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REVIEW

Cortical progenitor biology: key features mediating proliferation versus differentiation

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Abbreviations used: 3-D, three dimensional; AGS3, activator of G protein signaling 3; AIPS, apical intermediate progenitors; AJ, adherens junction; APs, apical progenitors; aRGs, apical radial glial cells; ARHGAP11B, Rho GTPase-Activating Protein 11B; Arhgef2, Rho guanine nucleotide exchange factor 2; Aspm, abnormal spindle-like microcephaly associated protein homolog; Atf4-6, activating transcription factor 4-6; BAC, bacterial artificial chromosome; BPs, basal progenitors; bRGs, basal radial glia-like cells; Cdc42, cell division control protein 42 homolog; Cdk5rap2, CDK5 regulatory subunit-associated protein 2; Celsr3, cadherin EGF LAG seven-pass G-type receptor 3; Cenpj/CENPJ, centromere protein J; CITK, citron kinase; CP, cortical plate; Crb2, Crumbs2; CR, ciliary remnants; CSF, cerebrospinal fluid; Dlg1, disks large homolog 1; Dynlt1, dynein light chain Tctex-type 1; eIF2, eukaryotic initiation factor 2; Elp3, elongator complex protein 3; Eml1, echinoderm microtubule-associated protein-like 1; ER, endoplasmic reticulum; FGF, fibroblast growth factor; Flrt1/3, Fibronectin leucine-rich transmembrane protein 1/3; Fzd3, Frizzled-3; GE, ganglionic eminences; hES, human embryonic stem cells; hiPSCs, induced pluripotent stem cells; hNSC, human neural stem cells; IGF-1, insulin-like growth factor-1; INM, interkinetic nuclear migration; IPs, intermediate progenitors; IRE-1, inositol-requiring enzyme-1; iSVZ, inner subventricular zone; Jag1, Jagged 1; JAMS, junctional adhesion molecules; Lfc, Rho guanine nucleotide exchange factor 2; LGN, G-protein-signaling modulator 2, called LGN for its Leucine–Glycine–Asparagine repeats; Lis1/LIS1, lissencephaly-1 protein; Llgl1, lethal giant larvae homolog 1; MADM, mosaic analysis with double markers; mES, mouse embryonic stem cells; Mib1, Mindbomb1; miRNAs, microRNAs; MST, mitotic somal translocation; mtROS, mitochondrial-reactive oxygen species; Nde1, nuclear distribution protein homolog 1; NEs, neuroepithelial cells; NICD, notch intracellular domain; Nt3, neurotrophin-3; NuMA, nuclear-mitotic apparatus protein; oSVZ, outer subventricular zone; Pals1, protein associated with lin seven 1; Par3/6, partitioning defective 3/6 homolog; PDGFD, platelet-derived growth factor D; PDGFRβ, platelet-derived growth factor receptor β; Perk/PERK, PKR-like endoplasmic reticulum kinase; Prdm16, PR domain zinc finger protein 16; RhoA, ras homolog gene family, member A; Sas4, spindle assembly abnormal protein 4; Sip1, Smad-interacting protein 1; SNPs, short neural progenitors; SSA, spindle size asymmetry; SVZ, subventricular zone; TBC1D3, TBC1 domain family member 3; Tctex-1, T-complex testis-specific protein 1 homolog; TF, transcription factor; TMEM14B, transmembrane protein 14B; Trnp1, TMF1-regulated nuclear protein 1; UPR, unfolded protein response; Vangl2, vang-like protein 2; VS, ventricular surface; VZ, ventricular zone; Wdr62, WD repeat-containing protein 62; Wnt7, Wingless 7; Xbp1, X-box-binding protein 1; ZIKV, Zika virus; ZO-1, Zona occludens-1; αPKC, α protein kinase C.

Abstract

The cerebral cortex is a highly organized structure whose development depends on diverse progenitor cell types, namely apical radial glia, intermediate progenitors, and basal radial glia cells, which are responsible for the production of the correct neuronal output. In recent years, these progenitor cell types have been deeply studied, particularly basal radial glia and their role in cortical expansion and gyrification. We review here a broad series of factors that regulate progenitor behavior and daughter cell fate. We first describe the different neuronal progenitor types, emphasizing the differences between lissencephalic and gyrencephalic species. We then review key factors shown to influence progenitor proliferation versus differentiation, discussing their roles in progenitor dynamics, neuronal production, and potentially brain size and complexity. Although spindle orientation has been considered a critical factor for mode of division and daughter cell output, we discuss other features that are emerging as crucial for these processes such as organelle and cell cycle dynamics. Additionally, we highlight the importance of adhesion molecules and the polarity complex for correct cortical development. Finally, we briefly discuss studies assessing progenitor multipotency and its possible contribution to the production of specific neuronal populations. This review hence summarizes recent aspects of cortical progenitor cell biology, and pinpoints emerging features critical for their behavior.

Keywords: cell division (symmetric, asymmetric), cerebral cortex evolution, cortical neurogenesis, mouse mutant, neurodevelopment, progenitor cell.

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The cerebral cortex is the brain region responsible for higher cognitive functions. It is a highly organized structure, and its development depends on diverse progenitor cell types, which give rise to post-mitotic neurons that migrate extensively to find their appropriate positions in the developing cortical wall (for review see Kawauchi, 2015; Stouffer *et al*. 2016). This extremely dynamic yet highly regulated process leads to the formation of the six-layered adult neocortex. Toward understanding its development, invertebrate models such as *Drosophila* have provided valuable insights (Homem and Knoblich, 2012), and rodents (mice and rats) are commonly used model organisms for unraveling the complex mechanisms underlying cortex development and the cell biology of neuronal progenitors. Rodents are, however, lissencephalic species and the neuronal progenitor cell populations within their developing cortices are less diverse as compared to gyrencephalic species, such as the ferret and primates. Recent analyses of primate brains, including human postmortem brains, have allowed an improved discernment of progenitor cell diversity, as well as their contributions to increased cortical complexity, and folding of the brain (Fernandez *et al*. 2016).

The timing and duration of neurogenesis varies, and correlates with brain complexity, *vis-à-vis* the studied model organism. For instance, murine neurogenesis lasts around 2 weeks, whereas it takes 3 months in the developing human brain. This extended period of human neurogenesis relies on increased neuronal progenitor self-renewal, promoting the expansion of the germinal layers, and increasing the number of neurons produced throughout corticogenesis. This in turn will lead to expansion of the neocortex, contributing to the appearance of *gyri* and *sulci.* In addition, the advent of human embryonic stem cells (hES) and induced pluripotent stem cells (hiPSCs) has introduced novel *in vitro* systems to study diverse aspects of neuronal progenitor dynamics. Human organoids are three-dimensional (3-D) structures derived from hES or hiPSCs, and include cortical-like tissue containing ventricular lumen, germinal zones, and neurons. These *in vitro* models augment our ability to analyze and dissect the characteristics of corticogenesis in human brains, aiding dissection of the pathophysiology underlying brain disorders, as analogous processes in mutant mouse models are more limited (Eiraku *et al*. 2008; Lancaster *et al*. 2013, Lancaster and Knoblich 2014; Clevers 2016; Bershteyn *et al*. 2017; Di Lullo and Kriegstein, 2017; Iefremova *et al*. 2017). Understandably, a holistic appraisal of all steps of cortical development (e.g., proliferation, modes of cell division, cell differentiation, cell migration) is key to unraveling the pathophysiological mechanisms underlying cortical malformations such as microcephaly (small brain), lissencephaly (smooth brain), and heterotopia (abnormally positioned neurons), often associated with intractable epilepsy and intellectual disability (for a recent review of human cortical malformations see Romero *et al*. 2018). Genetic and environmental factors (e.g., viruses such as Zika) can perturb these critical steps. Elucidating the intrinsic and extrinsic mechanisms controlling progenitor cell proliferation versus neuronal differentiation will help shed light on cortical expansion, gyrification, and ultimately neocortical evolution.

In this review, we describe the different types of neuronal progenitor populating the developing cortex. We focus on the critical factors that control progenitor self-renewal and cell differentiation, emphasizing those either promoting a proliferative potential, or leading to cell cycle exit and neuronal commitment. Ultimately, we discuss how the broad variety of progenitors found in gyrencephalic species contributes to

increasing the final neuronal output, resulting in the highly expanded and folded neocortex characteristic of human brains.

Introduction to cortical progenitor types

The neocortex develops from the anterior-most region of the neural tube, the dorsal telencephalon. This region gives rise to glutamatergic excitatory neurons (principal or pyramidal neurons), which account for around 80% of the neurons of the neocortex (Lodato and Arlotta, 2015). The other 20% of cells of the neocortex are GABAergic interneurons. Murine cortical interneurons are generated in the ganglionic eminences (GE), located in the ventral telencephalon, and migrate long distances to integrate in the developing cortex (for a review of interneuron development see Marin, 2013; Bandler *et al*. 2017; Laclef and Metin, 2018). However, the origin of primate, human interneurons is still a controversial topic. Diverse studies support that in primates, including humans, the germinal zones located in the dorsal telencephalon are an important source of interneuron production (Letinic *et al*. 2002; Rakic and Zecevic, 2003; Jakovcevski *et al*. 2011; Al-Jaberi *et al*. 2015). However, two recent studies showed primate, human interneurons to be mainly produced in the GE, as it occurs in the mouse brain (Hansen *et al*. 2013; Ma *et al*. 2013). In this review, we focus on mechanisms controlling the behavior of progenitors responsible for the generation of projection neurons.

Apical progenitors (APs): neuroepithelial cells (NEs), apical radial glial cells (aRGs), and short neural progenitors (SNPs)

The walls of the neural tube are populated by neuroepithelial cells (NEs) that possess an apico-basal polarity with attachment of their basal processes to the basement membrane and junctional coupling at the apical side. These NEs undergo the distinctive process of interkinetic nuclear migration (INM) during the cell cycle: their nuclei move apico-basally within the neuroepithelium, entering M-phase at the apical surface (Bertipaglia *et al*. 2017). NEs are characterized by the presence of occludin-positive (+) tight junctions (Aaku-Saraste *et al*. 1996), as well as gap junctions for intercellular connections and the flux of small molecules, thus enabling cellular communication (Elias and Kriegstein, 2008). They have a prominin+ apical domain that accommodates a primary cilium protruding into the ventricles (Taverna *et al*. 2014), which senses signals from the cerebrospinal fluid (CSF) that will influence NE behavior (Lehtinen and Walsh, 2011; Arbeille *et al*. 2015).

NEs divide in a symmetric proliferative fashion, amplifying their numbers and hence the neural progenitor pool. Upon the onset of neurogenesis from embryonic day (E)10–12 of murine development, NEs transit from a purely proliferative to a neurogenic state (Gotz and Huttner, 2005; Matsuzaki and Shitamukai, 2015), while their tight junctions are gradually annexed by an adherens junction (AJ) belt that is characterized by the presence of proteins such as N-cadherin and zona occludens (ZO)-1 (Aaku Saraste *et al*. 1996) (AJs will be further discussed in the section 'Influence of the polarity complex and cell adhesion molecules'). Consequently, NEs become apical radial glial cells (aRGs) that comprise the predominant neuronal progenitor cell type within the developing neocortex (Malatesta *et al*. 2000; Noctor *et al*. 2001; Noctor *et al*. 2004). aRGs are also highly polarized cells, exhibiting basal processes attached to the basement membrane, and apical processes linked by adhesion forming a transition region [termed here the 'ventricular surface (VS)'] with CSF in the ventricles (Fig. 1a). As for NEs, aRG apical domains are prominin+ and contain a primary cilium (Taverna *et al*. 2014). Their basal process has been extensively described to be a scaffold for migrating neurons to reach their positions in the cortical plate (CP) (Borrell and Gotz, 2014). aRGs also undergo INM, although the nuclear oscillation is restricted to the ventricular zone (VZ), where their somata reside (Gotz and Huttner, 2005). Because of this highly dynamic cell cycle process, the VZ is often described as a pseudostratified neuroepithelium.

The transition of NEs to aRGs is critical for determining the size of the initial pool of progenitors available to generate neurons, thereby determining the final neuronal output and brain size (Fernandez *et al*. 2016). Several factors involved in this transition, for example, fibroblast growth factors (FGFs), are also key for maintaining aRG identity. Notably, FGF10 triggers expression of aRG markers in NEs (Sahara and O'Leary, 2009) and also supports an aRG fate by inhibiting their transition toward a more committed neuronal progenitor (Kang *et al*. 2009). The Notch signaling pathway has also been described to be important for the transition from NEs to aRGs (Gaiano *et al*. 2000; Hatakeyama *et al*. 2004; Martynoga *et al*. 2012). While they lose tight junctions and develop the AJ belt, aRGs acquire astroglial features (Florio and Huttner, 2014; Gotz *et al*. 2015) and become positive for a set of astroglial markers, such as the astrocyte-specific glutamate transporter and others. They also express key neurogenic transcription factors (TF), such as Pax6 (Gotz *et al*. 1998).

aRGs are more restricted in their proliferative potential than NEs. They mainly undergo asymmetric proliferative divisions in the rodent, self-renewing while producing post-mitotic neurons or neurogenic progenitors (see section 'Basal progenitors (BPs): intermediate progenitors (IPs) and basal radial glia-like cells (bRG), and their implication in cortical expansion' and Fig. 1). Direct neurogenesis involves the process by which neurons are produced directly from aRGs. On the other hand, indirect neurogenesis refers to the process by which aRGs produce other intermediary types of progenitor cells that will subsequently produce neurons, thereby increasing the net neuronal output. Neurons that are produced earlier during corticogenesis (e.g., E11.5–

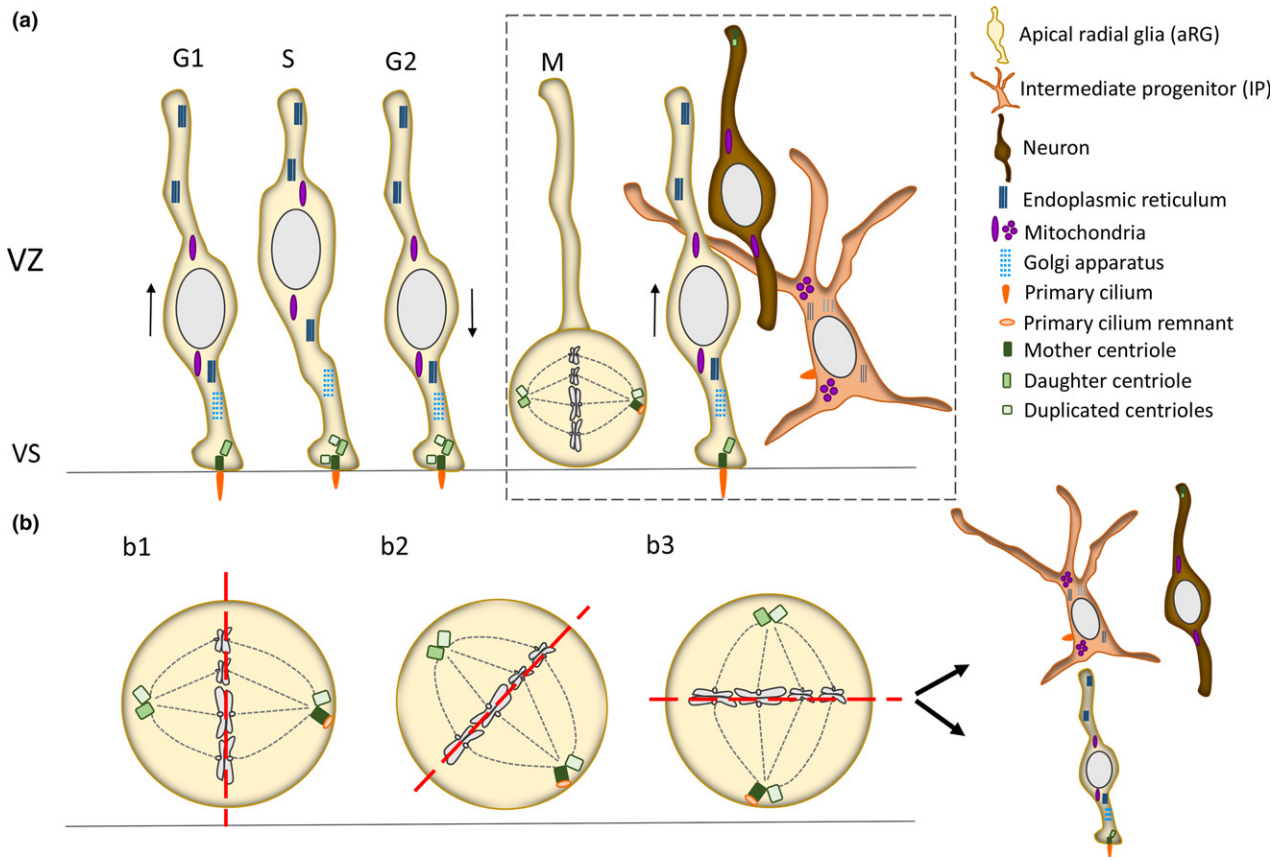


Fig. 1 Ventricular zone (VZ) dynamics and apical radial glia (aRG) behavior in the developing cortex a) VZ containing interphase and mitotic aRG, which divide to self-renew and produce intermediate progenitors (IP) or neurons. aRG nuclei move apico-basally through the different stages of the cell cycle, S-phase occurring in the most basal part of the VZ and mitosis at the ventricular surface (VS). Cellular organelles show a diverse distribution in interphase aRGs: the endoplasmic reticulum is localized in the basal and apical processes, whereas the Golgi apparatus is localized exclusively in the latter. Mitochondria present an elongated morphology. The older centriole, within the centrosome, constitutes the basal body docking a primary cilium, which becomes shorter as the cell cycle progresses. The mother and daughter centriole duplicate before M-phase. In IPs the Golgi acquires a more basal position and the mitochondria present a fragmented morphology. As it delaminates from the VZ, the IP docks a basolateral primary cilium. Newly born neurons migrate far from the VZ, while their mitochondria recover an elongated morphology. (b) aRG mitosis. For simplicity, the basal process of mitotic aRGs is not represented. aRGs mainly divide asymmetrically, self-renewing and producing a more committed cell in the neuronal lineage, for example, an IP or a neuron. Spindle orientation has been associated with daughter cell fate. Depending on the insertion of the cleavage furrow (red dashed line), divisions are defined as vertical (b1), oblique (b2), or horizontal (b3). The mother centriole as well as primary cilium remnants associated with it are inherited by the daughter cell acquiring an aRG fate thus remaining in the VZ. In this schema, the other daughter cell represents a multipolar IP or a newly born neuron.

E14.5 in mouse) are destined for the deeper layers of the neocortex. Later in corticogenesis, neurons are produced (e.g., E14.5–E17.5 in mouse) that migrate past the early born neurons to reach their appropriate positions in the upper layers of the CP. aRGs appear to be restricted over time in their potential to produce different neuronal subtypes. Thus, as neurogenesis proceeds, aRGs become more committed to a specific neuronal output (Luskin *et al*. 1988; McConnell and Kaznowski, 1991; Walsh and Cepko, 1993; Frantz and McConnell, 1996; Desai and McConnell 2000; Shen *et al*. 2006; Gao *et al*. 2014; Kaplan *et al*. 2017). This topic will be further discussed in section 'Multipotency and potential subpopulations of aRGs', and certain specific features of human aRGs in section 'Basal progenitors (BPs): intermediate progenitors (IPs) and basal radial glia-like cells (bRG), and their implication in cortical expansion'.

Another apical progenitor cell type that co-exists with aRGs in the VZ are the short neural progenitors (SNP), also known as apical intermediate progenitors (AIPS). SNPs have an apical process as aRGs, however, their basal process remains constrained within the VZ and hence does not span the entire cortical wall. SNPs located in the dorsal

telencephalon are mainly neurogenic progenitors, producing neurons upon symmetric division (Gal *et al*. 2006; Stancik *et al*. 2010). Nevertheless, SNPs found in the VZ of the GE are capable of self-renewing, as well as producing other types of progenitors (Pilz *et al*. 2013).

Basal progenitors (BPs): intermediate progenitors (IPs) and basal radial glia-like cells (bRGs), and their implication in cortical expansion

As corticogenesis progresses, aRGs produce basal progenitors (BPs), mainly intermediate progenitors (IPs) in the rodent, which are multipolar transit-amplifying progenitors. IPs are Tbr2+ (Englund *et al*. 2005) and they divide in a basally located germinal zone: the subventricular zone (SVZ). The Notch signaling pathway also plays a role in the transition from aRGs to IPs: activation of Notch signaling inhibits the production of IPs from aRGs (Mizutani *et al*. 2007; Martynoga *et al*. 2012). Another pathway modulating the switch between self-renewal and neurogenic commitment is the Wnt signaling pathway. In early corticogenesis it promotes aRG proliferation (Woohead *et al*. 2006), while in later stages it is crucial for IP and even neuronal production (Hirabayashi *et al*. 2004; Munji *et al*. 2011).

In the mouse, IPs are mainly neurogenic, dividing symmetrically to produce two neurons (Haubensak *et al*. 2004; Miyata *et al*. 2004; Noctor *et al*. 2004). Diverse mechanisms have been described to control this process (Borrell *et al*. 2012; Laguesse *et al*. 2015a, Haushalter *et al*. 2017). While murine IPs mainly undergo terminal divisions to produce neurons, in the primate–human brain they have a higher proliferative potential, being capable of self-renewing several times before their terminal division (Ostrem *et al*. 2017).

The initial pool of aRGs is larger in gyrencephalic species and these cells mostly promote indirect neurogenesis by giving rise to a diversity of basally located progenitors. This results in a higher neuronal production, which will have an effect on cortical size and folding (Fernandez *et al*. 2016).

While the developing murine cortex predominantly comprises aRGs and IPs, another type of progenitor can be found in very small numbers within the SVZ: the basal radial glia-like cells (bRGs), present at less than 1% of the cortical progenitors in mouse (Shitamukai *et al*. 2011; Wang *et al*. 2011). bRGs represent a large proportion of BPs in gyrencephalic species. They are produced from aRGs within a specific time-window early during corticogenesis (LaMonica *et al*. 2013; Gertz *et al*. 2014; Martinez-Martinez *et al*. 2016), and share similar features, as well as a wide range of characteristic markers with aRGs, such as Pax6, nestin, and vimentin (Ostrem *et al*. 2017). bRGs are considered essential for neocortical expansion and gyrification, and thus are mainly studied in human, ferret, and macaque brains (Fernandez *et al*. 2016; Ostrem *et al*. 2017). In these species, bRGs are localized in the most basal region of the SVZ, which is bisected by axons into an inner SVZ (iSVZ) and an outer SVZ (oSVZ). The oSVZ is the most proliferative germinal zone in the primate and human developing neocortex. bRGs are generally attached to the basement membrane by a basal process, although they lack the apical process characteristic of aRGs (Fietz *et al*. 2010; Hansen *et al*. 2010; Reillo *et al*. 2011; Wang *et al*. 2011). Although this is the classical morphological description of bRGs, it has been shown that they can present different combinations of progenitor markers and diverse shapes, and up to five different morphologies have been described in the primate SVZ (Betizeau *et al*. 2013). Upon cell division and prior to cytokinesis, their soma moves toward the developing CP in a process known as mitotic somal translocation (MST). In the mouse, they mainly divide in an exhaustive symmetric fashion to produce two neurons (Wang *et al*. 2011). However, in the ferret and primate (including human) brains, bRGs are capable of self-renewing while producing IPs and neurons (Hansen *et al*. 2010; Betizeau *et al*. 2013; Gertz *et al*. 2014; Martinez-Martinez, 2016). This phenomenon results in a greater number of progenitor cells, which in turn increases the final number of neurons produced. Activation of the Notch signaling pathway has been reported to be important for human bRG proliferation (Hansen *et al*. 2010).

bRGs have been shown to be produced by aRG horizontal cell divisions (LaMonica *et al*. 2013; Gertz *et al*. 2014; Martinez-Martinez *et al*. 2016), although direct delamination of aRGs has also been suggested (Gertz *et al*. 2014). In the mouse dorsal telencephalon, most aRG divisions are vertical, but in primate–human brains there is a shift toward horizontal divisions, which may be important for bRG production and oSVZ expansion (LaMonica *et al*. 2013). It is worth noting that the murine GE is the telencephalic region with the biggest diversity of progenitor types and a major production of BPs. GE-AP vertical divisions are less abundant, and proliferative SNPs divide mainly in a horizontal/oblique fashion (Pilz *et al*. 2013; Falk *et al*. 2017).

Two molecules were initially described to play a key role in temporally regulated bRG production and oSVZ seeding in the ferret: Cadherin 1 and Trnp1. Down regulation of Cadherin 1 and Trnp1 levels promotes horizontal aRG divisions and delamination from the VZ, resulting in bRG production. On the other hand, up-regulation of these proteins decreases bRG production, stopping the seeding of the oSVZ (Martinez-Martinez *et al*. 2016) Knockdown of Trnp1 in mouse aRGs had been previously correlated with a switch toward horizontal divisions and the production of bRG-like cells resulting in the appearance of folds in the otherwise smooth mouse neocortex. (Stahl *et al*. 2013). Related to this study, other molecules and signaling pathways have been shown to induce bRG production in the mouse developing cortex. For example, activation of Shh signaling in mouse aRGs during early corticogenesis leads to

the expansion of both IP and bRG-like cell populations, resulting as well in the presence of folds in the mouse cortex (Wang *et al*. 2016a). Forced expression of Pax6 in mouse aRGs and their progeny also induces the generation of primate-like bRGs (Wong *et al*. 2015).

In addition to these molecules, other studies have revealed certain genes only present in human aRGs that seem to be crucial for BP production. This is the case for the platelet-derived growth factor D (PDGFD). Although the latter is expressed in murine GE-aRGs it is exclusively expressed in dorsally located aRGs in human. Expression of PDGFD or ectopic expression of its receptor PDGFRβ in the mouse dorsal neuroepithelium induces production of bRG-like cells (Lui *et al*. 2014). Similarly, Florio and colleagues (2015) found the gene Rho GTPase-Activating Protein 11B (ARHGAP11B) to be preferentially expressed in human aRGs, and when over-expressed in the mouse neuroepithelium, it led to the generation of BPs (both IP- and bRG-like cells) (Florio *et al*. 2015). In line with these studies, Ju and colleagues (2016) revealed a novel function for *TBC1D3*, a great ape-specific gene which has undergone segmental duplications during evolution, in brain development and bRG production. Expression of TBC1D3 in the mouse VZ led to the production of primate-like bRGs, indicating the potential role of this gene in neocortical evolution (Ju *et al*. 2016). Very recently, Liu and colleagues (2017) identified the primate-specific gene TMEM14B as a marker for bRGs. Expression of this gene by *in utero* electroporation in the mouse VZ promoted neuronal progenitor production and expansion of the SVZ. In addition, Nestin-Cre-mediated knock-in mice for TMEM14B exhibited cortical folding (Liu *et al*. 2017). Taken together, these studies open the gate to the discovery of more primate and/or human-specific RG genes that could mediate the switch from aRGs toward BP production, particularly bRGs, as well as shed light into the cellular and molecular mechanisms that promote a bRG-like identity (Heide *et al*. 2017). Lastly, it would be of great interest to unravel why in the mouse developing cortex, Pax6+ progenitors located outside the VZ sometimes lead to cortical anomalies (Cappello *et al*. 2012; Insolera *et al*. 2014; Kielar *et al*. 2014), while other times they behave as proper bRG-like cells leading to expansion of the SVZ, increased number of upper layer neurons and/or cortical area, and ultimately the appearance of fold-like structures (Stahl *et al*. 2013; Florio *et al*. 2015; Wong *et al*. 2015; Ju *et al*. 2016; Liu *et al*. 2017).

The VZ contains aRGs, which move apico-basally through different stages of cell cycle by INM, with mitosis occurring at the VS. It is believed that INM occurs in order to create space in the VZ so more aRGs can undergo M-phase at the VS. It has been hypothesized that aRGs that eventually become bRGs may leave the VZ to escape this space constraint, moving basally to the oSVZ while maintaining several aRG traits (Florio and Huttner, 2014). Although they lack apical anchoring, bRGs maintain their basal processes which are likely to be important for their self-renewal potential. In addition, integrin signaling through basal contact has been shown to be involved in bRG proliferation and amplification. When integrin signaling was blocked in ferret brain organotypic slices, the pool of cycling progenitors both in the VZ and SVZ was decreased, the effect being more prominent in the SVZ (Fietz *et al*. 2010). The basal process of bRGs is important for MST, and the length, frequency and directionality of MST have been proposed to be associated with brain evolution. In human fetal brain, MST appears to be more frequent than in other species, and the distance travelled by the nuclei seems to be greater, following a straighter pattern of movement toward the pial surface (Ostrem *et al*. 2017).

Although we have focused on the importance of generating a large quantity of progenitors to increase neuronal output, bRGs also contribute to the tangential expansion of the neocortex by providing more basal fibers, without corresponding apical attachments, which act as a scaffold for migrating neurons to reach their final position (Florio and Huttner, 2014; Borrell and Gotz, 2014). These cells hence allow a 'fan-like' pattern of neuronal migration, which was recently proposed to contribute to cortical folding (del Toro *et al*. 2017). By using a mouse model in which the adhesion molecules Flrt1 and Flrt3 were deleted, the authors observed the appearance of gyri and sulci in the mouse cortex. Strikingly, this effect was not because of progenitor amplification, but rather a change in the pattern of neuronal migration (Del Toro *et al*. 2017). Therefore, although progenitor amplification and increased neuronal output are key for neocortical expansion, emerging studies are uncovering novel mechanisms which seem to be important for the cortical folding process (see also Heide *et al*. 2017).

Finally, it is worth mentioning that these primate–human-specific traits may be one of the main targets of brain diseases and cortical malformations, which may explain why mouse models often fail to recapitulate the patients' phenotype. The use of cerebral organoids will help to unravel human-specific mechanisms of brain development as well as generating novel *in vitro* human models of neurodevelopmental and neurodegenerative diseases (Di Lullo and Kriegstein, 2017). Lately, two different studies used brain organoids to study the mechanisms leading to Miller–Dieker syndrome (Bershteyn *et al*. 2017; Iefremova *et al*. 2017), a severe form of lissencephaly. While defects in aRG divisions could be detected (Bershteyn *et al*. 2017; Iefremova *et al*. 2017), potentially recapitulating what had already been found by using mouse models (Yingling *et al*. 2008), a defect in bRG division was also observed (Bershteyn *et al*. 2017). Hence, it is important to further develop human-like models to study certain diseases, since human-specific traits may be altered.

Intrinsic versus extrinsic influences on progenitor transitions

Diverse studies have shown the importance of feedback signaling from post-mitotic neurons to maintain the aRG proliferative state. Notch signaling is probably the most studied: differentiating cells express Notch ligands belonging to the Delta-like and Jagged families, which signal toward aRGs triggering the Notch signaling cascade and thus the repression of proneural factors (Agirman *et al*. 2017). The Wnt7-Celsr3-Fzd3 pathway was shown to mediate Jag1 expression in immature neurons (Wang *et al*. 2016b). Immature neurons lacking Celsr3 or Fzd3 do not respond to cortical Wnt7, which results in a down-regulation of Jag1 levels, and a consequent down-regulation of Notch signaling in aRGs (Wang *et al*. 2016b). The TF Smad-interacting protein 1 (Sip1) is highly and exclusively expressed in post-mitotic neurons throughout corticogenesis, and its deletion also impacts aRG behavior triggering a production of upper layer neurons at the expense of deep layer neurons, and eventually a precocious gliogenesis (Seuntjens *et al*. 2009). Related to this work, Parthasarathy and coworkers showed that neurotrophin 3 (Nt3), which is a target of Sip1, modulates the balance between aRG self-renewal and commitment to a more differentiated progenitor cell type (Parthasarathy *et al*. 2014). However, down-regulating Nt3 levels does not rescue the Sip1 mouse mutant phenotype, suggesting that in the latter there are additional mechanisms regulating progenitor fate switch (Parthasarathy *et al*. 2014). Signals derived from other neuronal populations also influence aRGs. Cajal Retzius cells are among the first neurons produced arising from different regions of the telencephalon and distributing superficially around the developing cortex. Changed distributions of these cells were shown to alter aRG proliferation (Griveau *et al*. 2010). Lastly, deletion of a transient tangentially migrating glutamatergic population derived from ventral regions of the cortex also alters aRG proliferation and enhances a precocious switch toward neuronal production (Teissier *et al*. 2010).

Extrinsic factors are also emerging to be crucial not only for aRG but also for bRG proliferation. Transcriptome analyses point toward a role for extracellular matrix components produced in a cell-autonomous manner in controlling bRG proliferation (Fietz *et al*. 2012; Florio and Huttner, 2014; Pollen *et al*. 2015). Additionally, extrinsic factors derived from neurons in different brain regions could affect the proliferation of SVZ progenitors. Particularly, thalamocortical axons invading the intermediate zone (IZ) have been proposed to be an important source of mitogens for progenitors located in the SVZ in gyrencephalic species (Dehay and Kennedy, 2007). Recent work by Reillo and colleagues (2017) supports the influence of growing axonal tracts on progenitor dynamics. By combining axonal and cell lineage tracing with immunostainings, the authors correlated progenitors with different axonal tracts, and illustrated the dynamic interactions occurring throughout development (Reillo *et al*. 2017).

Although extrinsic mechanisms mediate progenitor dynamics, other studies support an intrinsic control of the latter. For instance, Albert and colleagues (2017) have also recently reported that transitions between cell types are moderated by different histone methylation profiles. By isolating diverse progenitor populations in the mouse developing cortex (namely NEs, aRGs, bRGs, and neurons) followed by ChIP-seq, they traced different methylation patterns regulating the expression of TFs known to be involved in progenitor transitions (Albert *et al*. 2017). Thus, this paper proposes additional strategies controlling progenitor cell fate.

Factors influencing symmetric versus asymmetric division

Mitotic spindle and cleavage plane, inheritance of apical and basal processes

Invertebrate models have been used extensively to study the mechanisms controlling symmetric versus asymmetric cell division (for a review see di Pietro *et al*. 2016). Orientation of the cleavage plane may influence daughter cell fate and control the mode of division (Fig. 1). In these models, cell fate determinants are asymmetrically positioned within the cell prior to mitosis. When the mitotic spindle is oriented parallel to the apico-basal gradient of fate determinants, the cleavage furrow gives rise to an uneven repartition of these factors between the daughter cells, leading to an asymmetric cell division. On the contrary, when the mitotic spindle is positioned perpendicular to this gradient, the cleavage furrow will be oriented in the same direction of the gradient, leading to an even distribution of fate determinants: the progeny generated will acquire the same identity.

Initially it was thought that a similar situation occurs in mammalian aRGs. More precisely, symmetric divisions would be achieved by insertion of the cleavage furrow perpendicular to the VS (vertical divisions), while a parallel or oblique insertion of the latter (horizontal or oblique divisions, respectively) would lead to an asymmetric cell division producing two daughter cells with different fate (spindle orientations depicted in Fig. 1b) (Chenn and McConnell, 1995). However, association between orientation of the cleavage plane and daughter cell fate is not as straightforward, and this will be discussed further here.

Inheritance of the apical domain of aRGs was initially thought to be critical for daughter cell fate determination. A previous model proposed that the daughter cell inheriting the apical domain, would retain aRG identity, while the cell deprived of this apical structure would be committed to a different fate, for example, by being more restricted to the neuronal lineage (but see below). Since asymmetric inheritance of the apical structure may lead to differentiative

divisions, a slight tilt in spindle orientation, enough for the cleavage plane to bypass the apical domain, could trigger an asymmetric cell division (Kosodo *et al*. 2004; Marthiens and ffrench-Constant, 2009). Thus, it is important to carefully determine whether the cleavage furrow bypasses or divides the apical domain during aRG mitoses, since it may have an impact on the identity of the newly generated daughter cells. This domain was originally identified by immunohistochemistry (Kosodo *et al*. 2004; Marthiens and ffrench-Constant, 2009). More recently live-imaging techniques provide information concerning the dynamics of its cleavage through the last steps of M-phase (Konno *et al*. 2008; Asami *et al*. 2011; Matsuzaki and Shitamukai, 2015). These reports showed that only in a relatively low proportion of cell divisions during the neurogenic period of corticogenesis, the apical domain was uniquely inherited by one of the daughter cells (Konno *et al*. 2008; Asami *et al*. 2011; Shitamukai *et al*. 2011). Accordingly, these observations suggest that factor(s) other than apical domain inheritance determine cell fate, and this is in agreement with the fact that during mouse neurogenesis there is a majority of vertical divisions (Matsuzaki and Shitamukai, 2015) not all of them being proliferative and symmetric. Thus, although classically the inheritance of the apical epithelial structure, dependent on cleavage furrow insertion, was believed to be key to acquiring proliferating aRG identity, this vision is currently changing, indicating that other features are crucial to determine daughter cell identity and to provide self-renewal potential.

Inheritance of the aRG basal process has also been shown to contribute to cell fate determination. Basal process-dependent integrin signaling appears to be important for maintaining aRG and bRG proliferative potential (Fietz *et al*. 2010). In addition, Tsunekawa and coworkers (2012) described how the mRNA of cyclin D2, responsible for G1 progression, is mainly present in aRG basal processes. This implies that upon aRG cell division, only the daughter cell inheriting the basal process will progress through G1 while retaining proliferative potential (the relationship between cell cycle length, particularly G1, and proliferative potential is further addressed in section 'Cell cycle considerations'). Thus, inheritance of the basal process seems to promote a proliferative state and to maintain aRG self-renewal potential (Konno *et al*. 2008, Fietz *et al*. 2010; Tsunekawa *et al*. 2012). Emphasizing the importance of epithelial structure inheritance, in zebrafish it was described how upon aRG division, aRG-fated daughter cells often lose their apical endfoot, which then regrows allowing maintenance of the whole epithelial structure (i.e., apical and basal processes) (Alexandre *et al*. 2010). In addition, delaminating neurons in the chick spinal cord lose their apical structure while leaving the VZ (Das and Storey, 2014). Therefore, inheritance and maintenance of epithelial features (apical and basal processes) seem to be a key factor controlling cell fate upon aRG division (Matsuzaki and Shitamukai, 2015).

Related to the distribution of cell fate determinants and retention of epithelial features, mitotic spindle assembly and attachment to the cell cortex is also a tightly controlled process which can influence these processes. Centrosomes, which are localized in the apical process of aRGs during interphase, move a short distance basally before undergoing duplication and forming the spindle poles (Hu *et al*. 2013). The latter nucleate microtubules constituting the spindle, and they are also associated with astral microtubules, which link the mitotic spindle to the cell cortex. Mitotic spindle orientation is likely to be regulated by a complex of proteins involved in its attachment to the cell cortex by astral microtubules. This complex includes the GoLoco domain-containing protein LGN, which associates with the cell cortex through Gαi. In addition, LGN binds to nuclear-mitotic apparatus protein (NuMA), which binds to the dynein–dynactin complex. This motor complex exerts forces on the astral microtubules, thus orienting the mitotic spindle (for a review see di Pietro *et al*. 2016).

Several studies have shown the impact of altering the level of these proteins on spindle orientation and aRG progeny after cell division. Interfering with the levels of LGN leads to a randomization of spindle orientation, both in the chick (Morin *et al*. 2007) and in the mouse neuroepithelium (Konno *et al*. 2008). Both studies show that LGN is crucial to maintain vertical cell divisions, and randomization of the mitotic spindle leads to the presence of aberrantly positioned cycling progenitors (Konno *et al*. 2008). Despite the spindle fluctuations, there is no change in neuronal output or in brain size when deleting LGN in the mouse, suggesting that ectopic progenitors still generate neurons (Konno *et al*. 2008). Also, the LGN-interacting protein disks large homolog 1 (Dlg1) controls vertical divisions in the chick neuroepithelium (Saadaoui *et al*. 2014). Dlg1 ensures the proper localization of LGN to the cell cortex and it is necessary for correct spindle orientation (Saadaoui *et al*. 2014). The GoLoco-containing protein activator of G protein signaling 3 (AGS3), which also interacts with NuMA, was initially described to have similar functions to LGN and to be important for spindle orientation, since knockdown of AGS3 in mouse aRGs led to a decrease in oblique spindle orientations (Sanada and Tsai, 2005). However, this function of AGS3 is controversial, since a recent study has shown that despite its interaction with NuMA, AGS3 is not recruited to the cell cortex in mouse aRGs, and it does not control mitotic spindle orientation during cell division (Saadaoui *et al*. 2017). This study is in agreement with previous work showing no brain anomalies in AGS3 knockout mice (Blumer *et al*. 2008).

Several proteins associated with cortical malformations have been shown to have an impact on mitotic spindle

positioning (for a review of cortical progenitors and malformations see Bizzotto and Francis, 2015). Mutations in the dynein regulators Lis1 and nuclear distribution protein homolog 1 (Nde1) lead to spindle orientation defects, resulting in neuronal progenitor depletion, and thus affecting neuronal production (Feng and Walsh, 2004; Yingling *et al*. 2008). Tctex-1 (*Dynlt1*), the light chain component of the dynein motor complex, and the guanine nucleotide exchange factor Lfc also regulate spindle orientation in a convergent fashion (Gauthier-Fisher *et al*. 2009). The interplay between these two proteins regulates the balance between aRG proliferation and neuronal differentiation. More precisely, Tctex-1 inhibits the pro-neurogenic actions of Lfc and enhances aRG vertical divisions (Gauthier-Fisher *et al*. 2009). Many genes involved in centrosome-related functions are associated with microcephaly and have been reported to influence spindle orientation. For instance, knockdown of Aspm, the most frequently affected gene in autosomal recessive human primary microcephaly, leads to a decrease in vertical symmetric cell divisions in NEs, promoting asymmetric mitoses, and precocious cell delamination from the neuroepithelium (Fish *et al*. 2006). Citron kinase (CITK), another protein known to be responsible for microcephaly in humans, has been shown to be important for maintenance of vertical divisions in early corticogenesis (Gai *et al*. 2016). CITK possibly converges with Aspm function to promote symmetric cell divisions and NE amplification, since CITK over-expression rescues the mitotic spindle defects observed upon Aspm knockdown (Gai *et al*. 2016). Loss of Cdk5rap2 also perturbs spindle orientation of aRGs (Lizarraga *et al*. 2010), although other mitotic spindle defects were also observed in this study. Related to other diseases, mutations in echinoderm microtubule-associated protein-like 1 (Eml1), a microtubule-binding protein associated with subcortical band heterotopia, also lead to an increase in oblique divisions, as well as to increased mitotic spindle length (Kielar *et al*. 2014; Bizzotto *et al*. 2017). In addition, silencing of Huntingtin, the protein associated with Huntington's disease, also affects aRG mitotic spindle orientation (Godin *et al*. 2010). Additionally, mutant Huntingtin perturbs these processes by altering the localization of proteins such as NuMA and dynein (Molina-Calavita *et al*. 2014). Although these disease-associated proteins have been associated with spindle orientation defects, it still remains to be elucidated if this is a main and primary phenotype observed upon their deregulation. Other microtubule-associated processes, such as ciliogenesis, centrosome biogenesis, and correct mitotic spindle assembly could also be altered (Lizarraga *et al*. 2010; Insolera *et al*. 2014; Garcez *et al*. 2015; Gabriel *et al*. 2016; Jayaraman *et al*. 2016; Bizzotto *et al*. 2017). Thus, further work is required questioning if abnormal spindle orientation represents the direct cause and/or the consequences of other primary defects which eventually lead to the cortical anomalies.

Although still controversial, spindle orientation has been suggested to regulate NE and aRG progeny fate, and to ensure that the progenitor pool expands enough to produce the appropriate number of neurons. Spindle orientation changes toward a horizontal/oblique aRG division have been associated with the generation of daughter cells which differentiate prematurely (Fish *et al*. 2006, Gai *et al*. 2016). In addition, as mentioned above, bolstering horizontal and oblique divisions in the mouse neuroepithelium in early- to mid-corticogenesis has been associated with the production of bRGs (Shitamukai *et al*. 2011; Stahl *et al*. 2013; Wong *et al*. 2015), as well as in ferret and human corticogenesis (LaMonica *et al*. 2013; Martinez-Martinez *et al*. 2016). Thus, tilting the mitotic spindle resulting in an horizontal/oblique division can lead to depletion of the aRG pool and a decreased number of neurons (Feng and Walsh, 2004; Fish *et al*. 2006, Yingling *et al*. 2008; Gauthier-Fisher *et al*. 2009; Gai *et al*. 2016), to the presence of aberrantly positioned cycling progenitors (Konno *et al*. 2008; Insolera *et al*. 2014, and reviewed in Bizzotto and Francis, 2015), as well as being related to the production of IPs and bRGs (Fish *et al*. 2006; Shitamukai *et al*. 2011; LaMonica *et al*. 2013; Stahl *et al*. 2013; Wong *et al*. 2015; Gai *et al*. 2016, Martinez-Martinez *et al*. 2016). In addition, recently it has been reported that randomization of the mitotic spindle can also alter the ratios of different AP populations, decreasing aRG numbers while increasing the amount of SNPs (Falk *et al*. 2017). These combined data strongly support the idea that other factors contribute to daughter cell fate determination, and we need to further unravel the mechanisms by which spindle orientations become perturbed and impact cell fate.

Cellular organelle behavior in progenitors

Role of microtubule-based organelles in cortical progenitor dynamics: centrosome, primary cilium, and midbody

As discussed in the preceding section, centrosome-related proteins that are associated with cortical malformations, particularly with primary microcephaly, can affect spindle orientation (Feng and Walsh 2004; Fish *et al*. 2006; Gai *et al*. 2016). There are also other processes related to centrosomes that can be abnormal, leading to alterations in progenitors and neuronal production. Conditional deletion in aRGs of Centromere protein J (Cenpj) (also known as spindle assembly abnormal protein 4, Sas4), a critical protein for centrosome biogenesis, leads to progressive loss of centrosomes, causing mitotic delay and aRG delamination from the VZ. These ectopic proliferating Pax6+ progenitors eventually undergo p53-mediated apoptosis resulting in a thinning of upper cortical layers and microcephaly (Insolera *et al*. 2014). A more recent study also addressed the role of Cenpj

by performing acute knockdown of the protein at mid-corticogenesis (Garcez *et al*. 2015). The latter interferes with centrosome biogenesis, specifically affecting centriole duplication, which in turn results in a decrease of astral microtubules and spindle orientation defects. In this study, perturbing centriole duplication causes Cenpj-depleted aRGs to remain in M-phase, eventually leading to the appearance of ectopic Tbr2+ progenitors in the VZ (Garcez *et al*. 2015), a phenotype hence differing from that previously described for the Cenpj conditional knockout mice (Insolera *et al*. 2014). A role for Wdr62 and Aspm was shown interacting and localizing to the mother centriole, and showing gene dose-related centriole duplication defects which correlate with the severity of the reduced cortical thickness and brain size observed in mutant mice (Jayaraman *et al*. 2016). No spindle orientation defects were observed in the Wdr62 mouse model (Jayaraman *et al*. 2016). In different studies, the production of extra-numerary centrosomes and the formation of multipolar mitotic spindles were reported to deplete the aRG pool, which in turns results in a decreased neuronal output and a smaller brain (Marthiens *et al*. 2013). Increased numbers of mitotic spindle poles were also observed upon loss of Cdk5rap2, resulting in a decrease of aRGs and premature neurogenesis (Buchman *et al*. 2010; Lizarraga *et al*. 2010).

Therefore, centrosome-related proteins have a critical role in controlling aRG cell division, progeny fate, and neuronal output. In addition, it was elegantly shown that in mouse aRGs, upon asymmetric division, the daughter cell that inherits the eldest centriole, namely the 'old mother' centriole, acquires the self-renewal aRG fate remaining in the VZ, while the cell inheriting the 'new mother' centriole is committed to differentiate (Fig. 1b) (Wang *et al*. 2009). Recently, it was discovered that Mindbomb1 (Mib1), known for its role in the Notch signaling pathway, also acts as a fate determinant by associating with centriolar satellites. In asymmetric divisions in the chick spinal cord neuroepithelium, Mib1 is inherited by the daughter cell that differentiates into a neuron (Tozer *et al*. 2017). Inheritance of pericentriolar material components may hence be important for determining cell fate, potentially regulating signaling pathways such as Notch. In an unrelated study, Delaunay and coworkers (2014) showed that the mitotic spindle *per se* could as well determine the asymmetry of a cell division. Spindle size asymmetry (SSA), which appears to be mediated by Wnt signaling through Vangl2, has a role in determining cell fate upon aRG division: cells that become neurons preferentially arise from the daughter cell (often apical) receiving the pole with the larger spindle volume (Delaunay *et al*. 2014).

The centrosome is also a critical organelle during interphase in aRGs since the mother centriole acts as a basal body to dock the primary cilium (Fig. 1a). The primary cilium is an antenna-like structure located in the apical domain of aRGs, sensing signals from the CSF, and triggering signaling pathways (Shh, Wnt, mTOR) that can control diverse features of these cells, such as proliferation (Itoh, 2010; Lehtinen *et al*. 2011) and apical domain size (Foerster *et al*. 2017). The primary cilium is reabsorbed before mitosis, when the centrosomes constitute the spindle poles. Although reabsorption of the primary cilium prior to M-phase has been classically accepted, Paridaen and colleagues (2013) showed that ciliary membrane remnants (CR) remain attached to the mother centriole throughout mitosis in aRGs in the mouse neuroepithelium (Fig. 1b). The cell inheriting both the old mother centriole and the CR docks a primary cilium apically before its sister cell, thus being exposed to CSF signals first (Piotrowska-Nitsche and Caspary, 2012). This CR-inheriting cell tends to remain an aRG in the VZ, while the other cell acquires a neurogenic fate (Fig. 1b) (Paridaen *et al*. 2013). Tbr2+ differentiated cells leaving the VZ after cell division dock a primary cilium in the basolateral membrane while delaminating (Fig. 1b) (Wilsch-Bräuninger *et al*. 2012).

Although CR present in M-phase appear to have a role in the production and maintenance of aRGs, disassembly of the primary cilium prior to mitosis has also been reported to be critical for correct progression of the neuronal progenitor cell cycle, and generation of the appropriate number of neurons (Gabriel *et al*. 2016). By using organoids derived from hiPSCs, a role for CENPJ in promoting cilium disassembly was unraveled. This previously unknown function of CENPJ was found critical for cell cycle re-entry and progenitor proliferation: the neuronal progenitors of organoids derived from CENPJ-mutant hiPSCs showed impaired cell cycle re-entry, accompanied by premature neuronal differentiation (Gabriel *et al*. 2016), hence differing from data generated in mouse models. Related to primary cilium disassembly, in the chick neuroepithelium, neurons leaving the VZ upon cell division showed abscission of the primary cilium (Das and Storey, 2014). Shedding and excision of ciliary (and midbody, see below) particles into the ventricle was also previously identified (Dubreuil *et al*. 2007).

Therefore, the centrosome and primary cilium, which are tightly connected throughout the cell cycle, play a key role in controlling aRG proliferation. Disruption of proteins associated with these organelles can result in diverse aRG defects, such as spindle orientation abnormalities (Feng and Walsh, 2004; Fish *et al*. 2006; Gai *et al*. 2016), altered centrosomal biogenesis (Insolera *et al*. 2014; Garcez *et al*. 2015; Jayaraman *et al*. 2016) abnormal mitotic spindle assembly (Lizarraga *et al*. 2010; Marthiens *et al*. 2013), and disruption of primary cilium assembly/disassembly (Paridaen *et al*. 2013; Gabriel *et al*. 2016).

Apical cilium assembly in epithelial cells requires midbody remnants (Bernabé-Rubio *et al*. 2016). When the cleavage furrow grows toward the apical membrane, microtubules of the central spindle are compacted and give rise to

the midbody, which is formed at the bridge between apical cells during cytokinesis. Several midbody proteins are recruited prior to cytokinetic abscission, which are either inherited by one daughter cell or released into the extracellular space, to be engulfed by another cell (Dionne *et al*. 2015; Dwyer *et al*. 2016). As for the primary cilium, shedding and excision of midbody-derived particles has been previously described in the chick neuroepithelium (Dubreuil *et al*. 2007). Disruption of midbody morphology and abscission has been reported in mouse mutants for the microtubule motor protein Kif20b (Janisch *et al*. 2013). Remarkably, in this study, no mitotic defects were observed. However, in early stages of corticogenesis, misalignment of the midbody with the apical membrane and change in its morphology appear to trigger apoptosis, leading to a reduction in the progenitor pool, and a thinner cortex (Janisch *et al*. 2013). The relationship between the midbody and NEs and/or the apical membrane of aRGs, as well as the primary cilium, is still not well understood. However, the retention of this structure, known for its role in mediating the final steps of cell division, could also be important for progenitor proliferation and maintenance, and like CR inheritance, appears to be crucial for aRG fate acquisition (Paridaen *et al*. 2013).

Insights into mitochondria and Golgi apparatus function in APs

Mitochondria are crucial in post-mitotic neurons to provide energy, and abnormalities in this organelle are associated with neurodegenerative diseases. Additionally, several mitochondria-related diseases show developmental manifestations (Khacho and Slack, 2017). Although the role of mitochondria and the Golgi apparatus in early steps of corticogenesis are not extensively described, and their roles in cortical neuronal progenitors remain largely unknown, recent studies have shed light on their functions during progenitor proliferation and neuronal differentiation. Firstly, mitochondrial morphology has been reported to differ between aRGs, IPs, and neurons (Khacho *et al*. 2016). While mitochondria morphology is elongated both in aRGs and neurons (Fig. 1a), it has a fragmented appearance in IPs (Fig. 1a) (Khacho *et al*. 2016). Loss of mitochondrial function in aRGs leads to defects in proliferation, disruption of cell cycle exit, and inability of cells to differentiate into neurons, resulting in cortical thinning (Khacho *et al*. 2017). Related to this study, the level of mitochondrial reactive oxygen species was shown to be decreased in differentiating neurons as compared to APs progenitors (NEs and aRGs) (Inoue *et al*. 2017). Additionally, the acute loss or gain-of-function of PR domain zinc finger protein 16 (Prdm16), a TF that regulates mitochondrial reactive oxygen species levels, led to defects in aRG proliferation, whereupon differentiating cells showed aberrant morphologies and locations in the cortical wall (Inoue *et al*. 2017).

Taverna and colleagues (2016) described different behaviors of the Golgi apparatus in aRGs and committed IPs. The Golgi localizes in the apical process of aRGs, and is not associated with the centrosome (Fig. 1a). When an aRG commits to an IP fate, the Golgi translocates toward a more basal position, bypassing the nucleus and locating itself in a basally located process (Fig. 1a) (Taverna *et al*. 2016). Another role ascribed to Golgi is in symmetric versus asymmetric divisions, since a pool of the pericentriolar fate determinant Mib1 is stored in this organelle (Tozer *et al*. 2017). When a progenitor undergoes symmetric division, Mib1 is released from the Golgi to ensure its equal repartition among the progeny (Tozer et al. 2017). Thus, the location and potential storage function of the Golgi apparatus in progenitors seems to contribute to the mode of aRG division.

Contribution of the Unfolded Protein Response (UPR), within the endoplasmic reticulum (ER), to neurogenesis

As previously described, aRGs begin to divide asymmetrically at E12 in mice via a temporal progression from direct to indirect neurogenesis to give rise to IPs, a transient albeit crucial population of precursors (reviewed in Tiberi *et al*. 2012).

Although the molecular mechanisms that control this transition from direct to indirect neurogenesis during embryonic development remain to be clarified, accumulating reports have demonstrated the unfolded protein response (UPR) within the endoplasmic reticulum (ER) of aRGs to be a key modulatory factor (reviewed in Laguesse *et al*. 2015b, Dwyer *et al*. 2016). The ER is well-documented to be a specialized and dynamic tubular organelle that is a keystone in many metabolic processes, a reservoir of intracellular calcium, and also critical for proper folding of proteins (reviewed in Hetz, 2012). The latter is essential for synthesis and post-translational modifications of newly synthesized secretory and membrane proteins, as well as their 3-D conformation and eventual export. Upon induction of ER stress within cells, a series of complementary adaptive mechanisms are activated to reduce translation and manage the protein-folding alterations, hence collectively categorized as the UPR (reviewed in Hetz, 2012).

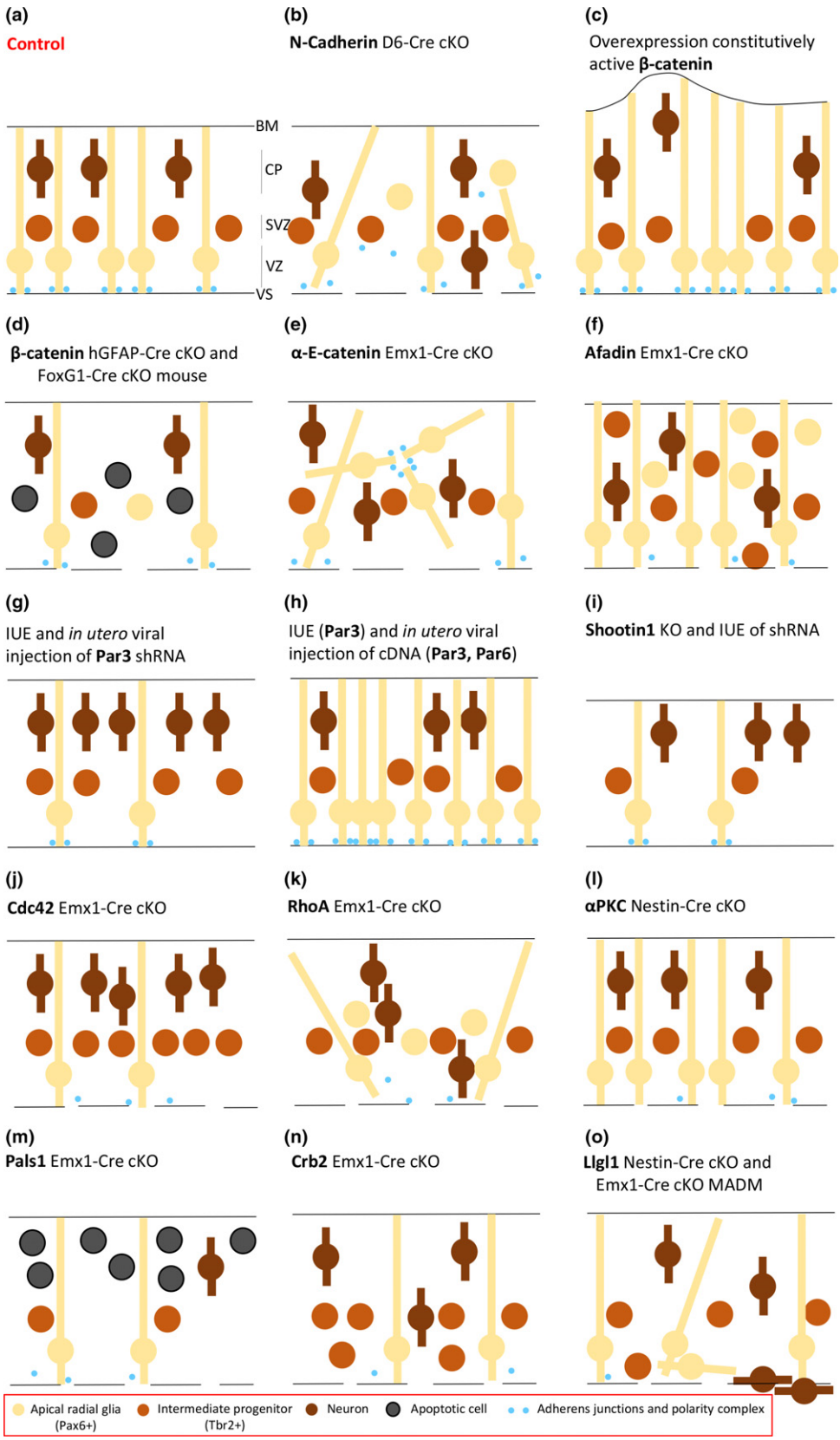
The regulation of UPR signaling during neurogenesis has been demonstrated in various *in vivo* (Shim *et al*. 2004; Zhang *et al*. 2007; Mimura *et al*. 2008; Laguesse *et al*. 2015a,b) and *in vitro* models (Hayashi *et al*. 2007; Firtina *et al*. 2009; Firtina and Duncan, 2011) with the activation of PERK and IRE-1 of the three-armed UPR pathway in murine embryonic stem cells (mES) (Cho *et al*. 2009) and human neural stem cells (hNSC) (Tseng *et al*. 2017). Furthermore, chemical induction of ER stress (by brefeldin A, thapsigargin, or tunicamyin) facilitates neurogenesis and neuronal differentiation at the expense of gliogenesis and glial differentiation, respectively, in P19 cells (Kawada *et al*. 2014), mES (Cho *et al*. 2009), and hNSC (Tseng *et al*.

2017). These findings are corroborated by accumulating *in vivo* reports of spatio-temporal expression of distinct UPR effectors that are associated with the regulation of UPR signaling, which correlates with milestones of murine corticogenesis that suggests physiological functions of UPR in neuronal commitment and cell fate acquisition (Atf4 (Frank *et al*. 2010; Laguesse *et al*. 2015a); Atf5 (Laguesse *et al*. 2015a); Atf6 (Zhang *et al*. 2007); Xbp1 (Hayashi *et al*. 2007; Zhang *et al*. 2007). Of interest is the progressive reduction of Atf4 signaling (the downstream effector of the PERK-mediated UPR pathway) in aRGs as corticogenesis proceeds, which is inversely correlated with the rate of indirect neurogenesis (Frank *et al*. 2010; Laguesse *et al*. 2015a). Together, these reports indicate that a gradual suppression of basal UPR promotes the transition from direct to indirect neurogenesis in aRGs during corticogenesis.

Furthermore, the physiological relevance of the UPR pathway in cell fate acquisition (Hetz and Papa, 2017) is increasingly supported by reports of neurodevelopmental phenotypes in genetic (Laguesse *et al*. 2015a) and infective (Gladwyn-Ng *et al*. 2018) mouse models, whereupon ER stress induction and UPR up-regulation in aRGs during mid-corticogenesis prolonged direct (at the expense of indirect) neurogenesis in embryonic mouse brains and hNSCs, with a subsequent decrease in the production of IPs (Laguesse *et al*. 2015a, Gladwyn-Ng *et al*. 2018). Laguesse and coworkers demonstrated in mice genetically deficient for the Elongator complex, specifically the loss of its Elongator complex protein 3 (Elp3), an impairment of speed of mRNA translation in aRGs, with an elevation in Perk–eIF2–Atf4 signaling throughout corticogenesis (Laguesse *et al*. 2015a). Gladwyn-Ng and colleagues reported a unique tropism of Zika virus (ZIKV) among the flaviviruses for cortical progenitors during fetal embryogenesis, and viral replication within the ER in various murine models of ZIKV infection (Gladwyn-Ng *et al*. 2018). Mice from both studies displayed severe microcephaly that resulted from a diminished rate of indirect neurogenesis that was linked to the triggering of ER stress and UPR up-regulation in aRGs. Crucially, suppressing the pathological up-regulation of Atf4 levels in either Elongator-deficient or ZIKV-infected progenitors rescued the neurogenic balance, as well as the microcephalic phenotype in the latter (Laguesse *et al*. 2015a, Gladwyn-Ng *et al*. 2018). These reports demonstrate a causal link between UPR dysregulation and protracted direct neurogenesis (at the expense of indirect neurogenesis) that is associated with microcephaly, and suggest this may underlie neurodevelopmental disorders.

Altogether, these reports uncovered the progressive and dynamic down-regulation of UPR in cortical progenitors that contributes to a homeostatic signaling network that

Fig. 2 Schematic representation of the developing cortex in polarity complex and cell adhesion molecule mouse mutants. (a) Wild type developing cortex: Apical radial glia (aRG, yellow cells) have their soma localized in the ventricular zone (VZ). They are attached to each other at the ventricular surface (VS) by their apical processes characterized by the presence of the polarity complex and adherens junctions (AJ) (altogether represented by blue dots). Their basal process is attached to the basement membrane (BM). Intermediate progenitors (IPs, orange cells) are localized in the subventricular zone (SVZ) and lack this polarized morphology. Newly generated neurons (brown cells) are positioned in the developing cortical plate (CP). First and second rows (b–f) depict mouse models for proteins mainly associated with AJs and cell adhesion. Third, fourth, and fifth rows (g–o) depict mouse models for polarity proteins. (b) Conditional deletion of N-cadherin disrupts aRG AJs and the VS. Mitotic cells, particularly Pax6+, were found scattered through the cortical wall and their processes showed an aberrant morphology, not spanning the entire developing cortex. Newborn neurons were also found in abnormal positions (Kadowaki *et al*. 2007). (c) Over-expression of constitutively active β-catenin in the neuroepithelium promotes aRG self-renewal increasing the tangential surface, as well as the final cortical area. This results in the appearance of fold-like structures (Chenn and Walsh, 2002). (d) Conditional deletion of β-catenin disrupts the VS and AJs. aRG decrease their self-renewing potential and they are found in ectopic positions. Mitotic cells (aRGs and IPs) undergo apoptosis, thus there is a decrease in aRG, IP, and neuronal numbers (Junghans *et al*. 2005; Gan *et al*. 2014). (e) Conditional deletion of α-E-catenin disrupts the VS and AJs. AJ-like structures are scattered in the cortical wall. aRG fibers are disorganized, and neurons are mislocalized forming a 'double-cortex' (Schmid *et al*. 2014). (f) Conditional deletion of afadin disrupts the VS and AJs. There is an increased proliferation of progenitor cells, accompanied by an increase in aRGs and IPs, localized throughout the entire developing cortex. Neurons are mislocalized, eventually forming a 'double cortex' (Gil-Sanz *et al*. 2014). (g) Par3 knockdown decreases aRG self-renewal potential resulting in a premature production of neurons (Costa *et al*. 2008, Bultje *et al*. 2010). (h) Over-expression of Par3 and Par6 promotes aRG proliferation (Costa *et al*. 2008, Bultje *et al*. 2009). (i) Deletion of shootin1 causes a decrease in mitotic cells (presumably aRGs and IPs), resulting in decreased neuronal numbers (upper layer neurons) and thinning of the cortical plate (Sapir *et al*. 2017). (j) Conditional deletion of Cdc42 leads to gradual loss of AJs and disruption of the VS. There is a decrease in aRG mitoses accompanied by an increase in mitotic IPs and neurons (Cappello *et al*. 2006). (k) Conditional deletion of RhoA results in the presence of aberrantly positioned aRGs and ectopic neurons, accompanied by the disruption of AJs (Cappello *et al*. 2012). (l) Conditional deletion of αPKC causes AJ and VS anomalies without major effects on corticogenesis (Imai *et al*. 2006). (m) Conditional deletion of Pals1 disrupts the VS and AJs. The aRG pool is depleted followed by a massive death of newly generated neurons (Kim *et al*. 2010). n) Conditional ablation of Crumbs2 (Crb2) disrupts the VS and AJs. It causes a depletion of the aRG pool and an increase in IPs, found mislocalized in the VZ. Neurons are transiently found in abnormal positions (Dudok *et al*. 2016). (o) Conditional deletion of Llgl1 disrupts the VS and AJs. aRG form rosette-like structures close to the ventricle, where IPs can be found as well. Neurons are found in ectopic positions, both close to the ventricle and forming a 'double cortex' (Beattie *et al*. 2017; Jossin *et al*. 2017).



modulates the neurogenic transition and cell fate acquisition of aRG progeny. While UPR is crucial within aRGs to dissipate the cellular expenses because of proliferation, continued efforts are crucial to elucidate the exact cellular and molecular mechanisms of how UPR signaling may function as a 'developmental rheostat' during corticogenesis.

Influence of the polarity complex and cell adhesion molecules

AJs are localized in a slightly more basolateral position than the centrosome-containing apical endfoot (Fig. 2a), linked to an F-actin belt, and the combination of the two are responsible for the anchoring of aRGs to each other to form the VS. These intercellular complexes are made up of cadherins and its downstream actors (e.g., β-catenin), junctional adhesion molecules and nectins (Aaku-Saraste *et al*. 1996; Manabe *et al*. 2002; Junghans *et al*. 2005; Kadowaki *et al*. 2007; Singh and Solecki, 2015). The adhesion molecules making up the AJs are linked to the cytoskeleton and they coordinate different signaling pathways triggered in neighboring cells (Singh and Solecki, 2015). Multiple studies have shown that AJ components are critical for aRG proliferation and proper cortical development (Fig. 2).

Conditional deletion of N-cadherin in the mouse cortex leads to AJ disruption, aberrant aRG morphology and polarity, and consequently, to abnormal corticogenesis. Under these conditions, both mitotic and post-mitotic cells were found to be randomly scattered throughout the cortical wall (Fig. 2) (Kadowaki *et al*. 2007). Expression of a stabilized form of β-catenin in mouse APs promotes their self-renewal and amplification, resulting in an enlarged cortical area and the appearance of fold-like structures (Fig. 2c) (Chenn and Walsh, 2002). On the other hand, ablation of β-catenin causes disruption of the VS and AJs, leading to a decrease in the proliferative potential of aRGs, apoptosis, and aberrant position in the developing cortex of this progenitor cell type (Fig. 2d) (Junghans *et al*. 2005; Gan *et al*. 2014). This results in a severe decrease in cycling progenitors and reduced neurogenesis (Gan *et al*. 2014). Importantly, most of these effects on proliferation and production of the correct number of neurons are seemingly because of Wnt/β-catenin signaling independent of AJ maintenance as shown by a β-catenin mutation only affecting signaling, but sparing AJ function (Draganova *et al*. 2015). Conditional deletion of α-E-catenin results in the uncoupling of AJs from F-actin, leading to the presence of scattered AJ-like structures throughout the cortical wall. The latter present a rosette-like structure and are positive for N-Cadherin and β-catenin, suggesting that individual aRGs maintain some degree of polarity although randomized (Fig. 2e). In addition, the pattern of actin and RC2 staining suggest defects in the aRG cytoskeleton and radial morphology. These defects in aRGs ultimately lead to the appearance of a 'double cortex'-like structure (Schmid *et al*. 2014). Inactivation in the mouse dorsal telencephalon of the AJ component afadin also results in a 'double cortex'-like phenotype (Gil-Sanz *et al*. 2014). The absence of afadin disrupts AJs and results in an increase in proliferating progenitors (both aRGs and IPs) found scattered throughout the developing cortex. Although post-mitotic neurons were produced, their laminar distribution was disrupted (Fig. 2f) (Gil-Sanz *et al*. 2014). Additionally, a recent study suggests that aRGs lacking afadin could have impaired primary cilia and spindle orientations (Rakotomamonjy *et al*. 2017). All these studies highlight the essential role of AJs and actin in maintaining aRG morphology, polarity, and localization. In addition, deregulation of these proteins severely impacts aRG proliferative potential, as well as proper neuronal production and position (Fig. 2b–f).

In close vicinity to the AJ belt are localized another set of molecules critical for aRG polarity, the Par polarity complex, αPKC, cell division control protein 42 homolog (Cdc42), and the Crumbs complex (Singh and Solecki, 2015). When NEs become aRGs and the AJ belt is assembled, the polarity protein Par3 dissociates from the tight junctions characteristic of NEs and associates with the newly formed AJs. This triggers the recruitment of other polarity proteins such as αPKC and Par6 to the proximity of AJs (Manabe *et al*. 2002). Polarity proteins are not only essential for maintaining aRG morphology, but in some cases they also act as fate determinants upon cell division.

Costa and colleagues (2008) showed that Par3 levels are critical for aRG proliferation: knockdown of Par3 in aRGs promoted premature cell cycle exit and the acquisition of early neuronal fates (Fig. 2g). On the other hand, over-expression of both Par3 and Par6 enhanced aRG self-renewal (Fig. 2h) (Costa *et al*. 2008). Therefore, the Par complex is critical to mediate progenitor self-renewal at the expense of neurogenic differentiation. While in interphase Par3 is localized basolaterally in the apical domain, during mitosis it shows a dynamic distribution in dividing aRGs (Bultje *et al*. 2009). Decreased expression of Par3 in aRGs leads to a switch toward symmetric neurogenic divisions and the subsequent exhaustion of the aRG pool (Fig. 2g). Ectopic expression of Par3 leads to an increase in Notch activity, whereas knockdown of Par3 has the opposite effect (Bultje *et al*. 2009). Bultje and colleagues (2009) suggested that Protein Numb homolog (Numb)/Numb-like protein influences Par3 activation of Notch signaling. Upon Numb/Numb-like knockdown, over-expression of Par3 no longer triggers Notch signaling. In addition, Numb/Numb-like knockdown rescued the aRG pool depletion induced by Par3 knockdown (Bultje *et al*. 2009). Therefore, in mammalian aRGs the interplay between Par3 and Numb/Numb-like may be important for Notch activity, which strongly influences daughter cell fate after division.

A recent study has shown that the neuronal polarity protein Shootin1 may have a similar function to Par3 in the mouse neuroepithelium (Sapir *et al*. 2017). Shootin1 is localized at the apical surface and plays a role in daughter cell fate determination upon aRG division. (Sapir *et al*. 2017). Shootin1 increases polyubiquitination of Numb and reduces polyubiquitination of Notch intracellular domain, both of which enhance Notch signaling. In agreement with this, both knockdown by *in utero* electroporation and mice lacking Shootin1 show a decrease in mitotic cells and as a result, Shootin1 knockout mice have a thinner CP (Fig. 2i) (Sapir *et al*. 2017). This study not only uncovers a role for the apically located protein Shootin1 but it also provides insight into potential mechanisms influencing Numb–Notch interplay in the VZ. While Notch signaling appears to be indeed crucial to maintain aRG-proliferative potential, it is important to bear in mind that as well as asymmetric fate determinants there are also other factors as described above (e.g., epithelial structure and oldest-centriole/CR inheritance) which have recently been described to be crucial to determine cell fate and that could also be involved in mediating Notch activation in basal as well as in these mutant conditions.

Other components of the polarity complex have been described to be crucial for aRG morphology and behavior. Conditional deletion of the small Rho-GTPases Cdc42 and RhoA results in the disruption of AJs. In the case of Cdc42, this leads to an immediate increase in basal mitoses accompanied also by an increase in neuronal numbers (Cappello *et al*. 2006) (Fig. 2j), while deletion of RhoA causes also ectopic Pax6+ cells and the formation of a double cortex (Cappello *et al*. 2012) (Fig. 2k). Strikingly, conditional deletion of αPKC at mid-corticogenesis disrupts apical complexes causing no major effect on corticogenesis (Fig. 2l) (Imai *et al*. 2006). Pals1, another component of the apical domain, was shown to be critical to maintain the pool of cycling aRGs (Kim *et al*. 2010). Deletion of this protein leads to cell cycle exit and premature neuronal production followed by cell death, eventually resulting in the absence of cortical structures (Fig. 2m) (Kim *et al*. 2010). Recently, the role of Crumbs2 (Crb2) was described in apical complex maintenance. Conditional deletion of Crb2 in the mouse telencephalon impaired the maintenance of the apical complex and aRG morphology: upon Crb2 deletion, nestin staining was barely present and appeared disorganized in the developing cortical wall. This was accompanied by a decrease in the aRG pool and an increase in IPs (Fig. 2n) (Dudok *et al*. 2016). The outcome of deregulating aRG apical complex proteins often converges in the disruption of the aRG apical domain, which in most cases results in a decrease of the aRG pool and altered distribution of both cycling progenitors and post-mitotic neurons.

In addition to the above-mentioned studies, the WD40 domain-containing protein lethal giant larvae homolog 1 (Llgl1) was described to be crucial for aRG polarity and proliferation, and ultimately proper cortical development (Beattie *et al*. 2017). Conditional deletion of Llgl1 in aRGs results in an increase in proliferating cells that form rosette-like structures in the VZ, accompanied by the disruption of the VS (Fig. 2o). This eventually leads to impaired neuronal positioning and a subcortical band heterotopia-like phenotype (Beattie *et al*. 2017). A similar work proposed that Llgl1 acts as a link between AJs and polarity complex proteins (Jossin *et al*. 2017). In this paper, the authors use a model in which Llgl1 is specifically deleted in progenitors from E12.5 onward. These mice present AJ abnormalities, disruption of the neuroepithelium, and aberrantly located post-mitotic neurons, situated close to the ventricles (Fig. 2o) (Jossin *et al*. 2017). Interestingly, the authors showed that Llgl1 is necessary to stabilize N-cadherin at the AJs. When Llgl1 is not phosphorylated by the polarity protein αPKC, it internalizes N-cadherin from the cell membrane. Upon αPKC phosphorylation of Llgl1, N-cadherin is no longer internalized and accumulates at the basolateral part of the apical domain (Jossin *et al*. 2017). Studies such as this emphasize the importance of the localization of adhesion molecules within the aRG wall.

Cell cycle considerations

Cell cycle length in progenitors is associated with their neurogenic potential. Early studies already suggested that progenitors located in diverse brain regions had different cell cycle lengths, and that the cell cycle lengthens as development proceeds, while switching toward a more neurogenic state (Borrell and Calegari, 2014). Later studies confirmed this initial discovery (Calegari *et al*. 2005; Lukaszewicz *et al*. 2005; Arai *et al*. 2011), pinpointing G1 as the phase responsible for these changes in cell cycle duration (Calegari and Huttner, 2003; Lange *et al*. 2009; Pilaz *et al*. 2009; Salomoni and Calegari, 2010). In relative terms, aRG-proliferative divisions were found to have a shorter cell cycle length when compared to neurogenic divisions (Calegari *et al*. 2005; Arai *et al*. 2011).

The direct implication of cell cycle length in neural progenitor commitment was shown by pharmacologically inhibiting G1-specific Cdk/Cyclins in mouse embryos. This leads to longer cell cycle, and precocious neurogenesis (Calegari and Huttner, 2003). The same result was obtained by performing RNAi-mediated silencing of Cyclin D1/Cdk4 (Lange *et al*. 2009). Accordingly, Pilaz and colleagues (2016) showed recently that mitotic delay promotes neuronal production from aRGs. In this study, two different approaches were used: first, mitotic progression of aRGs was analyzed in the Magoh heterozygous mouse mutant, described as a model for microcephaly with altered neurogenesis (Silver *et al*. 2010). In this mutant, a proportion of aRGs presented delayed mitotic progression, and these aRGs preferentially produced neurons (Pilaz *et al*. 2016). Secondly, a pharmacological approach was used to induce

mitotic delay, reproducing the phenotype observed in Magoh heterozygous mice (Pilaz *et al*. 2016). Another set of experiments demonstrated that shortening the cell cycle of progenitors indeed bolsters their proliferative capacity. Over-expression of Cdk4 and Cyclin D1, or exclusively cyclins, leads to shorter cell cycle, accompanied by a higher proliferation rate, and an increase in the progenitor pool (Lange *et al*. 2009, Pilaz *et al*. 2009, Nonaka-Kinoshita, *et al*. 2013). Thus, all these studies support the idea that cell cycle length is a parameter regulating progression of neurogenesis and progenitor fate.

Different factors regulate cell cycle duration and hence influence cell fate. Thus, triggering FGF signaling leads to a shorter G1, promoting progenitor proliferation and expansion (Lukaszewicz *et al*. 2002). Similarly, insulin-like growth factor 1 (Igf-1) also reduces G1 length, bolstering progenitor cell cycle re-entry (Hodge *et al*. 2004; Mairet-Coello *et al*. 2009). This highlights the fact that factors known to play a role in determining cell fate may do so by impacting cell cycle parameters. Therefore, cell fate determinants and cell cycle progression are probably highly interlinked (Borrell and Calegari, 2014).

MicroRNAs (miRNAs) have also been shown to be involved in controlling the expression of cell cycle proteins promoting proliferation vs differentiation, or *vice versa* (for a review on miRNAs and neuronal cell fate see Meza-Sosa *et al*. 2014; see also Ghosh *et al*. 2014; Govindan *et al*. 2017). The miRNA let7b regulates aRG proliferation through targeting, among others, cyclin D1 (Zhao *et al*. 2010). Using *in utero* electroporation, let7b was over-expressed in aRGs in early stages of mouse corticogenesis. Let7b over-expression delayed cell cycle progression, promoting neuronal differentiation. This defect in neurogenesis was rescued by co-expressing a cyclin D1 vector resistant to the miRNA (Zhao *et al*. 2010). miR-15b promotes cell cycle exit and neuronal differentiation by indirectly regulating cyclin D1 (Lv *et al*. 2014). The regulation of Cdk7 by miR-210 also appears to be critical for aRG cell cycle progression: over-expression of miR-210 promotes aRG cell cycle exit and cell differentiation (Abdullah *et al*. 2016). Another example is the higher primate-specific miR-1290, whose over-expression in progenitors slows down the cell cycle and promotes neuronal differentiation (Yelamanchili *et al*. 2014).

Most of the studies mentioned previously were performed in the mouse neuroepithelium, in which G1 clearly appears to be critical in controlling cell cycle duration. However, a recent study performed in ferret, indicates that cell cycle duration of progenitors in this species is mainly mediated by

Table 1 Factors affecting apical radial glia proliferation

Factor	Model	Progenitor and/or neuronal phenotype	References
Apical domain	Analysis of apical domain components in coronal sections and *en face* imaging of the mouse neuroepithelium	Maintenance of the apical domain promotes aRG fate	Kosodo *et al.* (2004), Marthiens and ffrench-Constant, (2009)
Integrins (basal process)	Blockage by antibodies and snake venom (echistatin)	Reduced proliferative potential of aRGs and bRGs	Fietz *et al.* (2010)
Cyclin D2 mRNA (basal process)	IUE of reporter mRNAs carrying Cyclin D2 3′UTR in the mouse neuroepithelium	Maintenance of the basal process promotes aRG fate	Tsunekawa *et al.* (2012)
Lfc	IUE of shRNA in the mouse neuroepithelium at early to mid corticogenesis	Increase in the number of cycling aRG and decreased number of newly formed neurons	Gauthier-Fisher *et al.* (2009)
Eldest centriole inheritance	IUE of tagged and photosensitive centrosomal proteins	Inheritance of the eldest centriole is associated with aRG fate acquisition	Wang *et al.* (2009)
Ciliary remnants inheritance	IUE of tagged-centrosomal and ciliary proteins	Inheritance of ciliary remnants is associated with aRG fate acquisition	Paridaen *et al.* (2013)
Par3 and Par6	IUE and *in utero* viral injection	Increased aRG proliferation and numbers	Costa *et al.* (2008), Bultje *et al.* (2009)
miR-15b	miR-15b inhibitor in the mouse neuroepithelium	Enhanced proliferation of aRG at the expense of cell differentiation	Lv *et al.* (2014)
miR-210	miR-210 sponge in the mouse neuroepithelium	Enhanced aRG proliferation and delayed neuronal production	Abdullah *et al.* (2016)
bFGF	Exposure of cortical progenitor cultures to bFGF	Increase in proliferative divisions	Lukaszewicz *et al.* (2002)
Igf-1	Nestin-*Igf-1* transgenic mouse	Increased cell-cycle re-entry and proliferation (VZ), and increased neuronal numbers in postnatal stages	Hodge *et al.* (2004)
let7b miR	IUE in the mouse neuroepithelium	Decreased neuronal progenitor cell cycle re-entry (VZ and SVZ)	Zhao *et al.* (2010)

Table 2 Factors affecting basal progenitor production (Intermediate progenitors and basal radial glia)

Factor	Model	Progenitor and/or neuronal phenotype	References
Nt3	IUE in the mouse neuroepithelium	Decrease in IPs concomitant with a decrease in aRGs	Parthasarathy *et al*. (2014)
Citk	KO mouse	Premature aRG cell cycle exit and increased generation of IPs	Gai *et al*. (2016)
Cdk5rap2	IUE of shRNA in the mouse neuroepithelium	Increased production of IPs at expenses of aRGs, and premature neuronal differentiation	Buchman *et al*. (2010)
Cenpj	IUE of shRNA in the mouse neuroepithelium	Increased production of IPs aberrantly positioned in the VZ and decreased neuronal production	Garcez *et al*. (2015)
Wdr62	Gene-trap mouse	Increase in IPs at the expense of aRGs, decreased cortical thickness (mainly upper layers)	Jayaraman *et al*. (2016)
Aspm	KO mouse	Increase in IPs at the expense of aRGs, decreased cortical thickness (mainly upper layers)	Jayaraman *et al*. (2016)
Elongator complex (*Elp3*)	FoxG1-Cre cKO mouse	Impaired production of IPs from aRG, decreased neuronal numbers	Laguesse *et al*. (2015a,b)
Cdc42	Emx1-Cre cKO mouse	Altered aRG cell cycle, increase in IPs and neurons	Cappello *et al*. (2006)
Crumbs2	Emx1-Cre cKO mouse	Decrease in aRG and increase in IPs, followed by subtle cortical lamination defects	Dudok *et al*. (2016)
CyclinD1 and CyclinE1	IUE in the mouse neuroepithelium	Increased apical and basal mitoses and enlarged SVZ Higher neuronal density (mainly upper layers)	Pilaz *et al*. (2009)
CyclinD1/Cdk4	IUE in the mouse neuroepithelium and cKI mouse	Increased Tbr2+ progenitor production and enlarged SVZ. Increased neurogenesis, cortical surface and brain size	Lange *et al*. (2009), Nonaka-Kinoshita *et al*. (2013)
miR-15b	IUE in the mouse neuroepithelium	Decreased Pax6+ progenitors and increase in the number of IPs	Lv *et al*. (2014)
Retinoic acid	*Rdh10* KO mouse	Decreased cycling aRGs, decreased cycling IPs, decreased neuronal numbers	Haushalter *et al*. (2017)
Robo/Slit	*Robo 1/2* and *Slit 1/2 KO* mice (null allele)	Decrease in cycling aRGs and increase in IPs. No immediate increase in neuronal production	Borrell *et al*. (2012)
PDGFRβ	Embryonic ventricular injection of recombinant PDGFD and IUE of constitutively active *PDGFR*β	Production of bRG-like progenitors	Lui *et al*. (2014)
ARHGAP11B	Microinjection of *ARHGAP11B* in organotypic slice culture and IUE in the mouse neuroepithelium	Increase in BPs (IPs and bRG-like progenitors), increased cortical area and folding	Florio *et al*. (2015)
TBC1D3	Nestin-*TBC1D3* transgenic mouse and IUE	Production of bRG-like progenitors and cortical folding	Ju *et al*. (2016)
Pax6	IUE in the neuroepithelium of Tis21-CreERT2 mice	Increased production of bRG-like cells and increased upper layer neuron production	Wong *et al*. (2015)
Trnp1	IUE of shRNA in the mouse neuroepithelium	Increase in IPs and generation of bRG-like progenitors. Appearance of folds	Stahl *et al*. (2013)
TMEM14b	Nestin-Cre cKI mouse	Production of bRG-like progenitors, cortical thickening and gyrification	Liu *et al*. (2017)

changes in S-phase, since little variation was observed in G1 when comparing different progenitor types (Turrero-Garcia *et al*. 2016). Additionally, the discovery of a broad diversity of progenitor types in different species (Fietz *et al*. 2010; Hansen *et al*. 2010; Reillo *et al*. 2011; Shitamukai *et al*. 2011; Wang *et al*. 2011; Betizeau *et al*. 2013) led to the study of their cell cycle parameters. In these studies, the proliferative potential was found to be decreased from mother to daughter cell, as neurogenesis proceeds (Borrell and Calegari, 2014). Along with the importance of S-phase length in ferret progenitors (Turrero Garcia *et al*. 2016), Wong and coworkers (2015) reported a longer S-phase in bRG-like cells, suggesting that regulation of this phase is important for the cell cycle of bRGs, which are characteristic of gyrencephalic species, such as the ferret.

As described above, there are multiple studies supporting a correlation between cell cycle length in progenitors with their neurodifferentiation potential. Nevertheless, a recent study showed that upon cell cycle arrest of undifferentiated aRGs, these cells still turn on a transcriptional program associated with the neuronal output they are meant to produce (Okamoto *et al*. 2016). In this study, single-cell

Table 3 Factors affecting neuronal production

Factor	Model	Progenitor and/or neuronal phenotype	Reference
Celsr3	KO mouse	Increased number of neurons and decreased number of aRGs	Wang *et al.* (2016a,b)
Fzd3	KO and Nex-Cre cKO mouse	Increased number of neurons and decreased number of aRGs	Wang *et al.* (2016a,b)
Sip1	Nestin-Cre and Nex-Cre cKO mouse	Premature generation of upper layers neurons at expense of deep layers	Seuntjens *et al.* (2009)
Lis1	hGFAP-Cre cKO mouse	Premature reduction in the aRG population, precocious neuronal production, and thinning of the cortex	Yingling *et al.* (2008)
Nde1	KO mouse	Premature reduction in the aRG population, precocious neuronal production, and thin cortex mainly upper layers	Feng and Walsh (2004)
Tctex-1	IUE of shRNA in the mouse neuroepithelium at early- to mid-corticogenesis	Decrease in the number of cycling aRG and increased number of newly formed neurons	Gauthier-Fisher *et al.* (2009)
Aspm	IUE of shRNA in the mouse neuroepithelium and KO mouse	Premature aRG cell cycle exit, exhaustion of their pool and production of neurogenic Tis21+ cells Decreased neuronal numbers	Fish *et al.* (2006), Jayaraman *et al.* (2016)
Cdk5rap2	Hertwig's anemia mutant mouse	Increased aRG cell cycle exit and apoptosis. Decreased number of neurons and cortical thickness	Lizarraga *et al.* (2010)
Huntingtin	IUE of shRNA in the mouse neuroepithelium and Nestin-Cre cKO mouse	Decreased cycling progenitors (aRGs and IPs) and premature increase in neuronal production	Godin *et al.* (2010), Molina-Calavita *et al.* (2014)
Spindle size asymmetry	3-D spindle shape quantification, knockdown by RNAi of Vangl2 in the mouse neuroepithelium	aRG daughter cells inheriting the larger spindle become neurons. Vangl2 KD increases SSA, results in precocious neurogenesis, and decrease in upper layer neurons	Delaunay *et al.* (2014)
CENPJ	hIPSC-derived organoids	Delayed aRG cell cycle re-entry and premature neuronal differentiation	Gabriel *et al.* (2016)
Mib1 inheritance	*In ovo* electroporation of the chick neuroepithelium of a tagged-Mib1 followed by videomicroscopy	The daughter cells inheriting Mib1 are likely to become neurons	Tozer *et al.* (2017)
Impairment of mitochondria function	*Aif* Emx1-Cre cKO mouse	aRG inability to exit cell cycle and failed neuronal differentiation	Khacho *et al.* (2017)
Pals1	Emx1-Cre cKO mouse	Premature aRG cell cycle exit and precocious deep layer neuron production, followed by apoptosis	Kim *et al.* (2010)
Par3	IUE and *in utero* viral injection of shRNA	Decrease in cycling aRG and premature neuronal production	Costa *et al.* (2008), Bultje *et al.* (2009)
Shootin1	*Shtn1* KO and IUE of shRNA in the mouse neuroepithelium	Decrease in mitotic cells, decreased neuronal numbers (mainly upper layer neurons) and thinner CP	Sapir *et al.* (2017)
Magoh	Mos2 heterozygous mutant mouse	Decreased neuronal progenitor production, enhanced neurogenesis, and increased apoptosis	Silver *et al.* (2010), Pilaz *et al.* (2016)
CyclinD1/Cdk4	IUE of shRNA in the mouse neuroepithelium	Generation of IPs is inhibited and neuronal production enhanced	Lange *et al.* (2009)
miR-210	IUE in the mouse neuroepithelium	aRG cell cycle exit and premature neuronal differentiation	Abdullah *et al.* (2016)
mir-1290	Lentiviral vector-mediated over expression in SH-SY5Y cells and H9-ESC-derived neural progenitors (H9-NPC)	Decreased proliferation and increased neuronal production	Yelamanchili *et al.* (2014)

transcriptomics revealed a set of genes whose expression changes over time in undifferentiated aRGs at different developmental stages. In order to address if these transcriptional changes could be altered by defects in cell cycle progression, the authors induced cell cycle arrest of aRGs while maintaining them undifferentiated, and performed the same type of single-cell transcriptomics analysis. Strikingly, the pattern of expression of the genes previously identified was not altered (Okamoto *et al*. 2016), implying that unrelated to cell cycle progression, aRGs at different stages can produce particular neuronal cell types (McConnell and Kaznowski, 1991; Kawaguchi and Matsuzaki, 2016). For several years, it has been debated how and why aRGs produce the appropriate neuronal cell type and if there are aRG subtypes which produce neurons destined for a specific cortical layer. These discussions are resumed in the next section.

Multipotency and potential subpopulations of aRGs

As described throughout this review, aRGs are the main progenitor cell in the developing cortex, responsible for the production of other progenitors as well as neurons. aRGs arise at approximately E10–12 (mouse) from NEs, and although their numbers decrease throughout corticogenesis, they populate the VZ until the late stages of this process.

Deep layer neurons (layers V–VI) are produced during E11.5–E14.5 in the mouse, while upper layers neurons (layers II–IV) are produced at E14.5–E17.5. The latter migrate past the former to reach their final location. Principal neurons are classified by their axonal target, although this is a highly broad and simplistic categorization. They are classified as intracortical neurons (commissural and associative), projecting to other parts of the cortex, and mainly located in upper layers, and corticofugal neurons (corticothalamic and subcerebral), sending projections outside of the cortex, and mostly found in deep layers (Lodato *et al*. 2015). It is frequently questioned if there are different aRG populations that give rise to specific neuronal subtypes, belonging to different cortical layers, and with different axonal targets.

Pioneer heterochronic transplantation studies in the 1990s suggested that as development proceeds, aRGs become more restricted in their fate potential (McConnell and Kaznowski, 1991; Frantz and McConnell, 1996; Desai and McConnell 2000). When transplanting early aRGs into brains already generating upper cortical neurons, these progenitors were capable of generating this neuronal type. However, the opposite experiment showed that aRGs from later stages of corticogenesis failed to generate early neuronal fates (McConnell and Kaznowski, 1991; Frantz and McConnell, 1996; Desai and McConnell 2000). Other studies confirmed this hypothesis by performing fate-mapping studies: retroviral-mediated labeling of individual aRGs showed that early progenitors could generate neurons belonging to all layers of the cortex (Luskin *et al*. 1988; Walsh and Cepko 1993). Recent work has made use of novel fate-mapping techniques, taking advantage of Mosaic Analysis with Double Markers (MADM), where aRG behavior and progeny can be followed accurately at the single-cell level, which strongly confirms that aRG multipotency decreases as corticogenesis proceeds (Gao *et al*. 2014; Kaplan *et al*. 2017).

Previous work focused on addressing if subtypes of aRGs could be committed to the production of a specific neuronal fate. This topic is still controversial, and no solid conclusions have yet been established. Franco and colleagues (2012) showed that aRGs expressing the TF Cux2 mainly produce upper layer neurons. A knock-in mouse (Cux2-CreERT2) was used to fate-map Cux2-positive aRGs and their progeny from early stages of neurogenesis. This approach showed that aRGs expressing Cux2 are present in the VZ from as early as E10.5, and while deep layers are generated, they mainly divide, until they produce upper layer-fated neurons at a later time-point. In addition, when forced to differentiate in an early environment, Cux2-positive aRGs still produced upper layer neurons (Franco *et al*. 2012). Following this study, work from a different group failed to obtain the same results, using the same Cux2-CreERT2 line, as well as a Fezf2-CreERT2 bacterial artificial chromosome (BAC) line (Guo *et al*. 2013; Eckler *et al*. 2015). The fact that these studies were performed in mice with different genetic backgrounds provided a potential explanation for different outcomes (Eckler *et al*. 2015; Gil-Sanz *et al*. 2015).

Although we focus on aRG progeny, a large amount of research has been performed to understand the contribution of IP progeny to cortical layers. The neuronal subtype produced by IPs has also been a controversial topic: while some studies support that IPs mainly generate upper layer neurons (Tarabykin et el., 2001; Zimmer *et al*. 2004), other more recent studies indicate that they produce neurons belonging to all cortical layers (Vasistha *et al*. 2015). Recently, Mihalas *et al*. (2016) showed that IPs have the potential to generate neurons of all layers. Early generated IPs mainly produce deep layer neurons, while later neuronal fates are produced from IPs generated throughout corticogenesis, therefore, depending on both early and late produced IPs (Mihalas *et al*. 2016).

Finally, it is worth noting that progenitor cells characteristic of the primate–human brain, namely bRGs located in the oSVZ, are thought to be directly involved in the production of neurons belonging to layers II–III, which are increased in size and complexity in primates (Ostrem *et al*. 2017). These cells are hence likely to lead to neuronal production at later stages (Martinez-Martinez *et al*. 2016).

Conclusions

Neuronal progenitor cell division, that is, symmetric proliferative, asymmetric, or symmetric neurogenic divisions, as

well as daughter cell fate acquisition are tightly controlled processes and many factors ranging from microtubule-based organelles to cellular metabolism have an impact on the mode of cell division. Thus, they contribute to regulating aRG proliferation, the switch toward a BP identity as well as their neuronal output (see Tables 1–3).

Classically spindle orientation was proposed to be key to determine the type of cell division and the identity of aRG progeny. However, this view may be changing since there is no straightforward relationship between spindle orientation, apical and basal process inheritance, and daughter cell identity. However, the inheritance and/or maintenance of processes seems to be crucial to maintain aRG-proliferative potential. Thus, the presence of the apical domain has been correlated with aRG proliferative identity, even if aRGs often first lose an apical endfoot, which is regenerated to recover the entire epithelial structure. In addition, several studies support that aRG-proliferative potential is defined by the inheritance of the basal process upon cell division: daughter cells lacking this structure are more committed to the neuronal lineage.

Other cellular features critical for correct aRG proliferation, polarity, and position are the AJ and the polarity complex components. Disruption of the latter has been shown extensively to perturb not only aRG dynamics but also other steps of corticogenesis. This emphasizes that aRG morphology, polarity, and close contact at the VS and with the CSF are critical not only for aRG behavior and its progeny but also for subsequent neuronal migration and positioning in the developing cortical wall.

Lately, many studies have unraveled the importance of centrosome-related processes in aRG behavior: centrosome and primary cilium dynamics during the cell cycle appear to have a strong influence on cell division and fate acquisition. The 'old mother' centriole, which in interphase acts as a basal body docking the primary cilium, appears to be critical for aRG fate acquisition after cytokinesis. Additionally, this organelle is associated with CR that also appear to be essential for daughter cell fate determination. Recently, the role of other organelles is beginning to be addressed, pinpointing the importance of aRG metabolism for proliferation, accurate timing of neurogenesis, and ultimately correct cortical development. For instance, ER-dependent UPR contributes to the switch to indirect neurogenesis, in order to produce BPs at the correct developmental time-point: impairment of this process prompts direct neurogenesis, inducing premature neuronal production and eventually resulting in decreased neuronal numbers and a small brain. Other aRG features, such as their activity pattern, may also influence the mode of cell division and progeny fate.

Finally, related to BPs, studies in ferret, primates, and postmortem human tissue have broadened our knowledge about progenitor diversity in the developing neocortex. Work further characterizing the SVZ of gyrencephalic species and its progenitor composition, as well as the discovery of primate-specific genes mediating the switch from aRGs to bRGs has opened a window to better understand neocortical expansion, folding, and evolution. In addition, the use of state-of-the art approaches such as human-based 3-D organoids will shed light not only on the biology of these progenitor cell types but also on their contribution to cortical malformations.

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