Review

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Neurogenesis during development of the vertebrate central nervous system

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Abstract

During vertebrate development, a wide variety of cell types and tissues emerge from a single fertilized oocyte. One of these tissues, the central nervous system, contains many types of neurons and glial cells that were born during the period of embryonic and post-natal neuro- and gliogenesis. As to neurogenesis, neural progenitors initially divide symmetrically to expand their pool and switch to asymmetric neurogenic divisions at the onset of neurogenesis. This process involves various mechanisms involving intrinsic as well as extrinsic factors. Here, we discuss the recent advances and insights into regulation of neurogenesis in the developing vertebrate central nervous system. Topics include mechanisms of (a)symmetric cell division, transcriptional and epigenetic regulation, and signaling pathways, using mostly examples from the developing mammalian neocortex.

Keywords central nervous system; development; neural progenitors; neurogenesis

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See the Glossary for abbreviations used in this article.

Introduction

During early development of the vertebrate embryo, neural fate is induced in the ectoderm by the underlying notochord. Subsequently, the neural plate undergoes patterning of the future distinctive CNS regions as well as neurulation to form the neural tube. The neural tube wall constitutes a pseudostratified epithelium as it is made up of NECs that move their nuclei depending on the cell cycle phase. Prior to division, NECs move their nuclei to the ventricular surface for mitosis to occur. At the onset of neurogenesis, these cells switch their identity and turn into RGCs that will generate, directly or indirectly, all neurons and later in development, glial cells (Fig 1).

Transition from neuroepithelial to radial glial cells

NECs and RGCs, collectively referred to as APs, portray apicobasal polarity, with apical and basal processes that span the neuroepithelium. As NECs turn into RGCs, they downregulate Golgi-derived apical trafficking, lose tight junctions but maintain adherens junctions. Also, they initiate the expression of astroglial markers such as GLAST and BLBP. The mechanisms underlying NEC to RGC transition are only partially understood. Expression of members of the bHLH transcription factor Hes family, as well as transient expression of Fgf10, is necessary for this transition [1,2].

At the onset of neurogenesis, RGCs switch from symmetric to asymmetric divisions, giving rise to an RGC daughter cell and a differentiating cell (Fig 2A, B). This latter cell constitutes a neuron, or in certain areas of the brain such as the neocortex, a more fate-restricted type of progenitor that is called IP and is one of the types of BPs. IPs divide mainly symmetrically to yield two neurons, thus doubling the neuron output. In some more expanded brain regions, such as the neocortex in mammals, there are additional BPs present with glial characteristics that are capable of self-renewal (see below). These progenitors are proposed to mediate cortical expansion in some mammals during evolution [3] (see below).

Cellular features of neural progenitors

Neural progenitor cells (NPCs) such as NECs and RGCs are highly polarized, with their apical membrane exposed to the ventricle and their basal side contacting the pial basal membrane (Fig 1).

Apical domain

The apical domain of RGCs contains several features that are important for RGC function. Just basal to the apical and subapical plasma membrane, the AJs mediate cell–cell adhesion. AJs consist of cadherins and catenins that connect to the intracellular actin network. Importantly, polarity proteins such as Par3, Par6, and aPKC are associated with the subapical cell cortex and are important for RGC proliferation [4]. The Rho GTPases RhoA, cdc42, and Rac1 have important roles in the maintenance of AJs and apical mitoses by the regulation of actin [5–9]. The apical plasma membrane is

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Glossary

AJ	adherens junctions
AP	apical progenitor
aPKC	atypical protein kinase C
Ascl1	Achaete-scute homolog 1
bHLH	basic helix-loop-helix
BLBP	brain lipid-binding protein
BMP	bone morphogenetic protein
BP	basal progenitor
bRG	basal radial glia
CNS	central nervous system
CSF	cerebrospinal fluid
Cux2	Cut-like homeobox 2
ECM	extracellular matrix
Fezf2	forebrain embryonic zinc finger-like protein 2
Fgf	fibroblast growth factor
GFAP	glial fibrillary acidic protein
GLAST	glial high-affinity glutamate transporter
Hes	hairy/enhancer of split
IGF	insulin growth factor
INM	interkinetic nuclear migration
Insc	Inscuteable
IP	intermediate progenitor
LGN	Leu-Gly-Asn repeat-enriched protein
lncRNA	long non-coding RNA
miRNA	microRNA
Myc	myelocytomatosis oncogene
Ndel1	NudE neurodevelopment protein 1-like 1
NEC	neuroepithelial cell
Nfia	Nuclear factor Ia
Ngn	Neurogenin
NICD	Notch intracellular domain
NPC	neural progenitor cell
oRG	outer radial glia
OSVZ	outer subventricular zone
Par	partition defective complex protein
RGC	radial glial cell
saRGC	subapical radial glial cell
Shh	Sonic Hedgehog
SNP	short neural precursor
Trim32	tripartite motif containing 32
Trnp1	TMF1-regulated nuclear protein 1

characterized by a specific composition of membrane constituents. The resulting apical polarity is essential for NPC function.

Newborn neurogenic daughter cells need to withdraw their apical endfoot from the apical belt of AJs in order to migrate basally and differentiate. Proneural genes expressed in the differentiating daughter cell induce downregulation of cadherins to mediate delamination from the ventricular surface, in a manner similar to epithelial–mesenchymal transition in other epithelia [10,11]. An alternative mechanism for delamination recently observed in chick and mouse neural tube is abscission of the apical endfoot that is similarly regulated by proneural genes acting upstream of cadherin and other factors [12]. In this process, actomyosin-dependent constriction of the apical process, preceded by dissociation of the centrosome from the apical primary cilium, leads to abscission of the apical process from the apical-most portion of the apical endfoot [12]. In this way, the cell loses its apical polarity and ciliary proteins, which contributes to its subsequent cell cycle exit and differentiation.

At the apical side, the centrosome is docked at the apical plasma membrane. Here, it functions as the basal body in nucleation of the primary cilium, an important sensory organelle that detects signals in the ventricular fluid/CSF such as IGF and Shh [13,14]. Primary cilium activity is required for maintaining proper apicobasal polarity as NECs transform into RGCs [15]. Upon disruption of Arl13b, a small ciliary GTPase, during NEC to RGC transition, the polarity of the cortical wall is inverted, with mitoses occurring at the pial surface and neurons migrating to the ventricular surface [15]. After onset of neurogenesis, primary cilium function in processing of the transcriptional repressor Gli3R is involved in the regulation of RGC proliferation [16] (see also below).

Basal process

The basal process of RGCs stretches all the way to the basal lamina at the pial surface. Recent studies have shown that the basal process is important in the maintenance of proliferative capacity through integrin signaling from the basal lamina and via the specific basal localization of the G1-S-phase regulator CyclinD2 [17–19]. It is hypothesized that the presence of a basal process is involved in the continued proliferative capacity of bRGs that are present in gyrencephalic brains [17].

Cell cycle kinetics of RGCs

Prior to mitosis, in G2, the RGC nucleus moves to the ventricular surface where the centrosome is docked. This nuclear movement is part of INM in which the NEC/RGC nucleus moves in concert with the cell cycle using actomyosin and microtubule motor proteins [20]. It has been proposed that INM functions to maximize the number of RGC mitoses at the small ventricular surface [20]. Another possible function of INM is to differentially expose the RGC nucleus to signals that are present along an apical–basal gradient, such as Delta-Notch signaling (see below). Recently, it was demonstrated that dynein recruitment to the nuclear pore through two consequential mechanisms is required for apical nuclear movement and mitotic entry of rat RGCs [21]. Interestingly, nuclear pore complexes were also necessary for the basal movement of the centrosome, which occurs just prior to prophase [21,22].

Changes in cell cycle length have been implicated in cell fate determination during neurogenesis [23]. The duration of the RGC cell cycle changes during brain development, with an increased G1 phase length being linked to neurogenic divisions [24–26]. Interestingly, the S-phase of RGCs that undergo proliferative divisions is longer than that of RGCs undergoing neurogenic divisions, suggesting that careful control of DNA replication takes place during the S-phase of expanding RGCs [24]. Conversely, one may speculate that somatic mutations that occur in RGCs after their switch to asymmetric self-renewing/neurogenic divisions due to the lack of correction of DNA replication errors may be a means of increasing neuronal diversity.

Regulation of symmetric versus asymmetric divisions

Mitotic spindle orientation

After onset of neurogenesis, RGCs divide mainly asymmetrically yielding one RGC daughter and a differentiating daughter cell (Fig 2B). In invertebrates such as *Drosophila*, asymmetric division has been shown to result from unequal division of cellular

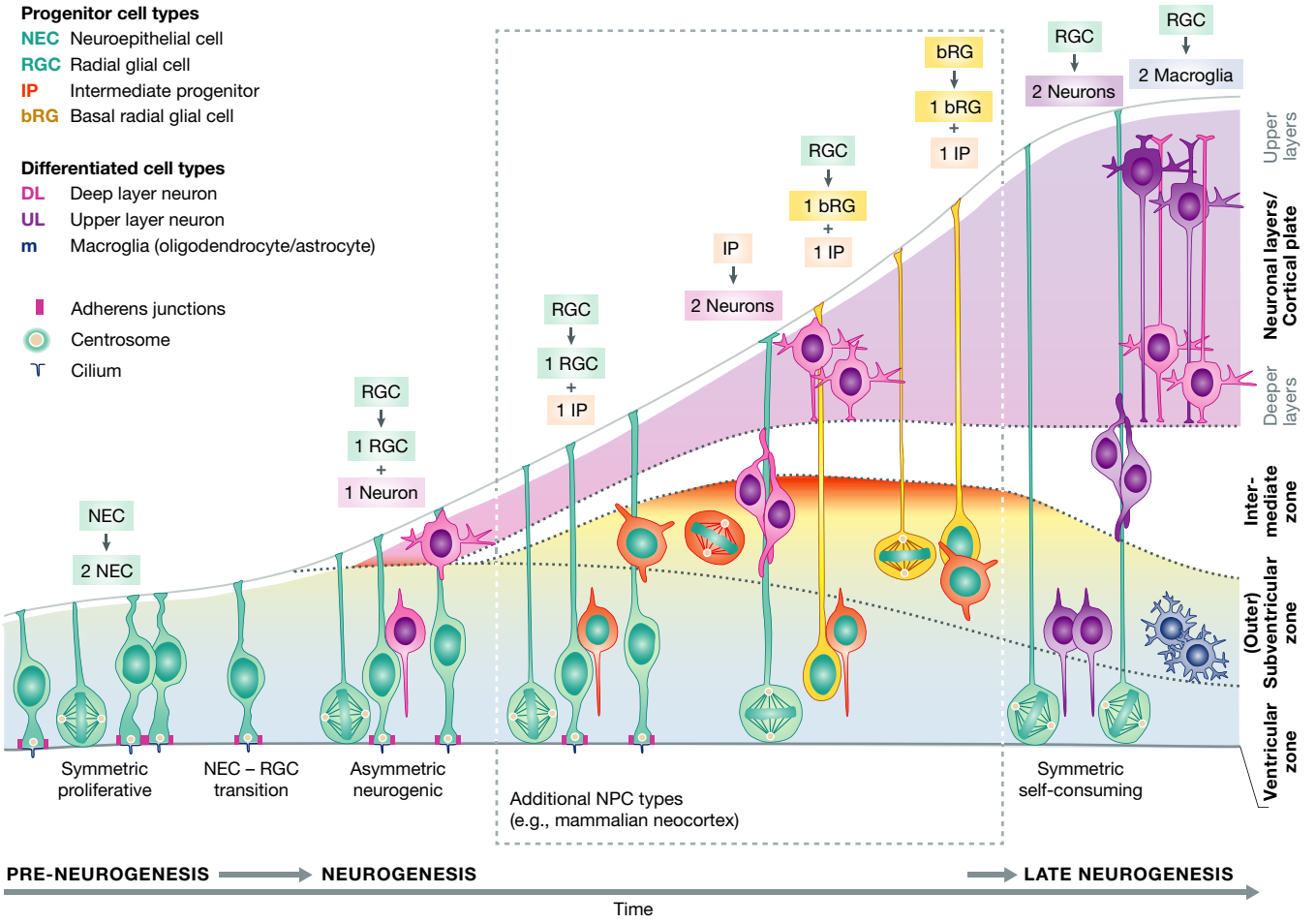

Figure 1. Schematic overview of neurogenesis in the embryonic vertebrate CNS.
The principal types of NPCs with the progeny they produce are indicated by different colors. Additional NPC types that are typically found in mammalian neocortex are indicated in the box; note that only some of the possible daughter cell outcomes are depicted.

components and cell fate determinants through horizontal cleavage planes (Fig 2C, right).

In the vertebrate developing brain, early RGC divisions feature cleavage planes perpendicular to the ventricular surface (vertical cleavage, Fig 2B, C left). The spindle orientation of symmetric RGC divisions is tightly regulated by mechanisms involving the centrosomes, astral microtubule positioning, and interaction with proteins present at the cell cortex [27]. The mitotic spindle is anchored to the cell cortex by astral microtubules via dynein and the LGN/Gαi/NuMa complex. Localization of the LGN complex components to the lateral membrane of NECs/RGCs is essential for maintaining early symmetric RGC divisions in vertebrate neurogenesis (Fig 2C, left) [28–30]. In addition, Lis1, a gene that causes lissencephaly ("smooth" brain) in humans when mutated, mediates capture of the astral microtubules by the cell cortex through interaction with dynein and Ndel1 [31]. Perturbation of the Lis1/Ndel1 complex severely disrupts the expansion of the NEC/RGC pool by inducing random cleavage planes [31–33].

In asymmetric divisions in *Drosophila*, Insc induces horizontal cleavage planes through recruitment of the LGN complex to the apical domain by interaction of Insc with polarity proteins (Fig 2C, right). However, horizontal cleavages are less common in vertebrate developing brains. For example, in the mammalian neocortex, oblique and horizontal cleavage planes appear only in later developmental stages (Fig 2C, middle) [34,35]. These cleavages generate basal progenitors such as IPs and bRG that are proposed to be important during evolutionary cortical expansion [36,37]. Disruption of mInsc at later stages of neurogenesis interferes with the spindle orientation of these asymmetric divisions [35], suggesting that release of the tight regulation of spindle orientation is important for inducing basal progenitors.

Indeed, mutations in genes regulating spindle orientation cause brain disorders such as lissencephaly and microcephaly in humans [38]. Interestingly, most known microcephaly genes encode centrosomal proteins, which often have a role in regulating spindle orientation, such as Aspm, Cdk5rap2, and MCPH1 [38–40]. Centrosome overduplication in mouse RGCs leads to multipolar mitotic spindles, eventually causing microcephaly due to RGC apoptosis and subsequent reduction in NPCs [41]. In general, besides regulating spindle orientation, the function of microcephaly genes is related to control of centriole duplication, centrosome maturation, and/or entry into mitosis. However, it is still unclear how disruption of

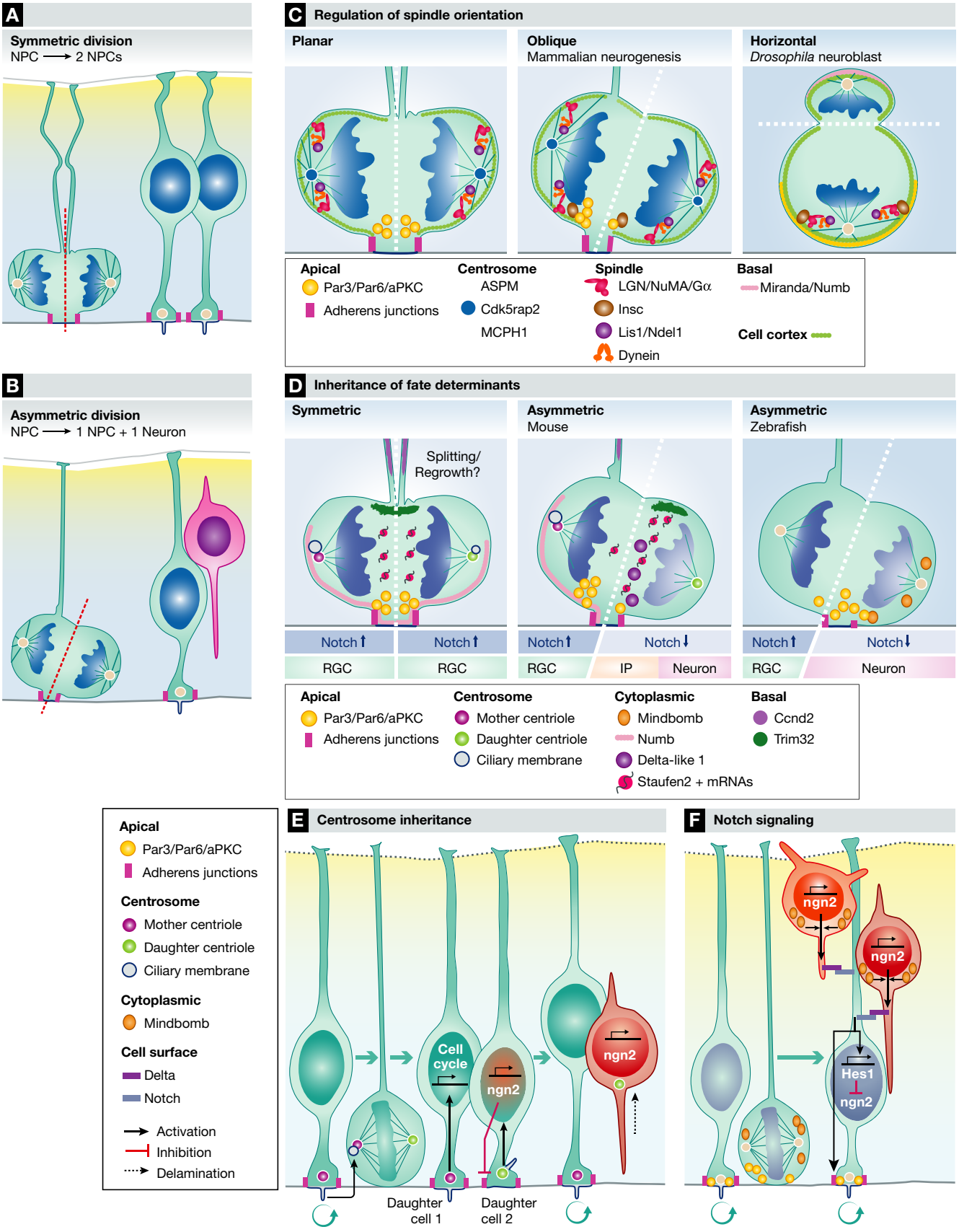

Figure 2. Division types of NPCs are determined by spindle orientation and inheritance of cell fate determinants.
(A, B) Symmetric division yields two NPCs, whereas asymmetric NPC division yields one NPC daughter and one differentiating daughter cell. (C) Spindle orientation in symmetric versus asymmetric divisions is regulated by centrosomal protein and spindle orientation complexes in vertical and oblique divisions of vertebrate NPCs (left and middle) and horizontal neuroblast divisions in *Drosophila*. (D) Cell fate determinants may be equally (symmetric division, left) or unequally (middle, mouse; right, zebrafish) distributed between daughter cells. (E, F) Examples of asymmetries between daughter cells that were introduced by asymmetric inheritance of differently aged centrioles and ciliary membrane (E), and Par3 and Notch signaling components (F).

these centrosomal functions leads to reduced brain size (see, e.g., [42]).

Asymmetric segregation of cellular components and cell fate determinants

As discussed above, the apical domain of RGCs contains important features such as the AJs and the centrosome. One previous model suggests that the cleavage furrow bypasses the apical domain, leading to its inheritance by only one daughter cell [34,43]. However, recent studies have shown equal division of the apical domain even in asymmetric divisions [28,37]. In this case, both daughter cells have inherited an apical domain initially, but the differentiating daughter will withdraw its apical process from the ventricular surface (Fig 2D, middle).

The basal process is thought to be important for the maintenance of NPC proliferation. In symmetric divisions occurring during early neurogenesis, the basal process of NECs can either be split and divided among the daughter cells [44], or inherited by one daughter cell with the other daughter re-extending it [45]. In contrast, in asymmetric divisions, the basal process is inherited by one daughter cell that retains self-renewing properties [37,45]. The daughter cell without the basal process is not able to re-establish it and becomes a differentiating cell such as a neuron or IP [28,37,46]. Taken together, these findings suggest that inheritance of both the apical and basal domain is required for maintaining RGC fate [28,37].

Recent studies have shown an intriguing link between centrosome asymmetries, ciliogenesis, and daughter cell fate (Fig 2E). In interphase cells, the centrosome contains one mother and one daughter centriole. The mother centriole is the oldest centriole within the cell and mediates nucleation of the primary cilium. Interestingly, older centrioles are preferentially inherited by daughter cells maintaining stem cell identity in the mouse neocortex [47]. A recent study shows that in mitotic RGCs, the mother centriole is able to retain ciliary membrane, which is subsequently asymmetrically inherited by one daughter cell that reforms a new cilium before its sister cell [48]. This earlier cilium reformation results in earlier ciliary signaling in this cell, which is proposed to contribute to its adoption of RGC daughter cell fate. In addition, nascent differentiating daughter cells show reformation of primary cilia at their basolateral instead of their apical membranes prior to their delamination [49]. These temporal and spatial asymmetries in ciliogenesis are proposed to lead to differential exposure of daughter cells to proliferative signals present in the CSF, such as IGF-1 [15,50], thus leading to asymmetrical daughter cell behavior.

In *Drosophila*, asymmetric division of neuroblasts is mediated through unequal division of polarity proteins and fate determinants. Similarly, in asymmetrically dividing RGCs of vertebrates, polarity proteins such as Par3 are asymmetrically segregated into one daughter cell [34,46,51,52]. At the same time, Notch signaling components such as the Notch ligand Delta-like 1, the regulator of Delta internalization, Mindbomb, and the Notch antagonist Numb are differentially segregated between daughter cells, leading to differential Notch signaling between daughter cells (Fig 2D, F) [51–53]. Interestingly, the cell fate related to Par3 inheritance appears to vary between species. In the mouse, Par3 segregates asymmetrically into the daughter cell that inherits both apical domain and basal processes and that remains an RGC (Fig 2D, middle) [51]. In contrast, in the zebrafish brain, the daughter cell inheriting the apical domain, including Par3, also inherits the Notch inhibitor Mindbomb and differentiates (Fig 2D, right) [46,52]. The other daughter cell quickly re-expresses Par3, re-establishes apical contact, and remains an RGC. At present, the mechanisms underlying these differences between species are unknown.

In addition to polarity proteins, other cytoplasmic proteins also show unequal inheritance in asymmetric divisions of neural progenitors. For example, the double-stranded RNA-binding protein Staufen binds a range of mRNAs that induce cell cycle exit and differentiation and segregates these into the differentiating daughter cell during mitosis of RGCs (Fig 2D, middle) [54,55]. One of these RNAs encodes Trim32 (Brat1 in *Drosophila*) that is asymmetrically segregated in both *Drosophila* neuroblasts and mammalian RGCs. Trim32 stimulates cell cycle exit through ubiquitination of c-Myc and activation of differentiation-inducing microRNAs such as Let-7 [56] (see also below).

Regulation of daughter cell fate specification

Transcription factors

During early development, the central nervous system is subdivided into the prospective different areas by gradients of morphogens such as Fgfs, Wnts, Shh, and BMPs. This patterning leads to regional expression of homeodomain and bHLH transcription factors that instruct NPCs to produce specific cell types during neurogenesis [57]. One of the master regulators of neurogenesis is the paired box containing homeodomain transcription factor *Pax6* that is expressed in several CNS regions, such as the forebrain, retina, and hindbrain [58]. In addition to the regulation of regional patterning, Pax6 promotes RGC proliferation and spindle orientation [59], but also promotes neurogenesis through the induction of bHLH proneural genes such as Neurogenins [60]. These partially opposing effects appear to be mediated through alternative splicing of Pax6 [61] and its interaction with other transcription factors such as Sox2 and Hes1 [58,60]. Neuronal differentiation is induced through the expression of region-specific proneural genes, Pou-homeodomain transcription factors such as Brn1/2, and SoxC transcription factors such as Sox4 and Sox11 that initiate specific neuronal programs and repress other regional identities [57,62]. For example, NPCs in the dorsal telencephalon express the bHLH proneural factors *Neurogenin*

(*Ngn*) 1/2. These factors instruct the generation of glutamatergic pyramidal neurons that make up the six-layered neocortex in mammals and repress ventral telencephalic genes. In contrast, the ventral telencephalon expresses *Gsh1/2*, *Nkx2.1,* and the bHLH proneural factor *Ascl1* that instructs the generation of GABA-ergic basal ganglia neurons and cortical interneurons, and represses dorsal identity.

The different types of neurons and glial are born sequentially from a pool of seemingly identical RGCs. Surprisingly, there is a significant stochasticity in RGC cell fate choices in individual RGC lineages in the developing retina, although there is a clear temporal order in neuronal subtype specification [63,64]. In analogy to findings made in *Drosophila*, the temporal order of neuronal specification by neural progenitors is thought to depend on sequential expression of transcription factors [65]. In the developing neocortex, neurons are born in an "inside-out" manner, with earlier-born neurons destined for the deep layers and later-born neurons for the upper layers. Contradicting observations with regard to the existence of fate-restricted RGCs in the developing cortex have been reported [66,67]. One study reports that a subpopulation of $Cux2^+$ RGCs generates only upper-layer neurons during later stages of neurogenesis [66]. However, recently, it was reported that $Fezf2^+$ RGCs sequentially produce deep and upper neurons, as well as oligodendrocytes and astrocytes [67]. Also, in this work, $Cux2^+$ RGCs contributed to both deep and upper layers. More studies will be needed to resolve the question whether fate-restricted RGCs constitute a relevant proportion of the progenitor pool and contribute specifically to the diversity of produced neurons.

Epigenetic modifications

In recent years, evidence has emerged that epigenetic modifications such as DNA methylation and histone modifications are involved in the control of temporal and spatial gene expression during neurogenesis, and the switch from neuronal to glial production [68]. Early-stage NPCs show high expression of regulators of epigenetic modifications. Examples of such regulators are HMG proteins that regulate the chromatin state and methyltransferases such as Ezh2 that function in histone modifications [69–71]. Therefore, the chromatin of early-stage neocortical NPCs is in a more open state (less condensed) than that of late-stage NPCs [70]. Global chromatin condensation as well as epigenetic modification of certain genes seems to be involved in the switch of NPC from producing neuronal to glial progeny during neocortical development [69,70,72]. For example, DNA methylation of glial genes such as *Gfap* prevents a premature switch from neuro- to gliogenesis [73]. Activated Notch signaling induces demethylation of the *Gfap* promoter through the induction of Nfia that dissociates DNA methyltransferases [74]. Conversely, at late stages of neurogenesis, proneural genes such as *Ngn1* are repressed through the action of Polycomb proteins [69].

The activity of specific transcription factors is also modified by epigenetic mechanisms. In the developing cortex, Pax6 mediates transcription of a range of genes that regulate patterning, NPC proliferation, but also instruction of IPs and late progenitor fates. Pax6 interacts with BAF155 and BAF170, which are components of ATP-dependent multi-subunit mSWI/SNF nucleosome remodeling complexes [75]. During early neurogenesis, BAF170 competes with the BAF155 subunit and modifies euchromatin structure. This results in the recruitment of Pax6/REST-corepressor complex to repress expression of *Pax6* target genes, such as *Tbr2*, *Cux2,* and *Tle2,* that instruct the generation of IPs and late cortical progenitors [75]. In this way, switching BAF complex subunits at some point during neurogenesis could release the repression of *Pax6* target genes, and the generation of IPs and late cortical neuronal types would follow. Another example of epigenetic control of transcription factor activity is transcriptional repression of the forkhead homeodomain transcription factor *Foxg1* through the chromatin remodeling protein Snf2 l at mid-neurogenesis. Repression of *Foxg1* leads to de-repression of the cell cycle exit regulator p21, thereby promoting cell cycle exit and neuronal differentiation of NPCs [76].

Post-transcriptional regulation of gene expression

Alternative pre-mRNA processing results in the generation of different proteins from one primary transcript. Alternative splicing plays a role in differentiation and development and has recently also been implicated in neurogenesis [77]. For example, alternative splicing of the transcriptional repressor REST by the splicing factor nSR100 leads to de-repression of neuron-specific genes and neuronal differentiation [78]. Furthermore, the polypyrimidine tract RNA-binding protein Ptbp2 inhibits splicing of exons that are typical for the splice variant expressed in adult tissues [79]. For example, Ptbp2 induces alternative splicing of proteins that are involved in RGC adhesion [79]. Deletion of Ptbp2 induces premature neurogenesis. Sequence-specific RNA-binding proteins such as Rbfox3 were shown to mediate alternative splicing of Numb, an important regulator of Notch signaling involved in the induction of neuronal differentiation [80].

An additional post-transcriptional mechanism for regulating gene expression in RGCs is through miRNAs, highly conserved non-coding RNAs of 18–24 nucleotides that bind to the 3′ UTR of mRNAs to silence their expression through degradation or suppressed translation [81]. In the developing brain, groups of miRNAs regulate either RGC proliferation or neuronal differentiation, suggesting that miRNAs play a crucial role in determining neuron numbers. For example, in the developing mouse cortex, miR-92 suppresses the transition of RGC into IPs by silencing the transcription factor *Tbr2* that induces IP fate [82,83]. Besides direct silencing of target genes, some miRNAs form a regulatory loop together with their targets. The HMG-box transcription factor *Sox2* that is expressed by NPCs and directs their self-renewal regulates expression of the RNA-binding protein LIN28 through epigenetic modifications [84]. LIN28 regulates the biogenesis of the let-7 miRNA family by inhibiting their maturation. In turn, let-7 miRNA suppresses expression of LIN28 and inhibits both proliferation and neuronal commitment through silencing of the cell cycle regulators *Ccnd1*, *Cdc25a*, and proneural genes *Ngn1* and *Ascl1*, respectively [84].

Recently, long non-coding RNAs (lncRNAs) have been implicated in the regulation of developmental processes including neurogenesis [85]. LncRNA loci encode RNA transcripts of >200 nucleotides that modulate gene expression through chromatin modifications and translational control such as alternative splicing. The lncRNA Rmst regulates neurogenesis in the midbrain through co-transcriptional interaction with Sox2 to activate proneural target genes such as *Ascl* and *Ngn1* [86]. In RGCs that are committed to neurogenic divisions, several lncRNAs such as Miat are expressed that regulate proliferation versus differentiation [87].

Signaling pathways

As already mentioned, a variety of signaling pathways triggered at the plasma membrane, notably the Notch, Wnt, Shh, and Fgf pathways, are known to act during the process of neurogenesis. Many of these signaling pathways have an effect on RGC proliferation and undergo considerable crosstalk (see also below).

Notch The Notch signaling pathway plays essential roles in the regulation of both embryonic and adult neurogenesis [88]. As first elucidated in *Drosophila*, Delta-Notch signaling regulates neurogenesis through the process of lateral inhibition. The Notch ligands Delta or Jagged activate Notch receptors on directly adjacent cells, leading to release of NICD that mediates the transcription of Hes genes. These in turn repress the expression of bHLH proneural genes such as *Ngn* and *Ascl* and thus keep this cell in a proliferative state. In the developing mouse cortex, the expression of Hes1 in RGCs oscillates with 2- to 3-h periods due to an autoinhibitory feedback loop [89]. These Hes1 oscillations induce oscillations in *Delta* and *Ngn2* expression. Therefore, it has been proposed that the differential expression levels of Hes1 could mediate differential responses of RGCs to incoming signals that regulate proliferation versus differentiation.

Pairs of daughter cells derived from asymmetric RGC divisions show asymmetries in Delta-Notch signaling components and activity (Fig 2D, F). For example, in asymmetric RGC divisions in the developing zebrafish as well as mouse telencephalon, the daughter cell with higher Notch signaling remains an RGC, while the daughter cell with low Notch signaling shows high expression of Delta and proneural genes and initiates delamination from the ventricular surface and neural differentiation (Fig 2F) [52,89,90]. In the developing mouse cortex, Notch ligands as well as the E3 ubiquitin ligase Mindbomb that promotes Notch signaling are expressed by neurons and IPs [91–94], which signal back to RGC via dynamic and transient processes (Fig 2F) [93]. One important question is how the response of cells to Notch signaling changes during neurogenesis, as Notch signaling is also active in newborn neurons. Some general repressors of Notch have been identified, but it is unclear whether these factors are specifically upregulated during neurogenesis [95,96]. Recently, a transcriptional repressor, Bcl6, was identified with increased expression during neurogenesis. Bcl6 changes the composition of the Notch-dependent transcriptional complex at the *Hes5* promoter and leads to histone modifications that permanently silence *Hes5* through recruitment of the deacetylase Sirt1 [97]. This epigenetic switch results in stable Hes5 inactivity despite active Notch signaling in differentiating cells, thereby stabilizing neuronal differentiation.

Wnt Wnt/β-catenin signaling is important in patterning of, and regulation of proliferation and differentiation in, the developing brain [98]. After binding of Wnt ligands to their Frizzled/LRP5/6 receptors, cytoplasmic β-catenin is stabilized and translocates to the nucleus where it mediates gene transcription through LEF/TCF transcription factor activity. Wnt signaling activity plays dual roles during neurogenesis. During early neurogenesis, Wnt signaling promotes symmetric RGC divisions and delays IP formation [99]. Later at neurogenesis, however, Wnt activity promotes IP formation and neuronal differentiation through upregulation of N-myc [100–102]. A recent study reports that N-myc is expressed in RGCs that are undergoing neurogenic division in the chick neural tube [103]. N-myc increases non-vertical cleavage planes and represses Notch signaling to stimulate neuronal differentiation [103]. Although it is not yet understood how the differential Wnt signaling responses are mediated, it is likely that the targeted genes change during neurogenesis through context- and cell-type-dependent mechanisms such as epigenetic modifications.

Hedgehog Sonic hedgehog (Shh) signaling is essential for proper dorsoventral patterning of the vertebrate central nervous system. Shh signaling is activated through binding of Shh ligand to the Patched receptor, followed by ciliary accumulation of Smoothened and processing in the primary cilium of the Gli transcription factors into their activator forms that mediate downstream gene transcription. In the absence of Shh, the Gli proteins are processed into repressor forms. In addition to its roles in patterning, Shh signaling also has important roles in the regulation of the RGC cell cycle kinetics through cell cycle regulators, as well as in the production of IPs [14,16,104]. During neurogenesis, active Shh signaling decreases, whereas activity of the Gli3 repressor increases, which is necessary for IP production and neuronal differentiation [16].

A recent study provides mathematical modeling of spinal cord neurogenesis to predict that decreasing Shh signaling mediates the switch from symmetric proliferative and asymmetric self-renewing divisions to symmetric neurogenic divisions by changing RGC cell cycle kinetics [105]. In the developing neocortex, Shh activity promotes symmetric proliferative divisions of RGCs through transcription of the Notch transcription factor Hes1 [106], thus showing that there is a significant crosstalk between different signaling pathways in the regulation of RGC proliferation.

Fgf Such interplay between pathways has also been observed for Fgf and Notch. Fgfs are important for anterior–posterior patterning of the brain as well as for expansion of RGCs by symmetric division through downstream activation of Hes1-mediated transcription [107].

NPC environment

In addition to the above-mentioned extracellular signals, numerous other factors in the NPC environment influence NPC behavior (Fig 3).

At the ventricular surface, several ECM molecules such as laminin and syndecan-1 are present that regulate, via integrin receptors, the apical adhesion and proliferation of RGCs [108,109]. Apical adhesion of RGCs and apical localization of integrin β1 are also controlled by ephrin B1 [110]. At the basal side, the interaction of the NPC basal process with basal lamina ECM is thought to be important for the self-renewing potential of RGCs and bRGs [17]. Another important signal from the basal side, retinoic acid, is produced by the meninges. Retinoic acid is essential for the switch of RGCs from symmetric proliferative to asymmetric neurogenic divisions at the onset of neurogenesis [111].

In addition to signals derived from the apical or basal side, environmental cues present within the developing neural tube wall also exert important effects on NPCs. For example, the presence of blood vessels near IPs appears to regulate their proliferation [112,113]. This resembles NPC regulation by blood vessels in the stem cell niche of adult neural progenitors. In addition, non-neuronal cells such as

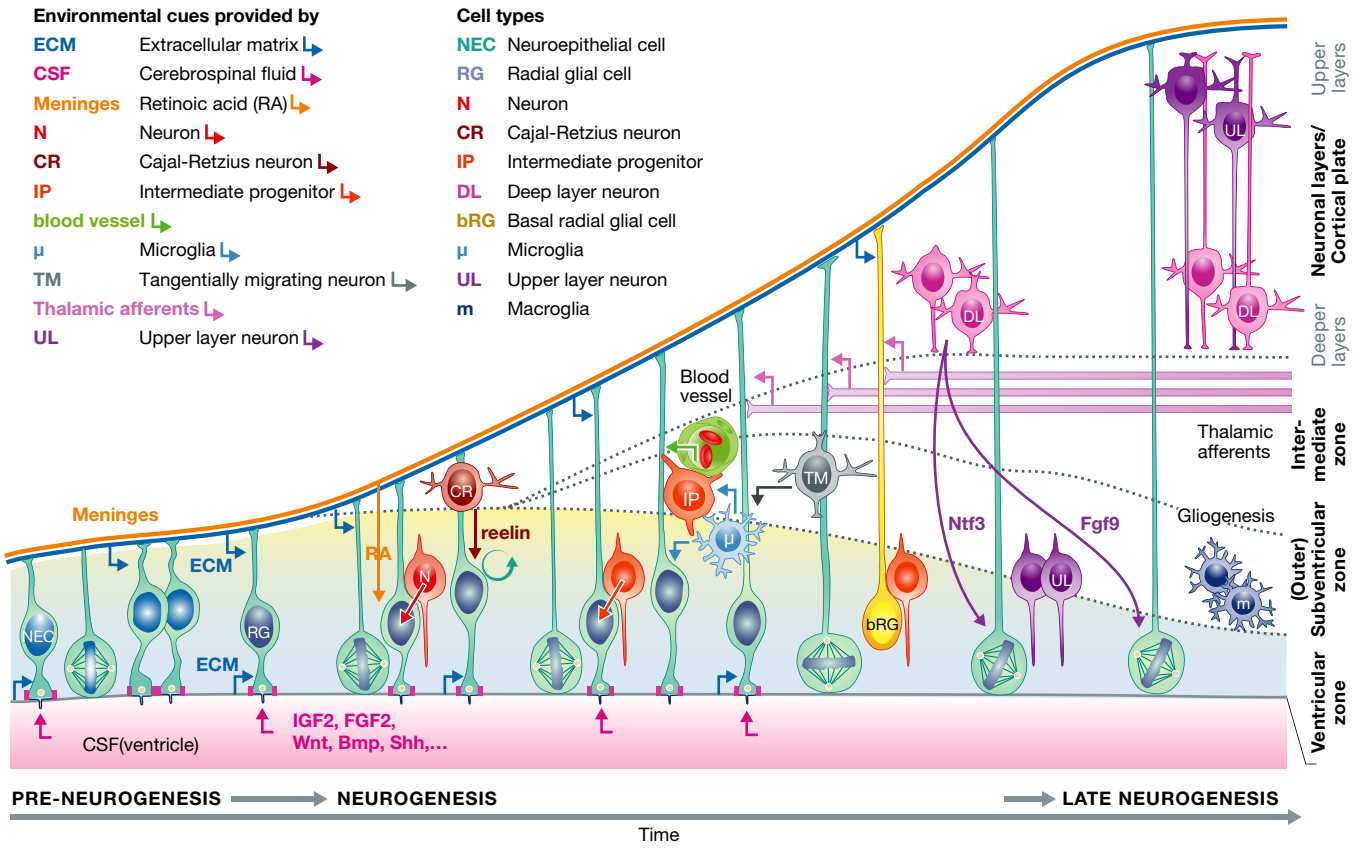

Figure 3. Environmental cues regulating NPC proliferation and differentiation.
For details, see text.

microglia that are present already during neurogenesis have been shown to regulate maintenance of the RGC population [114,115].

Post-mitotic neurons produce molecules that provide feedback information to RGCs. The Cajal–Retzius cells are the first type of neuron to be born in the neocortex. These cells secrete the glycoprotein reelin and express the cell adhesion molecules nectins that mediate neuronal migration. In addition, these cells play a role in modulating regionalization within the developing cortex by the secretion of signaling factors [116]. Furthermore, Cajal–Retzius cells influence RGC proliferation through the action of reelin that amplifies Notch signaling in early RGCs, thus promoting symmetric proliferative divisions and postponing neurogenesis [117]. In contrast, later-born cortical neurons express signaling molecules such as neurotrophin 3 and Fgf9 that regulate cell fate choices and the switch of dividing RGCs to astrogenesis [118]. Feedback signals to RGCs are also derived from neurons born in other brain regions, such as transient glutamatergic neurons born in the ventral telencephalon that migrate tangentially into the dorsal telencephalon [119].

Regional and species differences in neurogenesis

Neural progenitor type diversity

Timing of neurogenesis as well as the total neuronal output differs between CNS regions and between species [120]. One of the most expanded brain regions in mammals is the neocortex that enables many higher cognitive functions [121]. Such regional expansion could result from: (i) a greater initial pool of RGCs at the onset of neurogenesis, (ii) increased neuronal production through increased number of RGC cell cycles or the addition of "intermediate" transiently proliferating progenitor types, and (iii) a prolonged neurogenic period. Indeed, all of these parameters seem to be involved in expansion of the neocortex, especially in primates [120]. The developing mammalian telencephalon shows a large diversity of neural progenitor subtypes, as judged by their morphology, their mode of divisions, and their progeny (Fig 1; [3]). However, there is considerable heterogeneity in progenitor behavior, making it difficult to determine links between specific progenitor subtypes and their downstream lineages. In species with gyrencephalic brains, the OSVZ characteristically contains bRG (also oRG), previously called OSVZ progenitors. bRGs keep radial glial characteristics such as apically directed and/or basal processes and can divide repeatedly [17,122–124]. Lissencephalic species such as mice show only low numbers of bRGs in the developing dorsal telencephalon [37,125,126]. bRGs appear to be born from divisions with oblique and horizontal cleavages of apical RGCs in mouse and human [36,37]. Recent data have shown that bRGs in macaque and human OSVZ can contain either or both apically directed and basal processes and that these different morphological types can freely transition back and forth, showing remarkable dynamics in bRG

characteristics and lineages [36,122]. Additional apical RGC types, named short neural precursors (SNPs) [127], and subapical RGCs (saRGCs) [128] have also been identified. SNPs divide apically like apical RGCs, but have only short basal processes and undergo mainly neurogenic divisions [127]. saRGCs were identified in the developing ventral telencephalon of lissencephalic rodents and in the dorsal telencephalon of gyrencephalic species. Therefore, saRGCs are proposed to add to cortical expansion through increased production of neurons [128].

These observations show that depending on the CNS region and species, different types of neural progenitors exist with a wide variety of morphologies, division modes, and lineages to generate diverse neuronal outputs. Furthermore, neural progenitor types and their lineages are by no means strictly separated and unidirectional.

Differential molecular control of cell fate decisions

Although many general principles and mechanisms underlying neurogenesis have been identified, it is poorly understood how (subtle) differences in molecular mechanisms mediate the different neuronal outputs required for distinct brain regions. For example, only few molecular mechanisms in induction and maintenance of the diverse types of neural progenitors in the mammalian neocortex have been identified. Recently, it was shown that the nuclear Trnp1 protein maintains self-renewing RGCs, possibly through chromatin remodeling [129]. Interestingly, Trnp1 expression is reduced in areas of cortical expansion in human fetal brains. Also, deletion of Trnp1 in mouse leads to increased horizontal cleavages and increased bRG production [129].

As mentioned above, differences in early patterning events induce subtle intrinsic molecular and epigenetic differences between RGCs of different regions. Subsequently, RGCs of different CNS regions show different responses to signals. For instance, upon deletion of the small GTPase RhoA, RGCs in cortex, midbrain, and spinal cord show similar RGC polarity defects and migrate away from the ventricular surface. However, RGCs in more expanded regions such as cortex and midbrain respond by hyperproliferation, whereas RGCs in the spinal cord proliferate less [6,8,9]. Within tissues, RGC proliferative capacity is modulated through differential expression of transcription factors, possibly influenced by dorsoventral and anterioposterior gradients of morphogens. For example, maintained expression of the transcription factor PLZF modulates RGC response to FGF ligands in the central domain of the developing spinal cord through alterations in FGF receptor and subsequent downstream signaling component levels [130]. In this way, centrally localized RGCs maintain proliferative capacity, whereas their dorsal and ventral counterparts undergo differentiation. Future studies will certainly uncover new mechanisms that differentially regulate initial RGC pool expansion, regulation of cell cycle and progenitor diversity, and the length of the neurogenic period to understand how regional and species differences in neuronal output are mediated.

Conclusions

The generation of the proper amount of neurons in the various regions of the developing vertebrate central nervous system depends on a carefully regulated spatial and temporal balance between NPC proliferation and differentiation (Fig 4). This balance is controlled by the cumulative activities of numerous extracellular and intracellular factors. The timing of the switch of NPCs from proliferation to differentiation, as well as the sequential induction of specific NPC and neuron types, differs between central nervous system regions and vertebrate species. Recently, there has been a steep increase in the identification of molecules and mechanisms that govern specific aspects of neurogenesis. A challenge now is to integrate this knowledge into a coherent concept of NPC proliferation versus differentiation, to determine, at the cellular and molecular level, the principles that are

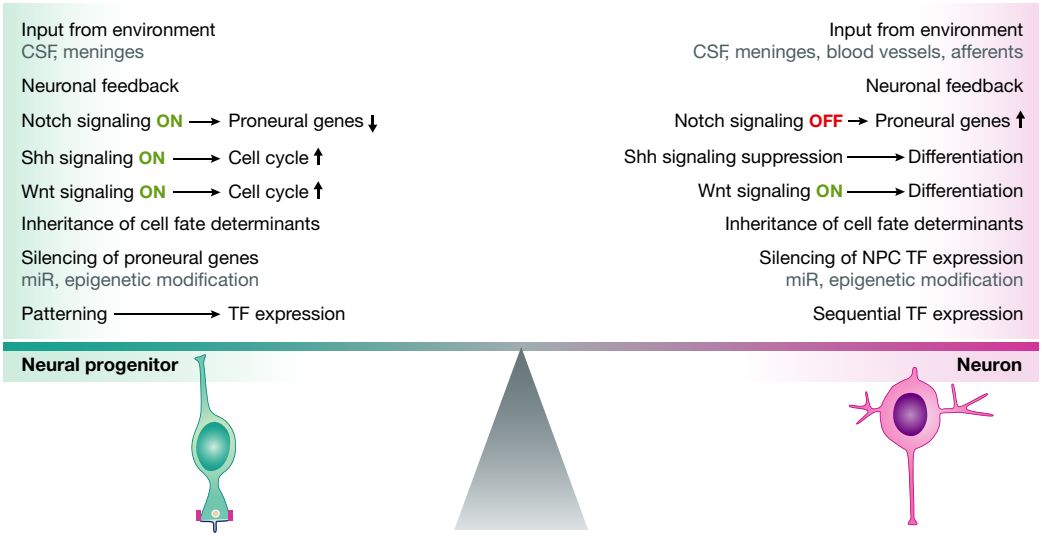

Figure 4. Extracellular and intracellular factors affecting the balance between NPC proliferation versus differentiation.
For details, see text. TF, transcription factor.

Sidebar A. In need of answers

Recent technological advances in live imaging and lineage reconstruction in both "old" model animals used to study neurogenesis such as mouse, zebrafish, Drosophila, and in "new" models with gyrencephalic brains such as macaque and ferret will hopefully allow answering of some of the important open questions in the field of neurogenesis:

(i) How is the input from signaling pathways integrated into a specific cell fate choice?
(ii) Do morphologically and molecularly distinct progenitor types have distinct and specific lineages? What is the level of stochasticity in these lineages?
(iii) Which molecular mechanisms mediate the induction of different progenitor types and how do these differ between species and CNS subregions?
(iv) Ultimately, which genomic changes account for the greater proliferative capacity of neural stem and progenitor cells that underlies the evolutionary expansion of the neocortex?
(v) What are the similarities and differences between embryonic and adult neurogenesis? What is the embryonic origin of adult neural stem cells?

conserved in vertebrate central nervous system development, and to identify the modifications that account for the differences between species.

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Conflict of interest

The authors declare that they have no conflict of interest.

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