Cell-cycle control and cortical development

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Abstract | The spatio-temporal timing of the last round of mitosis, followed by the migration of neuroblasts to the cortical plate leads to the formation of the six-layered cortex that is subdivided into functionally defined cortical areas. Whereas many of the cellular and molecular mechanisms have been established in rodents, there are a number of unique features that require further elucidation in primates. Recent findings both in rodents and in primates indicate that regulation of the cell cycle, specifically of the G1 phase has a crucial role in controlling area-specific rates of neuron production and the generation of cytoarchitectonic maps.

Arealization
The developmental process that leads to the breaking up of the cortical sheet into anatomically, functionally and connectionally distinct areas.

Interkinetic migration
The back and forth motion of the nucleus of cortical precursors in the ventricular zone during the cell cycle.

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Neuronal computation and architecture reach their highest level of sophistication in the mammalian cortex. The functional architecture of the six-layered cortex with its compartmentalization into discrete, specialized areas characterized by a particular connectivity and cellular composition, constitutes the framework in which this computation is implemented. Understanding the development of the cortex remains a major challenge at the heart of understanding what makes us human. Furthermore, dysfunction of the cortex is at the root of numerous neurological disorders, emphasizing the importance of research in this area.

Cortical precursor cells are heterogeneous in their proliferative features, molecular markers and the laminar fate of their progeny. Phenomena as diverse as migration and fate determination are integrated during corticogenesis but the mechanisms involved are not fully understood. Cell-cycle parameters affect rates of neuron generation and the extrinsic factors modulating the cell cycle determine the future cortical cytoarchitecture. Some of these extrinsic factors, and certain features of cortical development, are primate-specific.

In this Review, we describe the cell-cycle-related mechanisms that influence cortical lamination and arealization. We examine how cell-cycle parameters contribute to the emergence of the cortical cytoarchitecture by regulating the balance between proliferation and differentiation of cortical precursors, with a special emphasis on the role of the G1 phase. We focus on work carried out in primates given the unique features of corticogenesis in this order and its importance for understanding human neurological disorders.

Corticogenesis in mammals

During corticogenesis in mammals (from embryonic day 11 (E11) to E19 in the mouse) two germinal compartments — the ventricular zone (VZ) and the subventricular zone (SVZ) — lining the cerebral ventricles generate pyramidal neurons as well as a fraction of the inhibitory neurons of the cerebral cortex[1]. In the VZ, neuroepithelial progenitors divide at the apical surface and undergo interkinetic migration during G1 and G2 phases of their cycle[2]. Later, mitoses occur at the basal surface of the VZ to progressively form the subventricular zone (SVZ) (FIG. 1a) where precursor cells do not exhibit interkinetic migration.

Three main types of cortical precursor cells have been identified throughout corticogenesis: radial glial cells (RGCs), which are restricted to the VZ of the rodent[3–6] but not of the primate, short neural precursors (SNPs)[7] and intermediate progenitor cells (IPCs)[8,9] (FIG. 2). SNPs and RGCs both divide at the apical surface of the VZ and exhibit distinctive morphologies. Whereas the elongated bipolar RGCs span the full thickness of the embryonic cortical wall, SNPs are anchored by ventricular endfeet and are thought to have only a short basal process[7]. Although it cannot be excluded that SNPs are themselves derived from RGCs, it is thought that SNPs are committed to symmetrical neurogenic divisions[7,10]. IPCs are neuronal progenitors derived from RGCs that divide away from the ventricular surface in the VZ and in the SVZ[8,9].

Although the SVZ initially derives from VZ precursors both in primates and in non-primates, clear differences in gene expression between the two precursor pools resident in the VZ and the SVZ have been identified[11–17].

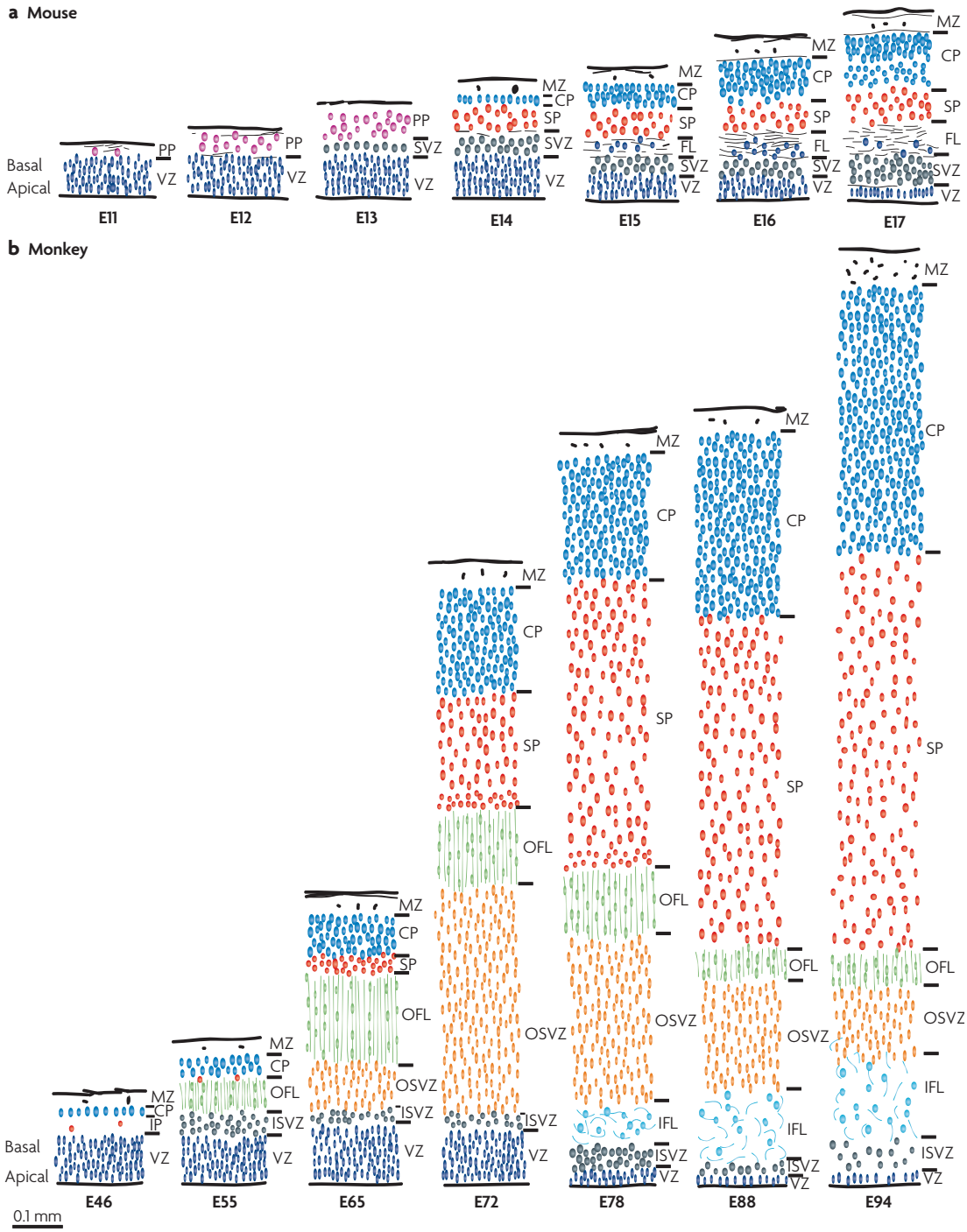


Figure 1 | **Differences in the anatomical organization of the rodent and primate embryonic cortex.** These schematics are transects through the presumptive area 17 of the embryonic cortex in mouse (**a**) and monkey (**b**) at comparable developmental stages. The depth of each layer is drawn to a common scale. Gestation period is 19 days in the mouse and 165 days in the monkey. Cortical neurogenesis lasts 8 days in the mouse (embryonic day 11 (E11) to E19) and spans 60 days in the monkey visual cortex (area 17). In primates, an early appearing (at approximately E55) outer fibre layer (OFL) forms a major landmark during development. The ventricular zone (VZ) declines progressively after E65. By contrast the subventricular zone (SVZ) increases progressively in depth and by E72 is divided into an inner subventricular zone (ISVZ) and outer subventricular zone (OSVZ) by an intruding inner fibre layer (IFL). The OSVZ exhibits a number of unique features. It is histologically similar to the VZ and also has a compact radial organization. This alone distinguishes the primate OSVZ from the loosely organized SVZ of rodents and primates. Contrary to what is observed in the rodent, where the VZ is the main germinal compartment throughout corticogenesis, the primate VZ declines rapidly during corticogenesis and is paralleled by the early appearance of the SVZ followed by the OSVZ. The monkey cortical plate (CP) appears as early as E46. The sub-plate (SP) is evident after E55. The marginal zone (MZ) is minimal before E65. FL, fibre layer; IP, inner plexiform layer; PP, pre-plate. Reproduced with permission from REF. 34 © (2002) Oxford Journals.

These differences correlate with distinct neuronal progeny; the VZ is involved in the generation of lower layer neurons, whereas the SVZ is involved in the generation of upper layer neurons. For instance, the transcription factors OTX1 and FEZ1 are expressed in VZ precursors, downregulated in SVZ precursors and subsequently upregulated in subsets of deep layer neurons[11–14]. Both OTX1 and FEZ1 have a crucial role in specifying the axonal projections of subsets of lower layer neurons. Several other transcription factors (CUX2, TBR2, SATB2 and NEX)[15–18] as well as the non-coding RNA *Svet1* (REF. 19) are selectively expressed in both the SVZ and in upper layer neurons (FIG. 2). This congruency of gene expression, first in SVZ progenitors and subsequently in supragranular neurons, as well as time-lapse microscopy studies suggest that the SVZ gives rise to upper layer neurons[8,9,16,17,19–21] (FIG. 2).

In agreement with these findings, distinct molecular mechanisms have been identified for the specification of infragranular and supragranular lineages. Studies from mutant mice show that the basic helix–loop–helix (bHLH) factors neurogenin 1 (NGN1) and NGN2 are required for the specification of a subset of deep layer neurons but not for the specification of upper layer neurons. PAX6 and TLX, two transcription factors required for the normal formation of the SVZ[16,18,22], are synergistically involved in the specification of upper layer neurons[23]. Conceivably, the selective expansion of the upper layer compartment in the primate cortex results from modifications of the PAX6/TLX-related specification without modification of the neurogenin specification mechanisms[23].

Observations in rodents show that the first neurons to be generated form a conspicuous pre-plate that is subsequently split by later-generated cortical plate neurons to form an outer marginal zone and an inner sub-plate[24,25] (FIG. 1a). As corticogenesis proceeds, newly generated postmitotic neurons migrate radially from the

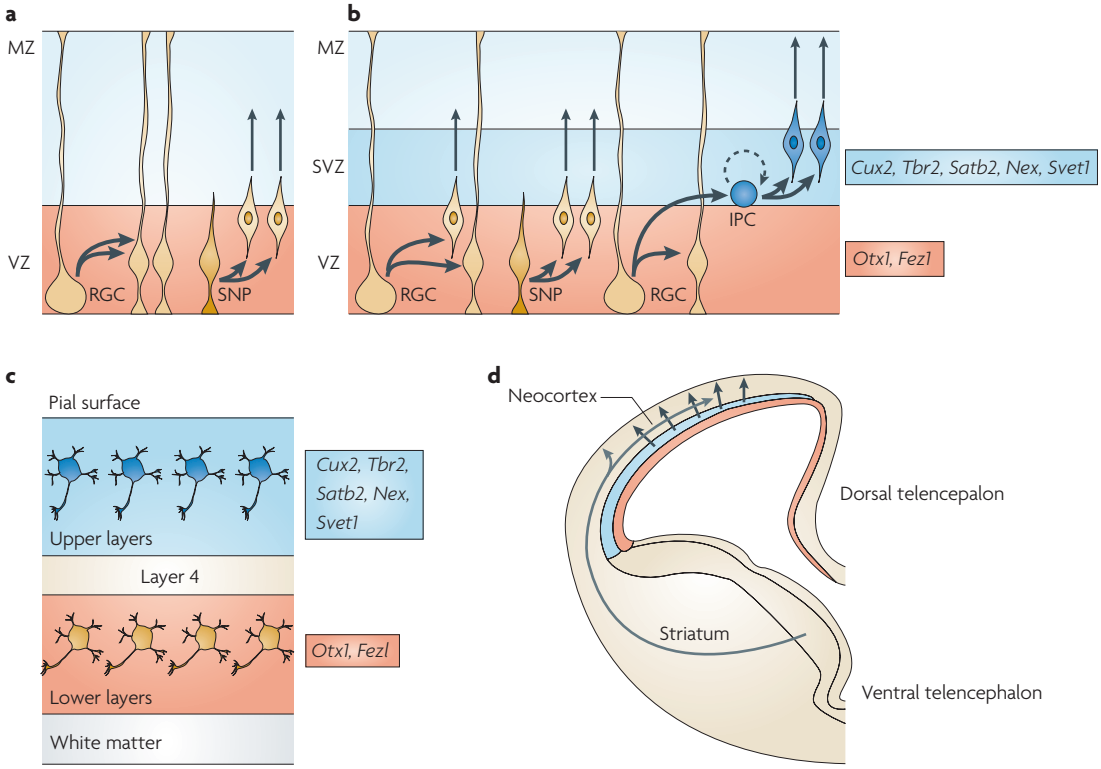


Figure 2 | **The proliferative behaviour and progeny of cortical progenitors.** This is a simplified summary of the types of division made by cortical precursors. Schematic transects of the rodent cortex at embryonic day 13 (E13) (**a**), at E17 (**b**) and in the adult (**c**). Cortical neurons are generated from three types of precursors: radial glia cells (RGCs), short neural precursors (SNPs) and intermediate progenitor cells (IPCs). RGCs and SNPs divide at the apical surface of the ventricular zone (VZ) (**a**,**b**). RGCs undergo several types of symmetrical and asymmetrical divisions[46,142], as indicated by the arrows, including self-renewing ones (**a**) or neurogenic divisions (**b**). SNPs are committed neural precursors (**a**,**b**). IPCs divide away from the ventricular surface in the VZ and in the subventricular zone (SVZ) (**b**). IPCs have been reported to undergo mostly neurogenic divisions with a small fraction undergoing symmetrical proliferative divisions (as indicated by the dotted circular arrow). Through asymmetrical divisions, RGCs give rise to IPCs that migrate to the SVZ (**b**). The VZ generates lower layer neurons (red) and the SVZ generates upper layer neurons (blue). Genes listed in red are expressed in both the VZ and lower layer neurons. Genes listed in blue are expressed both in the SVZ and upper layer neurons (**b**,**c**). A schematic view of a coronal section of mouse brain at E13.5 is shown (**d**). Interneurons generated in the ventral telencephalon migrate tangentially to the cortical plate of the dorsal telencephalon (grey arrow). Pyramidal neurons generated in the cortical VZ and SVZ migrate radially to the cortical plate (black arrows). MZ, marginal zone. Modified with permission from *Nature Rev. Neurosci.* REF. 1 © (2001) Macmillan Publishers Ltd.

germinal compartments to settle in the cortical plate, forming the six-layered cortex. The laminar fate of postmitotic neurons is determined by the timing of the terminal mitosis — the earliest-born neurons form the deep layers of the cortex and later-generated neurons occupy successively higher layers[26] as shown by birth-dating experiments using S-phase markers that label proliferating cells[25,27–31].

Early cortical patterning in the germinal zones. Signalling molecules (such as FGF8, SHH, WNTs and BMPs) (BOX1, FIG. 3a) that have an important role in early cortical patterning are found to act in the germinal zones[32, 33]. Cortical specification occurs during neurogenesis in the germinal zones in agreement with the protomap hypothesis of cortical development (BOX1). This has been elegantly demonstrated by experiments showing that the ectopic expression of FGF8 leads to a duplication of the rodent barrel-field[156] (FIG. 3b).

Primate-specific features of corticogenesis. The organization, developmental timing and relative dimensions of the germinal and postmitotic compartments of the primate cortex differ from those of rodents[34] (FIG. 1). In the monkey, cortical neurons are produced over a 60-day period from E40 to E100 (REF. 29). A number of transient neuron populations are unique to the primate preplate[35,36]. In contrast to rodents, there are few early born cells split by the cortical plate, and the sub-plate, which is generated later and over a more extended period compared to non-primates, is considerably enlarged in monkeys[34,37]. Whereas the vast majority of cortical interneurons are produced in the ventral telencephalon[1] and reach their final destination in the cortical plate via tangential migration in rodents (FIG. 2d), the germinal compartments of the dorsal telencephalon in primates generate a high proportion of the inhibitory neurons[38]. In primates there is also an important expansion of the SVZ to form the outer SVZ (OSVZ), which is not found in rodents[34,39] (FIG. 1). The OSVZ exhibits unique

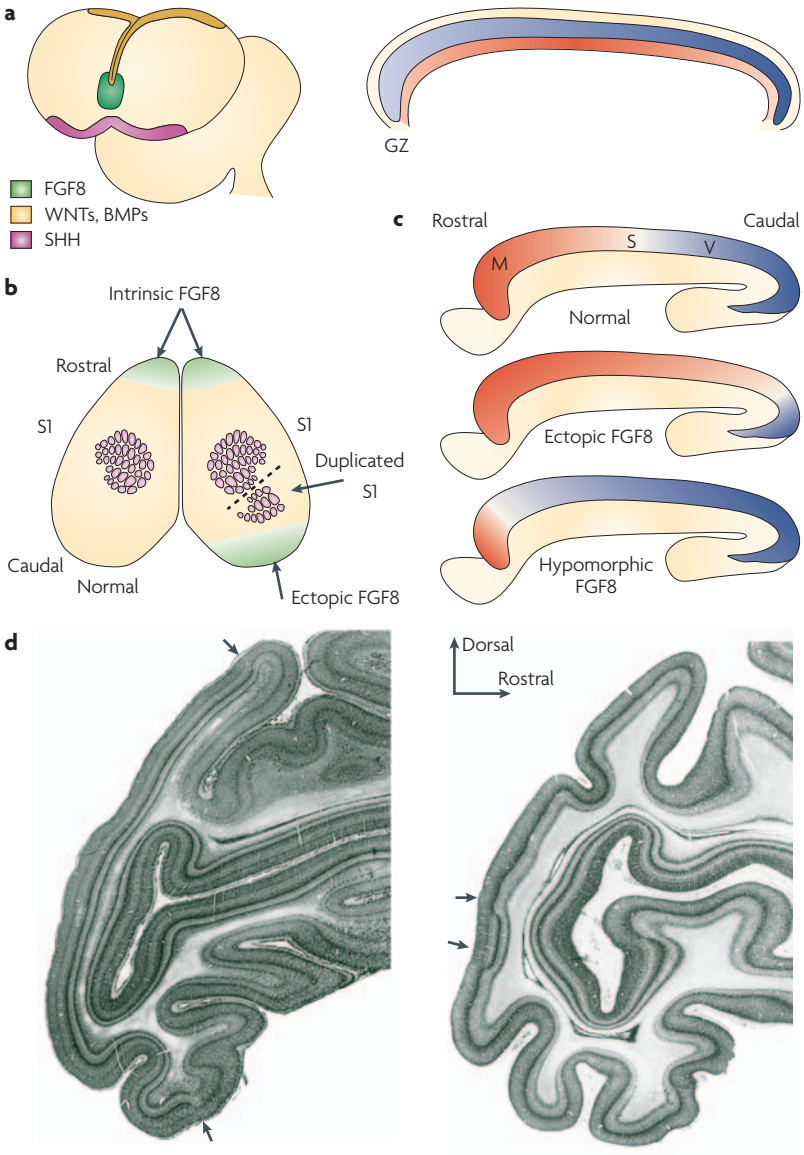


Figure 3 | **Mechanisms of arealization in the cortex.** **a** | The developmental stage E13 of the mouse embryo at the point of the formation of the two telencephalic vesicles. Proteins such as fibroblast growth factor 8 (FGF8), secreted from the anterior neural ridge and the commissural plate; WNTs and bone morphogenetic proteins (BMPs), secreted from the cortical hem; and sonic hedgehog (SHH), secreted from the medial ganglionic eminence[148–151], generate positional information, resulting in a graded tangential expression of transcription factors (represented by the blue and red shading) that translates into a molecular regionalization of the germinal zone (GZ) and the generation of proto-areas[32,154]. Modified with permission from REF. 173 © (2002) Elsevier Science. **b** | FGF signalling affects the relative dimensions and the identity of cortical regions. Ectopic expression of FGF8 in the caudal pole of the neocortex elicits a partial duplication of the S1 barrel-field[156], the receptive field for whisker responses. **c** | Increasing or decreasing the concentrations of certain signalling molecules modifies rostral–caudal growth and areal dimensions. Ectopic expression of FGF8 in the rostral cortex at embryonic day 11.5 leads to a caudal shift of areas, whereas a hypomorphic FGF8 cortex exhibits a rostral shift of areal borders with a rostral expansion of caudal regions[155,156]. M, motor; S, somatosensory; V, visual. **d** | Parasagittal Nissl–stained sections of the primate visual cortex showing the effect of the depletion of embryonic thalamocortical axons on the compartmentalization of the primary visual area 17 and the adjacent area 18. The axons of the primary thalamic visual relay nucleus (the lateral geniculate nucleus), which convey ascending information coming from the retina, converge exclusively onto the primary visual area 17. Depletion of the geniculocortical axons subsequent to early retinal ganglion cell ablation during the early stages of neurogenesis in the primate results in a drastic reduction of the tangential extent of the mature area 17 (right panel) compared with the same area of control brain (left panel) . This finding is compatible with the protocortex hypothesis (BOX 1). Black arrowheads indicate area 17 borders. Reproduced with permission from REF. 126 © (1996) Wiley–Liss.

Enucleation
Surgical removal of part of the eye.

histological features. It is the main site of neuron production in primates (this role is undertaken by the VZ in rodents) and here neurons destined for the upper layers of the cortex are generated. The enlargement of the SVZ in primates can be linked to the development of the supragranular layers[40]. The primate OSVZ enlargement could have occurred in response to evolutionary pressure to generate an enlarged population of sub-plate neurons and an increased fraction of cortical interneurons, and to accommodate the pronounced cytological complexity of the upper layers of the primate cortex[20,31,34,38,41]. Whereas the rodent SVZ is only partially self-sustaining, requiring a constant supply of precursors from the VZ[3,8,9,17,42], OSVZ self-renewal is considerably more pronounced in primates[20,34].

Cell cycle and neuronal production

Cell-cycle features. In cortical progenitors, as in other somatic cells, proliferation and growth arrest are regulated by a balance of extrinsic and intrinsic signals that direct entry, progression into and exit from the cell cycle[43]. The complex regulatory and signalling pathways that regulate cell-cycle progression are highly conserved (BOX 2).

Cortical progenitors generate a huge diversity of neuronal phenotypes. Asymmetrical division, where an unequal distribution of determining factors during mitosis results in two daughter cells with different fates, is a conserved mechanism for generating diversity in the CNS[44–46]. The expression of a number of cell-intrinsic factors is temporally related to the transition from symmetrical to asymmetrical divisions[47–50], although the mechanisms determining the mode of division and the switching between modes are not completely understood[46].

Mechanisms determining neuron number. The computations carried out by the cerebral cortex require specific patterns of connections between precise numbers of diverse types of neurons[51]. One possibility is that there is a tight spatio-temporal control of the number of neurons generated through cell-cycle regulation. Experimentally this is difficult to prove (BOX 3). It has been established that the number of neurons in individual layers and areas correlates with changes in the rate of neuron production[52,53]. In primates, it is possible to directly investigate the cell-cycle kinetics of precursors of a given area[31]. This work shows that the different rates of neuron production that characterize the upper layer neuronal precursor pools in visual areas 17 and 18 are associated with distinct cell-cycle kinetics[20]. The role of cell-cycle regulation in determining neuronal number in the adult cortex is consistent with findings elsewhere in the CNS[54].

Specifically, two cell-cycle parameters determine neuron number: the rate of cell-cycle progression and the balance between cell-cycle re-entry or exit. Whereas proliferative divisions generate two progenitors that re-enter the cell-cycle, differentiative divisions result in at least one daughter cell exiting the cell cycle to undergo differentiation.

Mathematical modelling has been used to explore how cell-cycle parameters influence neuron number[20,53,55]. Changing the rate of cell-cycle progression has a straightforward impact: in a steady-state precursor population a 50% increase in cell-cycle progression (that is, halving the length of the cell cycle) doubles the rate of neuron generation. The influence of the mode of division, proliferative or differentiative, on neuron number is altogether more complex. Increasing the frequency of differentiative division leads to only a transient increase in neuron production followed by a rapid exhaustion of the precursor pool and a subsequent drop in neuron production. This contrasts with increasing the rate of proliferative divisions which ultimately leads to an increase in the rate of neuron production through an amplification of the precursor pool. Experimental findings show just how the temporal changes in these cell-cycle parameters generate different numbers of neurons in the successive cortical layers[53].

G1 phase and mode of division. Although the molecular mechanisms that determine the tightly regulated occurrence of proliferative versus differentiative divisions are largely unknown, converging evidence suggests that the mode of division is correlated to cell-cycle components and, more specifically, to G1-phase regulation.

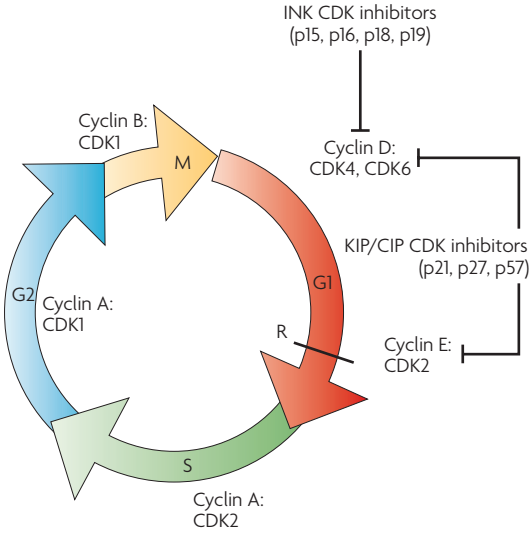
Box 1 | **Protomap and protocortex hypotheses: mechanisms of arealization in the cortex**

Evidence of early regionalization prior to the arrival of thalamic input supports the protomap model[32,146,147]. The neocortex is regionalized along the rostral–caudal axis: the rostral-most regions consist of areas involved in executive functions, whereas the caudal-most regions consist of areas involved in motor, auditory and visual functions. The protomap model states that the regional layout of the cortex is established at early stages of development in response to signalling centres located along the edges and midline of the neural plate and, at later stages, in the telencephalic vesicles[148–151] (FIG. 3a). The patterning centres generate regional and graded expression of transcription factors (FIG. 3a) that in turn mediate map formation by regulating cell proliferation, differentiation, migration and survival[146,152,153] (reviewed in REFS 32,33,154). Patterning molecules exhibit dose-dependent effects that determine the dimensions of frontal, parietal and occipital regions, suggesting a developmental origin for individual differences in brain size and providing insight into brain evolution[155–157] (FIG. 3b,c). Although early stages of cortical patterning have been mainly studied in the mouse, there is evidence of conservation in the role of some patterning genes (Wnts, for example) in humans[158].

The protocortex theory[118] postulates that an initially homogeneous cortex is divided into functional areas late in development by cues from incoming thalamic axons. Although the protocortex and protomap hypotheses of cortical development have often been cited as alternative and mutually exclusive, they are increasingly being viewed as complementary aspects of a single mechanism[32,33,115,159]. Indeed, the protomap hypothesis as proposed by Rakic did not exclude the possible role of the sensory periphery in shaping arealization, as shown by prenatal enucleation experiments in which depletion of thalamocortical axons during early corticogenesis leads to drastic changes in areal specification of the visual cortex[116,125,160,161] (FIG. 3d).

Box 2 | **Structure and regulation of the cell cycle**

The cell cycle of eukaryotic cells can be divided into four successive phases: M phase (mitosis), in which the nucleus and the cytoplasm divide; S phase (DNA synthesis), in which the DNA in the nucleus is replicated, and two gap phases, G1 and G2. The G1 phase is a critical stage, allowing responses to extracellular cues that induce either commitment to a further round of cell division or withdrawal from the cell cycle (G0) to embark on a differentiation pathway[162]. The G1 phase is also involved in the control of DNA integrity before the onset of DNA replication. Between S and M phases is the G2 phase during which the cell checks the completion of DNA replication and the genomic integrity before cell division starts. The transition from one phase of the cell cycle to the next is controlled by cyclin–CDK (cyclin-dependent kinase) complexes which ensure that all phases of the cell cycle are executed in the correct order. Terminally differentiated neurons cannot undergo cell-cycle re-entry. The cell-cycle components mentioned in this article that influence cortical neurogenesis are indicated in the figure together with the approximate position in the cycle at which they function.



The complex regulatory and signalling pathways that regulate cell-cycle progression are highly conserved in eukaryotes. Two cell-cycle checkpoints control the order and timing of cell-cycle transitions (G1–S and G2–M) and ensure that critical events such as DNA replication and chromosome segregation are completed correctly before allowing the cell to progress further through the cycle. A major cell-cycle restriction point (R) is located at the end of the G1 phase. Beyond this point, precursors will invariably complete the cell cycle.

Progression through the mitotic cycle is driven by the actions of CDKs and their activating cyclin subunits. CDK activity is suppressed through interactions with two main families of inhibitory proteins (CDK inhibitors or CDKIs): the INK4 family that exhibits selectivity for CDK4 and CDK6, and the CIP/KIP family that has a broader range of CDK inhibitory activity[163]. The rate of cell-cycle progression is determined by the relative abundance of positive and negative regulators. A number of cell-cycle regulators are expressed in cortical neuroblasts including cyclin D, cyclin E and CKIs from the CIP/KIP family and the INK4 family[20,164–168]. Work on mutant mice has shown that the overall size of the brain is governed by the molecular machinery of the cell cycle[169,170].

During mouse corticogenesis, there is a progressive increase in neuron production and in the frequency of differentiative divisions. Simultaneously, there is a slowing down of the rate of cell-cycle progression, which is mainly due to a lengthening of the G1 phase[56]. This phenomenon, together with the observation that markers selectively expressed in neuron-generating, differentiative divisions inhibit G1 progression (for example, *Tis21* (also known as *Btg2*)[48,57] and *BM88* (also known as *Cend1*)[50,58]) point to a link between G1 duration and the mode of division.

Mouse cortical precursors treated with differentiation-promoting factors show an increase in the duration of the G1 phase. Conversely, treatment with mitogenic factors decreases G1 length[59]. At the single-cell level, time-lapse videomicroscopy studies show that the G1 phase is long in differentiative divisions and short in proliferative divisions[59]. This and other work[20,60,61] suggest that concerted mechanisms control the progressive increase in duration of G1 and the proportion of differentiative divisions that is observed as corticogenesis proceeds[62]. However, the mechanisms that underlie the joint control of these parameters have not been elucidated and it remains to be determined whether these two processes are causally related. This requires selectively altering the regulation of G1 without affecting other signalling pathways, as occurs when using growth factors[59], and can be achieved through ectopic expression of selective G1–S regulators, such as cyclin-dependent kinase inhibitors (CDKIs). There is evidence, however, that the role of certain CDKIs that control the G1–S transition is not limited to cell-cycle regulation but can also affect cell fate[63] and migration[64]. Therefore, proof of a causal link between G1-phase progression and mode of division requires the demonstration that selectively shortening the length of G1 phase by overexpressing cyclins leads to an increase in proliferative divisions.

A prolonged G1 phase could be a characteristic feature of differentiative divisions, facilitating the integration of extrinsic signals that influence cell fate and/or allowing an unequally inherited cell-fate determining factor(s) to act over a sufficient time period[46,60] (FIG. 4). Studies on the cell cycle of embryonic stem (ES) cells have provided evidence that the regulation of G1 is related to the balance between differentiation and self-renewal. Both primate and murine self-renewing ES cells show a reduced duration of the G1 phase[65,66]. The length of the G1 phase corresponds to a window of increased sensitivity to differentiation signals[67], which is in agreement with results showing that a number of neuronal determination and proliferation-promoting signals exert their influence through factors of the G1phase[68–70]. It is tempting to speculate that shortening of the G1 phase might shield stem cells from signals that induce differentiation[71].

Mitotic history technique
Quantitative analysis of nuclear labelling in the adult following S phase labelling during development.

Cell-fate determination. Birth-dating experiments in rodents coupled with manipulation of the cellular environment suggested that cell fate is determined prior to migration[25,72–74]. In *reeler* (*Reln*) mutant mice, the profound disruption of the cortical environment has no influence on the timetable and areal differences in the generation rate of corticospinal neurons, indicating a causative link between birth date and cell fate[75,76]. Heterochronic transplantation experiments show that as corticogenesis proceeds there is a progressive restriction of cortical fate and imply that extrinsic factors during the final mitosis influence neuronal fate[77–79].

The factors responsible for the timed generation of different neuronal phenotypes have been reinvestigated in lineage studies of isolated cortical precursors[80]. Together with earlier findings, these results show that there is a cell-intrinsic programme[80] that is influenced by extrinsic factors[78,79,81,82] so that both extrinsic and intrinsic factors cooperate to determine cell fate. The temporal pattern of *in vitro* neuronal subtype generation matches that observed *in vivo* remarkably well[80]: Reln-positive Cajal–Retzius neurons are formed first, followed by cells expressing markers of initially lower (*Foxp2*, *Tle4*, *ER81* (also known as *Etv1*)) and subsequently upper cortical layers (*Cux1* (also known as *Cutl1*)), confirming earlier findings that there is a progressive restriction of cell-fate potential possibly as a consequence of chromatin rearrangement or changes in gene expression. Hence, as the developmental programme unfolds, progenitors lose the capacity to generate subtypes formed at earlier stages of the programme. Interestingly cell-cycle regulation appeared to be involved in the timing of neurogenesis *in vitro*; the lengthening of the cell-cycle *in vitro* may have evolved in a similar fashion as *in vivo* (BOX 3).

Box 3 | **Mitotic history of the neuron**

Measurements from the germinal zones provide data about the cell-cycle parameters for overall populations of precursor cells that generate multiple cell types. The mitotic history technique selectively monitors the proliferative behaviour of a single population of precursors that are generating a particular phenotype.

Injection of tritiated (^{3}H)-thymidine in the embryo labels precursors that are in S phase. Cells that exit the mitotic cycle shortly after the injection show maximum autoradiographic labelling whereas the autoradiographic signal is diluted during successive division in precursors that re-enter the cell cycle[171]. The number of autoradiographic grain counts per nucleus distinguishes neurons that exited the cell cycle following the first mitosis after the ^{3}H-thymidine injection (first generation; FG) and each successive generation of later-born neurons (subsequent generation; SG)[52,53]. Computation of the percentages of FG and SG neurons with respect to the total population (T) provides a powerful tool to investigate cell-cycle kinetic variations of cortical precursors. Assuming that the length of S phase is constant during corticogenesis[99,101–103,105,107], variation in the percentages of precursors labelled with ^{3}H-thymidine reflect changes in the rate of cell-cycle progression. Hence, variations in the SG/T ratio reflect changes in rate of cell-cycle progression of the founder populations[53,75,76]. Variations in the FG/SG ratio reflect changes in the proportion of precursors that exit the cell cycle (differentiative divisions) with respect to the proportion of precursors that continue cycling (proliferative divisions), indicating changes in the mode of division[53,75,76]. The comparison of the percentages of different categories of labelled neurons is unbiased by regressive phenomena as labelled and unlabelled precursors and their progeny undergo similar rates of cell death.

The percentage of FG neurons within a population of adult neurons (FG/T) defines a generation rate that reflects the rate of neuron production of that population[52,53,172,173].

The finding that cell-cycle regulation and the developmental programmes that generate sequential neuronal subtypes are maintained *in vitro* raises the possibility that cell-cycle control mechanisms are involved in fate determination[80,83,84] (for a different view, see REF. 85). Cell-cycle mechanisms could be responsible for determining both the numbers and the phenotype of cortical neurons generated in each layer.

Regulatory feedback mechanisms. There is evidence suggesting that the rates of proliferation and differentiation are influenced by signals from the cellular environment including postmitotic compartments of the cortex (FIG. 5). These signals can provide a regulatory feedback mechanism that adjusts both the dimensions of the proliferative precursor pool and the processes involved in fate determination.

It has been proposed that adherens junctions mediate a local feedback mechanism in the early VZ. Disruption of the apical junction complexes by deletion of αE-catenin results in increased proliferation through abnormal activation of the hedgehog (HH) signalling pathway[86]. The 'crowd-control model' postulates that during normal development increased densities of neuronal precursors are 'sensed' by an increase in the proportion of the cell surface that is occupied by adherens junctions and leads to a downregulation of HH signalling, resulting in decreased proliferation[86].

The cortical plate is thought to influence the rate of corticogenesis as well as cell-fate determination by descending axons to the germinal zones[87–89]. In the *Reln* mutant, which has an abnormal cortical environment, the generation rate of early produced neurons is strongly reduced[76]. Despite this decreased rate of neuron production, the newborn *Reln* mutant shows a paradoxical excess in the number of corticospinal neurons[75]. Birth-dating shows that this excess is due to an increase in the probability of newborn neurons acquiring the corticospinal phenotype[75]. These results confirm findings elsewhere in the CNS that postmitotic neurons exert an important feedback control over neurogenesis and cell fate[90,91].

We have only a very sketchy idea of the possible mechanisms underlying these feedback loops. The decreased rates of lower layer neuron production and the increased rates of upper layer production in *Reln* mice could be the consequence of a more precocious transition from the VZ to the SVZ in the mutant[75]. This transition is promoted by endogenous WNT molecules in a manner that is dependent on sonic hedgehog (SHH) and fibroblast growth factor 2 (FGF2)[92,93]. Because WNT7b is expressed in the early generated cortical plate neurons[94] there is the possibility of a feedback mechanism by which deep layer neurons signal back to the VZ precursors and promote their transition to an SVZ fate, leading to the cessation of deep layer neuron production and the initiation of upper layer neuron production[92]. This fits nicely with the observed reduced neurogenesis during lower layer generation in the *Reln* mutant and other findings that suggest that the cortical plate exerts inhibitory feedback on proliferation[95].

Newly generated neurons may also influence neuronal proliferation and therefore the size of the precursor pools by releasing the neurotransmitters GABA (γ-aminobutyric acid) and glutamate, which are known to promote proliferation in the VZ and inhibit proliferation in the SVZ[96]. In rodents, GABA-releasing interneurons generated in the ventral telencephalon (FIG. 2d) that migrate into the cortical SVZ could provide extrinsic signals regulating cortical proliferation (FIG. 5).

The cell cycle and cortical architecture

Regulation of cell-cycle parameters and corticogenesis. A rostral–caudal histogenic gradient is maintained throughout corticogenesis[30,97–99]. The mitotic history of phenotypically defined populations of neurons shows that there is a progressive lengthening of cell-cycle duration during corticogenesis that can be largely attributed to a lengthening of the G1 phase[53,100,101]. Similar trends are found when cell-cycle progression rates are measured directly in the germinal zones[99,102–105]. The lengthening of the cell cycle is accompanied by an increase in the fraction of cells that exit the cell cycle via differentiative divisions[53].

As discussed above, the seemingly paradoxical observation that corticogenesis is characterized by a slowing down of the cell cycle and an increase in the frequency of differentiative divisions but an increase in the rate of neuron production is explained by the fact that the size of the precursor pool shows important variations during development[20,53]. The increased rate of neuron production, peaking at mid-corticogenesis, is the consequence of the high frequency of proliferative divisions that occur at the onset of corticogenesis, leading to a progressive build-up of the precursor pool. Likewise, the slowing down of neuron production during the later parts of corticogenesis is not so much the consequence of the slowing down of cell-cycle progression as of the exhaustion of the precursor pool.

One possible explanation for the observed lengthening of the cell cycle during corticogenesis accompanied by increased rates of differentiative division is provided by the association of short G1 phases with proliferative divisions and long G1 phases with differentiative divisions. Hence, the rates of cell-cycle progression in a population could simply reflect underlying changes in the proportions of the two types of division. Until recently, the prevailing view was that the rate of cell-cycle progression in the mouse cortex was homogeneous amongst precursors of a given stage[106]. However, there is considerable evidence that there is heterogeneity in the precursor pool, not only in terms of phenotype but also in terms of proliferative behaviour and cell-cycle duration of individual precursors[53,107,108]. Because differentiative divisions are longer, the increasing fraction of differentiative divisions will lead to an increase in the mean cell-cycle duration of the population as a whole. This has been tested using a knock-in mouse line in which it was possible to distinguish the subpopulations of proliferative and neurogenic precursors before they entered S phase. This showed that, compared with proliferative divisions, differentiative divisions have a significantly longer cell cycle[62]. Thus it seems that although the length of the cell cycle for both neurogenic and proliferative divisions increases during corticogenesis, it is the increasing proportion of differentiative divisions that is largely responsible for the lengthening of cell-cycle progression at the population level.

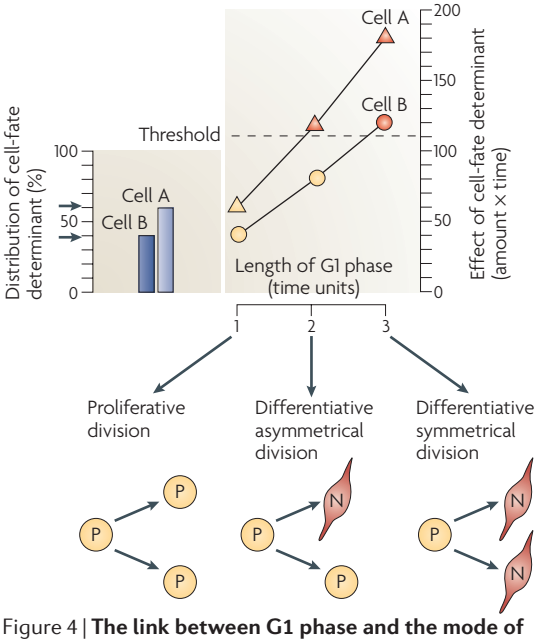


Figure 4 | **The link between G1 phase and the mode of division of cortical precursors.** The cell-cycle length hypothesis[60] has been proposed to explain the mechanisms linking the mode of division to the length of the G1 phase. It has been proposed that during cell division each daughter cell receives an unequal amount of a neurogenic cell-fate determining factor that sets the probability of the daughter cell fate according to the length of the G1 phase[46,60]. This is illustrated here, at 40% and 60% inheritance. At a very short cell-cycle length value, determined by a short G1-phase duration (time 1), neither daughter cell will become a neuron and the division is proliferative. At the same inheritance, but with intermediate cell-cycle length (time 2), cell A is above the threshold and becomes a neuron; this division is asymmetrical and differentiative. At the longest cell-cycle length (time 3) both daughter cells (A and B) are above threshold and become neurons; this division is symmetrical and differentiative. N, neuron; P, precursor cell. Modified with permission from *Nature Rev. Mol. Cell Biol.* REF. 46 © (2005) Macmillan Publishers Ltd.

Cell-cycle length in primates. In primates, the regulation of cell-cycle duration is temporally and structurally different from rodents[109]. In the primate VZ, there are variations in the duration of the S and G1 phases and, contrary to the situation in rodents, the cell cycle is shorter at mid-corticogenesis owing to changes in the length of both G1 and S phases[109]. Variations in S-phase duration during corticogenesis appears to be a primate-specific cell cycle control feature. Compared with rodents, the length of the cell cycle in primate cortical precursors is considerably extended[20,109]. It has been proposed that the prolonged cell cycle in monkey cortical precursors is an adaptative feature that is related to the evolutionary

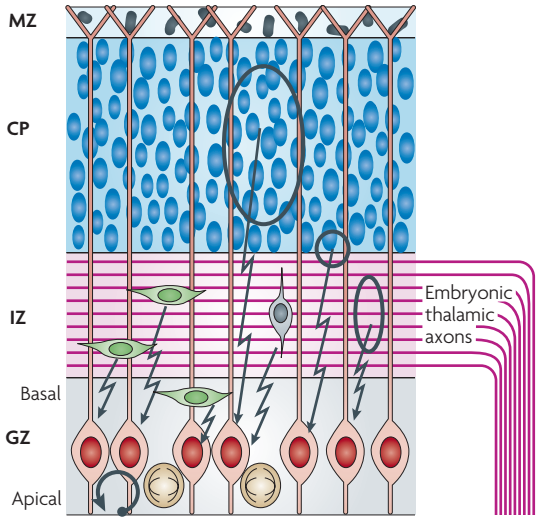


Figure 5 | **Schematic diagram of different extrinsic influences affecting precursor proliferation dynamics and cell fate.** The different cellular compartments of the embryonic cortex are thought to provide signals that modulate the proliferation and fate of cortical precursors in germinal zones (red cells). Regulatory feedback includes influences from the postmitotic pyramidal neurons of the cortical plate (CP)[75,76] (blue cells), specifically from the lower layers[92], from newborn cortical neurons migrating in the intermediate zone (IZ) compartment (grey cell) and local feedback signalling from the germinal zone (GZ) precursors (circular arrow)[86]. Extracortical extrinsic signals are provided by tangentially migrating interneurons generated in the ventral telencephalon (green cells) and by ingrowing embryonic thalamic axons (magenta) that have been shown to influence cell-cycle kinetics of precursors[111]. MZ, marginal zone.

expansion of the neocortex in primates[110]. Because environmental signals contribute to the fate specification of cortical precursors during the cell cycle and regulate the rate of precursor proliferation[75,76,78,111], the prolonged cell-cycle duration in primates might ensure fine control of the production rates of phenotypically defined neurons.

Areal and laminar specification. Superimposed on the broad regionalization set up by the early patterning centres (FIG. 3a) are the multiple cortical areas that constitute the cerebral cortex. Each area is defined by both its structure and connectivity, which together determine its sensory, motor or cognitive function. Structurally, cortical areas are defined by their cytoarchitecture which reflects the number and soma morphology of its constituent neurons arranged in six to eight layers running parallel to the cortical surface. Areal differences in cytoarchitecture and laminar neuron number are general features of the cortex across species[41,112,113]. The developmental processes that specify the cytoarchitecture of cortical areas are therefore instrumental in defining the cell fate and number of the cortical neurons that constitute the laminar structure of the cortex[52,53,114,115].

Linking the proliferative features measured in precursors to particular neocortical areas is not possible in rodents. However, studies of the mitotic history of adult cortical neurons in rodents show that there are marked differences in the generation timetable of homologous layers in neighbouring areas[52,75]. These studies also show that neurons forming the distinct cortical layers of neighbouring areas originate from precursors that differ markedly in their cell-cycle kinetics[53]. Furthermore, area-specific differences in the rate of cell-cycle progression, mode of division[53] and cell fate[75,76] show that cortical areas originate from a mosaic of distinct proliferative programmes in the various germinal compartments in rodents. Because these distinct proliferative programmes generate areal differences prior to the arrival of thalamic fibres, they imply the presence of an intrinsic, defined developmental programme and are compatible with the concept of the protomap theory[32,115–117] (BOX 1).

Area 17, the primary visual area of the primate, is of considerable interest as a developmental model for two main reasons. First, in the adult it has 50% more neurons in the upper layers with respect to its neighboring area, area 18, from which it is separated by a sharp border[41]. Second, in monkey embryos it is possible to identify the germinal zones that generate area 17, thereby providing a unique opportunity to experimentally relate events in the germinal zone to the final outcome in terms of neuronal production and cortical phenotype. Regional differences in proliferation that prefigure the areal map have been reported in the germinal zones of the embryonic primate cortex[31]. Interestingly, these area-specific differences in cell-cycle kinetics occur selectively at the time of upper layer neuron production[20,31], well after the arrival of the thalamic axons. Given the mitogenic effect of embryonic thalamic axons on cortical precursors[111], these areal differences in the germinal compartments that generate areas 17 and 18 could be initiated and/or maintained by extrinsic mechanisms, which is compatible with the protocortex theory[32,117,118] (BOX 1).

In vivo and *ex vivo* analysis of the cell-cycle regulation of OSVZ precursors in primates has shed light on the molecular correlates of area-specific differences in proliferation. Area 17 OSVZ precursors are characterized by both a shorter G1 phase and increased rates of cell-cycle re-entry compared with area 18 OSVZ precursors. These differences in cell-cycle regulation are underlined by differences in the levels of expression of the CDKI $p27^{kip1}$ and cyclin E, important regulators of S-phase entry. These results highlight the role of G1-phase regulation in corticogenesis[20]. Modelling the observed differences in both rates of cell-cycle re-entry and in the length of the G1 phase shows that the combined variation of these two parameters is sufficient to generate the enlarged supragranular layers that distinguish area 17 from the adjacent area 18.

Role of afferent axons on corticogenesis. There is evidence in favour of afferent axons influencing the proliferation of neuronal precursors in the CNS in vertebrates and invertebrates. In invertebrates, growing optic axons influence the proliferation of their target neurons[119] by promoting the G1–S transition[120]. For example, removal of the retina during early development in frogs results in lower mitotic rates in the tectum[121].

Corticopetal axons
Axons originating from neurons located outside the cortex that project to cortical areas.

Corticofugal axons
Axons originating from cortical neurons that project outside the cortex to subcortical structures.

Major axon tracts from dorsal thalamic nuclei innervate the developing cortex and provide an extrinsic source of factors that could influence proliferation and/or differentiation (FIG. 4). *In vitro*, embryonic thalamic axons release a mitogenic factor that promotes the proliferation of mouse cortical precursors by decreasing the length of the G1 phase[111]. Although the relative timing of thalamic development in monkeys is conserved, compared with non-primates corticogenesis is protracted[115], so thalamic axons reach the developing cortex relatively earlier in primates compared with rodents[122]. In the mouse, early thalamic axons are within 80 μm of cortical precursors[123]. Because corticopetal axons grow ventral to thalamic axons in the rodent, as corticogenesis proceeds thalamic axons are progressively distanced from cortical precursors and this will therefore limit the influence of thalamic axons on proliferative activity. In monkeys, thalamic axons are located in the OSVZ zone and immediately above it in the outer fibre layer (OFL), thus remaining in a position to potentially influence proliferation throughout corticogenesis[34]. The differences in the localization of corticofugal and corticopetal fibres between rodents and primates could reflect an adaptive feature that is related to the expansion of the cortex.

The lateral geniculate nucleus axons that target the OSVZ of area 17 in monkeys could be responsible for the temporally and spatially restricted stimulation of proliferation that results in the transient increase in size of the upper layer precursor pool in area 17 (REFS 20,31,34,111). There is also *in vivo* evidence in primates which suggests that embryonic thalamic axons might affect areal size and specification during cortical neurogenesis[116,124–126]. Because thalamic axons are precisely targeted to distinct areas[127], which is possibly due to early cortical expression of EphA receptors[128], they will be able to differentially influence the rate of proliferation across the germinal zones and, therefore, determine cytoarchitectural features. In this way, the thalamic axons are seen to act on the protomap[115,116] (BOX 1).

Conclusions and perspectives

During evolution the cerebral cortex underwent tangential expansion and an increase in the number of cortical areas. However, the genetic and cellular mechanisms that led to this expansion have only begun to be investigated[129–134]. The mode of division of cortical precursors is linked to the length of the G1 phase, signifying that the fine-tuning of a very basic biological mechanism — namely the G1 phase of the cell cycle — is the primary parameter that orchestrates the exquisitely ordered production of neurons during corticogenesis.

One important avenue of research is to identify the molecular reason for the uniqueness of the human brain. One possibility that needs to be further explored is that the small differences between humans and great apes concern regulatory processes[115,130,135]. There is considerable evidence that proliferation-related factors have contributed to the evolutionary enlargement of the cerebral cortex. Mutations of genes that interfere with the switch between proliferative symmetrical divisions and differentiative asymmetrical divisions in the VZ have been shown to affect cortical size[136–138]. Studies of human congenital microcephaly have identified several genes that specifically influence brain size through the regulation of neural progenitor division by altering microtubule organization at the centrosome[139,140]. The evolutionary patterns of genes responsible for primary microcephaly are consistent with the hypothesis that genes regulating brain size during development also have a role in brain evolution in primates, especially in humans[129,141].

Our knowledge of the proliferative behaviour of mouse precursors is sufficiently detailed to model corticogenesis in this species[5,8,142]. This is a crucial step towards the mechanistic understanding of corticogenesis. However, there are numerous primate-specific features in cortical development that regulate the cell cycle so it can be optimally tuned to the spatio-temporal production of phenotypically defined neurons. The existence of such primate-specific features means that the experimental investigation of the proliferative behaviour of cortical precursors in monkeys is particularly pressing, especially given that the OSVZ generates the supragranular layers. These are thought to house crucial computational components of the cortex[51], which are arguably not present in the rodent[143]. The challenge of understanding the unique features of human cortical development and of unravelling the self-organization principles of the most sophisticated computational device known[144] will be a demanding task we cannot shirk.

Future research on the early stages of corticogenesis will be required to explain how regionalized gene expression and proliferative programmes interact, and to shed light on the relationship between the molecular control of the cell cycle and fate determination[32,83,84]. Understanding how diverse phenomena including proliferation, cell-fate determination and migration are coordinated at later stages of corticogenesis is also becoming increasingly important[145].

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