuil et al., 2007; Hinds and Ruffett, 1971). Accordingly, the centrosome – which constitutes the basal body of the primary cilium in interphase and forms the poles of the mitotic spindle after duplication – is also apically located (Chenn et al., 1998). Hence, the apical mitosis of APs probably reflects the need for spatial coordination between the chromosomes and the apical centrosomes.

During cytokinesis in APs, the cleavage furrow proceeds unidirectionally from the basal to the apical plasma membrane (Kosodo et al., 2004) (Yoichi Kosodo, Kazunori Toida, Veronique Dubreuil, Judith Schenk, Emi Kiyokage, Alessio Attardo, Felipe Mora-Bermudez, Tatsuo Arai and W.B.H., unpublished). This allows cytokinesis to begin basally before the apical segregation of sister chromatids is complete. It also ensures that the segregation of the apical junctional complexes to the two daughter cells occurs very late in cytokinesis, and that abscission takes place at the apical surface. This ‘apical-last’ principle helps to preserve the integrity of the neuroepithelium and might be particularly important for the equal distribution of crucial apical and junctional constituents during symmetric, proliferative AP division.

Absence of spindle rotation and of horizontal cleavages in mammalian APs

In the *D. melanogaster* embryo, the switch from symmetric, proliferative divisions of neuroectodermal progenitors to asymmetric, differentiative divisions of neuroblasts results from a 90° rotation of the spindle away from the apicobasal axis of polarity

(Buchman and Tsai, 2007; Doe, 2008; Egger et al., 2008; Knoblich, 2008; Roegiers and Jan, 2004; Wang and Chia, 2005; Wodarz and Huttner, 2003). Spindle rotation in *D. melanogaster* neuroblasts is mediated by the specific expression of *Inscuteable* (*Insc*), which alters the cortical alignment of the mitotic spindle and thereby renders the cleavage plane horizontal (Kaltschmidt et al., 2000; Kraut et al., 1996). By contrast, the vast majority of APs in the mammalian neuroepithelium, even when undergoing asymmetric cell division, divide with a vertical or nearly vertical cleavage plane (Fig. 3Cii) (Huttner and Brand, 1997; Konno et al., 2008; Kosodo et al., 2004). These data suggest that an *Insc* homologue is either not expressed or has a modified function in mammalian neural progenitors.

Horizontal cleavages are common in other developing mammalian epithelia, such as the epidermis (Lechler and Fuchs, 2005; Smart, 1970b) and the oesophagus (Smart, 1970a). The mouse homologue of *Insc* has been cloned from the murine epidermis, in which it has been implicated in directing horizontal cleavages that are important for tissue stratification (Lechler and Fuchs, 2005). In addition, mouse *Insc* appears to have a role in spindle orientation in the developing rat retina (Zigman et al., 2005). In the mammalian cerebral cortex, however, several lines of evidence indicate that the predominantly vertical or near-vertical cleavages of asymmetrically dividing APs (Kosodo et al., 2004) reflect the lack of an *Insc*-like activity in these cells. First, in mice, there is little, if any, specific expression of *Insc* in the VZ at early stages of neurogenesis (Zigman et al., 2005), when an increasing proportion of APs switch from symmetric to asymmetric divisions (Haubensak et al., 2004; Kosodo et al., 2004). Second, the forced expression of *Insc* in the mouse telencephalon increases horizontal cleavage dramatically among APs (Konno et al., 2008). Thus, the function of the *Insc* protein, in principle, seems to be conserved between *D. melanogaster* and mammals. Importantly, however, mammalian cortical APs can switch from symmetric to asymmetric cell division without rotating the mitotic spindle for horizontal cleavage (Fig. 3C). This key difference between *D. melanogaster* neuroblasts and mammalian APs appears to indicate that the canonical function of *Insc* is absent in mammalian APs.

From an evolutionary perspective, the lack of *Insc* activity in mammalian APs might have important implications. In *D. melanogaster*, the switch from symmetric to asymmetric cell division is an active process that is brought about by the *Insc*-mediated 90° spindle rotation in the neuroblast but, in the highly elongated mammalian cortical APs, this switch might well occur by default. In mammals, the switch might entail the downregulation of the machinery that would normally ensure symmetric, proliferative AP division (i.e. cleavage that is exactly parallel to the apicobasal axis and enables the inheritance of apical plasma membrane and adherens junctions by both daughter cells). If this is the case, the lack of spindle rotation in mammalian cortical APs might have permitted the emergence of other mechanisms to increase the number of symmetric, proliferative divisions of APs during mammalian, and particularly primate, evolution. One such mechanism is an increase in the precision of cleavage.

Enhanced cleavage precision in APs – a role for *Aspm*

Symmetric cell division in increasingly elongated APs requires that cleavage continues to occur precisely along the apicobasal axis of the cell. The downregulation of the machinery that would normally ensure symmetric, proliferative AP division can be conceived as a reduction in cleavage precision (Fig. 3C). It follows that symmetric,

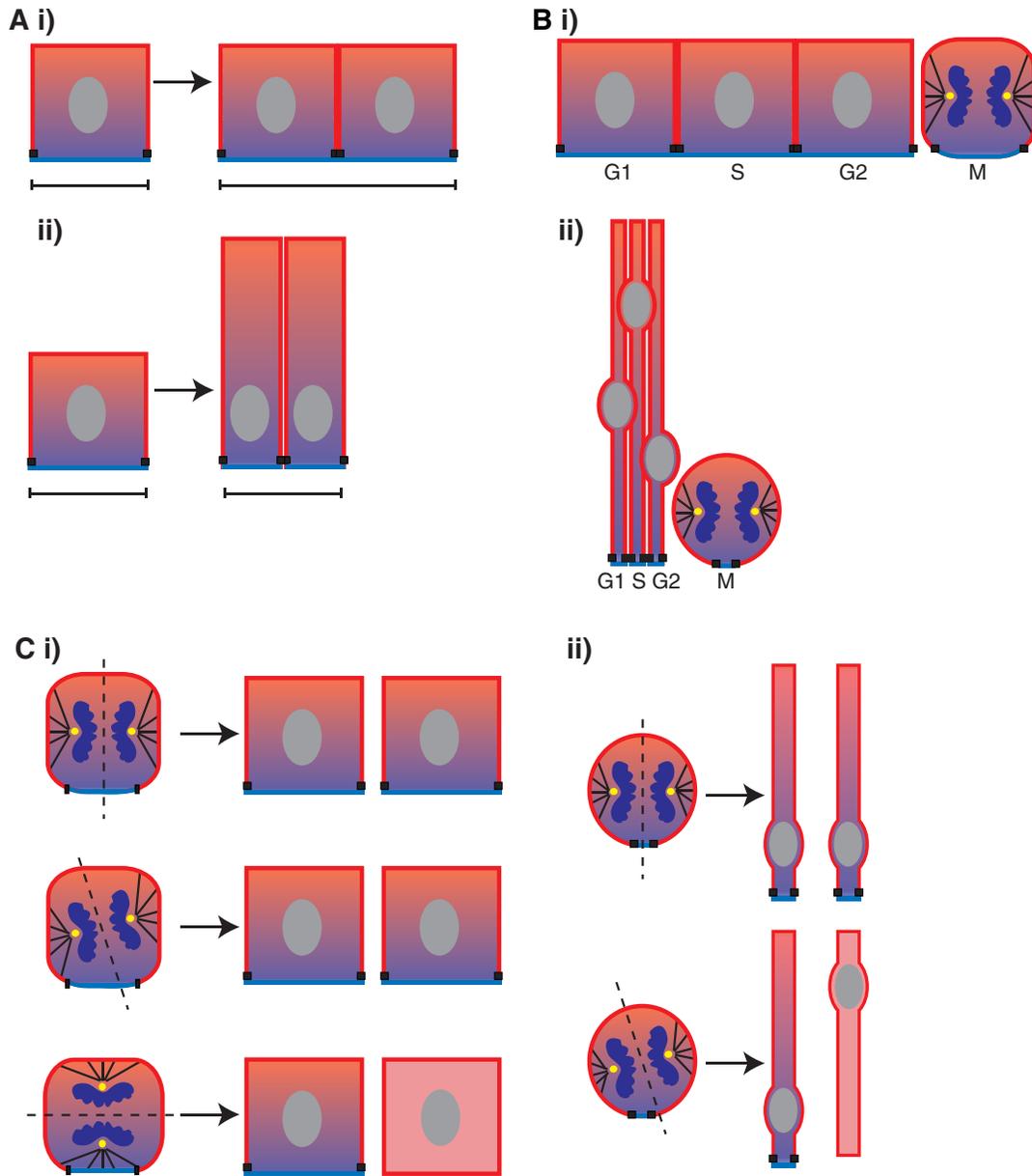
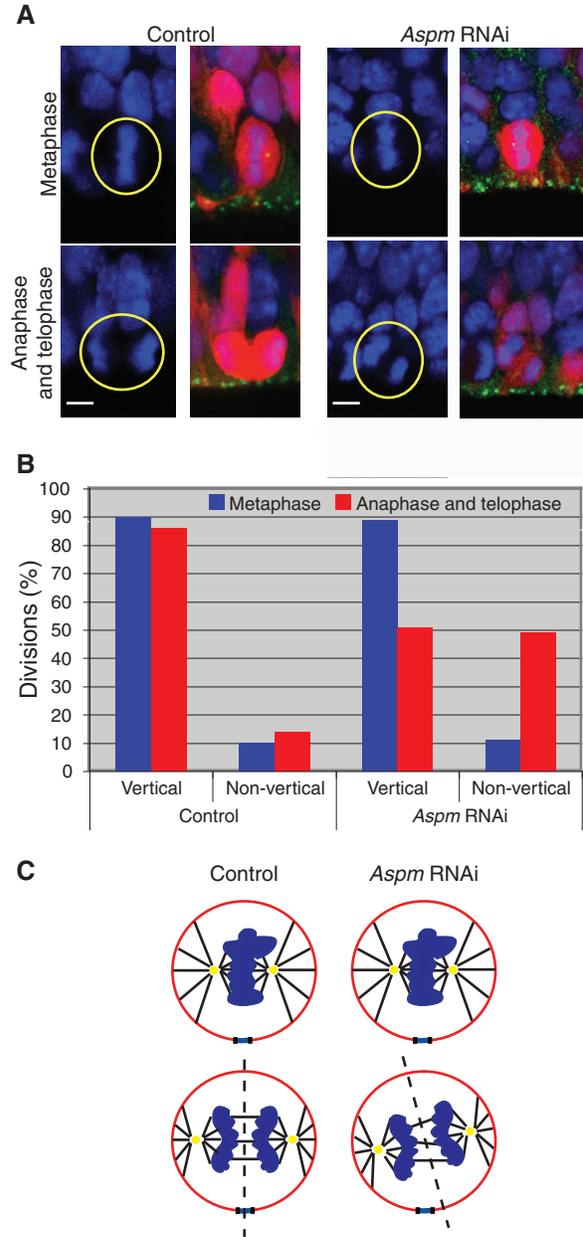


Fig. 3. Elongation, pseudostratification and cleavage precision of APs. The apical plasma membrane and corresponding cortical domains are represented by blue lines, and the basolateral plasma membrane and corresponding cortical domains are represented by red lines. Interphase nuclei are shown in grey and representative sister chromatids are in dark blue. Black rectangles represent junctional complexes and yellow dots indicate centrosomes or mitotic-spindle poles. For clarity, only the astral microtubules of the mitotic spindle are depicted (black lines). (A) Cell elongation enables more progenitors to inhabit each unit of epithelial surface area. (Ai) An example of a cuboidal epithelial progenitor generating two similar progenitors, which require twice the area of apical surface (bars). (Aii) An example of a cuboidal epithelial progenitor generating two elongated progenitors, which can be accommodated without an increase in apical surface area. (B) Pseudostratification enables more apical mitoses to occur per unit of ventricular space. (Bi) In a cuboidal epithelium, progenitors in interphase (G1, S, G2) and in mitosis (M) occupy approximately the same space. Hence, as the duration of M-phase typically constitutes only a small fraction of the total length of the cell cycle, only a minor proportion of the apical surface area is used for progenitors that are engaged in mitosis. (Bii) In a pseudostratified epithelium, interphase nuclei translocate away from the apical surface, so a much greater proportion of the space at the apical surface can be filled by progenitors that are engaged in mitosis. Hence, the proliferative potential of a pseudostratified epithelium increases until the progenitor interphase nuclei that can be accommodated in the cylindrical space basal to an apical mitotic progenitor have reached a number that is equal to the length of the cell cycle divided by the duration of mitosis. According to Smart (Smart, 1972a; Smart, 1972b), in the mammalian neuroepithelium, APs in mitosis occupy three times more space beneath the ventricular surface than AP interphase nuclei. Thus, as an example, if the cell-cycle length of APs is 12 hours and mitosis takes 30 minutes, proliferation is maximized when 24 (12/0.5) AP interphase nuclei reside basal to an apical mitotic progenitor, which is equivalent to a pseudostratified neuroepithelium with eight (24/3) nuclear layers basal to the apical mitotic layer. (C) In elongated apical progenitors, the relative frequency of symmetric and asymmetric division can be controlled by the regulation of cleavage precision rather than by mitotic-spindle rotation. (Ci) In a cuboidal epithelial progenitor with apicobasal polarity, symmetric cell division (the inheritance of apical plasma membrane and junctional complexes by both daughter cells) can result from both a perfectly vertical and a slightly non-vertical cleavage plane (top and middle, respectively; broken lines). Asymmetric cell division is typically achieved by a 90° rotation of the mitotic spindle, which leads to a horizontal cleavage plane (bottom). (Cii) With increasing pseudostratification and AP elongation, the apical domain shrinks correspondingly. Consequently, asymmetric cell division can result from a slight deviation of the cleavage plane away from the vertical apicobasal axis (bottom) (Huttner and Brand, 1997), and symmetric cell division requires a cleavage-precision machinery that ensures ingress of the cleavage furrow precisely along the apicobasal axis (top) (Fish et al., 2006).

Fig. 4. In mammals, *Aspm* maintains the orientation of the mitotic spindle, and hence promotes the precision of cleavage, in APs after the onset of anaphase. (A,B) Knockdown of *Aspm* in the dorsal telencephalon of E10.5 *Tis21*-GFP knock-in mice (Haubensak et al., 2004) by electroporation of endoribonuclease-prepared siRNA (esiRNA) followed by 24-hour whole-embryo culture was performed as described previously (Fish et al., 2006), except that in some experiments a pCAGGS-Cherry plasmid instead of a pCAGGS-mRFP plasmid was used to identify the targeted APs. (A) *Tis21*-GFP-negative APs [i.e. APs that have not yet switched to neurogenic or differentiative divisions (Haubensak et al., 2004)] in metaphase (upper panels), and anaphase and telophase (lower panels) were analyzed by confocal microscopy of 16- μ m cryosections (1 μ m optical section shown). Control, electroporation with pCAGGS-Cherry plasmid only; *Aspm* RNAi, co-electroporation of *Aspm* esiRNAs and pCAGGS-Cherry plasmid. The left panel of each pair shows DAPI staining (blue) of the metaphase plate (upper panels) and sister chromatids (lower panels); the right panel of each pair shows DAPI staining, Cherry intrinsic fluorescence (red) and γ -tubulin immunofluorescence of centrosomes (green); this reveals the apical surface, which is towards the bottom of this image. Scale bars: 5 μ m. (B) Quantification of the angle of the metaphase plate (blue), and of the predicted cleavage plane, as deduced from the position of sister chromatids in anaphase and telophase (red) relative to the apical surface of the neuroepithelium (determined by centrosome immunostaining and defined as 0°) in *Tis21*-GFP-negative control APs and APs subjected to *Aspm* RNAi [see the following references for methodological details (Fish et al., 2006; Kosodo et al., 2004)]. ‘Vertical’ was defined as an angle of 90–75°, and ‘non-vertical’ as an angle of 74–0°. Data are expressed as a percentage of all divisions for the control ($n=10$ for metaphase, $n=36$ for anaphase and telophase) or *Aspm* RNAi [$n=19$ for metaphase, $n=41$ for anaphase and telophase; anaphase and telophase data include 24 and 22 cases, respectively, that were published previously in Fig. 3B of Fish et al. (Fish et al., 2006)]. (C) Model for *Aspm* function after the onset of anaphase. The apical plasma membrane is represented by blue lines and the basolateral plasma membrane by red lines. Chromosomes and sister chromatids are shown in dark blue. Black rectangles represent junctional complexes, yellow dots indicate mitotic-spindle poles, black lines indicate the mitotic spindle and broken lines show the predicted cleavage plane. *Aspm* is not essential for correct positioning of the mitotic spindle, the axis of which in mammalian APs is typically oriented parallel to the apical surface by the end of metaphase (upper cells). Under control conditions, when *Aspm* is present (in particular in *Tis21*-GFP-negative APs), this orientation is maintained after anaphase onset and APs divide with a vertical cleavage plane, i.e. symmetrically (lower-left cell) (Fish et al., 2006; Kosodo et al., 2004). Upon *Aspm* knockdown, the axis of the mitotic spindle (particularly the central spindle) is increasingly likely to deviate from this orientation after anaphase onset, and hence the cleavage plane is increasingly likely to bypass the apical plasma membrane and junctional complexes (Fish et al., 2006), which results in the asymmetric division of APs (lower-right cell). This suggests an important role for *Aspm* in maintaining the precise alignment of the mitotic spindle perpendicular to the apicobasal AP axis after anaphase onset and throughout cytokinesis.

proliferative divisions of APs can be increased by maintaining cleavage precision (Fig. 3Cii, top). Previously, we described a role for the abnormal spindle-like microcephaly-associated protein (*Aspm*), which regulates brain size (Bond et al., 2002), in maintaining symmetric AP divisions (Fish et al., 2006). *Aspm* is more-highly expressed in rodent APs that are undergoing symmetric, proliferative divisions than in APs that are dividing in an asymmetric, neurogenic manner. This suggests that the downregulation of *Aspm* at the onset of neurogenesis promotes asymmetric divisions (Fish et al., 2006).

How might *Aspm* regulate cleavage precision? In mammals, *Aspm* is localized to the poles of the mitotic spindle (Bond and Woods, 2005; Fish et al., 2006; Kouprina et al., 2005) and the midbody (Paramasivam et al., 2007), and its midbody localization suggests that it is also present on the central spindle. The knockdown of *Aspm* in rodent APs results in the deviation of the cleavage plane (as predicted from the positions of the sister chromatids in anaphase



and telophase) from the normal vertical orientation; this knockdown leads to asymmetric division (Fish et al., 2006) (Fig. 4). Interestingly, we recently observed that the alignment of chromosomes at the metaphase plate, which, in the vast majority of cases, is perpendicular to the ventricular surface, is unaffected by *Aspm* knockdown; by contrast, sister chromatids deviate from the apicobasal axis after the onset of anaphase (Fig. 4). These observations suggest that *Aspm* is not necessary for the correct positioning of the mitotic spindle [which is determined by the end of metaphase (Buchman and Tsai, 2007; Haydar et al., 2003)], but that it has a crucial role in maintaining the orientation of the spindle axis after the onset of anaphase.

The precise mechanism by which *Aspm* maintains the orientation of the spindle axis is presently unclear, although it is likely to involve its predicted microtubule-bundling capacity (Bond et al., 2002; do Carmo Avides and Glover, 1999; Ripoll et al., 1985), which might help to buffer forces that act on the spindle poles as chromatids

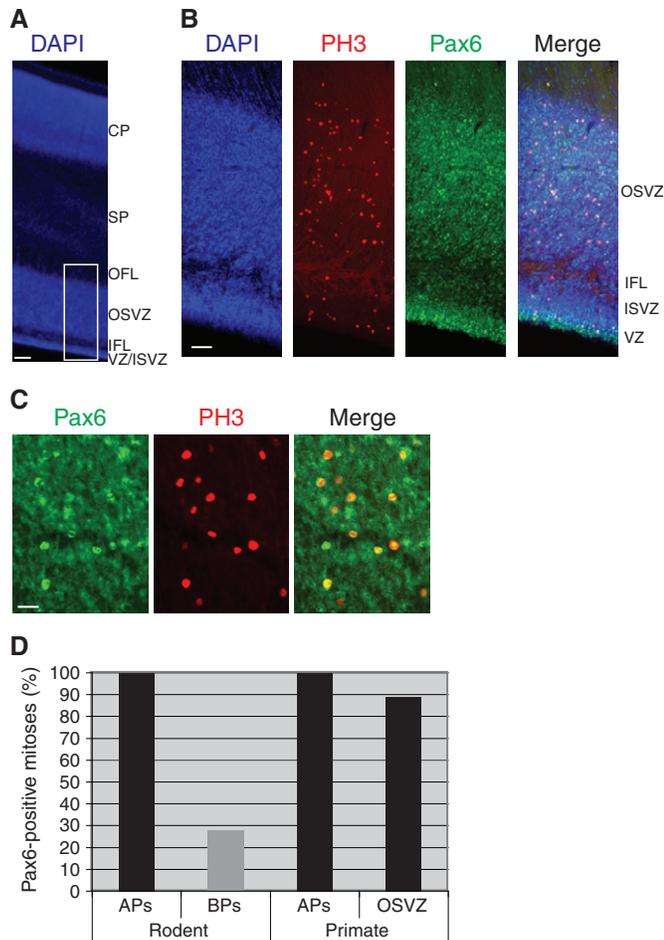


Fig. 5. Pax6 is present in mitotic progenitors of the primate OSVZ. Immunocytochemistry [technique modified from Kosodo et al. (Kosodo et al., 2004) to include an antigen-retrieval protocol] was performed on a 60- μ m cryosection of macaque E80 cerebral cortex [area 17/18, *Cynomolgus* monkey foetus (Lukaszewicz et al., 2005)]. (A) Low-power overview of the cortical wall stained with DAPI (blue). IFL, inner fibre layer; OFL, outer fibre layer; SP, subplate; CP, cortical plate. The boxed area is shown at higher magnification in B. Scale bar: 250 μ m. (B) Triple labelling of the area that contains the progenitor layers, as indicated by the boxed area in A. Blue, DAPI staining; red, phosphohistone H3 (PH3) immunofluorescence; green, Pax6 immunofluorescence. Note the presence of Pax6-positive mitotic cells in both the VZ and the OSVZ. Scale bar: 100 μ m. (C) Detection of Pax6 (green) and PH3 (red) within the OSVZ by immunofluorescence. Note the presence of Pax6 in almost all mitotic OSVZ progenitors. The ventricular surface is below the lowest extent of the image. Scale bar: 20 μ m. (D) Quantification of Pax6-positive mitotic rodent and primate APs of the VZ, rodent BPs of the SVZ and primate OSVZ progenitors. Rodent data are from 10- μ m cryosections of dorsal telencephalon of E12.5 and E13.5 mice (four embryos each, three to five cryosections per embryo) that were subjected to double immunofluorescence for Pax6 and PH3 as above, and analyzed by confocal microscopy (1- μ m optical sections). Primate data are from the same cryosection as is used in B and C. All mitotic rodent and primate APs were Pax6-positive (mouse 128/128, monkey 20/20). Less than 30% (9/32) of mitotic rodent BPs were Pax6-positive, and these Pax6-positive cells exhibited weak immunostaining relative to APs (indicated by the grey colour of the column). By contrast, almost 90% (94/106) of mitotic primate OSVZ progenitors were Pax6-positive and the level of immunoreactivity was equivalent to that of APs. Similar data were obtained when mitotic monkey OSVZ progenitors were analyzed for Pax6 expression without using an antigen-retrieval protocol [82% Pax6-positive (299/364), not shown].

separate during anaphase. In addition, the association of *Aspm* with the midbody (Paramasivam et al., 2007) is likely to be important, and might indicate that *Aspm* coordinates the positions of the mitotic spindle and the contractile ring. Importantly, the cleavage furrow is known to ingress perpendicularly to the axis of the mitotic spindle (Glotzer, 2001), and the direction of ingress depends on signals from the central spindle (Bringmann and Hyman, 2005). Thus, abscission at the apical plasma membrane of APs (Dubreuil et al., 2007; Kosodo et al., 2004) requires that the spindle axis, and particularly the central spindle, be precisely parallel to the apical membrane until the very end of cytokinesis. A small deviation from this orientation would cause abscission to occur at the apical-most end of the lateral plasma membrane (rather than at the apical membrane proper), which would lead to asymmetric division (Fig. 3Cii). It is intriguing to hypothesize that, in mammalian cortical APs, *Aspm* has evolved as a 'cleavage-precision protein' that helps to ensure the maintenance of spindle orientation after the onset of anaphase and throughout cytokinesis.

The maintenance of spindle-axis orientation is important for 'apical-last', symmetric, proliferative AP division, which suggests that genes that are involved in cleavage precision might have been particularly prone to evolutionary change. It is interesting to note that, during primate evolution, mutations in the *Aspm* locus that change the primary structure of the protein have occurred more frequently than expected relative to synonymous (neutral) mutations, which is indicative of positive selection (Kouprina et al., 2004;

Zhang, 2003). These changes in the *Aspm* protein might reflect its adaptation as a 'cleavage-precision protein'. If the precision of vertical cleavage along the apicobasal axis of APs increases in line with their elongation, the number of symmetric cell divisions would be likely to increase, and this is thought to be necessary for the lateral expansion of the cerebral cortex in the primate lineage. Indeed, mechanisms to maximize the precision of cleavage would probably be required to promote the proliferative division of elongated APs. Similar to *Aspm*, several other genes that encode centrosome-associated proteins (such as *CENPJ* and *CDK5RAP2*) have been implicated in the regulation of brain size (Bond et al., 2005), and might have a similar role to *Aspm* in modulating the orientation of the spindle and the precision of cleavage.

Basal location of progenitor-cell mitosis – overcoming apical constraint

The three evolutionary changes in neural-progenitor-cell biology that have been described above (pseudostratification, the absence of spindle rotation and the promotion of cleavage precision) all affect the cell division of APs of the VZ. However, as has also been noted, it is an increase in the thickness of the SVZ and the number of basally dividing progenitors that primarily distinguishes cortical development in primates from that in rodents. Therefore, understanding the origin and diversification of basally dividing progenitors is arguably the rate-limiting step in our understanding of how relative brain size varies among mammals. To this end, we must first consider what the advantage is of locating progenitor mitoses basally.

Apical constraint

As has been described above, the pseudostratified nature of the vertebrate neuroepithelium provides a proliferative advantage because it increases the number of APs per unit of apical area (Fig. 3A,B). However, this advantage is ultimately restricted by the fact

that APs divide apically. This limitation, or apical constraint, was first noted by Smart (Smart, 1972a; Smart, 1972b). Once pseudostratification has resulted in a sufficiently large number of abventricular interphase nuclei 'waiting' to undergo mitosis apically, further pseudostratification will not increase the number of apical mitoses per unit time, but will instead increase the length of the cell cycle of APs because of interkinetic nuclear migration. Thus, to maximize the number of apical mitoses, there is an optimal thickness for pseudostratification (see legend to Fig. 3Bii).

To achieve any further increase in progenitor number and divisions per unit time, it is necessary that "nuclei are free to go into mitosis away from the central canal [apical] surface" (Smart, 1972b). Indeed, this is what happens in the rodent telencephalon. Rather than generating a neuron directly, an AP can generate a BP that then produces two neurons, thereby doubling the number of neurons that are generated per AP division (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). These observations provide strong support for Smart's hypothesis, which states that basal mitoses are an adaptation to overcome the spatial constraint that is imposed by the confinement of AP mitoses to the apical surface of the pseudostratified neuroepithelium (Smart, 1972b).

The evolution of OSVZ progenitors

The principle of apical constraint can explain the advantage of locating neural-progenitor mitoses basally, but how have basal divisions increased in number during evolution, and especially in the developing primate cortex? This question remains open, but two contrasting hypotheses have been offered. The first, which is based on the observation of radially aligned nuclei within the primate SVZ, suggests that a novel population of neural progenitors, the OSVZ-progenitor population, has evolved in primates (Smart et al., 2002) (Fig. 2Ciii). Because OSVZ progenitors are morphologically similar to APs in the VZ, it was hypothesized that these cells are radial glia (APs) that have located their nucleus basally and no longer undergo interkinetic nuclear migration (Smart et al., 2002). The second hypothesis, which is based on the recent characterization of BPs in rodents (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004) (Fig. 2Bii), proposes that self-amplification of non-epithelial BPs (Fig. 2Cii) explains the increase in cortical size in primates (Kriegstein et al., 2006; Martinez-Cerdeno et al., 2006).

These two hypotheses generate fundamentally different predictions, both about the cell-biological attributes of basally dividing cells in primates and about the genetic changes that have given rise to these cells. The hypothesis by Smart and colleagues (Smart et al., 2002) predicts that APs (Fig. 2Ci) are the ancestral cell type to OSVZ progenitors. Importantly, this implies that basally dividing cells in primates maintain epithelial-like characteristics (e.g. apicobasal polarity and adherens junctions) (Fig. 2Ciii) and that the major cell-biological change in the transition from APs to OSVZ progenitors is the loss of the apically directed nuclear migration that is typical of the G2 phase of the cell cycle of APs. By contrast, the hypothesis by Kriegstein and colleagues (Kriegstein et al., 2006) predicts that BPs (which are non-epithelial; Fig. 2Cii) are the ancestral population. This hypothesis also predicts the extended proliferation of a neural progenitor that lacks an apical plasma membrane, adherens junctions and cell polarity; this would be a key difference between basally dividing progenitors in rodents (Fig. 2B) and primates (Fig. 2C).

We have tested the lineage relationship between basally dividing cells in primates, and rodent APs and BPs. In rodents, Pax6 is characteristically expressed in APs and downregulated in BPs

(Englund et al., 2005); having confirmed this observation (Fig. 5D, left), we investigated Pax6 expression in primate neural progenitors. In an embryonic day 80 (E80) monkey, the VZ and the OSVZ, and to a lesser extent the ISVZ, contained many Pax6-positive cells (Fig. 5B). Likewise, we observed Pax6-positive cells in the human SVZ (J.L.F. and W.B.H., unpublished), which is consistent with other recent reports (Bayatti et al., 2007; Mo and Zecevic, 2007). To confirm that the Pax6-positive cells in the monkey OSVZ included progenitors [rather than being neurons, which in primates can also be Pax6 positive (Mo and Zecevic, 2007)], we specifically examined mitotic cells by staining for phosphohistone H3. This revealed that >80% of progenitors in the OSVZ were Pax6 positive (Fig. 5C and Fig. 5D, right). Thus, primate OSVZ progenitors express Pax6 in a very similar, if not identical, manner to APs (Fig. 5D, right).

The lack of downregulation of Pax6 expression in primate OSVZ progenitors (Fig. 5), together with their radial morphology (Smart et al., 2002; Lukaszewicz et al., 2005), strongly suggests that they are closely related to APs, which is consistent with the hypothesis of Smart and colleagues (Smart et al., 2002). Moreover, given that Pax6 is characteristically expressed in neural progenitors that exhibit epithelial features (APs) (Costa et al., 2008; Englund et al., 2005; Quinn et al., 2007), these data also support the idea that OSVZ progenitors are epithelial in nature. In this context, it is important to note that, on the basis of their cell biology and marker expression, the primate ISVZ progenitors rather than the OSVZ progenitors are probably the equivalent of rodent BPs. In rodents, however, most cortical neurons are derived from BPs (Haubensak et al., 2004), whereas in primates most upper (supragranular)-layer neurons in the cortex are generated from OSVZ progenitors (Lukaszewicz et al., 2005).

The epithelial-progenitor hypothesis

On the basis of the data described in this Commentary, we hypothesize that the fundamental cell-biological difference between rodent and primate cortical neurogenesis is the expansion, in primates, of progenitors with epithelial characteristics (the 'epithelial-progenitor hypothesis'). This implies that the increase in SVZ thickness in primates reflects the lateral expansion of radial progenitors, i.e. radial units, which underlies the evolutionary expansion of the cerebral cortex (Rakic, 1995; Rakic, 2007). Another important implication is that the direct progenitor of most neurons in primates is epithelial, whereas in rodents it is not. The presence of Pax6 in human neurons (Mo and Zecevic, 2007), which might reflect inheritance from an epithelial, Pax6-positive progenitor, is fully consistent with this concept.

The full extent to which OSVZ progenitors retain epithelial characteristics remains to be investigated. We hypothesize that certain features of APs, such as apicobasal polarity and apical adherens junctions, are maintained in OSVZ progenitors (Fig. 2Ciii). Consistent with this, a radial-glia-like morphology has been observed for some OSVZ progenitors, which exhibited long apical and basal processes (Lukaszewicz et al., 2005). By contrast, it is unlikely that OSVZ progenitors maintain an apical centrosome in interphase, given that their mitosis occurs basally (Fig. 2Ciii).

Conclusions and perspectives

An increase in the neural-progenitor-cell population is considered to be fundamental to the evolutionary expansion of the cerebral cortex. Here, we have described several aspects of the cell biology of mammalian neural progenitors that, we hypothesize, have

contributed to increases in neural-progenitor number by increasing the number of symmetric divisions that these cells undergo. In particular, the pseudostratification of the progenitor layer, the lack of switching from vertical to horizontal cleavage, the evolution of precision proteins that ensure symmetric progenitor division and the translocation of radial progenitor nuclei to the OSVZ for mitosis in primates might all have been adaptations that have led to increases in cortical size. Importantly, each of these changes indicates that selection has favoured the maintenance of radial, epithelial neural progenitors.

Radial, epithelial neural progenitors might have had at least two possible selective advantages. First, the organization of the cortical plate into a highly structured, layered sheet, which is a developmental process that is crucial for proper neuron connectivity and function, requires radial progenitors (Rakic, 1988; Rakic, 1995; Rakic, 2007). Second, junctional complexes and cell polarity confer control on the proliferative process, whereas the loss of polarity is implicated in unregulated growth and cancer (Lee and Vasioukhin, 2008). This might explain why the vast majority of the non-epithelial BPs of rodents does not undergo more than one round of cell division. Nonetheless, this single round of division doubles the number of neurons that are present in each radial unit, which suggests an important role for BPs in generating radial thickness (Abdel-Mannan et al., 2008; Farkas et al., 2008; Pontious et al., 2008).

Following on from these observations and considerations, we have proposed the epithelial-progenitor hypothesis to explain the evolutionary expansion of the primate cerebral cortex. This hypothesis argues that the expansion of the primate SVZ, and consequently of the primate cerebral cortex, was largely driven by an increase in epithelial neural progenitors. This model generates many testable predictions about the largely unknown cell-biological features of OSVZ progenitors; for instance, how do these cells divide and can they self-amplify through symmetric, proliferative divisions?

The OSVZ shows a substantial increase in thickness before the onset of upper (supragranular)-layer neuron production, which would be consistent with the idea that OSVZ progenitors expand via symmetric, proliferative divisions within the OSVZ. However, in light of the presumptive apicobasal polarity of OSVZ progenitors, we hypothesize that these cells predominantly divide in an asymmetric, neurogenic manner (Fig. 2Ciii). Given the 'apical-last' nature of symmetric cell division of polarized neural progenitors, the most parsimonious explanation is that symmetric, proliferative divisions are reserved for APs in the VZ and that, once an AP is committed to asymmetric, neurogenic division, it translocates its nucleus basally. This would then ease apical congestion by allocating the apical 'slots' for symmetric cell division (Fig. 2Ci). Deciphering these unknowns and, in particular, finding the genetic changes behind their evolution, remains a primary goal.

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