

# Control of cerebral size and thickness

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**Abstract** The mammalian neocortex is a sheet of cells covering the cerebrum that provides the structural basis for the perception of sensory inputs, motor output responses, cognitive function, and mental capacity of primates. Recent discoveries promote the concept that increased cortical surface size and thickness in phylogenetically advanced species is a result of an increased generation of neurons, a process that underlies higher cognitive and intellectual performance in higher primates and humans. Here, we review some of the advances in the field, focusing on the diversity of neocortical progenitors in different species and the cellular mechanisms of neurogenesis. We discuss recent views on intrinsic and extrinsic molecular determinants, including the role of epigenetic chromatin modifiers and microRNA, in the control of neuronal output in developing cortex and in the establishment of normal cortical architecture.

**Keywords** Cortex · Growth · Surface/radial expansion · Progenitor diversity · Molecular determinants · Epigenetic regulation · MicroRNA

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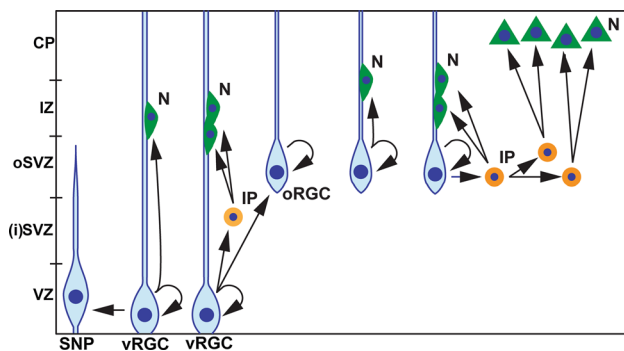
## Introduction

The neocortex (NCX), a brain structure specific to mammals, is a layered sheet of cells covering the cerebrum that is composed of multiple subtypes of neuronal and glial cells. In the NCX, neurons with specific morphological, connective, and transmission characteristics and functions are distributed radially into six laminae and tangentially in distinct functional domains, whose compositions increase progressively in phylogenetically recent species. During development, the lateral and radial growth of the cortex determines the size of the cortical surface and its thickness, respectively. The expansion of the size of the cerebral hemispheres, and the appearance of a highly complex multi-laminated and folded NCX, were critical events in mammalian evolution. Although the basic principles for NCX development in mammals with lissencephalic NCX (in general, showing a small cortical surface and no folding) or gyrencephalic NCX (an expanded cortical surface with folding) are similar, the modulation of the developmental processes in different species causes important variations in neocortical architecture, surface, size, and thickness. It is therefore still not possible to reconcile the available wide range of experimental data in one single model. Here, we introduce recently discovered key features of cortical neurogenesis including key cellular and molecular mechanisms involved in cortical tangential and radial expansion.

## Progenitor diversity

The majority of the NCX neurons are glutamatergic projection neurons generated by neuroepithelial (NE) stem cells and progenitors, located in the germinative ventricular (VZ) and subventricular zones (SVZ) of the dorsal telencephalon.

Based on their apicobasal polarized morphology during the interphase of the mitotic cycle, distinct types of neuronal progenitors have been identified: (1) bipolar progenitors, the NE cells, and the ventricular radial glial cells (vRGCs); (2) nonpolar progenitors, the intermediate progenitor cells (IPs, also known as basal progenitors, BP); and (3) monopolar progenitors, the short neural precursors (SNPs) and the outer radial glial cells (oRG) [1–4] (Fig. 1; Table 1).



**Fig. 1** Main progenitor subtypes in developing neocortex. The scheme illustrates the main cortical progenitor subtypes. The ventricular (apical) Pax6(+) radial glial cell (vRGC) self renews in the ventricular zone (VZ) and generates a neuron (dark green cell, N) either directly or through produced Tbr2(+) intermediate progenitor (IP) that after a symmetrical division in the subventricular zone (SVZ), amplifies the neuronal output. Mostly in the rodent cortex, vRGCs generate neurogenic short Pax6(+) radial glial progenitors (SNP), attached to the apical ventricular surface. Predominantly in gyrencephalic species, vRGCs produce abundant set of Pax6(+) outer radial glial cells (oRGC), located more densely in the outer subventricular zone (oSVZ) where they divide either symmetrically to expand their pool or asymmetrically to self-renew and generate IP-like cells, robustly amplifying the generated from oSVZ neurons. The IP cells (light orange) in the rodent cortex derive from vRGCs, and are located in SVZ (equivalent to inner, iSVZ, in human cortex) while the IP-like cells (dark orange) in ferret/human cortex originate predominantly from oRG located in oSVZ (based on literature data: [22, 38, 39, 43, 94, 196])

## Bipolar progenitors

The NE cells are polarized along their apicobasal axis showing apical and basal attachments to the ventricular surface and the pial (basal) surface, respectively. They specifically express the intermediate filament protein Nestin and show apical localization of the membrane protein prominin-1 (CD133) [5, 6]. Their tight and adherents junctions are associated with complexes such as the partition-defective Par protein complex [7], and they express intergrin alpha as basal lamina component [2].

After the onset of neurogenesis [in the mouse around embryonic day (E) 10.5], the NE cells in VZ progressively transform into bipolar radial glial cells (bpRGCs) that extend an apical process to the ventricular surface, and a long basal process across the entire thickness of the developing NCX, reaching the basement membrane at the pial surface. As the apical plasma membrane of the RGCs corresponds to the ventricular surface where they undertake mitosis, these progenitors are named ventricular radial glial cells (vRGCs) (or apical, aRGCs). While losing few early neuroepithelial features, vRGCs gain some astroglial characteristics, namely expression of the astrocyte-specific glutamate transporter Glast/Slc1A3, the calcium binding protein S100 $\beta$ , Vimentin, glial fibrillary acidic protein Gfap, brain-lipid-binding protein (Blbp), and transcription factor (TF) Pax6 [8, 9]. Extending their long, bipolar fibers between the VZ and the pial surface, vRGCs were considered to belong to the astroglial cell lineage. They were thought to provide only guidance for newly born neurons during their migration towards their final locations in the cortical plate (CP), and to generate astrocytes and oligodendrocytes at the end of neurogenesis [10]. Current evidence has shown, however, that vRGCs are pluripotent progenitors, generating both cortical neurons and astroglia [11–17]. During the mitotic cell cycle, NEs and vRGCs undergo interkinetic nuclear migration (INM), a mitosis

**Table 1** Progenitor subtypes in mouse cortex

Type	Stage	Location	Apicobasal morphology	Marker	Position at mitosis	Progeny	References
NE	E9.5–E13.5	VZ	Bipolar	Prominin 1 (CD133), Nestin	Apical	NEs, RGCs, IPs, Ns	[2, 23]
vRGCs	E10.5–P0	VZ	Bipolar	Pax6, Sox2, Glast, Blbp, S100 $\beta$ , Vimentin, RC2, Nestin	Apical	vRGCs, IPs, Ns, oRGCs	[2]
bpRGCs		SVZ/VZ	Bipolar	RC2, Glast, hGFAP, Sox2, Pax6	Sub apical	bpRGCs, oRGCs	[24, 25]
IPs	E10.5–P0	SVZ	Nonpolar	Tbr2	Basal	IPs, Ns	[4, 23, 26, 27]
oRGC	E12.5–P0	oSVZ/iSVZ	Monopolar	Pax6, Sox2, Blbp, Nestin, Glast	Basal	oRGCs, IPs, Ns	[35–39]
SNPs	E12.5–E16.5	VZ	Monopolar	Tubulin $\alpha$ 1 promoter	Apical	SNPs, Ns	[20, 22]

NE neuroepithelial cells, vRGCs ventricular radial glial cells, oRG outer radial glial cells, IPs intermediate progenitors, Ns neuron, SNPs short neural precursors, bpRGCs bipolar radial glial cells, LL lower layers, UL upper layers, VZ ventricular zone, SVZ subventricular zone, oSVZ outer SVZ, iSVZ inner SVZ

phase-dependent migration of the cell nucleus through which the nuclei end up at the apical or basal surface of the VZ during the M- or S-phase, respectively [18]. The apico-basal polarity and self-renewal of vRGCs is maintained by the Rho-GTPase *cdc42*, which activates the Par-complex to maintain adherent junction coupling and VZ progenitor fate. Upon disruption of *cdc42* gene function, the vRGC in VZ gradually convert into IPs [19].

Similar to the vRGCs, a small subset of the progenitors with radial morphology, the short neural progenitors, SNPs, also divide at the apical surface; however, they retract their basal processes during the M-phase of the mitotic cycle [20, 22], thus extending only one apical process to the ventricular surface. Collectively, the NE cells, vRG progenitors, and SNPs are referred to as apical progenitors [23]. The recently described bipolar radial glia cells (bpRGCs) in cortical VZ, and even more prominently in SVZ, of the gyrencephalic ferret/human cortex, do not contact the apical or pial surfaces, and divide at subapical positions, generating progeny intensively proliferating in species with higher gyrification [24, 25].

#### Nonpolar progenitors

The IPs are non-epithelial unpolarized cells assumed to be principal progenitors for the generation of cortical neurons. Being the progeny of vRGCs already produced during the early stages of neurogenesis [23], IPs migrate away from the VZ to form a secondary proliferative zone, the SVZ, that becomes visible after E12.5 in the mouse cortex [23, 26, 27]. Progenitors, dividing at the basal surface of the VZ (and in later stages of neurogenesis in the SVZ including oRG, as described below), are collectively referred to as basal progenitors [23]. The specification of IP fate depends on the sequential expression of a transcription factor cascade (*Pax6*–*Ngn2*–*Tbr2*) in vRGCs and in the IPs derived from them (reviewed in [30]). Differential expression of the TFs *Pax6* (*Paired box 6*) and *Tbr2* (*T-brain gene-2*)/*EOMES* identifies the IPs as *Tbr2*(+)/*Pax6*(–) cells, in contrast to vRGCs that are *Pax6*(+)/*Tbr2*(–) cells [1, 28, 29]. In addition, expression of molecular markers such as *Svet1* (intron region of the putative *Netrin* gene receptor *Unc5d*), *vGlut2*, *NeuroD1*/*NeuroD2* and TFs *Cux1*/*Cux2* [31, 32, 34] and *Insm1* (insulinoma-associated 1) distinguish the IPs from vRGCs [33].

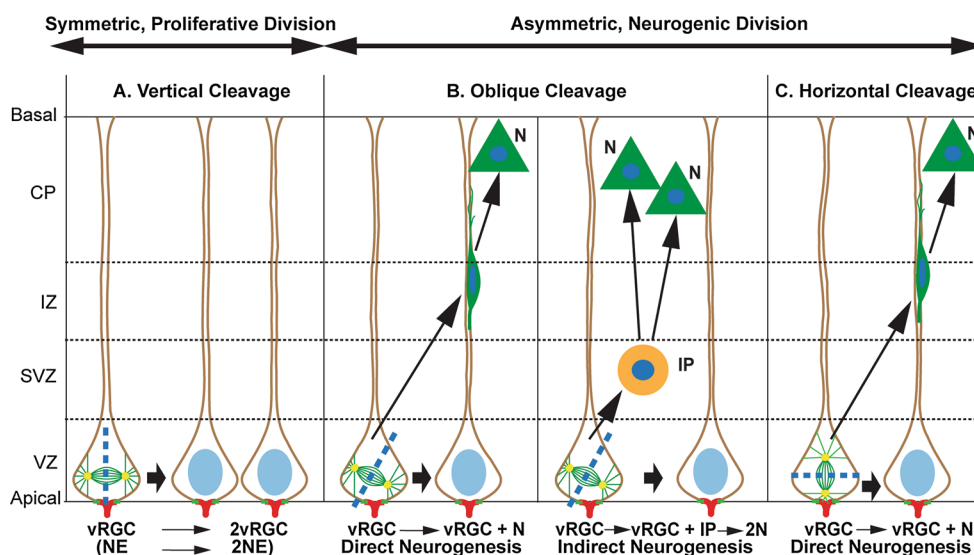
#### Monopolar progenitors

In mammals with enlarged and folded NCX, particularly in primates and especially in the human brain, the SVZ expands and becomes clearly subdivided into an inner (iSVZ) and outer (oSVZ) zone, delineated by fiber tracts [42–44]. The oRGC are a novel RGC subtype, identified

initially in the outer subventricular zone (oSVZ) of gyrencephalic species [24, 35–40]. Although scarce in number, oRGC (also named basal RGCs, bRGCs) have been detected in smooth brain species in the SVZ just above the band of *Tbr2*(+) IPs [35, 36, 41]. However, a substantial difference is that, in mouse, oRGC divide asymmetrically to self-renew and directly generate neurons, whereas in human cortex, oRGC in oSVZ generate transamplifying cells (IP-like cells) that in turn produce neurons [36]. The oRGC extend only one basal process that is used by the generated neurons in oSVZ to migrate by somal translocation into the CP. As a progeny of the vRGCs [36], oRG show characteristics similar to those of vRGCs, including the expression of vRGC markers such as *Pax6*, *Sox2*, *Nestin*, *Glast*, and *Blbp*. Despite that, oRGC do not express the vRGC markers *prominin1* (CD133), *Par3* or *aPKC* (atypical protein kinase C) and they do not undergo INM during mitosis [37–39]. Based on cellular and marker expression characteristics, it is assumed that, in primate brain, progenitors located in iSVZ are close to the rodent IPs, while the enlarged set of oSVZ progenitors (oRGC, and their transamplifying IP-like cells), particularly in the human cortex, contributes to increased thickness of upper layer (UL) neuronal identities [45] (Fig. 3). Since only a few oRGC exist in the SVZ of the rodent cortex, the pronounced presence of oRGC in iSVZ/oSVZ is considered to be a cellular mechanism underlying the cortical expansion and folding in phylogenetically advanced mammals [3, 38] (Figs. 1, 3).

#### Modes of progenitor division

During cortical development, progenitors undergo progressive cell divisions, leading from the expansion of the progenitor pool to the generation of neurons. It is the mode of cell division, as well as the role of the spindle rotation in the selection of the cleavage plane, which finally determines the size of the mammalian neocortex [11, 16]. Before initiation and during the early stages of neurogenesis, the NE/RGCs divide mostly in a symmetric manner to yield daughter cells with identical apical and basal components, thus supplying the progenitor pool and inducing the tangential growth of the cortex (Fig. 2a) [46, 47]. Although evidence from a fixed- and time-lapsed imaging study has convincingly demonstrated that basal processes of the dividing vRGCs in mouse, chick, and zebrafish embryonic cortex may split during the symmetric proliferative divisions (reviewed by Fietz and Huttner [1]), there are data indicating that, on symmetric divisions of RGCs, the basal process is inherited by only one of the RGC daughters, while the second one re-grows its basal process. However, whether, during the asymmetric RGCs division, the basal process is strictly inherited either by the RGC daughter



**Fig. 2** Division modes of cortical progenitors in rodent cortex. **A** Before beginning and during the early stages of neurogenesis, the neuroepithelial cells (NE) and ventricular radial glial cells (vRGCs), divide symmetrically through vertical (apicobasal) cleavage to self renew, enlarging the stem cells/progenitor pools. Consequently, the two daughter cells receive equal amount of apical membrane components. The basal process of symmetrically dividing RGC can either split or can be inherited by the older RGC while the new RGC daughter re-grows a basal process (reviewed in [1]). **B** vRGCs/NE cells have a small apical domain (red) and tight junctions (green) that anchor them to each other as well as to the ventricular surface. During neurogenesis, vRGCs divides asymmetrically, preferably through oblique/vertical cleavage, which results in unequal distribution of apical cell fate determinants. Consequently, vRGCs self-renew and generate either neuron (direct mode of neurogenesis) or another type of progenitor, the intermediate progenitor (IP) which produces two neurons after a symmetrical division in SVZ (rodent cortex) or

more neurons after multiple divisions in the iSVZ (ferret/human cortex, not presented in the schema). Thus, IPs act as amplifiers allowing through indirect mode of neurogenesis to increase the number of neurons generated from an RG unit in VZ at a particular developmental stage. Because of still controversial view on mode of inheritance of the basal processes especially in asymmetric division (reviewed in [1]), this issue is not included in the figure. **C** Rarely, vRGCs in VZ (rodents) can divide asymmetrically through horizontal (planar) cleavage generating RG progenitor and neuron. This is the preferable division mode for oRGC in oSVZ/iSVZ (ferret/human). Recent evidence suggests that the daughter cell that maintains apical contact inherits the basal fiber of the RGC through which it receives Notch and Integrin signalling and becomes a vRGC. The daughter cell that lacks a basal process express Notch ligands and activates Notch signalling in neighboring vRGCs, and starts expression of IP specific markers (based on literature data: [2, 3, 51, 196])

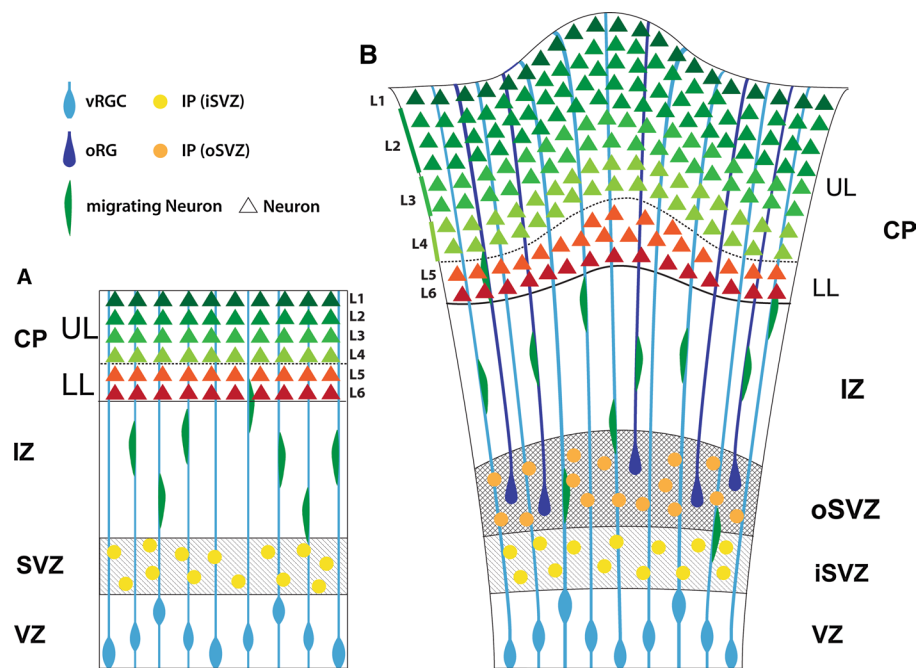
(and required for its self-renewal) or by the generated neuron (using it as a structure for migration), is still an unresolved issue.

The cortical thickness, on the other hand, depends on two different modes of progenitor proliferation. During early neurogenesis, although infrequently, the vRGCs undergo asymmetric divisions through which the apical and basal cell elements are unevenly distributed between the two daughter cells. This leads either to the production of one neuron or to a neurogenic basal progenitor (IPs/oRGC), in addition to the self-renewal of vRGCs [48, 50, 51] (Fig. 2c). Before entering terminal neurogenic divisions in the SVZ, these progenitors undergo 1–3 (IPs in rodents) or multiple (oRGC) subsequent proliferative divisions with horizontal or random cleavage planes to amplify their pools, thereby expanding their neurogenic output and thus leading to expansion of cortical size (surface and thickness) (Fig. 2b) [23, 26, 27, 52]. Later studies have strongly suggested that the acquisition of distinct cell fates after the RGC division critically depends on the inheritance

of the tiny apical domain containing putative cell fate determinants [49] (Fig. 2b).

### Mechanisms of cortical surface expansion

Mechanisms that may control specifically either surface area size or radial cortical thickness and folding are still not well understood. According to the classical radial unit hypothesis [46], proliferating vRG progenitors in the VZ generate neurons, which use the RGC processes as a scaffold to migrate into the cortex where they reach distinct functional areas (Fig. 3). Thus, neurons with the same ontogeny form radial columns with specific functions. It has been proposed that the size of cerebral surface critically depends on the number of radial units in the VZ, whereas the cortical thickness is determined by the neuronal output from the VZ progenitors of each column. Although being interconnected, certain cellular and molecular mechanisms show a tendency to preferably influence one or the other process.



**Fig. 3** Features of cortical neurogenesis in rodent and human brain. **A** In lissencephalic mouse brain, the ventricular radial glial cells (*vRGCs*) divide at the apical surface in the ventricular zone (*VZ*) to self-renew and generate either neurons or more often, intermediate (*IPs*) progenitors (*hell orange*) that divide in the subventricular zone (*SVZ*, rodents) or inner subventricular zone (*iSVZ*, ferret/human) to generate pairs of neurons. The neurons use the *RG* radial fibers as a scaffold to migrate into cortical plate (*CP*). In rodents, only few outer radial glial cells (*oRG*) are found at the basal surface of the *SVZ*, which undergo direct neurogenesis and generate neurons [36]. **B** In gyrencephalic folded human brain, the *SVZ* is augmented, and subdivided into inner (*iSVZ*) and outer subventricular zones (*oSVZ*). The *oSVZ* contains highly abundant outer radial glial cells (*oRGC*)

extending only one basal fiber towards the pia. The *oRG* cells self-renew and generate neurogenic *Tbr2*(+) *IPs*-like cells (*dark-orange*) which continue dividing for a limited number of cycles, before generating increased number of neurons. These neurons use the basal process of *oRGC* as additional scaffold, helping their lateral dispersion during cortical folding. Different from *IPs* in the mouse *SVZ*, the *IPs* in *iSVZ* and *oSVZ* undergo multiple mitotic cycle, increasing the output from the original *vRGCs*. The composition of *oSVZ* in different gyrencephalic species (ferret, macaque, human) is highly heterogeneous and requires more conclusive views on their molecular features, lineage relationships and functions. At the end of neurogenesis, the *vRGCs* generate cells from the glial cell lineage, astrocytes and oligodendrocytes (not shown in the figure; for a review, see [197])

#### Transition from NE to RGC fate and onset of neurogenesis

During development, the timing of the onset of the transition from NEs to *vRGCs* decides whether the progenitors will cease self-renewal and begin generation of neurons. The delayed initiation of the asymmetric mode of NE/*RGCs* division results in amplification of the progenitor pool [44]. Such a prolonged phase depends on cell-cycle lengthening [53, 54] and correlates with excessive expansion of cortical size and surface, as seen in phylogenetically advanced mammals [10, 55–57]. Maintenance of the stem-like properties of *RGCs* is critically dependent on Notch signaling. The transformation of NE cells into *vRGCs* is delayed in mice deficient for both receptors, *Notch1* and *Notch3* [58], while expression of activated *Notch1* in NE cells promotes acquisition of generation of premature *vRGC* identity [59]. Similarly, loss of Notch activation as seen in *Mib1* (*Mind bomb 1*) conditional KO mutant mice leads to depletion of the *RGC* number, and premature differentiation into *IPs* and neurons [60]. Interestingly, the

cellular source for the Notch signaling turned out to be the *SVZ* *IPs* as well as migrating neurons that express *Mib*, a RING-type E3 ubiquitin ligase which induces endocytosis of the Delta ligand in *RGCs* [60].

During mitosis of *vRGCs*, the apical polarity protein 3 (*Par3*) becomes dispersed in the proliferating cells. The mode of *Par3* distribution (even vs. polarized) in the daughters determines the symmetric or asymmetric inheritance of the polarized components. Consequently, the cell with the greater amount of *Par3* parallels high Notch signaling activity in the daughter cell that renews as *vRGC* [7]. In contrast, cells with inherited less *Par3* express Notch signaling activity and acquire cell fate of either neuron or *IP*.

#### Environmental signals regulate progenitor proliferation

Cortical growth and patterning depends on signaling molecules emanated from inductive centers of the cortical primordium, formed after closure of the neural tube. Such

**Table 2** Molecular determinants of cortical surface area (CS), thickness (CT) and folding (CF)

Genes/Mouse	Affected progenitors	Cortical phenotype	References
Signaling factors			
Lrp6 (KO)	IPs	CT, CS	[204]
$\beta$ -Catenin (cKO/cOE)	IPs	CT, CS, CF	[62, 205]
GSK-3 (cKO)	vRGCs, IPS	CT, CS, CF	[206]
Mib1 (cKO)	vRGCs, IPS	CT, CS	[60]
Fgfr1/2/3 (cKO)	IPs	CT, CS	[207]
Fgf10 (KO)	vRGCs, IPS	CT, CS	[66]
Smoothed (KO)	IPs	CT, CS	[208]
Shh (cKO)	IPs	CT	[209]
TFs affecting earlier vs. later born neuronal fate			
Foxg1 (KO, cKO)	IPs	CT, CS	[106, 115]
Ngn1/2 (KO)	IPs	CT	[110]
Pax6 (cKO, cOE)	IPS, oRGs	CT, CS	[86, 210–213]
Tlx1 (KO)	IPs	CT	[112]
Id4 (KO)	IPs	CT, CS	[113, 214]
Sall 1 ( <i>putative TF</i> ) (cKO)	IPs	CT, CS	[114]
TFs affecting the expansion of IP pool			
Tbr2 (cKO)	IPs	CT, CS	[90, 91]
Insm1 (KO)	IPs	CT	[33]
Arx (cKO)	IPs	CT, CS	[92]
Cux2 (KO)	IPs	CT	[120]
AP2y/Tcfap2 (KO)	IPs	CT	[95]
Trnp1 (KD)	IPS, oRG, bpRGCs	CF	[24, 25]
NFIB (KO)	oRG	CT	[126]
TFs involved in progenitor specification and layer identity			
Fezf2 (KO)		CT	[99, 215]
Otx1		CT	[118, 119]
Cux1/2 (KO)		CT	[31, 32, 120]
POU3F2/POU3F3 (KO)		CT, CS	[121–123]
TFs acting as postmitotic determinants			
Bcl11b (Ctip2)		CT	[117]
Sox5 (KO)		CT	[128, 129]
Tbr1 (KO)		CT	[216, 217]
Satb2 (KO)		CT	[164, 165]
Chromatin and epigenetic factors			
Brg1 (cKO)	vRGCs	CT, CS	[158, 219]
BAF170 (cKO, cOE)	IPs	CT, CS	[159, 160]
Ezh2 (cKO)	IPs, oRG	CT	[151]
Querkopf (cKO)		CT, CS	[141]
AF9 (KO)	vRGCs, IPs	CT	[218]
Dnmt1 (cKO)		CT, CS	[146]

CT cortical thickness (referring to affected upper layers, lower layers or both), CS cortical surface, CF cortical folding, KO knockout, cKO conditional knockout, cOE conditional overexpression, KD knock-down, IPs Intermediate progenitors, oRG outer radial glia, vRGCs ventricular radial glia cells, bpRGCs bipolar radial glia cells

determinants belong to morphogen families like Wnts, bone morphogenic proteins (Bmp), transforming growth factors (Tgfs), retinoic acid (RA), sonic hedgehog (Shh), and fibroblast growth factors (Fgfs) [61] (Table 2). For instance, in vRGCs,  $\beta$ -catenin is a constituent of the adherent junctional complexes and participates in Wnt signal transduction. Due to enforced re-entering of the progenitors

into mitosis, transgenic expression of a stabilized  $\beta$ -catenin in NE/RGCs results in highly increased progenitor number and folding of the VZ, enlarged and folded cortical surface area, and increased thickness [62]. Similar expansion of cortical surface was observed following a decrease in cell death through elimination of Caspase 9 [63] or EphrinA7 (reviewed in [64, 65]).

The timely transition from NE cells into vRGCs is also dependent on Fgfs and Notch signaling [66, 67] (Table 2). The powerful morphogen Fgf8, secreted from the anterior forebrain inductive center, controls area location across the antero-posterior cortical axis [68, 69]. Fgf8 specifies anterior cortical fate via transcriptional regulation of TFs *Er81*, *Foxg1*, *Nkx2.1*, *Pea*, and *SP8*, but inhibits caudal fate through repression of TFs *COUP-TF1*, *Emx2*, and *Wnt8b* [70–72]. Nonetheless, Fgf8 also plays a role in the maintenance of normal proliferation and survival of cortical progenitors [73]. Fgf10 exerts a similar function mostly within the frontal cortex [66, 67]. Targeted deletion of Fgf10 delays the transformation of NE cells into vRGCs, leading to profound increase of neuronal output, laminar thickness, and the expansion of cortical size [66]. On the other hand, Fgf15 and Fgf2 support the differentiation of NE cells to vRGCs, promoting neurogenesis [66, 67, 74]. Moreover, an increase in the Fgf signaling via Fgf2 before the beginning of neurogenesis (and without affecting the IP numbers) leads to gyrus formation in the smooth mouse cortex with largely preserved layering and morphology [75]. In contrast, loss of function of all Fgf receptors during the earliest stages of corticogenesis leads to the severe loss of vRGCs and premature termination of neurogenesis, involving the Notch signaling pathway [76].

### Spindle orientation and neurogenesis

Recent data suggest that spindle orientation exerts control on NE/RGC proliferation versus differentiation, thus influencing the progenitor pool and cortical size [51, 77–80]. While randomly orientated at the onset of mitosis, the spindle rotates during the metaphase to acquire planar orientation, thus becoming truly parallel to the surface of the NE/RGC until the anaphase of the cell cycle. After a vertical cleavage, the generation of identical daughter cells (self-renewal), and the enlargement of the progenitor pool are permitted, which is of crucial importance for maintenance of normal cortical size and surface.

Mutations of *MCPHI*, *CDK5RAP2*, and *NDE1* genes in human affect spindle orientation and progenitor proliferation [81, 82], causing microcephaly [83]. An important regulator of symmetric proliferative progenitor division during early neurogenesis is the protein phosphatase PP4c, which phosphorylates the microtubule binding protein Ndel1, assuring the planar orientation of mitotic spindle [88]. Consequently, deletion of PP4c specifically during early neurogenesis leads to random spindle orientation and premature asymmetric neurogenic division, diminishing the progenitor pool and the final neuronal output. Maintenance of a precise alignment of the mitotic spindle critically depends on *Aspm* (abnormal spindle-like microcephaly-associated protein) that is localized at the poles of the mitotic spindle

[84, 85]. Similarly, the microtubule-associated protein gene *Spag5* (or *Astrin*), a direct target of TF *Pax6*, is also localized in the spindle poles [86]. The elimination of *Spag5* function promotes non-vertical (differentiative oblique) instead of vertical symmetric (proliferative) divisions, depleting the progenitor pool for normal neurogenesis, and contributing to the reduced radial thickness of the cortex in *Pax6*-deficiency [86]. Spindle randomization in vRGCs has also been shown for the LGN protein complex, which, acting together with the dynein complex binding partner *lissencephaly1* (*Lis1*), gathers pulling force in order to control the plane of cell division [77, 79].

Other facts imply, however, that spindle orientation may influence predominantly cell fate decisions in the neurogenic progenitor population [87]. Thus, conditional knockout and overexpression of *Incuteable* (*Incs*) in mice revealed a profound effect on the fraction of RGCs undergoing oblique divisions: mutation of *mInsc* almost abolishes oblique mitotic spindle while *mInsc* overexpression promotes oblique divisions and specifically increases the number of *Tbr2*(+) IP cell lineage [87]. This results in premature transition of RGCs to IPs, and severely reduced cortical thickness, due to apoptotic death of the progenitors in VZ that contains prematurely differentiated neurons [88]. Overall, these new data support the view that a change in the spindle orientation in a critical phase during early neurogenesis is directly related to the transition between symmetric and asymmetric progenitor division and has a potential to control cortical surface growth.

## Mechanisms of radial cortical expansion

### Regulation of IP production

As a framework for understanding the developmental expansion of cortical surface and thickness, the modified radial unit hypothesis provides the basis to understand how a molecularly specified protomap of cortical arealization in VZ progenitors is transferred to the CP [46, 55, 70, 71]. Since IPs are already present at the beginning of neurogenesis, it has been predicted that alteration of IP numbers would mostly change radial thickness and not surface area [46]. The relative abundance of IPs and/or oRGC compared to vRGCs number is assumed to determine both cortical size and folding in gyrencephalic species [4, 57, 89].

The genesis of IPs from vRGCs and their proliferative capacity is dependent on both intrinsic and extrinsic factors, including diffusible morphogenes (Table 2) [21, 205–209]. Most of the studied intrinsic determinants of IP fate have turned out to be transcription factors. The specific expression of TF *Tbr2* in IPs is necessary and sufficient for IP specification: conditional ablation of

*Tbr2* leads to loss of IPs and decrease of cortical thickness affecting all cortical layers [90, 91]. Furthermore, TF *Insm1* controls the conversion of RG progenitors into IPs, and, accordingly, *Insm1* deletion profoundly diminishes the IP number leading to marked reduction of cortical radial thickness [33]. Expressed in a subset of vRGCs, and showing an enhanced level at the start of IP production, the TF *AP2gamma/Tcfap2c* has a crucial role in the upregulation of important IP molecular determinants (e.g., *Tbr2* and *NeuroD1*), placing this factor as an upstream regulator of IP progenitor fate, acting in a region-specific manner (in occipital cortex) [95]. Cortical progenitors in the VZ (RGCs) and SVZ (IPs) express either Cyclin D1 or CyclinD2, two G1-phase active cyclins, respectively [93, 94]. Although not required for IPs fate specification, Cyclin D2 plays a crucial role in maintaining normal size of the IP pool and cortical size and thickness [93].

#### Role of oRG progenitors

The selective influence of IPs on radial cortical growth and gyrification in primates has been challenged by Nonaka-Kinoshita et al. [94]. They have provided proof that a massive expansion of IPs is not sufficient to induce folding in transgenic mice overexpressing *cyclin D4* to promote IP proliferation, but instead leads only to surface expansion. However, in the gyrencephalic cortex of ferret, which shows abundant presence of oRG, the same manipulation causes both cortical surface area expansion and folding, indicating a specific contribution of oRGC over IPs in the radial complex organization in phylogenetically distinct species [94]. Moreover, the expanded set of oRG in iSVZ provided additional scaffold for the oRGC-neuronal progeny, also allowing lateral dispersion when reaching the CP. Overall, these findings support the view that the two types of abventricular progenitors, IPs (in SVZ) and oRGC (in iSVZ/oSVZ), have specific functions in the expansion of surface versus radial thickness/folding, respectively, across the mammalian phylogeny [3, 41, 43] (Fig. 3).

An important difference between the mouse smooth and gyrencephalic ferret cortex is the timely specific distribution of oRGC in SVZ. Although few oRGC (approximately 3–10 % of all progenitors) have been identified in the mouse cortex during early neurogenesis (at E12.5), in the ferret, a large set of oRG (approximately 50 %) and *Tbr2*(+) IPs are located in iSVZ [36, 98]. This results in a substantial increase of the progenitor pool during early neurogenesis, extensive radial growth with increased UL neuronal numbers, and cortical folding in the ferret brain [98]. It has therefore been proposed that cortical radial growth reflects specific expansion of the germinative

zones, containing distinct primary sources for neurogenesis: the VZ in the smooth cortex, and iSVZ or oSVZ in species with low-folded (ferret) or highly folded cortex (non-human primates and humans), respectively [40, 42, 98]. Along the same lines, despite the scarce presence of oRGC in the lysencephalic mouse brain [35, 36], the Götz laboratory have recently induced augmented radial cortical thickness and gyrification by manipulating the expression of the *Trnp1* (*TMF1-regulated nuclear protein 1*) gene, which encodes for a DNA-binding protein [24, 25], controlling the cell proliferation [96]. In vivo manipulation of the *Trnp1* expression level (normally high in vRGCs and low in IPs/oRGC) disclosed specific effects in cortical architecture. Overexpression of *Trnp1* promotes the NE/vRGC renewal and, accordingly, the tangential/lateral expansion of the cortical surface. Lowering the *Trnp1* level causes radial expansion due to increased numbers of both IPs and oRGC in SVZ and folding of the lysencephalic cortex. These interesting findings support the view that establishment of the capacity for cortical folding during mammalian evolution may involve superimposed layers of cellular decisions including modulation of the pool size of not only oRGC but also IPs, as predicted in the IPs hypothesis [97].

Reminiscent to IPs that are a source of Notch signaling for vRGCs [60], the oRGC express *Hes1*, the main effector of Notch [38]. Pharmacological inhibition of Notch signaling in cultured human cortical tissue induces premature neuronal differentiation, implying that Notch signaling prevents oRG differentiation, thereby regulating the neuronal output [38]. Similarly, integrin signaling, via contact between the basal fiber of oRGC and cortical basal lamina, maintains the oRGC identity [37].

#### Selective expansion of distinct cortical layers affect cortical thickness

Neurons with distinct layer identities are produced following the intrinsically encoded “inside-first–outside-last” developmental program, according to which early born neuronal subtypes populate the lower (infragranular) cortical layers (LL, L6, L5) while later born neurons progressively reach the upper (supragranular) layers (UL, L4–L2) (reviewed in [99]). Early work proposed that expansion of the thickness of the ULs compared to LLs in the human brain contributes to the increase of the NCX surface and formation of gyri [100]. In the macaque cortex, the period of neurogenesis includes 28 cell cycles compared to only 11 cycles in the rodent brain [101, 102]. This causes a substantial enlargement of the progenitor pool and increased production of UL neurons, mostly from iSVZ/oSVZ progenitors coordinated with an enlarged cortical surface in gyrencephalic species [103].



A distortion of the balance between the available progenitor pools (apical vs. basal progenitor subtypes) leads to specific alterations in cortical thickness and surface area [55, 56, 104, 105]. A recent report suggests that, even before the start of neurogenesis, expression of TF *Cux2* delineates two sets of *Cux2*(−) and *Cux2*(+) RGCs that produce LL and UL neuronal identities, respectively [107]. Even when forced to differentiate prematurely, the *Cux2*(+) RGCs still produce neurons with only UL identities [107, 108]. Nevertheless, this issue is still controversial since transgenic lineage analyses have indicated that *Cux2* + RGCs could generate both UL and LL neurons [109].

A wealth of experiments have demonstrated that TFs play important intrinsic roles in many aspects of corticogenesis, including specification of neuronal layer identity. One molecular mechanism that determines earlier versus later born neuronal fate includes TF *Foxg1*, which prevents the generation of Cajal–Retzius neurons after their normal birthday (at E9.5), allowing subsequent generation of normally sized LL and UL [106]. Expression of proneuronal bHLH TFs *Ngn1* and *Ngn2* in the progenitors seems to determine predominantly LL neuronal fate, while TFs *Pax6* and *Tlx* regulate the generation of UL neuronal fates by maintaining RGCs proliferation during early or late neurogenesis, respectively [110–112] (Table 2). Interestingly, at different stages of corticogenesis, TF *Id4* exerts specific functions, at early stages for early stem cell proliferation and at later stages for differentiation of UL neuronal fate, thereby controlling both lateral expansion and thickness of NCX [112, 113]. Likewise, expression of TF *Sall1* in early RGCs promotes proliferative over neurogenic division, whereas, later on, *Sall1* supports both the exit of IPs from mitosis and the differentiation of UL neuronal identities [114]. Other studies indicate that TFs *FoxG1* [106, 115], *Tbr2* [90, 91], and *Insm1* [33] promote cortical growth/thickness mostly by enabling the expansion of the IPs pool (Table 2). Acting as a direct regulator of *Cdkn1*, an inhibitor of cell cycle progression, the TF *Arx* (Aristaless-related homeobox) controls the proliferation of IPs. Following targeted mutation, the *ArxKO* brains show strong reduction of radial thickness, due mostly to UL misbuilding [92].

Cortical thickness is also influenced by TFs that specify sets of neuronal layer identities [99, 110]. Both the zinc-finger TF *Fezf2* (formerly *Fez1* or *Zfp312*) [116, 117] and the orthodenticle homeobox TF *Otx1* [118], are expressed primarily in early vRGCs, and subsequently in subsets of their L6/L5 or L5 neuronal progenies. Despite this correlation, *Otx1* expression does not affect the establishment of LL neuronal fate [119], while *Fezf2* is required for the generation and specification of corticospinal motor neurons (CSMNs) [116]. In the adult brain of *Fezf2* null mutant

mice, CSMNs are absent; however, the total cortical thickness is identical with control brains due to the expanded generation of L6 neurons and L6 thickness, suggesting a fate switch (L5 into L6) of the early cortical progenitors upon abolishment of *Fezf2* function [116, 125].

Expression of TFs *Cux1/Cux2* and *Svet1-RNA* label progenitors in SVZ and UL neurons, whilst *POU3F2/POU3F3* (formerly *Brn1* and *Brn2*) and *Tle1* (*Groutcho1*) are expressed in subsets of late RG progenitors and L3–L2 neurons [31, 32, 34, 99]. Notably, targeted deletion of *Cux2* leads to increased progenitor proliferation in SVZ and increased UL cortical density and cortical thickness [120]. In contrast, double disruption of *POU3F2/POU3F3* in mice causes a dramatic reduction in cortical thickness due to the specific loss of UL neurons and defective neuronal migration [121–123]. The Nuclear Factor One B (*Nfib*) is expressed in both vRGCs and LL neurons [124]. Upon targeted mutation of the *Nfib* gene, oRG progenitors and late basal progenitors are absent, the ULs become thinner, and later born UL neurons show delayed migration towards the CP, thus contributing to the reduction of cortical thickness [126].

Concomitant with their role in regulating layer-specific patterns of molecular identity, a number of TFs are required for correct neuronal migration from the germinative zones into the CP (reviewed in [127]). Such a role has been proposed even for TFs acting only as post-mitotic determinants in neuronal sets in distinct layers, e.g., *Bcl11b* (formerly *Ctip2*) expressed in L5, *Sox5* expressed in ULs [128, 129], and TF *Tbr1*, which co-regulates multiple aspects of both early and later born neurons [127]. Similarly, targeted mutation of the adhesion molecule *Mdga1* is necessary for L2/L3 neurons to migrate and “build” the normal thickness of the ULs in the cortex [130].

### Emerging roles of epigenetic control in cortical development

The generation of the astonishing variety of neuronal subtypes in the developing cortex is coordinated by distinct transcriptional programs, in which epigenetic and chromatin regulations are believed to play decisive roles [131–133]. There is increasing evidence that a number of genetic neurodevelopmental disorders, which lead to intellectual disability, are caused by mutations in the genes encoding epigenetic factors and chromatin remodeling proteins [133, 134]. Here, only some key epigenetic mechanisms related to the determination of cortical size, thickness, and neural fate determination are considered, focusing mainly on the role of chromatin status modification and the recently discovered functions of microRNAs.

## Chromatin modifiers

Epigenetic machinery controls heritable changes in gene expression programs in NSCs without altering the genomic sequence, thereby mediating a switch in the state of chromatin structure [135]. Epigenetic modifications and chromatin regulation display dual roles: they exert direct effects on gene transcription, and serve as platforms for the recruitment of TFs, allowing for persistent changes in chromatin states [131–133].

The basic structural unit of the eukaryotic chromatin is the nucleosome that consists of 145–147 base pairs DNA in 1.8 helical turns around an octamer of four evolutionary highly conserved histone proteins [136, 137]. The accessibility of TFs to their regulatory elements in downstream target genes is achieved by two main mechanisms: (1) covalent histone modification, and DNA methylation; (2) non-covalent, energy-dependent chromatin modifications involving ATP-dependent chromatin remodeling complexes, such as SWI/SNF (SWIItch/Sucrose NonFermentable) [131–133]. Histone acetylation and deacetylation is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Histone acetylation results in relaxing chromatin condensation and activation of gene transcription while decrease of histone acetylation causes heterochromatin condensed state and suppression of transcription [138, 139].

Proliferation and self-renewal of NSCs depends on interaction of TF TLX-HDAC, that lead to transcriptional repression of its target genes, *p21* (cyclin-dependent kinase inhibitor) and *PTEN* (a tumor suppressor gene) [140]. The first in vivo data that histone acetylation controls cortical development came from phenotypic analyses of the *Querkopf* knock-out mice, in which the gene encoding for K (lysine) acetyltransferase 6B (*Kat6b*) was deleted [141]. *Querkopf*-deficient mutants display severe defects in the central nervous system, specifically displaying a small-sized and thin cortex, and predominant dysgenesis of *Otx1*(+) L5 neurons [141]. Several recent studies have shown that mouse embryonic stem cell (mESCs) can recapitulate the sequential appearance of cortical layering in vitro; however, with a deficiency in generation of UL neuronal fate [142, 143]. Transient inhibition of HDAC by valproic acid (VPA) in mESC-derived neural progenitors not only induces neuronal differentiation but also selectively enriches the *Cux1*(+) UL vs *Ctip2*(+) LL neuronal fate, suggesting that histone acetylation promotes a temporal progression of neurogenesis leading to a fate switch from LL to UL producing progenitors [144].

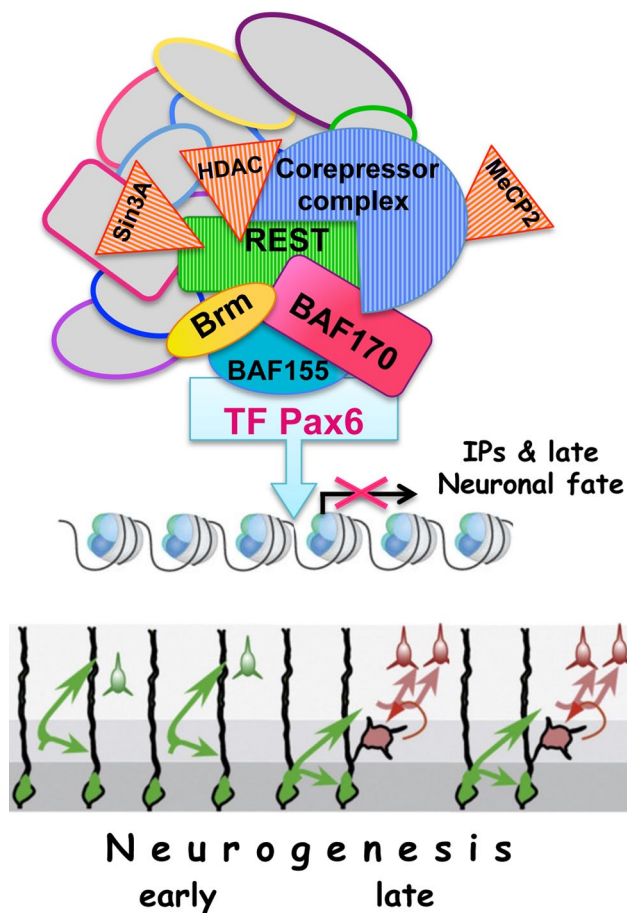
Covalent chemical modification of DNA (more specifically methylation of the fifth position cytosine, 5mC) by DNA methyltransferases (*Dnmt1*, *Dnmt2*, *Dnmt3*) plays important roles in the regulation of gene expression,

chromatin structure, gene imprinting, X-chromosome inactivation, and genomic stability [145]. Conditional deletion of *Dnmt1* in both dividing neural precursor cells and post-mitotic neurons in mouse brain resulted in severe thinning of CP [146]. *Dnmt3b* is robustly expressed during early neurogenesis (E10.5–13) whereas *Dnmt3a* is expressed in neural stem cells in postnatal brains [147]. However, their individual roles in cortical neurogenesis are still not clear. Additionally, simultaneous elimination of the proteins TET2 and TET3 involved in DNA demethylation causes impairment of progenitor differentiation and cell accumulation [148].

The polycomb group (PcG) proteins function within multiprotein complexes (named Polycomb repressive complexes, PRC1 and PRC2), which modify histones and generally repress transcriptional target activity [149]. Recent studies have disclosed that PRC2 controls the balance between progenitor self-renewal versus differentiation in the developing cortex. An enhancer of the *Zeste* homolog (*Ezh2*) is a catalytic component of PRC2, which catalyzes the addition of methyl groups in histone H3 at Lys27 (H3K27) in target gene promoters, causing epigenetic silencing [150]. More specifically, conditional abolishment of *Ezh2* function before the onset of neurogenesis in the developing mouse cortex causes removal of the H3K27me3 repressive mark, and a shift towards differentiation affecting both the direct and indirect mode of neurogenesis [151]. Specifically, upon *Ezh2* deletion, enhanced generation of IPs/early born LL neurons and depletion of IPs/late born UL neurons was found. This is most probably due to acceleration of the timing of developmental program that triggers glia differentiation inappropriately early [151].

In contrast to the PcG protein complex, the trithorax (TrxG) chromatin-remodeling complex mostly activates gene expression [152], regulating different developmental processes including stem cell proliferation [153]. Activation of gene expression via the methylation of lysine 4 on histone H3 is the main mechanism involved [154]. Recently, TF *ZNF335/NIF-1* was identified as a gene disrupted in severe microcephaly in humans, exerting an important role in progenitor self-renewal and differentiation [155]. As a part of TrxG complex, ZNF335/NIF-1 interacts with the H3K4 chromatin methyltransferase complex, activating brain-specific genes that promote neuronal differentiation through control of REST repressor element 1 (RE1)-silencing transcription factor [156], acting through recruitment of HDACs [157]. Accordingly, brain-specific knockout leads to severely reduced forebrain structures [155].

The vertebrate mSWI/SNF chromatin remodeling complex, also known as the BAF (Brg1/Brm associated factor) complex, contains at least 15 different subunits, including two interchangeable core ATPase subunits (Brg1 or Brm), invariant core subunits (BAF47, BAF155, BAF170), and



**Fig. 4** Hypothetical model proposing how epigenetic mechanism based on BAF chromatin remodeling complex controls the modes (direct vs. indirect) of cortical neurogenesis defining the cortical size, thickness and neuron layer subtype composition in the adult brain (according to [159, 160]). In the mouse cortex, coupling between TF Pax6 and the BAF complex subunits (BAF170, BAF155, Brm) and the REST–corepressor complex controls expression of genes involved in specification of IPs and late neurogenesis. During early stages of cortical neurogenesis, the restricted expression of BAF170 in non-neurogenic vRGCs recruits the REST–corepressor complex to promoters of TF Pax6 target genes (*Tbr2*, *Cux1*, *Tle1* and other still unknown targets) that are involved in specification of both *Tbr2*(+) IPs, and genes specifying late (UL) neuronal fate. In *BAF170* deficiency, BAF170 is replaced by additional BAF155 subunit, leading to loss of the REST–corepressor complex and induction of euchromatin state that relieves the repression of these Pax6 target genes, inducing a premature (heterochronic) induction of late (mostly indirect) neurogenesis. Bisulfite sequencing analysis of CpG methylation on *Cux1* and *Tle1* promoters using genomic DNA isolated from *BAF170cKO* and control cortices revealed a substantial decrease of CpG methylation in *BAF170cKO* and increase of these marks upon *BAF170* overexpression in vivo. In opposite, *BAF170* loss of function resulted in an increase in active H3K9Ac chromatin marks on *Cux1* and *Tle1* promoters (and reduction of repressive modifications H3K27Me3), *BAF170cOE* had opposite effects. *BAF170cKO*: cortex-specific conditional knock-out of *BAF170*; *BAF170cOE*: cortex specific conditional overexpression of *BAF170*. The differently colored circles indicate subunits of the BAF complex

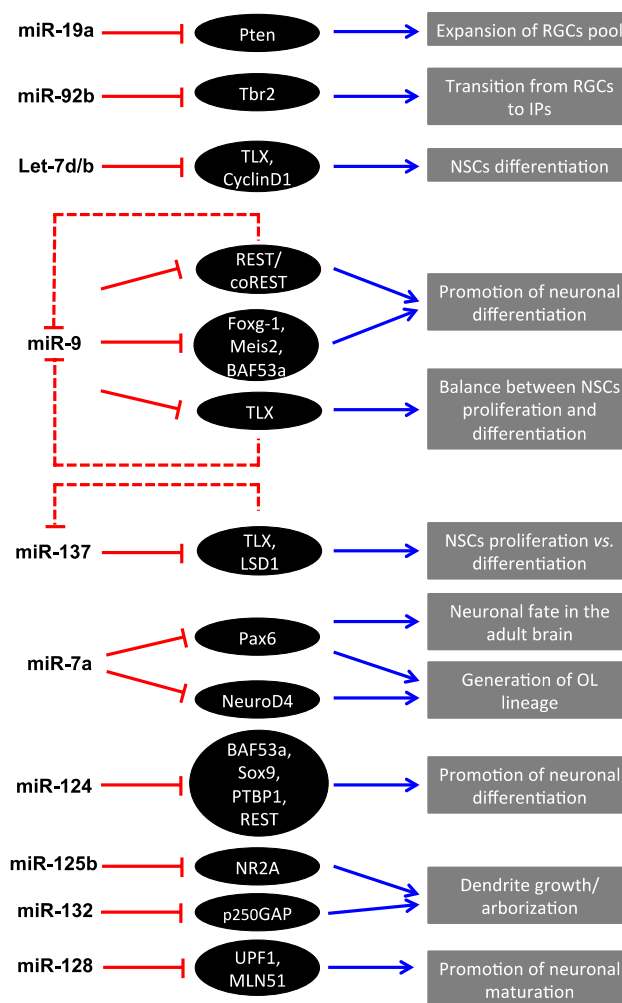
a variety of lineage-restricted subunits [133]. By using the energy of ATP to disrupt nucleosome–DNA contact, chromatin remodeling moves, removes, or exchanges nucleosomes along the DNA, and makes DNA–chromatin interactions accessible to transcriptional regulators allowing binding to their targets. Biologically distinct BAF assemblies are active in distinct neural cell types, e.g., the npBAF complex in neural progenitors and the nBAF complex in differentiated neurons [158]. Furthermore, the progressive transition from embryonic stem cells (ESCs) to neural progenitors is accompanied by the exchange of one of the two BAF155 subunits of the (esBAF) complex for BAF170 in the npBAF complex [133]. In the developing mammalian cortex, the BAF170 subunit is only transiently expressed in non-neurogenic (Pax6+/prominin–) vRGCs during the period of early neurogenesis (E10.5–E14.5) when neurons with LL identities are generated [159]. Analyses of the functional consequences of the in vivo ablation or overexpression of *BAF170* in transgenic mice have revealed that BAF170 is an important player in the control of cortical surface size and thickness [159]. During early (E12.5–E13.5) neurogenesis, the loss of *BAF170* function leads to a transient decrease in early born neuronal progenies (L6, L5 neuronal subsets) in conjunction with a surplus of generated *Tbr2*(+) IPs as well as premature (heterochronic) activation of UL fate specification genes (e.g., *Cux1*, *Tle1*) [159]. At later stages, however, the enlarged pool of IPs produces a modest surplus of LL neurons and large excess of UL neuronal fates. Together, the available data indicate that a dynamic expression of the BAF170 subunit in the BAF chromatin remodeling complex may control distinct aspects of cortical development, including temporal specification of a subpopulation of vRGCs as non-neurogenic, and maintaining an accurate timing for the initiation of the indirect (via IPs) mode of neurogenesis. Mechanistically, it was found that, during the early cortical neurogenesis (E10.5–E14.5) in the Brm-based BAF complex, TF Pax6 binds to BAF170 subunits, which recruit the REST–corepressor complex to the Pax6 downstream target gene promoters that are involved in the specification of IPs (*Tbr2*) and upper layer neuronal fates (*Cux1*, *Tle1/Groucho1*) [159] (Fig. 4). In addition, the loss of BAF170 in the BAF complex results in its substitution by the BAF155 subunit inducing euchromatin state, thus facilitating the accessibility of TF Pax6 to corresponding targets [160]. Consequently, upon elimination of the BAF170 form of the complex, the ultimate biological outcome of the BAF subunit exchange is the premature promotion of the indirect (via augmented IP pool) mode of neurogenesis. Interestingly, in a recent report, Ninkovic et al. [161] described that, during neurogenesis in adult olfactory bulb, instead of interacting

with the Brm subunit, TF Pax6 recruits specifically Brg1 (the alternative ATPase subunit in the BAF chromatin remodeling complex), which promotes the Pax6-mediated program for adult neurogenesis. Thus, specific interactions of Pax6 and BAF chromatin modifiers in distinct cellular contexts of developing and adult brain facilitate transcriptional programs of neurogenesis.

Recent studies have elucidated some interesting molecular mechanisms that specify the projection trajectory of the two major classes of cortical pyramidal neurons: cortico-cortical (callosal) projection neurons located mostly in ULs and subcortical projection neurons having LL identity [99, 162]. Notably, chromatin remodeling involving the BAF complex also controls the projection subtype identity. TFs *Ctip2/Bcl11b*, a newly identified BAF chromatin remodeling subunit [133, 163] that labels all L5 SCMN, and *Satb2* (AT-rich sequence-binding protein 2 (*Satb2*), that binds to DNA, are essential for the specification of subcortical (L5) and callosal (L3–L2) projection neurons, respectively [117, 164, 165]. In the absence of *Satb2*, active chromatin markers (H3K4me2) and histone H3 acetylation in the *Ctip2* locus were markedly increased, permitting the ectopic activation of *Ctip2* in *Satb2*-deficient callosal neurons [164]. Mechanistically, TF *Satb2* promotes callosal projection fate by antagonizing the expression of *Ctip2* in an epigenetic-like manner [164, 165], mediated by the proto-oncogene *Ski* [166] involving nucleosome chromatin remodeling [165]. Overall, the available, yet scarce, data suggest that interplay between TFs and epigenetic chromatin modifications is an important regulatory mechanism of different aspects of fate specification in neural stem and progenitor cells during cortical neurogenesis, profoundly affecting cortical size/thickness.

#### MicroRNA as regulators of corticogenesis

MicroRNA (miRNAs) are endogenous single-stranded noncoding RNA molecules of 22–24 nucleotides in length that are involved in posttranscriptional regulation of protein expression during various developmental processes including cortical neurogenesis [167–172]. In the cell nucleus, cleavage of the primary-miRNA by RNase III enzyme Droscha and DGCR8/Pasha (a binding partner) generates a 70-nucleotide-long precursor (pre-miRNA) that is exported into the cytoplasm, and is further processed into the mature double-stranded miRNA by RNase III enzyme Dicer. Upon unfolding, one strand is included in a silencing complex and guided to seed sequences usually located in the 3'-untranslated regions of target mRNAs causing transcript degradation or translational repression [173, 174].



**Fig. 5** miRNAs involved cortical neurogenesis. MiRNAs target multiple mRNAs in order to implement their regulatory action, also known as “fine-tuning”, in a variety of processes, as indicated. Some miRNAs establish feedback circuits. An example of this is miR-9 and Tlx, which form a negative feedback loop to induce precise spatiotemporal orchestration of NSC proliferation/differentiation in mouse telencephalon. At the same time, miRNA let-7d regulates the Tlx–miR-9 cascade to control neural cell fate and neurogenesis. MiR-19a and miR-92b, which belong to the same cluster (miR-17-92), control the expansion of RGCs pool and the transition to IPs by negatively regulating *Pten* and *Tbr2*, respectively. In embryonic neurogenesis, miR-7a inhibits the neurogenic factors *Pax6* and *NeuroD1* to promote the generation of oligodendroglial (OL) lineage. In the forebrain niche for adult neurogenesis, miR-7a-mediated regulation of *Pax6* expression promotes the dopaminergic neural fate of the interneurons of olfactory bulb. The expression of miR-124 is neural-specific and increases upon differentiation of NSCs into neurons. Both miR-124 and miR-9\* repress REST, which restricts the expression of neuronal genes in non-neuronal cells as well as the progenitor-specific BAF complex subunit BAF53a. This triple negative genetic circuit is specifically involved in the regulation of mitotic exit of cortical progenitors and the beginning of neuronal differentiation (based on data from [177–204])

Because miRNAs target multiple mRNAs, and the encoding genes are often included in clusters or families, generation of genomic miRNA loss- and gain-of-function transgenic models are challenging procedures. At present, our knowledge about the role of specific miRNAs in the regulation of cortical size and thickness in transgenic mice *in vivo* is still fragmentary. Global loss of miRNA regulation through conditional knockout of Dicer at different stages of corticogenesis driven by the Cre-strains, *Emx1*-Cre [175, 176], *Nestin*-Cre [176], *Foxg1*-Cre [177, 178], *CamKII*-Cre [179], and *Nex*-Cre [180] have revealed specific phenotypes as a result of time-dependent regulation of cell-fate determination of the progenitors (reviewed in [172, 181]). Deletion of Dicer function at the onset of neurogenesis (via *Emx1*-Cre line) leads to the most severe cortical phenotype at postnatal stages, affecting the cortical thickness and lateral cortical expansion and showing overproduction of early born LL neuronal fates (as a result of precocious LL differentiation), that leads to a severe reduction of later born UL neurons [176]. However, slightly later elimination of Dicer activity (via the *Nestin*-Cre line) results in a milder reduction of cortical thickness showing affected generation and migration of the later born neurons [176]. In addition, ablation of Dicer in neuronal progenitors [175, 176] triggers severe apoptosis and differentiation defects, while loss of Dicer activity in postmitotic neurons [179, 180] leads to mild apoptosis, suggesting that the role of miRNAs for cell survival is stage- and cell type-specific.

As discussed, growth of cortex with normal size and thickness critically depends on positive and negative factors for NSCs, RGCs/IPs self-renewal, and subsequent differentiation. Conditional deletion of miR-17-19 family (consisting of miR-17, miR-18, miR-19, and miR-92 subfamilies with distinct seed sequences) at the onset of neurogenesis in mouse cortex leads to pronounced reduction of cortical thickness [182]. Interestingly, miR-19a positively influences the expansion of RGC pool and regulates the transition RGC-IPs by inhibiting the tumor suppressor gene *Pten*, thus controlling the RGCs expansion [182]. As recently shown by Nowakowski et al. [178], the miR-92b, another member of the cluster, plays a restrictive role for generation of *Tbr2*(+) IPs from RGCs in the developing mouse cortex (Fig. 5) (reviewed in [182]).

Recent application of techniques for focal overexpression or inhibition of specific miRNAs indicates important roles in neuronal differentiation (reviewed in [172, 181]). By targeting both the nuclear receptor *TLX* that promotes NSC-fate and the *Cyclin D1*, the miRNA *Let-7b* regulates neural stem cell differentiation [183]. Several studies indicate that, in vertebrates, miR-124 promotes neuronal differentiation and cell cycle exit of NSC cells in the SVZ of adult brain, a niche for postnatal neurogenesis. Forced expression of miR-124 in SVZ promotes neuronal differentiation while knockdown

of miR-124 maintains the NSC fate (Fig. 5) [184, 185]. Although the majority of studies indicate that miRNAs are in general more important for induction of neuronal differentiation, a recent work has discovered a role of miR-7a in fore-brain neuronal subtype specification, promoting dopaminergic neural fate of the interneurons of olfactory bulb, due to inhibition of TF *Pax6* expression (Fig. 5) [186].

It should be noted that the function of only a few of these miRNAs as possible regulators of cortical size and thickness has so far been studied in transgenic mouse models. Targeted deletion of miR-9-1 together with miR-9-3, two precursors of miR-9/miR-9\* (\*miRNA synthesized from the opposite strand) show a marked reduction of the generative VZ and diminished thickness of all cortical layers. In fact, miR-9 suppresses NSC proliferation and promotes neuronal differentiation by modulation of the expression of several important factors, *Foxg1*, *Meis2*, *Nr2e1*, and *TLX* (Fig. 5) [187, 188].

Interestingly, when involved in cell fate control, many miRNAs forms feedback regulatory loops with their target's genes. The mechanism of miR-9-dependent promotion of neuronal differentiation includes a negative feedback regulation of the REST complex by silencing *REST/CoREST* genes [189–191]. Such regulatory interactions have been reported for miR-9 and *TLX* in determination of neural stem fate [183, 200], and for miR-137 with *TLX* and histone deacetylase *LSD1* in controlling the proliferation versus differentiation during corticogenesis [192]. Similarly, in NSCs, miRNA *let-7d* binds the 3'UTR of *TLX* and acts upstream of the *TLX*/miRNA-9 cascade, controlling neural cell fate and neurogenesis (Fig. 5) [183]. Recent data also suggest that a double-negative feedback loop of miR-9 and *Hes1*, a Notch signalling effector that exerts short-period expression oscillations, is essential for maintenance of progenitor proliferation and cells exit from mitosis [193].

Among the targets of miR-9/9\* and miR-124 are REST, which restricts the expression of neuronal genes in non-neuronal cells [189], and BAF53a, a component of the npBAF complex [158]. Notably, before NSCs start to exit from mitosis, miR-9\* and miR-124 are activated and bind to the BAF53a 3' UTR to fully repress BAF53a activity which is replaced by BAF53b subunit in the neural-specific BAF complex (nBAF) (Fig. 5) [177, 194]. Together, these data suggest that regulation of mitotic exit of cortical progenitors and the beginning of neuronal differentiation is under a triple-negative genetic circuit involving miR-9\*, miR-124, and REST as the main players [185]. Even more intriguingly, the synergistic effect of the expression of miR9/miR9\* and miR-124 was found to convert human fibroblasts to neurons [195]. More detailed analyses in transgenic models in future are expected to shed light on the specific roles of individual miRNAs in determining cortical size and thickness.

## Conclusions and future perspectives

The expansion of cortical surface and thickness in primate brain have been linked to the adoption of complex cognition. In past years, fundamental knowledge has accumulated on the progenitor diversity and specific proliferation behavior during cortical neurogenesis in phylogenetically different species. Although showing striking conservation, the mechanisms controlling the cortical size appear to act in species-specific manners which await further clarification. Future studies should help to elucidate the lineage relationships between distinct progenitor subtypes with respect to their cell polarity, asymmetry, spindle orientation, mode of division, and acquired fates of the daughter cells during specific stages of cortical neurogenesis in different species. Available data suggest important roles of transcription factors and morphogenes in different aspects of these processes; however, the underlying molecular mechanisms are still largely unknown. As multiple cortical developmental processes seem to be co-regulated by the same transcriptional program, future efforts should be aimed at the identification and regulation of specific pathways by particular TF. Epigenetic mechanisms mediated by various TFs could be of key importance for the establishment of transcriptional programs covering the enormous diversity of cortical progenitor and neuronal subtypes identities. An interesting prospective for future experiments will be to uncover gene regulatory pathways established by epigenetic machinery and miRNAs during cortical neurogenesis. Deep elucidation of the factors that control these processes will be essential for understanding the pathogenesis of neurodevelopmental diseases with a great significance for the human mental health.

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