Growth and folding of the mammalian cerebral cortex: from molecules to malformations

Tao Sun[1] *and Robert F. Hevner*[2]

Abstract | The size and extent of folding of the mammalian cerebral cortex are important factors that influence a species' cognitive abilities and sensorimotor skills. Studies in various animal models and in humans have provided insight into the mechanisms that regulate cortical growth and folding. Both protein-coding genes and microRNAs control cortical size, and recent progress in characterizing basal progenitor cells and the genes that regulate their proliferation has contributed to our understanding of cortical folding. Neurological disorders linked to disruptions in cortical growth and folding have been associated with novel neurogenetic mechanisms and aberrant signalling pathways, and these findings have changed concepts of brain evolution and may lead to new medical treatments for certain disorders.

The cerebral cortex is a central region in the mammalian brain that controls complex cognitive behaviours[1,2]. The growth of the cortex relies on the expansion of neural stem cells (NSCs) and neural progenitors (NPs), and the subsequent generation of postmitotic neurons. Cortical size varies markedly among mammalian species, and the brain-to-body mass ratio does not always closely correlate with behavioural complexity and intelligence[3,4]. However, at least in humans, cortical size is crucial for normal brain function, as patients with microcephaly or macrocephaly (that is, small or enlarged brains, respectively) show a range of cognitive deficits.

Based on cortical folding, mammals can be divided into lissencephalic species (such as mice), which have smooth-surfaced cortices, and gyrencephalic species (such as ferrets and most primates), which exhibit convolutions in the cortex. However, gyrification can vary considerably between and within mammalian orders, although it largely correlates with brain size[4]. For example, lissencephalic brains are found in small rodents and small primates (such as marmosets), whereas gyrencephalic brains are found in large rodents (such as capybaras) and large primates[5]. During evolution, cortical folding has enabled the mammalian brain to grow markedly in volume and to expand in surface area despite being housed in a confined skull.

In this article, we review the molecular regulation of cortical growth, explore the impact of recent findings on concepts of gyral formation and discuss cellular and genetic bases of cortical malformations that are associated with abnormal cortical size and folding. We first examine cortical growth and specifically discuss the characterization of different types of cortical progenitor cells, the molecular mechanisms of progenitor expansion, novel cellular and molecular regulators of neurogenesis (for example, primary cilia and microRNAs (miRNAs)), and genetic causes of human microcephaly and megalencephaly. These topics are followed by a consideration of important new findings pertaining to the formation of gyri and sulci. Gyrogenesis involves a complex sequence of events[6], and we focus on the following: the role of basal progenitor cells that detach from the ventricular surface and proliferate to augment cortical growth locally; the role of axons in cortical folding; molecules that regulate gyrus formation; and other, less prominent but nevertheless important mechanisms of gyrus formation, such as ventricular surface expansion, pial invagination and meningeal signalling. Last, we briefly discuss the relevance of gyrification to neurological functions, including the possibility that some gyral structures might be associated with cortical patterning, arealization and cognitive abilities.

Neural progenitors and cortical growth

The cerebral cortex is specified in the most rostral region of the early embryonic mammalian neural tube, which consists of neuroepithelial (NE) cells[7]. NE cells

[1]*Department of Cell and Developmental Biology, Weill Medical College of Cornell University, 1300 York Avenue, BOX 60, New York, New York 10065, USA.*
[2]*Department of Neurological Surgery and Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, Washington 98101, USA.*
e-mails: tas2009@med.cornell.edu; rhevner@uw.edu
doi:10.1038/nrn3707

are NSCs that can give rise to both neurons and glia[8]. Radial glial cells (RGCs) are progenitors that are derived from NE cells, reside in the ventricular zone (VZ) and form bipolar radial fibres between the ventricular and pial surfaces in the cortex (FIG. 1). RGCs display features of glia, which include serving as scaffolds for migrating neurons, expressing glial markers such as glial fibrillary acidic protein (GFAP) and astrocyte-specific glutamate transporter (GLAST; also known as SLC1A3), and giving rise to astrocytes[9–11]. More-recent studies have shown that RGCs can produce neurons and, subsequently, astrocytes and oligodendrocytes[9,10,12]. Conceptually, the radial unit hypothesis postulates that the cortex is assembled from radial progenitor units that consist of proliferative RGCs and more differentiated daughter cells, including neurons, which ultimately migrate radially along RGC fibres to form the characteristic six-layered cortical structure, from the inside out[10,11,13] (FIG. 1).

RGCs usually undergo asymmetrical division, giving rise to one RGC and one postmitotic neuron, or one RGC and one intermediate progenitor (IP) that resides in the subventricular zone (SVZ)[14]. More-recent studies suggest that IPs may be classified into two subpopulations — the apical IPs (aIPs) and basal IPs (bIPs) — that have distinct molecular profiles. Whereas aIPs reside in the VZ and have short radial attachments to the apical (ventricular) surface, bIPs delaminate from the VZ and migrate into the SVZ[15,16] (FIG. 1). IPs usually divide symmetrically to generate two postmitotic neurons and, like RGCs, are a major neurogenic cell population[17–19]. The molecular mechanisms that underlie IP divisions and the transition of RGCs to IPs are still unclear, although several transcriptional regulators, including insulinoma-associated protein 1 (INSM1), T-box brain protein 2 (TBR2; also known as EOMES) and TMF-regulated nuclear protein 1 (TRNP1), seem to be involved.

The SVZ in some mammals (primates, ferrets and others) can be subdivided histologically into the inner SVZ (ISVZ) and outer SVZ (OSVZ) by an inner fibre layer[20]. Recently, a new type of RGC, named basal RGCs (bRGCs; also called outer radial glia-like cells), was identified in the OSVZ of developing cortices in humans as well as in ferrets and other mammals, including lissencephalic species such as mice[16,21–25]. Morphologically, bRGCs are unipolar, as they have one basal fibre that ascends towards the pial surface but no apical fibre that projects to the ventricular surface[23]. The bRGCs behave like classic ventricular surface-attached apical RGCs (aRGCs): they divide asymmetrically to produce neurons or IPs and express RGC markers such as PAX6 and SOX2 (FIG. 1). It has been suggested that bRGCs are an additional source of progenitors that contribute to cortical growth and folding, indicating that bRGCs are important in gyrogenesis[26,27]. Interestingly, a histologically distinct OSVZ has also been described in marmosets, which are relatively lissencephalic, suggesting that neither bRGC abundance nor OSVZ histology is directly correlated with gyrencephaly[28].

The control of cortical size and folding thus depends on the balanced proliferation and differentiation of at least four types of progenitors: aRGCs and aIPs in the VZ, and bRGCs and bIPs mainly in the SVZ (FIG. 1). Several studies have shown that many molecules and signalling pathways, which are often highly conserved in mammals, have crucial roles in the regulation of cortical growth and folding[29–32]. It is no surprise that perturbation of any of these molecules or pathways would result in brain malformations, especially in the human brain, which contains an expanded geometric assembly of aRGCs, bRGCs, IPs and differentiating neurons that have much longer migration paths than those in species with smaller brains, such as mice, rats and ferrets[26,33,34].

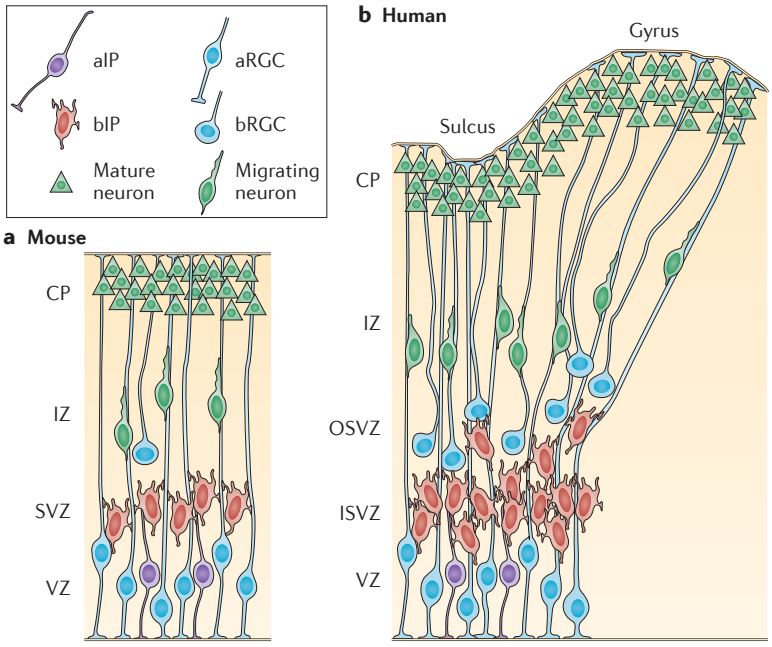


Figure 1 | **Multiple progenitors in the mouse and human developing cerebral cortex.** Progenitors are termed according to their apical or basal position in relation to the apical (ventricular) surface of the developing cortex. Apical radial glial cells (aRGCs) and apical intermediate progenitors (aIPs) reside in the ventricular zone (VZ). Basal IPs (bIPs) are mostly positioned in the subventricular zone (SVZ) in the mouse cortex and in the inner SVZ (ISVZ) in the human cortex. Basal RGCs (bRGCs) are identified in the SVZ in the mouse cortex and the outer SVZ (OSVZ) in the human cortex. Migrating neurons mostly appear in the intermediate zone (IZ), whereas mature neurons form an inside-out six-layered structure in the cortical plate (CP). Cortical surface expansion in the human cortex results in folded structures called sulci and gyri. The ISVZ and OSVZ are histologically separated by an inner fibre layer. It is unclear how the cytoarchitectonically distinct OSVZ and the characteristics of bRGCs in the OSVZ are related to cortical size and folding[28].

Molecular mechanisms of cortical growth

Cortical size depends on the expansion of the NP pool. Modest disruption of early NP development, such as during proliferation and survival, can subsequently be amplified and result in a markedly altered cortical size. Here, we summarize several essential mechanisms that regulate NP expansion and cortical size.

Cell cycle progression regulation. Early studies using ^{3}H-thymidine labelling, which indicates DNA synthesis, revealed the presence of proliferating progenitors in the VZ of cultured human fetal cortical slices[35]. Nuclei of VZ progenitors are positioned at basal (abventricular)

locations in the VZ during S phase, move towards the apical surface (the ventricle side) during G2 phase, undergo mitosis (M phase) at the apical (ventricular) surface and return towards basal positions in G1 phase; this oscillation process is called interkinetic nuclear migration (INM)[10,36,37] (FIG. 2a).

Both gap junctions and transcription factors have an impact on proper regulation of INM. Blocking gap junctions in RGCs causes delayed INM towards the apical surface[38]. Nuclei of RGCs in *Pax6*-mutant rat cortices often fail to reach the apical surface and, consequently, RGCs divide ectopically in basal locations[39]. Moreover, it

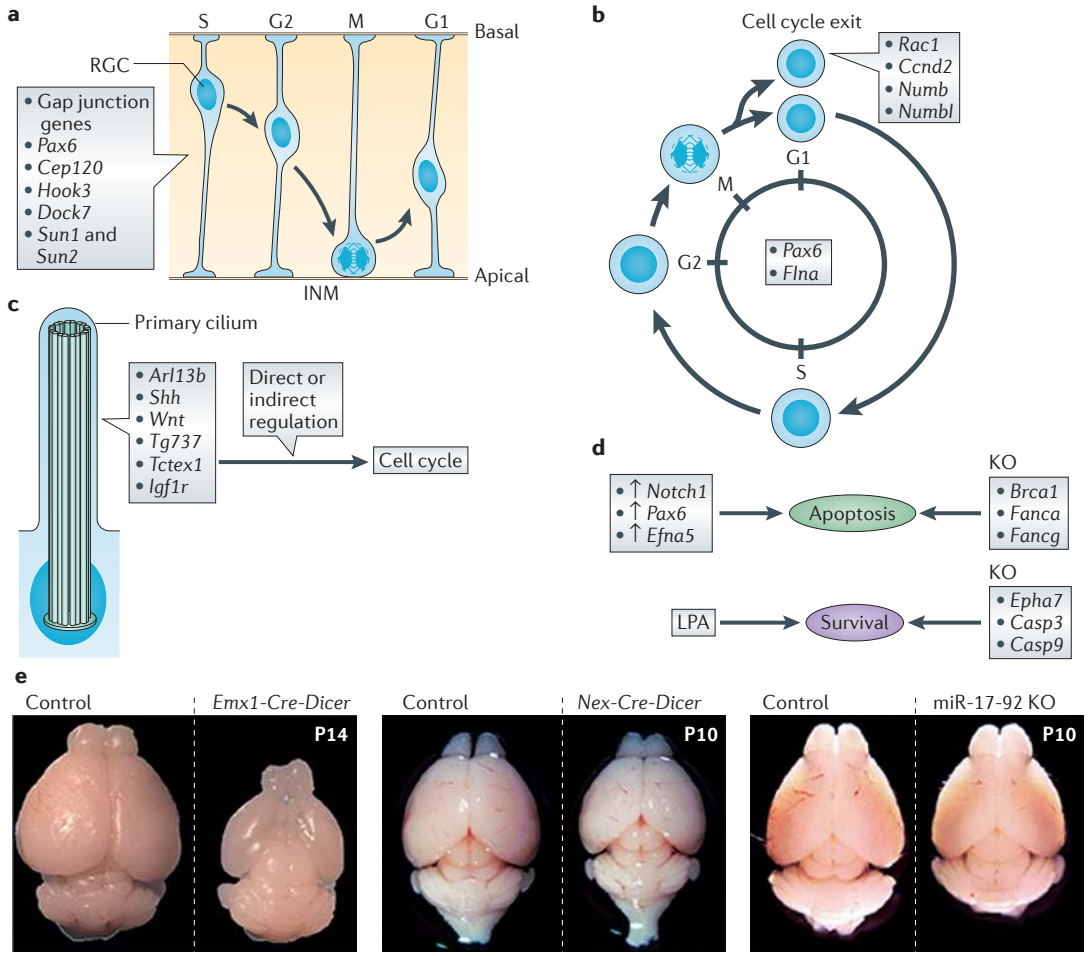


Figure 2 | **Molecular mechanisms of cortical growth.** Various mechanisms regulate neural progenitor (NP) expansion and cortical size. **a** | The expansion of radial glial cells (RGCs) in the ventricular zone (VZ) is dependent on interkinetic nuclear migration (INM) within these cells, and various genes have been identified that regulate this process. In INM, the nuclei of VZ progenitors (RGCs) are positioned at basal (abventricular) locations in the VZ during the S phase of the cell cycle and move towards the apical surface (the ventricle side) during the G2 phase. Subsequently, they undergo mitosis (the M phase) at the apical (ventricular) surface and return towards basal positions in the G1 phase. **b** | NP proliferation is controlled by genes that regulate the cell cycle length (*Pax6* and filamin A (*Flna*)) and cell cycle exit (*Rac1*, cyclin D2 (*Ccnd2*), numb homologue (*Numb*) and numb-like (*Numbl*)). **c** | Genes that are associated with primary cilia have direct or indirect roles in the cell cycle control of NPs. A mutation in *Tg737* causes dysfunctional ciliogenesis. Trafficking of molecules such as sonic hedgehog (SHH) and WNT by intraflagellar transport (IFT) relies on proper ciliogenesis. Knockout of ADP-ribosylation factor-like 13B (*Arl13b*), which encodes a protein that is abundant in cilia, causes reversal of the apical–basal polarity of RGCs in the mouse cortex. TCTEX-type 1 (*Tctex1*) and insulin-like growth factor 1 receptor (*Igf1r*) encode proteins that are directly involved in both ciliary disassembly and cell cycle re-entry. **d** | NP apoptosis and survival are regulated by multiple genes and are crucial for cortical size control. Whereas overexpression of *Notch1*, *Pax6* or ephrin A5 (*Efna5*) or knockout (KO) of breast cancer 1 (*Brca1*), Fanconi anaemia complementation group A (*Fanca*) or *Fancg* promotes apoptosis of NPs, the addition of lysophosphatidic acid (LPA; a mitogen) or knockout of ephrin receptor type A receptor 7 (*Epha7*), caspase 3 (*Casp3*) or *Casp9* increases the survival of NPs. **e** | MicroRNAs (miRNAs) are essential for regulating cortical size. Ablation of Dicer, an enzyme that processes miRNA precursors, in mice using *Emx1-Cre* or *Nex-Cre* lines results in smaller cortices[76,81] (left and middle panels). Mice in which the specific miRNA cluster miR-17-92 is knocked out also exhibit a small cortex[83] (right panel). *Cep120*, centrosomal protein 120; *Dock7*, dedicator of cytokinesis 7; *Hook3*; hook microtubule-tethering protein 3; P10, postnatal day 10; *Sun*, SUN-domain-containing. The middle panel of part **e** is reproduced from REF. 81. The right-hand panel of part **e** is reproduced, with permission, from REF. 83 © (2013) Elsevier.

has been shown that the dynamic centrosome–nucleus interaction is crucial for INM and cortical size control (FIG. 2a). Knockdown of centrosomal protein 120 (CEP120) impairs migration of RGC nuclei towards the apical surface, increases cell cycle exit and reduces the size of progenitor pools[40]. Disruption of a microtubule-binding protein, hook microtubule-tethering protein 3 (HOOK3), results in a reduced progenitor population owing to altered INM motility and speed[41]. Ectopic expression of dedicator of cytokinesis 7 (DOCK7) causes delayed INM and reduced numbers of cortical progenitors[42]. Furthermore, mice in which the genes encoding SUN-domain-containing protein 1 (SUN1) and SUN2 are knocked out have smaller cortices, and this is probably caused by a failure of nucleus movement towards the apical surface owing to defects in the coupling of the centrosome and the nucleus[43].

Direct evidence has been obtained showing that cell cycle progression is important in controlling NP expansion (FIG. 2b). In *Pax6*-mutant cortices, the length of the cell cycle, especially S phase, is markedly longer than it is in control cortices, and this lengthened cell cycle contributes to the decrease in the size of mutant cortices[44]. Mechanistic studies have shown that PAX6 regulates the G1-to-S phase transition in cortical NPs by repressing the cyclin-dependent kinase 6 (CDK6), cyclin D1 and cyclin D2 signalling pathways[45]. Knockout of the cytoskeleton-associated gene filamin A (*Flna*) causes cell cycle prolongation and a reduction in the number of NPs, resulting in a decrease in cortex size[46]. Numb homologue (NUMB) and numb-like protein (NUMBL) are important regulators of progenitor division. Whereas early embryonic ablation of *Numb* causes deletion of NPs, late embryonic inactivation of *Numb* and *Numbl* results in the formation of neurogenic cellular rosettes and folding of the cortex owing to hyperproliferation and delayed cell cycle exit, suggesting that NUMB has distinct functions in NP development[47,48]. Mutations in the gene encoding zinc-finger protein 335 (ZNF335) cause defects in NP self-renewal and neurogenesis and, consequently, a reduction in brain size in both humans and mice[49]. Moreover, loss of cyclin D2 and RAC1 (a RHO-family small GTPase) promotes cell cycle exit and causes a reduction in cortical size[50–52]. These studies indicate that cell cycle progression determines the behaviour, expansion and differentiation of RGCs and IPs in the cortex and, in turn, regulates cortical growth (FIG. 2b).

Although many cell cycle regulators have been identified and their functions have been determined, the mechanisms underlying the precise control of the number and duration of divisions of progenitors in normal mammalian cortical development remain unclear. Understanding the internal and external determinants of the number and duration of progenitor divisions may help to explain the variations in brain-to-body mass ratio among species.

Ciliogenesis and NP proliferation. The primary cilium, which is found in most mammalian cells, is an antenna-like microtubule-based organelle emanating from the cell surface. It contains an axoneme that consists of a ring of nine peripheral microtubule pairs but no central pair (a '9 + 0' arrangement) and uses intraflagellar transport (IFT) to facilitate signalling molecule migration along this structure[53]. Studies have shown that cilia play a crucial part in protein trafficking in NPs and neurons, and impairments in cilia function are associated with several neurodevelopmental disorders, including Joubert syndrome[54,55] (FIG. 2c). Mutations in genes involved in ciliogenesis (such as the IFT genes) in developing cortices cause severe brain malformations and probably affect the trafficking of molecules of the sonic hedgehog (SHH) and WNT signalling pathways[56]. Moreover, ADP-ribosylation factor-like 13B (*Arl13b*)-knockout mice (*Arl13b* encodes a small GTPase that is abundant in cilia and is mutated in Joubert syndrome) exhibit a reversal of the apical–basal polarity of RGCs in the cortex, suggesting an underlying pathogenic mechanism in Joubert syndrome[57].

A role for cilia in cell cycle progression is emerging. Primary cilia are normally present in G0–G1 phases and are resorbed before M phase in mammalian cell division[58,59]. TCTEX-type 1 (TCTEX1; also known as DYNLT1), a dynein light-chain protein that is phosphorylated at Thr94 and recruited to ciliary transition zones by phosphorylated insulin-like growth factor 1 receptor (IGF1R) before S phase entry, promotes both ciliary disassembly and cell cycle re-entry, and positively regulates the proliferation of RGCs[60,61]. Moreover, it has been shown that the ciliary membrane in NPs is associated with the mother centriole and is asymmetrically inherited by one daughter cell at the apical surface in M phase, suggesting a new cilium-mediated mechanism of maintaining the NP pool[62].

The secondary cilium, which is typically found in epithelial cells, is motile and is composed of an axoneme containing an extra central pair of microtubules (a '9 + 2' arrangement) compared with the primary cilium. Motile cilia may also play a part in regulating NP proliferation. Proper beating of cilia in ependymal cells and choroid plexus epithelial cells is essential for the flow of cerebrospinal fluid (CSF), and indeed a mutation in the IFT gene *Tg737* (also known as *Ift88*) causes dysfunctional ciliogenesis and hydrocephalus[63]. The CSF contains growth factors, such as IGF2, that promote NP proliferation and thus are important for controlling cortical size[31].

The interaction between ciliary dynamics and cell cycle progression remains an interesting research topic. Determining whether there is a correlation *in vivo* between ciliogenesis and cortical NP proliferation requires further investigation using imaging tools as well as cellular and genetic approaches (FIG. 2c).

Neuronal cell death in the regulation of cortical size. Widespread apoptosis has been described in the VZ and SVZ of mouse cortices during embryonic development, especially around the peak of neurogenesis at embryonic day 14 (E14)[64,65], indicating that apoptosis has an important role in cortical development. Indeed, cortical size is determined by the balance between NP expansion and cell death[66].

SUN-domain-containing protein
A protein containing SUN (Sad1p and UNC-84) domains in the carboxy-terminal regions. These proteins are often involved in positioning of the nucleus in a cell.

Joubert syndrome
A genetic disorder that affects the cerebellum. The most common features include ataxia and abnormal eye and tongue movements. Abnormal functions of cilia are associated with this disorder.

Centriole
A cell structure that is composed mainly of tubulin. A centrosome is made up by a pair of centrioles. Centrioles are involved in the organization of the mitotic spindle in dividing cells.

Hydrocephalus
A medical condition in which there is an abnormal accumulation of cerebrospinal fluid in the ventricles or cavities of the brain.

Various studies have shown that increased apoptosis is linked to decreased progenitor number and/or cortical size. Constitutive activation of Notch homologue protein 1 (NOTCH1) and PAX6 in NPs induces apoptosis and reduces the size of the progenitor pool[67,68], and breast cancer 1 (*Brca1*)-knockout mice show reductions in both cortical thickness and surface area because of the apoptosis of embryonic NPs[69]. Mice with mutations in Fanconi anaemia complementation group A (*Fanca*) and *Fancg*, which are implicated in Fanconi anaemia and cause chromosomal instability, also show a marked increase in NP apoptosis and exhibit a small brain size[70]. Moreover, mice with ectopic expression of ephrin A5 in early cortical progenitors (that express ephrin type A receptor 7 (EPHA7)) exhibit a reduced cortical size because of increased NP apoptosis[71].

Complementing the findings described above, decreased apoptosis has been linked with opposite effects on progenitors and the cortex. *Epha7*-knockout mice display enlarged cortices, indicating that the ephrin signalling pathway has an important role in controlling cortical size by regulating apoptosis[71]. Moreover, caspase 3 (*Casp3*)- and *Casp9*-knockout mice display markedly enlarged and malformed cortices because of reduced apoptosis[72,73]. Cortical explants cultured with lysophosphatidic acid (LPA), a phospholipid signalling molecule that is generated by G protein-coupled receptor activation, show increases in cortical wall thickness and folding, which are caused by reduced cell death and increased terminal mitosis of NPs[74]. Together, the results described above indicate that the expansion of cortical NPs is orchestrated by molecules involved in proliferation, cell survival and apoptosis (FIG. 2d).

MicroRNA regulation of cortical growth. The role of miRNAs in cortical development has been shown through the use of cortex-specific *Cre* lines that delete Dicer, which blocks miRNA biogenesis (FIG. 2e). *Dicer* deletion in cortical NPs using *Emx1-Cre*, *Nes-Cre* or *Foxg1-Cre* lines results in smaller cortices because of reductions in the sizes of the NSC and NP pools, increased apoptosis and impaired neuronal differentiation[75–79]. *Dicer* deletion from postmitotic neurons in the cortex using a calcium/calmodulin protein kinase II promoter-driven *Cre* line or a *Nex-Cre* line also causes reduced cortical size, probably through impairment of neurite outgrowth and increased neuronal packing density in the cortical plate[80,81].

Recent studies have determined which specific miRNAs and their targets are involved in NP development. The miR-17-92 cluster, which is located on chromosome 13 in humans and chromosome 14 in mice, is an important miRNA polycistron that is involved in the generation of many types of tumours[82]. The miRNAs in the miR-17-92 cluster are highly expressed in the VZ and SVZ of the mouse embryonic cortex, and mice in which the locus encoding the miR-17-92 cluster is conditionally knocked out using the *Emx1-Cre* line have small cortices[83] (FIG. 2e). miR-19 in the miR-17-92 cluster promotes NSC proliferation and RGC expansion by targeting phosphatase and tensin homologue (*Pten*), a gene that is crucial for controlling cortical size[83,84]. Furthermore, another miRNA in this cluster, miR-92, inhibits the transition of RGCs to IPs by targeting *Tbr2*, indicating that the miR-17-92 cluster controls cortical size by ensuring that the right proportions of RGCs and IPs are generated[83,85]. In addition, nuclear receptor TLX (also known as NR2E1) promotes cortical NSC proliferation, and brain-enriched miR-9 negatively regulates NSC expansion by targeting *Tlx*[86]. Interestingly, TLX also represses the expression of the miR-9 primary transcript, suggesting a feedback loop by which TLX and miR-9 control the size of the NSC pool[86]. By contrast, miR-134 maintains cortical NPs by targeting the expression of the neuronal migration protein doublecortin (DCX) and/or chordin-like protein 1 (a bone morphogenetic protein antagonist), which are normally expressed in differentiated neurons[87].

The functions of specific miRNAs in neuronal morphogenesis have also been explored. miR-134 plays a part in promoting cortical neurite outgrowth by targeting a GTPase-activating protein p250GAP (also known as ARHGAP32)[88]. Moreover, miR-124 promotes neurite outgrowth by suppressing RHOG, cell division control protein 42 homologue (CDC42) and RAC1 expression[89,90]. Last, miR-9 and miR-132 induce neurite outgrowth by silencing forkhead box P2 (*Foxp2*) in the cortex[91]. The direct effects of the miRNAs described above on cortical size control remain to be determined.

miRNAs normally act like transcription factors and can regulate many target genes that control various aspects of cortical development[92]. Hemizygous deletions of the locus encoding the miR-17-92 cluster in humans have been mapped in patients with Feingold syndrome, which is characterized by microcephaly, short stature and digital anomalies[93]. This association points to a potential role of miRNAs in human brain malformations. Although direct genetic evidence is lacking in humans, it seems that miRNAs form a network with protein-coding genes to regulate progenitor expansion, differentiation and neuronal morphogenesis in mammalian developing cortices.

Symmetrical–asymmetrical division of NPs. Cortical NPs consist of highly polarized cells (such as aRGCs and bRGCs) and unpolarized cells (such as bIPs), which undergo asymmetrical and symmetrical divisions, respectively[94]. NP divisions in the VZ can be characterized as symmetrical divisions if they occur in the vertical cleavage plane or asymmetrical divisions if they occur in either an oblique or a horizontal cleavage plane[95,96] (FIG. 3a). Live imaging has shown that, during neurogenesis, RGCs usually divide asymmetrically to generate one RGC and one IP or postmitotic neuron[97–99]. The highly conserved apical partition defective protein (PAR) complex, including PAR3, PAR6 and atypical protein kinase C (aPKC), plays a crucial part in orienting mitotic spindles and in controlling asymmetrical division in RGCs[100,101]. Indeed, overexpression of PAR3 or PAR6 promotes the generation of PAX6-expressing RGCs[102]. Furthermore, PAR3 interacts with NUMB and induces Notch activity, which is essential for maintaining RGC fate in the developing cortex[103,104].

Dicer
An RNAase III enzyme that cleaves double-stranded RNA and microRNA precursors into short (20–25 base pairs) double-stranded RNA fragments. It facilitates the formation of the RNA-induced silencing complex (RISC) and participates in the RNAi pathway and microRNA-mediated gene silencing.

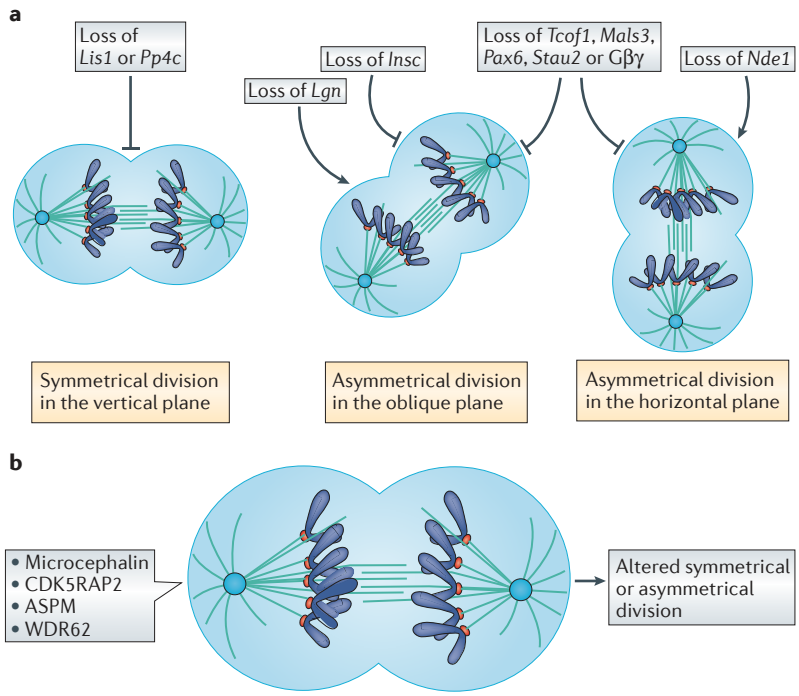


Figure 3 | **Symmetrical–asymmetrical cell division, centrosome associated proteins and neural progenitors.** **a** | According to spindle orientation and cleavage plane, neural progenitor (NP) divisions can be classified as being vertical (that is, symmetrical), oblique or horizontal (that is, asymmetrical). Various gene mutations can affect the orientations of cleavage planes, symmetrical versus asymmetrical division of NPs and, in turn, cortical size. Loss of lissencephaly 1 (*Lis1*) or protein phosphatase 4 catalytic subunit (*Pp4c*) in NPs disrupts vertical division, and loss of nuclear distribution E homologue 1 (*Nde1*) promotes horizontal division. Inactivation of *Lgn* or inscuteable (*Insc*) promotes or decreases oblique division, respectively. Loss of *Tcof1*, *Mals3*, *Pax6*, Staufen homologue 2 (*Stau2*) or Gβγ disrupts asymmetrical division in NPs. **b** | All identified autosomal recessive primary microcephaly (MCPH)-linked genes encode centrosome-associated proteins, and the mutations in these genes affect proper divisions of NPs and cause microcephaly. ASPM, abnormal spindle-like microcephaly-associated; CDK5RAP2, cyclin-dependent kinase 5 regulatory subunit-associated protein 2; WDR62, WD-repeat-containing protein 62.

Conversely, cortex-specific *Cdc42* deletion causes reduced apical localization of the PAR complex and leads to an increase in the number of IPs[105].

Various molecules that are involved in spindle orientation have been shown to control symmetrical and asymmetrical cell divisions in the developing cortex[95] (FIG. 3a). Mutation of the gene encoding LGN (also known as PINS or GPSM2), a nuclear mitotic apparatus protein 1 (NuMA)- and Gα-binding protein that is excluded from the apical side of dividing cells, causes randomized orientation of spindles and leads to an increase in the number of oblique divisions in NPs[106,107]. Inscuteable (*Insc*)-conditional-knockout and overexpression mice have thinner and thicker cortical walls caused by decreased and increased oblique divisions of RGCs, respectively[108]. Loss of *Tcof1*, which encodes the centrosome-associated protein treacle, disrupts spindle orientation in NPs and thereby causes a reduction in the size of the NP pool, which results in a smaller cortex[109]. Mice lacking dynein-associated nuclear distribution E homologue 1 (NDE1), which directs microtubule organization and positions the mitotic spindle, exhibit smaller cortices owing to an increase in the frequency of horizontal divisions in RGCs and a decrease in the size of the progenitor pool[110]. Likewise, loss of lissencephaly 1 (LIS1; also known as PAFAH1B1), which controls spindle orientation through NDE-like 1 and dynein, disrupts vertical division of NE cells and causes a decrease in cortical size[111].

Molecules that are not directly associated with spindle orientation have also been found to direct symmetrical and asymmetrical divisions of NPs. Inactivation of *Mals3* (also known as *Lin7c*) in the cortex disrupts NP polarity and promotes the differentiation of such cells[112]. In *Pax6* mutants, an increased number of NPs undergo divisions in a non-vertical plane, leading to premature delamination and more asymmetrical divisions[113]. Knockdown of the double-stranded RNA-binding protein Staufen homologue 2 (STAU2) in NSCs, which is normally asymmetrically expressed in TBR2-expressing daughter IPs, promotes differentiation, suggesting that STAU2 has a role in specifying NP subtypes[114]. A G protein subunit, Gβγ, controls spindle orientation, and disrupting Gβγ promotes differentiation of cortical NPs[115]. Finally, mice lacking the protein phosphatase PP4C show decreased vertical divisions of NE cells and RGCs, leading to reduced cortical size[116].

Together, these findings indicate that the symmetrical versus asymmetrical division of NPs not only specifies the fate of daughter cells but also controls progenitor expansion and differentiation, and eventually cortical size. Polarity proteins and molecules that control spindle orientation are important determinants of this process (FIG. 3a). Moreover, in two daughter cells of an RGC in slice cultures, inheritance of the basal (pia-directed) process from the RGC is associated with self-renewal, whereas non-inheritance of this process is associated with differentiation[24]. Interestingly, studies have shown that most RGCs undergo divisions in a vertical cleavage plane, even though they usually divide asymmetrically, whereas most IPs undergo division in a horizontal cleavage plane, even though they usually divide symmetrically[117,118]. Thus, it seems that endogenous molecules in NPs and environmental cues may play a more substantial part in determining symmetrical versus asymmetrical divisions in RGCs than the cleavage plane itself. Furthermore, the angle of the cleavage plane seems to be less critical than inheritance of the apical cell surface membrane[119].

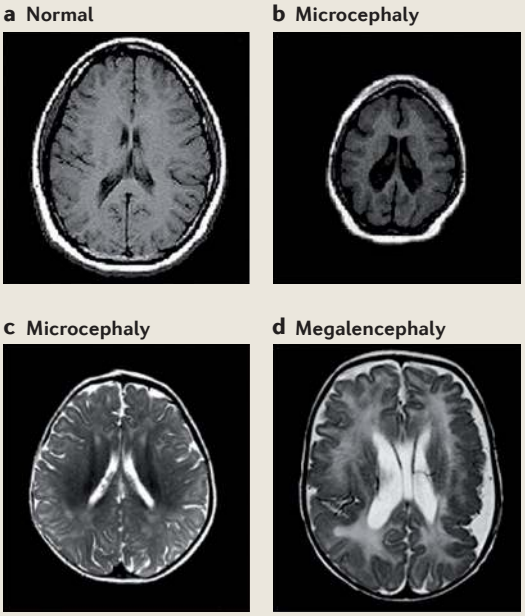
NP divisions and microcephaly

Several gene mutations that are associated with autosomal recessive primary microcephaly (MCPH) have been identified using linkage mapping[120,121] (BOX 1). *MCPH1* (also known as *BRIT1*), which encodes the protein microcephalin, was the first gene to be associated with MCPH and is highly expressed in human fetal and developing mouse brains[122]. Depletion of *MCPH1* increases the accumulation of chromosomal aberrations, suggesting that it is a DNA damage regulator and a tumour suppressor gene[123]. The functions of microcephalin in brain development have been further studied in an *Mcph1*-knockout mouse model, which also shows microcephaly[124]. In *Mcph1*-deficient NPs, the proportion

Box 1 | **Human microcephaly and macrocephaly**

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder that is characterized by a reduction in head circumference, a thin cortex and a decrease in brain surface area[121]. Individuals with MCPH exhibit intellectual disability but show no obvious motor control deficits. Linkage mapping has identified genes that are associated with MCPH[120,121], including abnormal spindle-like microcephaly-associated (*ASPM*) (see the figure, parts **a** and **b**, MRIs of normal-sized and mircocephalic brains, respectively[129]). Interestingly, many of these genes are involved in mitotic spindle assembly, suggesting that defects in the division of neural progenitors is involved in MCPH[96,136].

In contrast to microcephaly, macrocephaly refers to an abnormally large head, which can be caused by enlargement of the brain (megalencephaly) or other conditions such as hydrocephalus or cranial hyperostosis[204]. Macrocephaly has been linked to several cognitive disorders, including fragile X syndrome and autism[205]. Indeed, studies have shown that over 20% of autism cases are associated with macrocephaly[206,207]. Loss-of-function and gain-of-function mutations in *AKT3* cause microcephaly and hemimegalencephaly, respectively (see the figure, parts **c** and **d**, MRIs of microcephalic and megalencephalic brains, respectively[138,139]). Parts **a** and **b** are reproduced, with permission, from REF. 129 © (2002) Macmillan Publishers Ltd. All rights reserved. Images in parts **c** and **d** courtesy of W. Dobyns, Seattle Children's Hospital, USA.



of vertical divisions is reduced, and loss of *Mcph1* affects CHK1 (a kinase involved in cell cycle control) localization to the centrosomes in G2 phase and promotes NPs to enter M phase earlier[124].

Mutations in CDK5 regulatory subunit-associated protein 2 (*CDK5RAP2*; also known as *CEP215*), a centrosome-associated protein, have also been mapped in MCPH[125]. Loss of *Cdk5rap2* in mice alters centriole replication and causes increased numbers of daughter–daughter centriole pairs, cell death and premature cell cycle exit, resulting in smaller cortices[126,127]. Mutations in abnormal spindle-like microcephaly-associated (*ASPM*) have been mapped in patients with MCPH[128,129] (BOX 1). Loss of *Aspm* in mice promotes asymmetrical division by altering the cleavage plane in NPs and causes a reduction in the number of NPs and in cortical size[130,131]. Mutations in the gene encoding another centrosome-associated protein — namely, WD-repeat-containing protein 62 (WDR62) — are also associated with MCPH[132–134]. A functional study has shown that knockdown of *Wdr62* in mouse cortices causes delayed mitotic progression and disrupts centrosome integrity and spindle attachment, resulting in decreased NP proliferation[135].

Interestingly, all of the MCPH-associated genes that have been identified to date encode centrosome-associated proteins[96,136] (FIG. 3b). Moreover, a recent study has shown asymmetrical inheritance of the old mother centriole in RGCs, suggesting an important role for centrosomes in RGC maintenance[137]. Thus, during NP division, proper centrosome duplication and positioning are crucial for spindle organization and orientation. This in turn controls symmetrical versus asymmetrical divisions, determines the fate of daughter cells and cell cycle progression, and eventually dictates cortical size (FIG. 3b).

Expansion of NPs and megalencephaly

Megalencephaly with cellular dysplasia and its variants (hemimegalencephaly and focal cortical dysplasia) are caused by brain overgrowth and cellular defects that affect the entire brain, one hemisphere or a specific region. Recent progress has highlighted the importance of dysregulated phosphatidylinositol 3-kinase (PI3K)–AKT signalling in this subset of megalencephaly syndromes. Several megalencephaly-associated mutations have been identified by linkage mapping or exome sequencing, and detailed analyses have revealed that some of these mutations are mosaic (arising after fertilization). Interestingly, whereas loss-of-function deletions of *AKT3* lead to microcephaly, mosaic gain-of-function mutations in *PIK3CA*, *PIK3R2*, *MTOR* or *AKT3* result in hemimegalencephaly, suggesting that the PI3K–AKT pathway has a crucial role in controlling brain size[138–142] (BOX 1).

PTEN may also regulate brain size. Well-characterized mutations in *PTEN*, which is a tumour suppressor gene, have been associated with megalencephaly[143]. Moreover, PTEN normally suppresses the PI3K–AKT pathway and thus inhibits cell survival and growth[144]. *Pten*-knockout mice have large brains, which result from increased proliferation of NPs because of their shortened cell cycle and increased G0–G1 cell cycle entry[84,145]. Interestingly, megalencephaly-associated *PTEN* mutations are also related to autism[146–148]. Indeed, mice in which *Pten* is deleted in differentiated neurons show megalencephaly, abnormal social interaction and exaggerated responses to sensory stimuli, all of which are reminiscent of certain autistic traits[149].

Altered expression of cell cycle regulators can also lead to brain overgrowth, as shown in mouse models. Overexpression of CDK4 and cyclin D1 prevents G1 lengthening and thereby causes an expansion of the IP pool and an increase in cortical surface area[150]. Deletion of CDK inhibitor p57KIP2 (also known as CDKN1C) promotes cell cycle re-entry in NPs and an increase in cortical size[151]. Similarly, ectopic expression of stabilized β-catenin promotes cell cycle re-entry in NPs and leads to enlarged and folded brains in mice[29]. Moreover, deletion of fibroblast growth factor 10 (*Fgf10*) delays the transition of NE cells to RGCs and results in an increased number of progenitors and an enlarged brain[152].

In summary, evidence from human genetics and mouse models illustrates the importance of expansion of the NP pool during development — which is regulated by cell cycle progression, cell survival, symmetrical versus asymmetrical divisions and miRNAs — in controlling cortical growth.

Gyrencephaly: a mammalian trait

Gyrencephaly — that is, anatomical folding of the neocortex to form gyri and sulci — evolved as an effective way to pack large cortical surface areas into limited skull volumes[3]. As highlighted earlier, gyrencephaly is not limited to primates; it occurs in all mammalian orders[3,153,154]. Indeed, molecular and phenomic analyses suggest that the common ancestor of placental mammals had a gyrencephalic brain[155]. As gyrencephaly also occurs in some non-placental mammals, including echidnas (monotremes) and kangaroos (marsupials), it seems likely that this trait arose in the ancestor of all mammals. Thus, contrary to previous assumptions, the smooth (lissencephalic) neocortex seen in some modern species, such as manatees and mice, might have evolved secondarily[5,156].

Rather than a dichotomy, gyrencephaly and lissencephaly actually define a continuum of gyrification, in which bigger brains tend to be more gyrencephalic (FIG. 4). Accordingly, the gyrification index (GI), which is defined as the ratio of total neocortical surface area (including cortex 'buried' in sulci) to superficially exposed neocortical surface area, shows a strong positive relation with brain mass[4,154,157–159]. Measurements of GI range from pure lissencephaly (GI = 1.00, European hedgehog) to extreme gyrencephaly (GI = 5.55, Pacific pilot whale). Humans (GI = 2.56) rank highest among primates but are less gyrencephalic than some animals in other orders, such as zebras (GI = 2.94) and elephants (GI = 3.81). However, the relationship between the GI and brain mass is not strict; some species, such as beavers, manatees and (indeed) humans, have a smaller-than-expected GI going by brain mass (FIG. 4c). However, humans have an unusually thick neocortex (3.4 mm average), which is exceeded only by the manatee cortex thickness (4.0 mm). Debates on the significance of the GI and cortical thickness for intelligence remain unresolved, but greater cortical surface area may be essential for the elaboration of complex associational areas and new functions[1] (such as language).

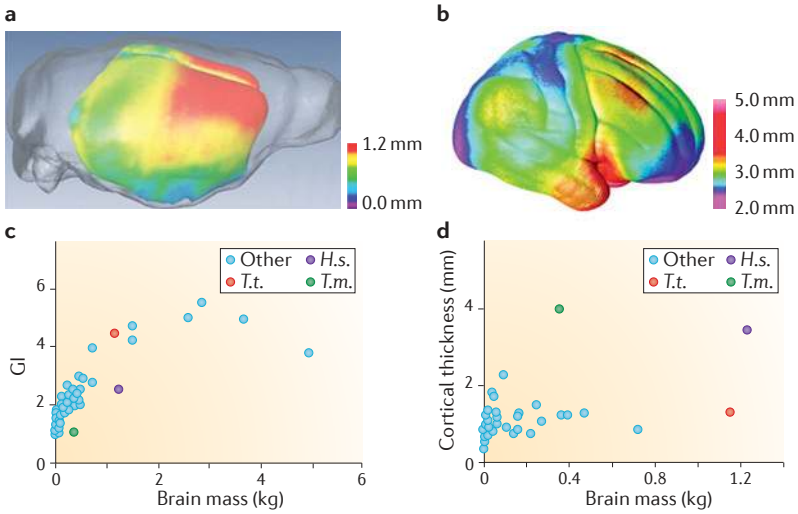


Figure 4 | **Brain mass, cortical thickness and gyrification.** **a** | The mouse brain is small (0.65 g) and is characterized by a smooth neocortex (gyrification index (GI) = 1.03). The thickness of the mouse cortex is indicated by the colour scale. **b** | By contrast, the human brain is considerably larger (1,230 g), and its cortex is gyrified (GI=2.56). Its cortical thickness also varies but is, on average, thicker than that of mice. **c** | The GI generally increases with brain mass across mammalian species for which data are available (n = 103). Manatees (*Trichechus manatus* (*T.m.*)) and humans (*Homo sapiens* (*H.s.*)) have relatively low GI scores for their brain mass compared with other mammalian species, such as the Atlantic bottlenose dolphin (*Tursiops truncates* (*T.t.*)). Other mammalian species not mentioned here are denoted by the blue data points. **d** | Cortical thickness varies little with brain mass across species for which data are available (n = 42), except in manatees and humans, which have unusually thick cerebral cortices on average. Part **a** is reproduced, with permission, from REF. 159 © (2008) Elsevier. Part **b** is modified, with permission, from REF. 158 © (2005) Oxford Journals. Data for panels **c** and **d** from REFS 4,154,157.

Gyrogenesis and basal progenitors

The development of gyri, which is called gyrogenesis, has been the subject of many classic studies, which together identified multiple mechanisms of gyrus formation (reviewed in REF. 6). Recently, interest in gyrogenesis has undergone a renaissance, which has been fuelled by progress in the characterization of various types of cortical progenitor cells. Indeed, the characterization of IPs led to the 'intermediate progenitor hypothesis', which proposes that gyral growth is mediated by differential regional proliferation of IPs[160]. More recently, the discovery of bRGCs as neurogenic progenitors that accrete in the cortical plate prompted the consideration of bRGCs as mediators of gyrification that remodel the radial glia scaffold[26,154]. In particular, regional differences in bRGC production could potentially account for the 'fan-like' divergence of radial fibres atop gyral crowns and 'parenthesis-like' convergence of fibres at sulcal depths, both of which are seen in classic descriptions of ferret and monkey gyrogenesis[161,162]. Indeed, the hypothesis that gyrus formation is dependent primarily on the local proliferation and growth of cortical tissue (or 'gyrus-building') is consistent with many previous observations, although the drivers of this growth are unknown[6]. Nevertheless, gyrus-building is not the only mechanism of gyrogenesis, and other mechanisms are discussed below.

Recently, several studies have investigated the role of basal progenitors (bIPs and bRGCs) in gyrogenesis. To locally perturb the genesis of basal progenitors in the mouse cortex, one group focused on *Trnp1*, a gene previously detected at higher levels in self-amplifying radial glia than in basal progenitor-producing radial glia[163,164]. Forced, high-level expression of *Trnp1* in the embryonic neocortex (through *in utero* electroporation) induced selective RGC self-amplification and decreased basal progenitor genesis, leading to tangential expansion of the neuroepithelium. By contrast, short hairpin RNA-mediated knockdown of *Trnp1* induced approximately twofold greater proliferation of basal progenitors, leading to radial growth and subsequent folding of the perturbed cortex. However, the extent to which the cortical folds resembled normal gyri (with a six-layered neocortex) was unclear. Interestingly, high expression of *TRNP1* also seems to correlate with ventricular surface expansion in some regions of the fetal human brain: for example, the parahippocampal cortex[163].

Another study, focusing on FGFs in cortical development, found that gyri were induced in the normally lissencephalic mouse cortex by intraventricular injection

of FGF2 during early cortical development[165]. Notably, FGF2 was not delivered to a focal cortical region but diffused throughout the ventricles bilaterally. Surprisingly, the effects of FGF2 were highly localized to the lateral neocortex, where increased tangential and radial growth led to the formation of a new gyrus, flanked by aberrant sulci. Interestingly, one of the aberrant sulci corresponded positionally to the lateral sulcus (also called the Sylvian fissure) in gyrencephalic species (a region previously identified as a 'cryptosulcus' in rodents on the basis of myeloarchitecture[166]). The FGF2-induced gyrus-forming neocortex displayed a thicker SVZ at E13.5, with twice the usual number of bIPs, but interestingly showed no apparent increase in the number of bRGCs. The induced gyri and sulci displayed a normal six-layered morphology at postnatal ages and were visible macroscopically in adult mice. The treated mice also showed reduced hippocampal growth and reduced expression of *Couptf1* (also known as *Nr2f1*), a caudolateral patterning-related gene. The gyrification response to FGF2 was ligand- and timing-specific, as FGF8B did not have the same effect[165] and nor did FGF2 administered at a slightly later stage of cortical development[167].

A third group set out to probe the role of basal progenitors in gyrogenesis by experimentally augmenting their proliferation through overexpression of cell cycle regulators CDK4 and cyclin D1 (REF. 168) (together called '4D'). Pan-cortical overexpression of 4D in mice (using genetic or lentiviral methods) beginning at E11.5 or E13.5 led to increases in SVZ thickness, IP proliferation, cortical thickness and cortical surface area but not in cortical folding. By contrast, focal 4D overexpression in ferrets (by plasmid electroporation from the ventricles or retroviral vector injection into the OSVZ on postnatal day 1, when layer 2/3 neurons are being generated) caused not only increased basal progenitor proliferation and greater cortical surface area but also increased cortical folding, with the formation of anomalous sulci, and a higher local GI. The hyperconvoluted cortex displayed normal six-layered cytoarchitecture. The authors interpreted their findings to suggest that ferrets have a greater natural capacity for gyrification than do mice. However, another interpretation might be that gyri and sulci are most likely to form under conditions of differential local growth (as opposed to during homogeneous cortical expansion).

Together, the recent studies discussed above suggest that differential regional amplification of basal progenitors in the SVZ can be sufficient to drive gyrification, even in mice. In the case of FGF2-induced gyri, differential regional proliferation was attributed to intrinsic local differences in the response to FGF2 (REF. 165). Interestingly, the timing of augmented basal progenitor proliferation that leads to gyrification differed among recent studies, spanning early[165], middle[163] and late[168] stages of cortical neurogenesis. Such differences in timing suggest that gyrification may arise at multiple stages, and this seems to be consistent with the prolonged sequential emergence of primary, secondary and tertiary gyri in humans, which occurs over a period of several months.

Myeloarchitecture
The laminar and radial arrangement of myelinated fibres in cortical areas. Like cytoarchitecture (the organization of cells), myeloarchitecture reveals important structural features of cortical areas.

Although induced regional amplification of basal progenitors can cause gyrogenesis, the distinct roles of bIPs and bRGCs in this process remain unclear. In recent studies, no consistent pattern of a basal progenitor response to proliferation has been evident. Knockdown of *Trnp1* induced proliferation of both bRGCs and IPs[163]; FGF2 induced proliferation of IPs only[165]; and overexpression of 4D in ferrets induced proliferation of SVZ progenitors (bIPs and bRGCs were not separately assessed[168]). It is possible that the requirement for different progenitor types in gyrogenesis may vary across stages of development and among species. A reasonable working model of gyrogenesis is that bRGCs primarily expand the cortical plate tangentially, whereas IPs primarily amplify neuron numbers to 'fill in' the cortical layers that have been attenuated by tangential expansion. IPs generate the majority of projection neurons for all cortical layers[15], and they are well suited for this role[14]. The observations that the SVZ, where bRGCs and IPs are located, is thicker at sites of gyrus growth and thinner beneath developing sulci also seem to be consistent with this model[160].

Basal progenitors and the subplate

The basal progenitor mechanism of gyrogenesis seems to be compatible with human gyrogenesis in most cortical regions. During the late stages of neurogenesis, when primary sulci are beginning to appear on the previously smooth fetal cortex, an expanded OSVZ progenitor compartment develops in many species, including humans (reviewed in REF. 5). The OSVZ contains both bRGCs and bIPs and grows thicker under prospective gyri in some regions, such as the fetal occipital lobe. Histological and MRI studies in humans and non-human primates have also documented the rapid growth of the OSVZ during gyrogenesis[20,169,170].

During early gyrogenesis, the subplate, a highly synaptogenic zone in which afferent axons arrive and mix with subplate neurons (also called interstitial cells) to form transient networks, also exhibits accelerated growth[20,162,169,170]. Perturbation of early subplate networks can have profound consequences for cortical development, including gyral patterns[6]. The selective growth of the subplate, a non-progenitor zone, during early gyrogenesis in humans and other species points to the importance of additional mechanisms besides basal progenitor proliferation in gyrogenesis.

Other regulators of gyrogenesis

Gyrogenesis encompasses a sequence of events including neurogenesis, cell migration, afferent innervation, dendrite growth, synaptogenesis and gliogenesis[6]. In this context, basal progenitor proliferation is only the first crucial step in the overall gyrus-building process (that is, differential radial growth of the cerebral wall). In subsequent steps, gyrogenesis also depends critically on afferent fibres from the thalamus and other sources, and axonal interactions between neurons and progenitors. Moreover, gyrus-building is not the only mechanism of gyrogenesis: ventricular surface expansion, pial invagination and meningeal signalling are also important in some cortical regions.

Axons and gyrogenesis. The role of afferent innervation in gyrogenesis was initially discovered by fetal lesion experiments in monkeys, dogs, raccoons and other species (reviewed in REF. 6). Early focal ablation of the monkey developing cortex caused not only reorientation of sulci around the lesion but also distant effects on the development of gyri and sulci in different lobes, including the contralateral hemisphere[6,171]. By contrast, distant effects on gyral patterns did not occur when the cortex was ablated later in development, after axonal connections had been established. Even more remote effects were demonstrated by bilateral enucleation of fetal monkeys, which led to alterations in visual cortex (occipital lobe) gyral patterns, with aberrant sulci on the normally smooth area 17 operculum[13,172,173]. The sulcal changes were accompanied by a reduction in the size of area 17 and by an increase in the number of callosal projections from area 18 (REF. 172). Embryonic thalamic axons may regulate neurogenesis by stimulating the proliferation of cortical progenitor cells, possibly through FGF secretion[174]. Thus, afferent innervation profoundly influences many aspects of cortical development, and the altered gyral patterns may reflect changes in neurogenesis and arealization (FIG. 5). Interestingly, thalamocortical innervation was also recently shown to regulate arealization in mice, including the expression of area-specific molecular markers in the cortex[175,176].

It has also been proposed that axons may influence gyrogenesis by pulling together strongly interconnected regions of the cortex[177]. This 'axon tension' hypothesis seems to be compatible with classic observations that gyral development coincides temporally with afferent innervation and offers the attraction of optimized compact wiring, as the most abundant corticocortical connections would presumably be shortened together. This hypothesis continues to attract theoretical interest[178,179], but little experimental support for it has been forthcoming to date[4,154,180]. Indeed, in mice with gyri induced by FGF2 treatment, no evidence of exuberant axon connections between gyral surfaces was detected[165].

Ventricular surface expansion and gyrogenesis. Cortical surface area is thought to be determined by the number of progenitors in radial units in the embryonic VZ and by the size, shape and neuronal composition of the cortical columns derived from each radial unit[11,13]. In this framework, one obvious way to expand the cortical surface would be to increase the number of apical progenitors and therefore the ventricular surface area. Classic studies downplayed ventricular surface expansion as a factor in gyrogenesis, labelling it a 'passive' and 'mechanical' process for which there was scant evidence[6]. However, more recently, new observations and evidence suggest that ventricular surface expansion is important for the normal development of some human gyri and sulci (especially in the occipital and temporal lobes) and in some neuropathological conditions.

A few gyri in the normal human brain, such as the parahippocampal gyrus, seem to grow primarily by ventricular surface expansion, followed by folding, apposition and fusion of the ventricular surfaces to form the convex gyrus (FIG. 5b). Interestingly, the ventricular recess of the parahippocampal gyrus was noted as a site of high *TRNP1* expression, implying that there is a bias towards radial unit self-amplification in the developing parahippocampal gyrus[163]. A similar sequence of ventricular surface expansion, folding, apposition and fusion occurs in the development of the calcar avis, a macroscopic lobule of cortex that protrudes into the occipital horns[6,181]. This mechanism contrasts with gyrus-building, in which gyri grow outwards from ventricular surfaces that remain open and do not fuse. Ventricular surface expansion seems to be less prevalent than gyrus-building as a mechanism

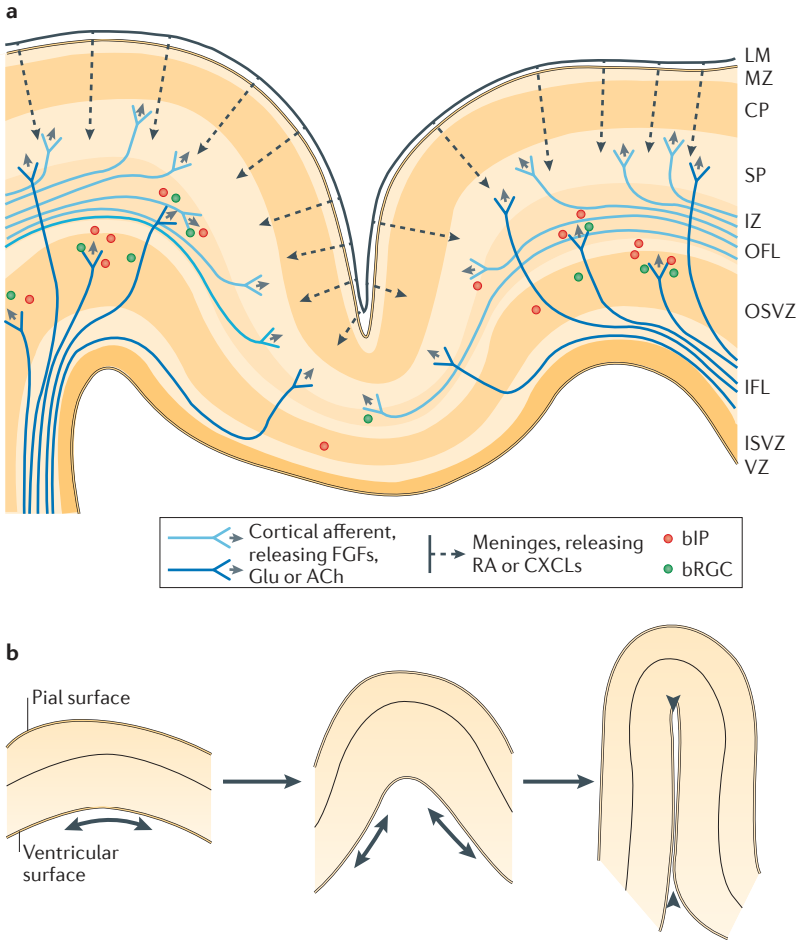


Figure 5 | **Cortical afferent axons and the meninges in gyrogenesis.** **a** | Afferent axons (dark blue and light blue, representing different fibre pathways) arrive in the cortex from many sources (including the thalamus, nucleus basalis, brainstem and contralateral cortex) while neurogenesis is still ongoing. Some axons may release neurotransmitters, such as glutamate (Glu) and acetylcholine (ACh) as well as peptides such as fibroblast growth factors (FGFs); these factors may influence gyrogenesis by regulating basal radial glial cell (bRGC) and basal intermediate progenitor (bIP) proliferation, and by contributing to neuropile growth. Indeed, the crowns of gyri receive more innervation than do sulcal depths. The leptomeninges (LM) (pia–arachnoid mater) produce additional diffusible factors, such as retinoic acid (RA) and chemokines (CXCLs), that can affect progenitor proliferation, cell migration and layer formation. **b** | Ventricular surface growth (arrows) and buckling can lead to obliteration of the enclosed ventricular recess (arrowheads), for example, during formation of the parahippocampal gyrus and some occipital gyri. CP, cortical plate; IFL, inner fibrous layer; ISVZ, inner subventricular zone; IZ, intermediate zone; MZ, marginal zone; OFL, outer fibrous layer; OSVZ, outer subventricular zone; SP, subplate; VZ, ventricular zone.

of gyrogenesis and may occur only in the temporal and occipital lobes, where the skull base forms a relatively resistant surface that might restrict cortical outward growth and cause the cerebral wall to buckle, compared with the cranial vault, which expands as necessary during fetal and neonatal life.

When excessive, ventricular surface expansion may also lead to pathological gyrification, as observed in genetic conditions in both mice and humans. Landmark studies in mice found that the cerebral cortex became remarkably convoluted and thin in mutants with either diminished NSC apoptosis[66] or increased NSC self-amplification[29]. However, such mutant mice did not represent models of normal gyrogenesis because subsequent cortical development was profoundly abnormal.

Pathological hypergyrification as a result of ventricular surface overgrowth in humans may be caused by gain-of-function mutations in *FGFR2* or *FGFR3*, which are associated with Apert syndrome and thanatophoric dysplasia, respectively. Both conditions cause brain malformations that are characterized by significantly increased mass (megalencephaly), cortical hyperconvolution and hippocampal dysgenesis[182,183]. In thanatophoric dysplasia, the inferior occipitotemporal regions are particularly affected: gyrification occurs prematurely and extensive ventricular recesses (known as 'diverticuli') become enclosed within the aberrant cortical folds[182,184]. Mouse models of this condition have been produced and likewise demonstrate selective expansion of occipitotemporal cortex and ventricular surface area but no cortical folding[185].

The meninges in gyrogenesis. The cortical meninges, which are derived from the cranial neural crest, have several critical roles in cerebral cortex development (FIG. 5). These include: maintaining the pial basement membrane; secreting retinoic acid, which causes differentiation of radial unit progenitors (NE cells and RGCs) at the expense of symmetrical expansion; and secreting chemokines, which attract and guide migrating interneurons and Cajal–Retzius cells[186]. Accordingly, defects in the meninges or their interactions with neural tissue often cause complex brain phenotypes, including abnormal gyral development, in humans and mice.

Defects in meningeal function are one cause of 'cobblestone' malformations, which are characterized in humans by pachygyria (also called 'type II lissencephaly') and/or polymicrogyria[187–189] (excessive, small, fused gyri). Cobblestone malformations arise when neural elements herniate through breaches in the pial basement membrane. The pial basement membrane is maintained by meningeal interactions with RGCs, and defects in either element (meningeal or neural) can cause cobblestone malformations, as demonstrated in various mouse models[190,191].

Growth factors and morphogens

FGF signalling has been implicated in regulating cortical growth, patterning and gyrification in mice and humans. The FGF signalling system can seem dauntingly complex owing to the large numbers of ligands (22) and receptors (4), and their promiscuous interactions. Several FGF ligands are expressed in the embryonic cortex or the rostral patterning centre (including FGF2, FGF8, FGF9, FGF10, FGF15, FGF17 and FGF18), together generating unique concentrations and combinations of FGFs at different coordinates in the cortical neuroepithelium. On the receptor side, three FGF receptors (FGFR1–FGFR3) are expressed in dynamic rostrocaudal and mediolateral gradients within the cortical neuroepithelium, such that responsiveness to FGFs also varies positionally in the cortex[30]. The FGF receptors are essential for cortical surface area and volume expansion in mice[192].

Positional variations in FGF ligand and receptor expression seem to influence the effects of FGF signalling perturbations on gyrogenesis. For example, *Fgfr3* is expressed in a low rostral-to-high caudal gradient during early cortical neurogenesis, and strong activating mutations in *FGFR3* (as occur in thanatophoric dysplasia or mouse models) cause occipitotemporal surface area expansion, which is sufficient to cause excessive gyrification in humans[182] but not in mice[185]. The selective effects of FGF2 on expansion and gyrification of the insula and the dorsolateral neocortex in mice following injection on E11.5 should also be interpreted in this context, although the precise basis of this selectivity is not understood yet[165]. FGFs may also mediate the effects of axons on cortical growth and gyrification[174].

The WNT–β-catenin pathway (also known as canonical WNT signalling) is also important in gyrification. Like FGF signalling, WNT signalling involves a multitude of ligands and receptors that may have different effects and interactions, many of which occur in the developing cortex and adjacent signalling centres such as the cortical hem (reviewed in REF. 32). In mice, sustained activation of β-catenin in NE cells and RGCs promotes their self-renewal, thus driving ventricular surface expansion and folding[29,193]. Interestingly, WNT signalling has the opposite effect on IPs, promoting their differentiation into neurons[194]. WNT signalling also regulates patterning of the neocortical primordium and is necessary to induce differentiation and growth of the hippocampus and dentate gyrus[195]. Thus, WNT signalling may contribute to gyrogenesis at multiple points in development, including cortical patterning, radial unit expansion and neuronal differentiation. Moreover, retinoic acid signalling regulates RGC proliferation and ventricular surface area by promoting the production of neurons and IPs from RGCs[196]. Other growth factors and morphogens that regulate cortical development, and that might potentially regulate gyrogenesis, include SHH, bone morphogenetic proteins and IGFs.

Gyral patterns in the human brain

Diverse anomalies of gyrification have been described in the human neuropathology and neuroimaging literature[197]. Some have known genetic or extrinsic aetiologies and are associated with neurological disorders such as intellectual disability and epilepsy. Lissencephaly (smooth brain) is caused by genetic defects in pial–glial interactions that disrupt the brain surface (cobblestone or type II lissencephaly) or by mutations in (mostly)

cytoskeletal-related genes that interfere with cell migration and proliferation (classic or type I lissencephaly). These cases illustrate the importance of cell migration in gyrus-building. Polymicrogyria (too many small gyri) is associated with insults such as viral infection and hypoxia–ischaemia as well as genetic syndromes such as peroxisome disorders; its morphogenesis is poorly understood but involves layer 1 fusion and resorption of meningeal cells[198]. Various other anomalies of gyrification have been associated with single-gene mutations (such as Apert syndrome and thanatophoric dysplasia, as described above) as well as more complex chromosomal disorders; examples include polymicrogyria-like dysgenesis in monosomy 1p36 (REF. 199) and narrowing of the superior temporal gyrus in Down syndrome.

The significance of gyral patterns in the brains of normal and exceptional individuals has been a subject of great interest and controversy (BOX 2). Some evidence suggests that gyral patterns are related to areal boundaries in humans[200]. One clear association links language functions to gyral asymmetries in and around the Sylvian fissure, where language cortices (including Broca's and Wernicke's areas) are located. Typically, language functions are lateralized (dominant) in the left hemisphere, where asymmetries point to differential growth of specific gyral complexes, including "a greater development of the posterior temporal region and of the parietal operculum on the left" (REF. 201) and other morphologic features. Asymmetrical growth of these cortical regions is detected in fetal brains *in vivo* by MRI as early as 20–23 gestational weeks[170]. Asymmetries in the expression of genes such as Lim domain only 4 (*LMO4*) are thought to determine some of these structural asymmetries, beginning in the 12th gestational week or earlier[202].

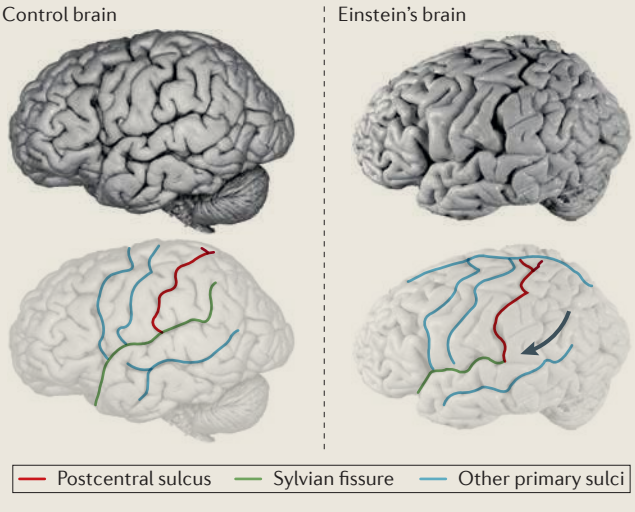
Future directions

Cortical growth and folding occur by the coordinated tangential and radial expansion of the cortex and its subdivisions, which provides a highly malleable framework for evolutionary change[11]. The morphologies of multiple progenitors are characterized in rodent and human cortices, but detailed cellular and molecular features still need to be identified, especially for IPs and bRGCs. Genetic perturbation in specific progenitor populations will help to dissect their distinct contribution to cortical growth. Linkage mapping or exome sequencing in humans will identify more mutations and dysregulation of coding genes and non-coding RNAs that are associated with malformations in cortical growth and gyrogenesis.

Recent progress in understanding cortical progenitor cells has elucidated mechanisms of gyral growth and radial fibre convergence and divergence. What remains unclear is how the regions of prospective gyral growth are defined and differentiated from regions of prospective sulcus formation. Analysis of *TRNP1* expression in the developing human cortex suggests that local differences in basal progenitor proliferation and SVZ growth are foreshadowed by differences in *TRNP1* expression among radial unit progenitors in the VZ[163].

Box 2 | **Unusual gyral morphology of Albert Einstein's brain**

Attempts to link gyral patterns to cognitive functions on an individual basis have been controversial. The most famous case concerns the brain of Albert Einstein, whose talents in visuospatial and mathematical thinking were combined with relatively late development of language and social skills. Analysis of Einstein's gyral patterns revealed significant differences from those in control brains in the size and configuration of gyri around the Sylvian fissure, especially in the parietal lobes[208]. In Einstein's brain (see the figure; left hemisphere shown, with anterior to left), the usual asymmetry of parietal gyri was lacking, and both sides exhibited right-sided morphology: the parietal opercula, defined as the region between the postcentral sulcus and the Sylvian fissure, were absent; the inferior parietal lobules were expanded; and the Sylvian fissure seemed to be truncated (arrow) and continuous with the postcentral sulcus. Considering the known functions of parietal cortex in visuospatial and mathematical thought, and the known links between brain asymmetries and language functions, it was proposed that Einstein's unique gyral patterns were related to his intellectual strengths (and weaknesses). This idea met some resistance, as it was suggested that Einstein's gyral pattern might not be very uncommon after all[209]. A more recent study, including newly released photographs of Einstein's brain, identifies additional uncommon features of his gyral and sulcal morphologies[210]. Links between unusual gyral patterns and cognitive profiles will probably prove difficult to establish conclusively until larger numbers are studied and anatomical variations within the population are better defined. The image of the control brain is reproduced, with permission, from REF. 211 © (1976) Oxford University Press. The image of Einstein's brain is reproduced, with permission, from REF. 208 © (1999) Elsevier.



Monosomy 1p36
A chromosomal deletion syndrome, in which the distal tip of the short arm of chromosome 1 (containing dozens of genes) is deleted. The syndrome is associated with neurological problems, such as epilepsy, and cortical malformations, including polymicrogyria.

Further studies will be necessary to better define how the VZ protomap contributes to gyrogenesis. Equally importantly, the role of afferent innervation in gyrogenesis remains poorly understood at the mechanistic level. The effects of not only thalamocortical axons but also, potentially, cortical efferent axons and their activity on basal progenitor cells[203], and the nature of the interactions between axons and basal progenitors (and other cortical cell types) have hardly been investigated. Finally, further investigations of gyral patterns in relation to cortical areas may eventually disclose links between gyrogenesis and areal formation.

1. Kaas, J. H. The evolution of brains from early mammals to humans. *Wiley Interdiscip. Rev. Cogn. Sci.* **4**, 33–45 (2013).
2. Geschwind, D. H. & Rakic, P. Cortical evolution: judge the brain by its cover. *Neuron* **80**, 633–647 (2013).
3. Striedter, G. *Principles of Brain Evolution* (Sinauer, 2005).
4. Zilles, K., Palomero-Gallagher, N. & Amunts, K. Development of cortical folding during evolution and ontogeny. *Trends Neurosci.* **36**, 275–284 (2013).
5. Kelava, I., Lewitus, E. & Huttner, W. B. The secondary loss of gyrencephaly as an example of evolutionary phenotypical reversal. *Front. Neuroanat.* **7**, 16 (2013).
6. Welker, W. in *Cerebral Cortex* Vol 8B (eds Jones, E. G. & Peters, A.) (Springer, 1990).
7. Rubenstein, J. L., Shimamura, K., Martinez, S. & Puelles, L. Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci.* **21**, 445–477 (1998).
8. Alvarez-Buylla, A. & Temple, S. Stem cells in the developing and adult nervous system. *J. Neurobiol.* **36**, 105–110 (1998).
9. Campbell, K. & Gotz, M. Radial glia: multi-purpose cells for vertebrate brain development. *Trends Neurosci.* **25**, 235–238 (2002).
10. Rakic, P. The radial edifice of cortical architecture: from neuronal silhouettes to genetic engineering. *Brain Res. Rev.* **55**, 204–219 (2007).
11. Rakic, P. Evolution of the neocortex: a perspective from developmental biology. *Nature Rev. Neurosci.* **10**, 724–735 (2009).
12. Gorski, J. A. *et al.* Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J. Neurosci.* **22**, 6309–6314 (2002).
13. Rakic, P. Specification of cerebral cortical areas. *Science* **241**, 170–176 (1988).
14. Pontious, A., Kowalczyk, T., Englund, C. & Hevner, R. F. Role of intermediate progenitor cells in cerebral cortex development. *Dev. Neurosci.* **30**, 24–32 (2008).
15. Kowalczyk, T. *et al.* Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb. Cortex* **19**, 2439–2450 (2009).
The authors demonstrate the morphology and molecular features of IPs that are positioned in the apical regions in the VZ using live-imaging approaches.
16. Hevner, R. F. & Haydar, T. F. The (not necessarily) convoluted role of basal radial glia in cortical neurogenesis. *Cereb. Cortex* **22**, 465–468 (2012).
17. Arnold, S. J. *et al.* The T-box transcription factor Eomes/Tbr2 regulates neurogenesis in the cortical subventricular zone. *Genes Dev.* **22**, 2479–2484 (2008).
18. Sessa, A. *et al.* Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. *Genes Dev.* **24**, 1816–1826 (2010).
19. Sessa, A., Mao, C. A., Hadjantonakis, A. K., Klein, W. H. & Broccoli, V. Tbr2 directs conversion of radial glia into basal precursors and guides neuronal amplification by indirect neurogenesis in the developing neocortex. *Neuron* **60**, 56–69 (2008).
References 17–19 demonstrate that transcription factor TBR2 has a crucial role in specifying and expanding cortical IPs.
20. Smart, I. H., Dehay, C., Giroud, P., Berland, M. & Kennedy, H. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* **12**, 37–53 (2002).
21. Reillo, I. & Borrell, V. Germinal zones in the developing cerebral cortex of ferret: ontogeny, cell cycle kinetics, and diversity of progenitors. *Cereb. Cortex* **22**, 2039–2054 (2012).
22. Fietz, S. A. *et al.* OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nature Neurosci.* **13**, 690–699 (2010).
23. Hansen, D. V., Lui, J. H., Parker, P. R. & Kriegstein, A. R. Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* **464**, 554–561 (2010).
References 22 and 23 demonstrate the existence of mitotically active bRGCs in human and ferret fetal cortices. Using time-lapse microscopy of living slices, the authors show that the bRGCs produce IPs and neurons, similarly to aRGCs.
24. Shitamukai, A., Konno, D. & Matsuzaki, F. Oblique radial glial divisions in the developing mouse neocortex induce self-renewing progenitors outside the germinal zone that resemble primate outer subventricular zone progenitors. *J. Neurosci.* **31**, 3683–3695 (2011).
25. Wang, X., Tsai, J. W., LaMonica, B. & Kriegstein, A. R. A new subtype of progenitor cell in the mouse embryonic neocortex. *Nature Neurosci.* **14**, 555–561 (2011).
26. Lui, J. H., Hansen, D. V. & Kriegstein, A. R. Development and evolution of the human neocortex. *Cell* **146**, 18–36 (2011).
27. Molnar, Z. & Clowry, G. Cerebral cortical development in rodents and primates. *Prog. Brain Res.* **195**, 45–70 (2012).
The authors of references 26 and 27 scholarly and systematically summarize the evolution of the rodent, primate and human cerebral cortices. They review morphological and molecular features of distinct progenitors in the neocortex.
28. Garcia-Moreno, F., Vasistha, N. A., Trevia, N., Bourne, J. A. & Molnar, Z. Compartmentalization of cerebral cortical germinal zones in a lissencephalic primate and gyrencephalic rodent. *Cereb. Cortex* **22**, 482–492 (2012).
29. Chenn, A. & Walsh, C. A. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* **297**, 365–369 (2002).
These authors demonstrate that expression of continuously activated β-catenin causes expansion of cortical NPs and folding of the cortex using mouse genetic tools. This paper suggests that cell cycle regulation of NPs is crucial for cortical growth and folding.
30. Iwata, T. & Hevner, R. F. Fibroblast growth factor signaling in development of the cerebral cortex. *Dev. Growth Differ.* **51**, 299–323 (2009).
31. Lehtinen, M. K. *et al.* The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. *Neuron* **69**, 893–905 (2011).
32. Harrison-Uy, S. J. & Pleasure, S. J. Wnt signaling and forebrain development. *Cold Spring Harb. Perspect. Biol.* **4**, a008094 (2012).
33. Ross, M. E. & Walsh, C. A. Human brain malformations and their lessons for neuronal migration. *Annu. Rev. Neurosci.* **24**, 1041–1070 (2001).
34. Walsh, C. A. Genetic malformations of the human cerebral cortex. *Neuron* **23**, 19–29 (1999).
35. Rakic, P. & Sidman, R. L. Supravital DNA synthesis in the developing human and mouse brain. *J. Neuropathol. Exp. Neurol.* **27**, 246–276 (1968).
36. Taverna, E. & Huttner, W. B. Neural progenitor nuclei IN motion. *Neuron* **67**, 906–914 (2010).
37. Reiner, O., Sapir, T. & Gerlitz, G. Interkinetic nuclear movement in the ventricular zone of the cortex. *J. Mol. Neurosci.* **46**, 516–526 (2012).
38. Liu, X., Hashimoto-Torii, K., Torii, M., Ding, C. & Rakic, P. Gap junctions/hemichannels modulate interkinetic nuclear migration in the forebrain precursors. *J. Neurosci.* **30**, 4197–4209 (2010).
39. Tamai, H. *et al.* Pax6 transcription factor is required for the interkinetic nuclear movement of neuroepithelial cells. *Genes Cells* **12**, 983–996 (2007).
40. Xie, Z. *et al.* Cep120 and TACCs control interkinetic nuclear migration and the neural progenitor pool. *Neuron* **56**, 79–93 (2007).
41. Ge, X., Frank, C. L., Calderon de Anda, F. & Tsai, L. H. Hook3 interacts with PCM1 to regulate pericentriolar material assembly and the timing of neurogenesis. *Neuron* **65**, 191–203 (2010).
42. Yang, Y. T., Wang, C. L. & Van Aelst, L. DOCK7 interacts with TACC3 to regulate interkinetic nuclear migration and cortical neurogenesis. *Nature Neurosci.* **15**, 1201–1210 (2012).
43. Zhang, X. *et al.* SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron* **64**, 173–187 (2009).
References 40–43 demonstrate that centrosomal proteins and microtubule-binding proteins control NP proliferation by affecting INM.
44. Estivill-Torrus, G., Pearson, H., van Heyningen, V., Price, D. J. & Rashbass, P. Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. *Development* **129**, 455–466 (2002).
45. Mi, D. *et al.* Pax6 exerts regional control of cortical progenitor proliferation via direct repression of Cdk6 and hypophosphorylation of pRb. *Neuron* **78**, 269–284 (2013).
46. Lian, G. *et al.* Filamin A regulates neural progenitor proliferation and cortical size through Wee1-dependent Cdk1 phosphorylation. *J. Neurosci.* **32**, 7672–7684 (2012).
47. Petersen, P. H., Zou, K., Hwang, J. K., Jan, Y. N. & Zhong, W. Progenitor cell maintenance requires numb and numblike during mouse neurogenesis. *Nature* **419**, 929–934 (2002).
48. Li, H. S. *et al.* Inactivation of Numb and Numblike in embryonic dorsal forebrain impairs neurogenesis and disrupts cortical morphogenesis. *Neuron* **40**, 1105–1118 (2003).
49. Yang, Y. J. *et al.* Microcephaly gene links trithorax and REST/NRSF to control neural stem cell proliferation and differentiation. *Cell* **151**, 1097–1112 (2012).
50. Chen, L., Melendez, J., Campbell, K., Kuan, C. Y. & Zheng, Y. Rac1 deficiency in the forebrain results in neural progenitor reduction and microcephaly. *Dev. Biol.* **325**, 162–170 (2009).
51. Leone, D. P. Srinivasan, K., Brakebusch, C. & McConnell, S. K. The rho GTPase Rac1 is required for proliferation and survival of progenitors in the developing forebrain. *Dev. Neurobiol.* **70**, 659–678 (2010).
52. Glickstein, S. B., Monaghan, J. A., Koeller, H. B., Jones, T. K. & Ross, M. E. Cyclin D2 is critical for intermediate progenitor cell proliferation in the embryonic cortex. *J. Neurosci.* **29**, 9614–9624 (2009).
53. Nigg, E. A. & Raff, J. W. Centrioles, centrosomes, and cilia in health and disease. *Cell* **139**, 663–678 (2009).
54. Lee, J. E. & Gleeson, J. G. Cilia in the nervous system: linking cilia function and neurodevelopmental disorders. *Curr. Opin. Neurol.* **24**, 98–105 (2011).
55. Louvi, A. & Grove, E. A. Cilia in the CNS: the quiet organelle claims center stage. *Neuron* **69**, 1046–1060 (2011).
56. Willaredt, M. A., Tasouri, E. & Tucker, K. L. Primary cilia and forebrain development. *Mech. Dev.* **130**, 373–380 (2013).
57. Higginbotham, H. *et al.* Arl13b-regulated cilia activities are essential for polarized radial glial scaffold formation. *Nature Neurosci.* **16**, 1000–1007 (2013).
58. Tucker, R. W., Pardee, A. B. & Fujiwara, K. Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells. *Cell* **17**, 527–535 (1979).
59. Gate, D., Danielpour, M., Levy, R., Breunig, J. J. & Town, T. Basic biology and mechanisms of neural ciliogenesis and the B9 family. *Mol. Neurobiol.* **45**, 564–570 (2012).

60. Li, A. *et al.* Ciliary transition zone activation of phosphorylated Tctex-1 controls ciliary resorption, S-phase entry and fate of neural progenitors. *Nature Cell Biol.* **13**, 402–411 (2011).
61. Yeh, C. *et al.* IGF-1 activates a cilium-localized noncanonical Gβγ signaling pathway that regulates cell-cycle progression. *Dev. Cell* **26**, 358–368 (2013).
62. Paridaen, J. T., Wilsch-Brauninger, M. & Huttner, W. B. Asymmetric inheritance of centrosome-associated primary cilium membrane directs ciliogenesis after cell division. *Cell* **155**, 333–344 (2013).
These authors demonstrate that the cilium membrane is asymmetrically inherited during divisions of apical progenitors, suggesting a new mechanism for the regulation of symmetrical versus asymmetrical divisions of cortical NPs.
63. Banizs, B. *et al.* Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development* **132**, 5329–5339 (2005).
64. Blaschke, A. J., Staley, K. & Chun, J. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. *Development* **122**, 1165–1174 (1996).
65. Thomaidou, D., Mione, M. C., Cavanagh, J. F. & Parnavelas, J. G. Apoptosis and its relation to the cell cycle in the developing cerebral cortex. *J. Neurosci.* **17**, 1075–1085 (1997).
66. Haydar, T. F., Kuan, C. Y., Flavell, R. A. & Rakic, P. The role of cell death in regulating the size and shape of the mammalian forebrain. *Cereb. Cortex* **9**, 621–626 (1999).
67. Berger, J. *et al.* Conditional activation of Pax6 in the developing cortex of transgenic mice causes progenitor apoptosis. *Development* **134**, 1311–1322 (2007).
68. Yang, X. *et al.* Notch activation induces apoptosis in neural progenitor cells through a p53-dependent pathway. *Dev. Biol.* **269**, 81–94 (2004).
69. Pulvers, J. N. & Huttner, W. B. Brca1 is required for embryonic development of the mouse cerebral cortex to normal size by preventing apoptosis of early neural progenitors. *Development* **136**, 1859–1868 (2009).
70. Sii-Felice, K. *et al.* Fanconi DNA repair pathway is required for survival and long-term maintenance of neural progenitors. *EMBO J.* **27**, 770–781 (2008).
71. Depaepe, V. *et al.* Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. *Nature* **435**, 1244–1250 (2005).
72. Kuida, K. *et al.* Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* **94**, 325–337 (1998).
73. Kuida, K. *et al.* Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* **384**, 368–372 (1996).
74. Kingsbury, M. A., Rehen, S. K., Contos, J. J., Higgins, C. M. & Chun, J. Non-proliferative effects of lysophosphatidic acid enhance cortical growth and folding. *Nature Neurosci.* **6**, 1292–1299 (2003).
75. De Pietri Tonelli, D. *et al.* miRNAs are essential for survival and differentiation of newborn neurons but not for expansion of neural progenitors during early neurogenesis in the mouse embryonic neocortex. *Development* **135**, 3911–3921 (2008).
76. Kawase-Koga, Y., Otaegi, G. & Sun, T. Different timings of dicer deletion affect neurogenesis and gliogenesis in the developing mouse central nervous system. *Dev. Dyn.* **238**, 2800–2812 (2009).
77. Nowakowski, T. J., Mysiak, K. S., Pratt, T. & Price, D. J. Functional dicer is necessary for appropriate specification of radial glia during early development of mouse telencephalon. *PLoS ONE* **6**, e23013 (2011).
78. Kawase-Koga, Y. *et al.* RNAase-III enzyme Dicer maintains signaling pathways for differentiation and survival in mouse cortical neural stem cells. *J. Cell Sci.* **123**, 586–594 (2010).
79. Saurat, N., Andersson, T., Vasistha, N. A., Molnar, Z. & Livesey, F. J. Dicer is required for neural stem cell multipotency and lineage progression during cerebral cortex development. *Neural Dev.* **8**, 14 (2013).
80. Davis, T. H. *et al.* Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. *J. Neurosci.* **28**, 4322–4330 (2008).
81. Hong, J., Zhang, H., Kawase-Koga, Y. & Sun, T. MicroRNA function is required for neurite outgrowth of mature neurons in the mouse postnatal cerebral cortex. *Front. Cell. Neurosci.* **7**, 151 (2013).
82. Tanzer, A. & Stadler, P. F. Molecular evolution of a microRNA cluster. *J. Mol. Biol.* **339**, 327–335 (2004).
83. Bian, S. *et al.* MicroRNA cluster miR-17-92 regulates neural stem cell expansion and transition to intermediate progenitors in the developing mouse neocortex. *Cell Rep.* **3**, 1398–1406 (2013).
The authors demonstrate that proper expression of miR-17-92 is required for the expansion of RGCs and for their transition to IPs using mouse genetic tools. They further show that essential target genes *Pten* and *Tbr2* are regulated by miR-17-92 using miRNA sponges.
84. Groszer, M. *et al.* Negative regulation of neural stem/progenitor cell proliferation by the *Pten* tumor suppressor gene *in vivo*. *Science* **294**, 2186–2189 (2001).
85. Nowakowski, T. J. *et al.* MicroRNA-92b regulates the development of intermediate cortical progenitors in embryonic mouse brain. *Proc. Natl. Acad. Sci. USA* **110**, 7056–7061 (2013).
86. Zhao, C., Sun, G., Li, S. & Shi, Y. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. *Nature Struct. Mol. Biol.* **16**, 365–371 (2009).
87. Gaughwin, P., Ciesla, M., Yang, H., Lim, B. & Brundin, P. Stage-specific modulation of cortical neuronal development by *Mmu-miR-134*. *Cereb. Cortex* **21**, 1857–1869 (2011).
88. Vo, N. *et al.* A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Proc. Natl. Acad. Sci. USA* **102**, 16426–16431 (2005).
89. Yu, J. Y., Chung, K. H., Deo, M., Thompson, R. C. & Turner, D. L. MicroRNA miR-124 regulates neurite outgrowth during neuronal differentiation. *Exp. Cell Res.* **314**, 2618–2633 (2008).
90. Franke, K. *et al.* miR-124-regulated RhoG reduces neuronal process complexity via ELMO/Dock180/Rac1 and Cdc42 signalling. *EMBO J.* **31**, 2908–2921 (2012).
91. Clovis, Y. M., Enard, W., Marinaro, F., Huttner, W. B. & De Pietri Tonelli, D. Convergent repression of Foxp2 3'UTR by miR-9 and miR-132 in embryonic mouse neocortex: implications for radial migration of neurons. *Development* **139**, 3332–3342 (2012).
92. Bian, S., Xu, T. L. & Sun, T. Tuning the cell fate of neurons and glia by microRNAs. *Curr. Opin. Neurobiol.* **23**, 928–934 (2013).
93. de Pontual, L. *et al.* Germline deletion of the miR-17 approximately 92 cluster causes skeletal and growth defects in humans. *Nature Genet.* **43**, 1026–1030 (2011).
94. Fietz, S. A. & Huttner, W. B. Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. *Curr. Opin. Neurobiol.* **21**, 23–35 (2011).
95. Lancaster, M. A. & Knoblich, J. A. Spindle orientation in mammalian cerebral cortical development. *Curr. Opin. Neurobiol.* **22**, 737–746 (2012).
96. Megraw, T. L., Sharkey, J. T. & Nowakowski, R. S. Cdk5rap2 exposes the centrosomal root of microcephaly syndromes. *Trends Cell Biol.* **21**, 470–480 (2011).
97. Chenn, A. & McConnell, S. K. Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. *Cell* **82**, 631–641 (1995).
98. Noctor, S. C., Martinez-Cerdeno, V., Ivic, L. & Kriegstein, A. R. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature Neurosci.* **7**, 136–144 (2004).
99. Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. & Kriegstein, A. R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**, 714–720 (2001).
100. Wang, Q. & Margolis, B. Apical junctional complexes and cell polarity. *Kidney Int.* **72**, 1448–1458 (2007).
101. Insolera, R., Chen, S. & Shi, S. H. Par proteins and neuronal polarity. *Dev. Neurobiol.* **71**, 483–494 (2011).
102. Costa, M. R., Wen, G., Lepier, A., Schroeder, T. & Gotz, M. Par-complex proteins promote proliferative progenitor divisions in the developing mouse cerebral cortex. *Development* **135**, 11–22 (2008).
103. Bultje, R. S. *et al.* Mammalian Par3 regulates progenitor cell asymmetric division via notch signaling in the developing neocortex. *Neuron* **63**, 189–202 (2009).
104. Gaiano, N., Nye, J. S. & Fishell, G. Radial glial identity is promoted by Notch1 signaling in the murine forebrain. *Neuron* **26**, 395–404 (2000).
105. Cappello, S. *et al.* The Rho-GTPase cdc42 regulates neural progenitor fate at the apical surface. *Nature Neurosci.* **9**, 1099–1107 (2006).
106. Konno, D. *et al.* Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. *Nature Cell Biol.* **10**, 93–101 (2008).
107. Zheng, Z. *et al.* LGN regulates mitotic spindle orientation during epithelial morphogenesis. *J. Cell Biol.* **189**, 275–288 (2010).
108. Postiglione, M. P. *et al.* Mouse inscuteable induces apical-basal spindle orientation to facilitate intermediate progenitor generation in the developing neocortex. *Neuron* **72**, 269–284 (2011).
109. Sakai, D., Dixon, J., Dixon, M. J. & Trainor, P. A. Mammalian neurogenesis requires Treacle-Plk1 for precise control of spindle orientation, mitotic progression, and maintenance of neural progenitor cells. *PLoS Genet.* **8**, e1002566 (2012).
110. Feng, Y. & Walsh, C. A. Mitotic spindle regulation by Nde1 controls cerebral cortical size. *Neuron* **44**, 279–293 (2004).
111. Yingling, J. *et al.* Neuroepithelial stem cell proliferation requires LIS1 for precise spindle orientation and symmetric division. *Cell* **132**, 474–486 (2008).
112. Srinivasan, K. *et al.* MALS-3 regulates polarity and early neurogenesis in the developing cerebral cortex. *Development* **135**, 1781–1790 (2008).
113. Asami, M. *et al.* The role of Pax6 in regulating the orientation and mode of cell division of progenitors in the mouse cerebral cortex. *Development* **138**, 5067–5078 (2011).
114. Kusek, G. *et al.* Asymmetric segregation of the double-stranded RNA binding protein Staufen2 during mammalian neural stem cell divisions promotes lineage progression. *Cell Stem Cell* **11**, 505–516 (2012).
115. Sanada, K. & Tsai, L. H. G protein βγ subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. *Cell* **122**, 119–131 (2005).
116. Xie, Y., Juschke, C., Esk, C., Hirotsune, S. & Knoblich, J. A. The phosphatase PP4c controls spindle orientation to maintain proliferative symmetric divisions in the developing neocortex. *Neuron* **79**, 254–265 (2013).
117. Noctor, S. C., Martinez-Cerdeno, V. & Kriegstein, A. R. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. *J. Comp. Neurol.* **508**, 28–44 (2008).
118. Smart, I. H. Proliferative characteristics of the ependymal layer during the early development of the mouse neocortex: a pilot study based on recording the number, location and plane of cleavage of mitotic figures. *J. Anat.* **116**, 67–91 (1973).
119. Kosodo, Y. *et al.* Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. *EMBO J.* **23**, 2314–2324 (2004).
120. Mochida, G. H. & Walsh, C. A. Molecular genetics of human microcephaly. *Curr. Opin. Neurol.* **14**, 151–156 (2001).
121. Woods, C. G., Bond, J. & Enard, W. Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings. *Am. J. Hum. Genet.* **76**, 717–728 (2005).
122. Jackson, A. P. *et al.* Identification of microcephalin, a protein implicated in determining the size of the human brain. *Am. J. Hum. Genet.* **71**, 136–142 (2002).
123. Rai, R. *et al.* BRIT1 regulates early DNA damage response, chromosomal integrity, and cancer. *Cancer Cell* **10**, 145–157 (2006).
124. Gruber, R. *et al.* MCPH1 regulates the neuroprogenitor division mode by coupling the centrosomal cycle with mitotic entry through the Chk1–Cdc25 pathway. *Nature Cell Biol.* **13**, 1325–1334 (2011).
125. Bond, J. *et al.* A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. *Nature Genet.* **37**, 353–355 (2005).
126. Barrera, J. A. *et al.* CDK5RAP2 regulates centriole engagement and cohesion in mice. *Dev. Cell* **18**, 913–926 (2010).
127. Lizarraga, S. B. *et al.* Cdk5rap2 regulates centrosome function and chromosome segregation in neuronal progenitors. *Development* **137**, 1907–1917 (2010).
References 124, 126 and 127 demonstrate molecular mechanisms of dysregulation of the centrosome cycle caused by altered expression of MCPH1 and CDK5RAP2 in mice, indicating the genetic basis of microcephaly in humans.

128. Bond, J. *et al.* Protein-truncating mutations in *ASPM* cause variable reduction in brain size. *Am. J. Hum. Genet.* **73**, 1170–1177 (2003).
129. Bond, J. *et al. ASPM* is a major determinant of cerebral cortical size. *Nature Genet.* **32**, 316–320 (2002).
130. Fish, J. L., Kosodo, Y., Enard, W., Paabo, S. & Huttner, W. B. Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells. *Proc. Natl. Acad. Sci. USA* **103**, 10438–10443 (2006).
131. Pulvers, J. N. *et al.* Mutations in mouse *Aspm* (abnormal spindle-like microcephaly associated) cause not only microcephaly but also major defects in the germline. *Proc. Natl. Acad. Sci. USA* **107**, 16595–16600 (2010).
132. Bilguvar, K. *et al.* Whole-exome sequencing identifies recessive *WDR62* mutations in severe brain malformations. *Nature* **467**, 207–210 (2010).
133. Yu, T. W. *et al.* Mutations in *WDR62*, encoding a centrosome-associated protein, cause microcephaly with simplified gyri and abnormal cortical architecture. *Nature Genet.* **42**, 1015–1020 (2010).
134. Nicholas, A. K. *et al. WDR62* is associated with the spindle pole and is mutated in human microcephaly. *Nature Genet.* **42**, 1010–1014 (2010).
135. Bogoyevitch, M. A. *et al.* WD40-repeat protein 62 is a JNK-phosphorylated spindle pole protein required for spindle maintenance and timely mitotic progression. *J. Cell Sci.* **125**, 5096–5109 (2012).
136. Kaindl, A. M. *et al.* Many roads lead to primary autosomal recessive microcephaly. *Prog. Neurobiol.* **90**, 363–383 (2010).
137. Wang, X. *et al.* Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. *Nature* **461**, 947–955 (2009).
138. Riviere, J. B. *et al. De novo* germline and postzygotic mutations in *AKT3*, *PIK3R2* and *PIK3CA* cause a spectrum of related megalencephaly syndromes. *Nature Genet.* **44**, 934–940 (2012).
139. Mirzaa, G. M., Riviere, J. B. & Dobyns, W. B. Megalencephaly syndromes and activating mutations in the PI3K–AKT pathway: MPPH and MCAP. *Am. J. Med. Genet. C Semin. Med. Genet.* **163**, 122–130 (2013).
140. Boland, E. *et al.* Mapping of deletion and translocation breakpoints in 1q44 implicates the serine/threonine kinase AKT3 in postnatal microcephaly and agenesis of the corpus callosum. *Am. J. Hum. Genet.* **81**, 292–303 (2007).
141. Lee, J. H. *et al. De novo* somatic mutations in components of the PI3K–AKT3–mTOR pathway cause hemimegalencephaly. *Nature Genet.* **44**, 941–945 (2012).
142. Poduri, A. *et al.* Somatic activation of *AKT3* causes hemispheric developmental brain malformations. *Neuron* **74**, 41–48 (2012).
References 138, 141 and 142 demonstrate the crucial role of the PI3K–AKT3 pathway in controlling cortical growth and megalencephaly using linkage mapping and exome sequencing.
143. DiLiberti, J. H. Inherited macrocephaly-hamartoma syndromes. *Am. J. Med. Genet.* **79**, 284–290 (1998).
144. Song, M. S., Salmena, L. & Pandolfi, P. P. The functions and regulation of the PTEN tumour suppressor. *Nature Rev. Mol. Cell Biol.* **13**, 283–296 (2012).
145. Groszer, M. *et al.* PTEN negatively regulates neural stem cell self-renewal by modulating G0-G1 cell cycle entry. *Proc. Natl. Acad. Sci. USA* **103**, 111–116 (2006).
146. Klein, S., Sharifi-Hannauer, P. & Martinez-Agosto, J. A. Macrocephaly as a clinical indicator of genetic subtypes in autism. *Autism Res.* **6**, 51–56 (2013).
147. O'Roak, B. J. *et al.* Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* **338**, 1619–1622 (2012).
148. Zhou, J. & Parada, L. F. PTEN signaling in autism spectrum disorders. *Curr. Opin. Neurobiol.* **22**, 873–879 (2012).
149. Kwon, C. H. *et al.* Pten regulates neuronal arborization and social interaction in mice. *Neuron* **50**, 377–388 (2006).
150. Lange, C., Huttner, W. B. & Calegari, F. Cdk4/cyclinD1 overexpression in neural stem cells shortens G1, delays neurogenesis, and promotes the generation and expansion of basal progenitors. *Cell Stem Cell* **5**, 320–331 (2009).
151. Mairet-Coello, G. *et al.* $p57^{KIP2}$ regulates radial glia and intermediate precursor cell cycle dynamics and lower layer neurogenesis in developing cerebral cortex. *Development* **139**, 475–487 (2012).
152. Sahara, S. & O'Leary, D. D. Fgf10 regulates transition period of cortical stem cell differentiation to radial glia controlling generation of neurons and basal progenitors. *Neuron* **63**, 48–62 (2009).
153. Borrell, V. & Reillo, I. Emerging roles of neural stem cells in cerebral cortex development and evolution. *Dev. Neurobiol.* **72**, 955–971 (2012).
154. Lewitus, E., Kelava, I. & Huttner, W. B. Conical expansion of the outer subventricular zone and the role of neocortical folding in evolution and development. *Front. Hum. Neurosci.* **7**, 424 (2013).
155. O'Leary, M. A. *et al.* The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* **339**, 662–667 (2013).
156. Kelava, I. *et al.* Abundant occurrence of basal radial glia in the subventricular zone of embryonic neocortex of a lissencephalic primate, the common marmoset *Callithrix jacchus*. *Cereb. Cortex* **22**, 469–481 (2012).
157. Reep, R. L. & O'Shea, T. J. Regional brain morphometry and lissencephaly in the Sirenia. *Brain Behav. Evol.* **35**, 185–194 (1990).
158. Narr, K. L. *et al.* Mapping cortical thickness and gray matter concentration in first episode schizophrenia. *Cereb. Cortex* **15**, 708–719 (2005).
159. Lerch, J. P. *et al.* Cortical thickness measured from MRI in the YAC128 mouse model of Huntington's disease. *Neuroimage* **41**, 243–251 (2008).
160. Kriegstein, A., Noctor, S. & Martinez-Cerdeno, V. Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. *Nature Rev. Neurosci.* **7**, 883–890 (2006).
161. Rakic, P. Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* **145**, 61–83 (1972).
162. Smart, I. H. & McSherry, G. M. Gyrus formation in the cerebral cortex of the ferret. II. Description of the internal histological changes. *J. Anat.* **147**, 27–43 (1986).
163. Stahl, R. *et al.* Trnp1 regulates expansion and folding of the mammalian cerebral cortex by control of radial glial fate. *Cell* **153**, 535–549 (2013).
This paper shows that gyri can be induced in the mouse cortex by focal knockdown of *Trnp1*, a negative transcriptional regulator of basal progenitor genesis. Regional variations of *TRNP1* expression were also found to correlate with prospective gyri and sulci in fetal human cortex.
164. Pinto, L. *et al.* Prospective isolation of functionally distinct radial glial subtypes—lineage and transcriptome analysis. *Mol. Cell. Neurosci.* **38**, 15–42 (2008).
165. Rash, B. G., Tomasi, S., Lim, H. D., Suh, C. Y. & Vaccarino, F. M. Cortical gyrification induced by fibroblast growth factor 2 in the mouse brain. *J. Neurosci.* **33**, 10802–10814 (2013).
This paper demonstrates that gyri with a well-formed, six-layered cortex could be induced in the mouse cortex by early exposure to increased expression of FGF2 in the ventricles. The formation of new gyri correlates with increased production of TBR2-expressing IPs.
166. Kreiner, J. Fissural cortex in the brain of the mouse. *Acta. Anat.* **86**, 23–33 (1973).
167. Vaccarino, F. M. *et al.* Changes in cerebral cortex size are governed by fibroblast growth factor during embryogenesis. *Nature Neurosci.* **2**, 246–253 (1999).
168. Nonaka-Kinoshita, M. *et al.* Regulation of cerebral cortex size and folding by expansion of basal progenitors. *EMBO J.* **32**, 1817–1828 (2013).
This paper shows that the pattern of gyri and sulci in ferrets could be altered, and made more complex, by local amplification of basal progenitor genesis. The increased production of basal progenitors was induced by overexpression of CDK4 and cyclin D1.
169. Kostovic, I. & Rakic, P. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J. Comp. Neurol.* **297**, 441–470 (1990).
170. Rajagopalan, V. *et al.* Local tissue growth patterns underlying normal fetal human brain gyrification quantified *in utero*. *J. Neurosci.* **31**, 2878–2887 (2011).
This paper shows that zones of the human cerebral cortex undergo differential growth during gyrus formation, with greatest growth in the OSVZ, IZ and SP. The data were collected by MRI imaging of live foetuses of different ages.
171. Goldman, P. S. & Galkin, T. W. Prenatal removal of frontal association cortex in the fetal rhesus monkey: anatomical and functional consequences in postnatal life. *Brain Res.* **152**, 451–485 (1978).
172. Dehay, C., Horsburgh, G., Berland, M., Killackey, H. & Kennedy, H. Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input. *Nature* **337**, 265–267 (1989).
173. Dehay, C., Giroud, P., Berland, M., Killackey, H. & Kennedy, H. Contribution of thalamic input to the specification of cytoarchitectonic cortical fields in the primate: effects of bilateral enucleation in the fetal monkey on the boundaries, dimensions, and gyrification of striate and extrastriate cortex. *J. Comp. Neurol.* **367**, 70–89 (1996).
174. Dehay, C., Savatier, P., Cortay, V. & Kennedy, H. Cell-cycle kinetics of neocortical precursors are influenced by embryonic thalamic axons. *J. Neurosci.* **21**, 201–214 (2001).
175. Vue, T. Y. *et al.* Thalamic control of neocortical area formation in mice. *J. Neurosci.* **33**, 8442–8453 (2013).
176. Chou, S. J. *et al.* Geniculocortical input drives genetic distinctions between primary and higher-order visual areas. *Science* **340**, 1239–1242 (2013).
177. Van Essen, D. C. A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature* **385**, 313–318 (1997).
178. Hilgetag, C. C. & Barbas, H. Role of mechanical factors in the morphology of the primate cerebral cortex. *PLoS Comput. Biol.* **2**, e22 (2006).
179. Mota, B. & Herculano-Houzel, S. How the cortex gets its folds: an inside-out, connectivity-driven model for the scaling of mammalian cortical folding. *Front. Neuroanat.* **6**, 3 (2012).
180. Ronan, L. *et al.* Differential tangential expansion as a mechanism for cortical gyrification. *Cereb. Cortex* http://dx.doi.org/10.1093/cercor/bht082 (2013).
181. Gross, C. G. Huxley versus Owen: the hippocampus minor and evolution. *Trends Neurosci.* **16**, 493–498 (1993).
182. Hevner, R. F. The cerebral cortex malformation in thanatophoric dysplasia: neuropathology and pathogenesis. *Acta Neuropathol.* **110**, 208–221 (2005).
This paper shows that aberrant occipitotemporal gyri in thanatophoric dysplasia, a disorder caused by activating mutations in *FGFR3*, are produced by tangential expansion of the VZ early in cortical development, before gestational week 18. The excessive growth also causes megalencephaly.
183. Cohen, M. M. Jr & Kreiborg, S. The central nervous system in the Apert syndrome. *Am. J. Med. Genet.* **35**, 36–45 (1990).
184. Yamaguchi, K. & Honma, K. Autopsy case of thanatophoric dysplasia: observations on the serial sections of the brain. *Neuropathology* **21**, 222–228 (2001).
185. Thomson, R. E. *et al.* Fgf receptor 3 activation promotes selective growth and expansion of occipitotemporal cortex. *Neural Dev.* **4**, 4 (2009).
This paper shows in mice that an activating mutation in *Fgfr3*, which is identical to the mutation in humans with thanatophoric dysplasia, causes selective tangential expansion of the occipitotemporal cortex, thus mimicking the human condition. In mice, however, the overgrowth is not sufficient to induce the formation of new gyri and sulci.
186. Siegenthaler, J. A. & Pleasure, S. J. We have got you 'covered': how the meninges control brain development. *Curr. Opin. Genet. Dev.* **21**, 249–255 (2011).
187. Bahi-Buisson, N. *et al.* GPR56-related bilateral frontoparietal polymicrogyria: further evidence for an overlap with the cobblestone complex. *Brain* **133**, 3194–3209 (2010).
188. Devisme, L. *et al.* Cobblestone lissencephaly: neuropathological subtypes and correlations with genes of dystroglycanopathies. *Brain* **135**, 469–482 (2012).
189. Radmanesh, F. *et al.* Mutations in *LAMB1* cause cobblestone brain malformation without muscular or ocular abnormalities. *Am. J. Hum. Genet.* **92**, 468–474 (2013).
190. Myshrall, T. D. *et al.* Dystroglycan on radial glia end feet is required for pial basement membrane integrity and columnar organization of the developing cerebral cortex. *J. Neuropathol. Exp. Neurol.* **71**, 1047–1063 (2012).
191. Zarbalis, K. *et al.* Cortical dysplasia and skull defects in mice with a *Foxc1* allele reveal the role of meningeal differentiation in regulating cortical development. *Proc. Natl. Acad. Sci. USA* **104**, 14002–14007 (2007).

192. Rash, B. G., Lim, H. D., Breunig, J. J. & Vaccarino, F. M. FGF signaling expands embryonic cortical surface area by regulating Notch-dependent neurogenesis. *J. Neurosci.* **31**, 15604–15617 (2011).
193. Wrobel, C. N., Mutch, C. A., Swaminathan, S., Taketo, M. M. & Chenn, A. Persistent expression of stabilized β-catenin delays maturation of radial glial cells into intermediate progenitors. *Dev. Biol.* **309**, 285–297 (2007).
194. Munji, R. N., Choe, Y., Li, G., Siegenthaler, J. A. & Pleasure, S. J. Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors. *J. Neurosci.* **31**, 1676–1687 (2011).
195. Lee, S. M., Tole, S., Grove, E. & McMahon, A. P. A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* **127**, 457–467 (2000).
196. Siegenthaler, J. A. *et al.* Retinoic acid from the meninges regulates cortical neuron generation. *Cell* **139**, 597–609 (2009).
This paper shows that the meninges regulate cortical development by producing retinoic acid. This is one of several mechanisms by which the meninges regulate cortical growth and, ultimately, gyrification.
197. Aronica, E., Becker, A. J. & Spreafico, R. Malformations of cortical development. *Brain Pathol.* **22**, 380–401 (2012).
198. Judkins, A. R., Martinez, D., Ferreira, P., Dobyns, W. B. & Golden, J. A. Polymicrogyria includes fusion of the molecular layer and decreased neuronal populations but normal cortical laminar organization. *J. Neuropathol. Exp. Neurol.* **70**, 438–443 (2011).
199. Shiba, N. Neuropathology of brain and spinal malformations in a case of monosomy 1p36. *Acta Neuropathol. Commun.* **1**, 45 (2013).
200. Fischl, B. *et al.* Cortical folding patterns and predicting cytoarchitecture. *Cereb. Cortex* **18**, 1973–1980 (2008).
201. Galaburda, A. M. & Geschwind, N. Anatomical asymmetries in the adult and developing brain and their implications for function. *Adv. Pediatr.* **28**, 271–292 (1981).
202. Sun, T. & Walsh, C. A. Molecular approaches to brain asymmetry and handedness. *Nature Rev. Neurosci.* **7**, 655–662 (2006).
203. Lickiss, T., Cheung, A. F., Hutchinson, C. E., Taylor, J. S. & Molnar, Z. Examining the relationship between early axon growth and transcription factor expression in the developing cerebral cortex. *J. Anat.* **220**, 201–211 (2012).
204. Williams, C. A., Dagli, A. & Battaglia, A. Genetic disorders associated with macrocephaly. *Am. J. Med. Genet. A* **146A**, 2023–2037 (2008).
205. Olney, A. H. Macrocephaly syndromes. *Semin. Pediatr. Neurol.* **14**, 128–135 (2007).
206. Fombonne, E., Roge, B., Claverie, J., Courty, S. & Fremolle, J. Microcephaly and macrocephaly in autism. *J. Autism Dev. Disord.* **29**, 113–119 (1999).
207. Lainhart, J. E. Increased rate of head growth during infancy in autism. *JAMA* **290**, 393–394 (2003).
208. Witelson, S. F., Kigar, D. L. & Harvey, T. The exceptional brain of Albert Einstein. *Lancet* **353**, 2149–2153 (1999).
In this paper, the sulcal anatomy of Einstein's brain, which was evaluated from newly released autopsy photos, was compared with control brains. The comparisons show that Einstein's brain had an unusual macroscopic anatomy that might have contributed to his unique talents as well as autistic traits in childhood.
209. Galaburda, A. M. Albert Einstein's brain. *Lancet* **354**, 1821; author reply 1822 (1999).
210. Falk, D., Lepore, F. E. & Noe, A. The cerebral cortex of Albert Einstein: a description and preliminary analysis of unpublished photographs. *Brain* **136**, 1304–1327 (2013).
211. DeArmond, S. J., Fusco, M. M. & Dewey, M. M. *Structure of the Human Brain: A Photographic Atlas* 2nd edn (Oxford Univ. Press, 1976).

Acknowledgements
The authors thank J. Knauss for critical reading of the manuscript. The authors thank W. Dobyns at Seattle Children's Hospital, Washington, USA, for sharing the unpublished images used in BOX 1. This work was supported by the Hirschl/Weill-Caulier Trust (T.S.), R01-MH083680 (T.S.), R21-MH087070 (R.F.H.) and R01-NS085081 (R.F.H.) grants from the US National Institutes of Health.

Competing interests statement
The authors declare no competing interests.