

Published in final edited form as:

*Nat Rev Neurosci.* 2014 April ; 15(4): 217–232. doi:10.1038/nrn3707.

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

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### 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.

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The cerebral cortex is a central region in the mammalian brain that controls complex cognitive behaviours<sup>1,2</sup>. 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<sup>3,4</sup>. 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<sup>4</sup>. 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<sup>5</sup>. 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<sup>6</sup>, 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<sup>7</sup>. NE cells are NSCs that can give rise to both neurons and glia<sup>8</sup>. 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<sup>9–11</sup>. More-recent studies have shown that RGCs can produce neurons and, subsequently, astrocytes and oligodendrocytes<sup>9,10,12</sup>. 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<sup>10,11,13</sup> (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)<sup>14</sup>. 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<sup>15,16</sup> (FIG. 1). IPs usually divide symmetrically to generate two postmitotic neurons and, like RGCs, are a major neurogenic cell population<sup>17–19</sup>. 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<sup>20</sup>. 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<sup>16,21–25</sup>. 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<sup>23</sup>. 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<sup>26,27</sup>. 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<sup>28</sup>.

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<sup>29–32</sup>. 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<sup>26,33,34</sup>.

## 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 <sup>3</sup>H-thymidine labelling, which indicates DNA synthesis, revealed the presence of proliferating progenitors in the VZ of cultured human fetal cortical slices<sup>35</sup>. 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)<sup>10,36,37</sup> (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<sup>38</sup>. Nuclei of RGCs in *Pax6*-mutant rat cortices often fail to reach the apical surface and, consequently,

RGCs divide ectopically in basal locations<sup>39</sup>. Moreover, it 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<sup>40</sup>. 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<sup>41</sup>. Ectopic expression of dedicator of cytokinesis 7 (DOCK7) causes delayed INM and reduced numbers of cortical progenitors<sup>42</sup>. 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<sup>43</sup>.

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<sup>44</sup>. 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<sup>45</sup>. 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<sup>46</sup>. 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 *Numb1* 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<sup>47,48</sup>. 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<sup>49</sup>. Moreover, loss of cyclin D2 and RAC1 (a RHO-family small GTPase) promotes cell cycle exit and causes a reduction in cortical size<sup>50–52</sup>. 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<sup>53</sup>. 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<sup>54,55</sup> (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<sup>56</sup>. 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<sup>57</sup>.

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<sup>58,59</sup>. 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<sup>60,61</sup>. 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<sup>62</sup>.

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<sup>63</sup>. The CSF contains growth factors, such as IGF2, that promote NP proliferation and thus are important for controlling cortical size<sup>31</sup>.

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)<sup>64,65</sup>, indicating that apoptosis has an important role in cortical development. Indeed, cortical size is determined by the balance between NP expansion and cell death<sup>66</sup>.

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<sup>67,68</sup>, and breast cancer 1 (*Brcal*)-knockout mice show reductions in both cortical thickness and surface area because of the apoptosis of embryonic NPs<sup>69</sup>. 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<sup>70</sup>. 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<sup>71</sup>.

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<sup>71</sup>. Moreover, caspase 3 (*Casp3*)- and *Casp9*-knockout mice display markedly enlarged and malformed cortices because of reduced apoptosis<sup>72,73</sup>. 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<sup>74</sup>. 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<sup>75-79</sup>. *Dicer* deletion from postmitotic neurons in the cortex using a calcium/calmodulin protein kinase II promoter-driven *Cre* line or a *Nes-Cre* line also causes reduced cortical size, probably through impairment of neurite outgrowth and increased neuronal packing density in the cortical plate<sup>80,81</sup>.

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<sup>82</sup>. 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<sup>83</sup> (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<sup>83,84</sup>. 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<sup>83,85</sup>. 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*<sup>86</sup>. 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<sup>86</sup>. By contrast, miR-134 maintains cortical NPs by targeting the expression of the neuronal migration protein double-cortin (DCX) and/or chordin-like protein 1 (a bone morphogenetic protein antagonist), which are normally expressed in differentiated neurons<sup>87</sup>.



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)<sup>88</sup>. Moreover, miR-124 promotes neurite outgrowth by suppressing RHO G, cell division control protein 42 homologue (CDC42) and RAC1 expression<sup>89,90</sup>. Last, miR-9 and miR-132 induce neurite outgrowth by silencing forkhead box P2 (*Foxp2*) in the cortex<sup>91</sup>. 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<sup>92</sup>. 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<sup>93</sup>. 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<sup>94</sup>. 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<sup>95,96</sup> (FIG. 3a). Live imaging has shown that, during neurogenesis, RGCs usually divide asymmetrically to generate one RGC and one IP or postmitotic neuron<sup>97–99</sup>. 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<sup>100,101</sup>. Indeed, over-expression of PAR3 or PAR6 promotes the generation of PAX6-expressing RGCs<sup>102</sup>. Furthermore, PAR3 interacts with NUMB and induces Notch activity, which is essential for maintaining RGC fate in the developing cortex<sup>103,104</sup>. Conversely, cortex-specific *Cdc42* deletion causes reduced apical localization of the PAR complex and leads to an increase in the number of IPs<sup>105</sup>.

Various molecules that are involved in spindle orientation have been shown to control symmetrical and asymmetrical cell divisions in the developing cortex<sup>95</sup> (FIG. 3a). Mutation of the gene encoding LGN (also known as PINS or GPSM2), a nuclear mitotic apparatus protein 1 (NuMA)- and G $\alpha$ -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<sup>106,107</sup>. Inscuteable (*Insc*)-conditional-knockout and overexpression mice have thinner and thicker cortical walls caused by decreased and increased oblique divisions of RGCs, respectively<sup>108</sup>. 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<sup>109</sup>. 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<sup>110</sup>. 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<sup>111</sup>.

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<sup>112</sup>. In *Pax6* mutants, an increased number of NPs undergo divisions in a non-vertical plane, leading to premature delamination and more asymmetrical divisions<sup>113</sup>. 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<sup>114</sup>. A G protein subunit, G $\beta\gamma$ , controls spindle orientation, and disrupting G $\beta\gamma$  promotes differentiation of cortical NPs<sup>115</sup>. Finally, mice lacking the protein phosphatase PP4C show decreased vertical divisions of NE cells and RGCs, leading to reduced cortical size<sup>116</sup>.

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<sup>24</sup>. 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<sup>117,118</sup>. 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<sup>119</sup>.

## NP divisions and microcephaly

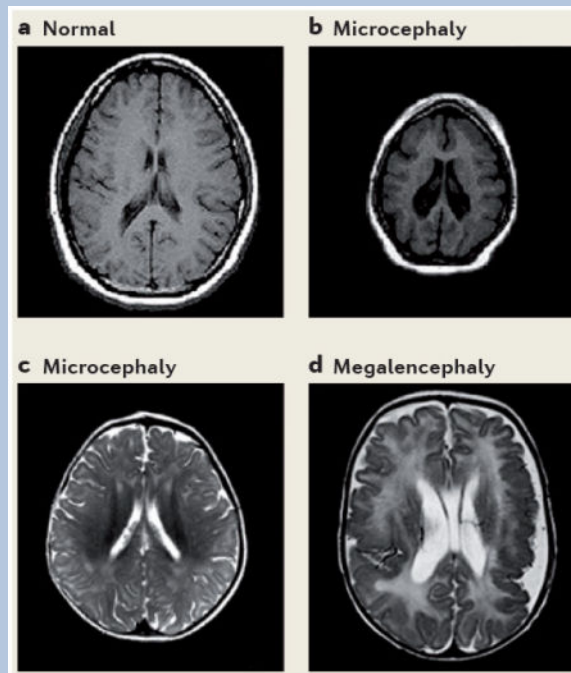
Several gene mutations that are associated with autosomal recessive primary microcephaly (MCPH) have been identified using linkage mapping<sup>120,121</sup> (BOX 1). *MCPH1* (also known as *BRIT1*), which encodes the protein microcephalin, was the first gene to be associated with MCPH and is highly expressed in human fetal and developing mouse brains<sup>122</sup>. Depletion of *MCPH1* increases the accumulation of chromosomal aberrations, suggesting that it is a DNA damage regulator and a tumour suppressor gene<sup>123</sup>. The functions of microcephalin in brain development have been further studied in an *McpH1*-knockout mouse model, which also shows microcephaly<sup>124</sup>. In *McpH1*-deficient NPs, the proportion 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<sup>124</sup>.



**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<sup>121</sup>. Individuals with MCPH exhibit intellectual disability but show no obvious motor control deficits. Linkage mapping has identified genes that are associated with MCPH<sup>120,121</sup>, including abnormal spindle-like microcephaly-associated (*ASPM*) (see the figure, parts **a** and **b**, MRIs of normal-sized and microcephalic brains, respectively<sup>129</sup>). Interestingly, many of these genes are involved in mitotic spindle assembly, suggesting that defects in the division of neural progenitors is involved in MCPH<sup>96,136</sup>.

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<sup>204</sup>. Macrocephaly has been linked to several cognitive disorders, including fragile X syndrome and autism<sup>205</sup>. Indeed, studies have shown that over 20% of autism cases are associated with macrocephaly<sup>206,207</sup>. 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<sup>138,139</sup>). 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.



Mutations in CDK5 regulatory subunit-associated protein 2 (*CDK5RAP2*; also known as *CEP215*), a centrosome-associated protein, have also been mapped in MCPH<sup>125</sup>. 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<sup>126,127</sup>. Mutations in abnormal spindle-like microcephaly-associated (*ASPM*) have been mapped in patients with MCPH<sup>128,129</sup> (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<sup>130,131</sup>. Mutations in the gene encoding another centrosome-associated protein — namely, WD-repeat-containing protein 62 (*WDR62*) — are also associated with MCPH<sup>132–134</sup>. 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<sup>135</sup>.

Interestingly, all of the MCPH-associated genes that have been identified to date encode centrosome-associated proteins<sup>96,136</sup> (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<sup>137</sup>. 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<sup>138–142</sup> (BOX 1).

PTEN may also regulate brain size. Well-characterized mutations in *PTEN*, which is a tumour suppressor gene, have been associated with megalencephaly<sup>143</sup>. Moreover, *PTEN* normally suppresses the PI3K–AKT pathway and thus inhibits cell survival and growth<sup>144</sup>. *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<sup>84,145</sup>. Interestingly, megalencephaly-associated *PTEN* mutations are also related to autism<sup>146–148</sup>. 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<sup>149</sup>.

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<sup>150</sup>. Deletion of CDK inhibitor p57KIP2 (also known as CDKN1C) promotes cell cycle re-entry

in NPs and an increase in cortical size<sup>151</sup>. Similarly, ectopic expression of stabilized  $\beta$ -catenin promotes cell cycle re-entry in NPs and leads to enlarged and folded brains in mice<sup>29</sup>. 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<sup>152</sup>.

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<sup>3</sup>. As highlighted earlier, gyrencephaly is not limited to primates; it occurs in all mammalian orders<sup>3,153,154</sup>. Indeed, molecular and phenomic analyses suggest that the common ancestor of placental mammals had a gyrencephalic brain<sup>155</sup>. 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<sup>5,156</sup>.

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<sup>4,154,157–159</sup>. 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<sup>1</sup> (such as language).

## 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<sup>160</sup>. 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<sup>26,154</sup>. 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<sup>161,162</sup>. 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<sup>6</sup>. 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<sup>163,164</sup>. 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<sup>163</sup>.

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<sup>165</sup>. 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<sup>166</sup>). 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<sup>165</sup> and nor did FGF2 administered at a slightly later stage of cortical development<sup>167</sup>.

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<sup>165</sup>, middle<sup>163</sup> and late<sup>168</sup> 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.

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<sup>163</sup>; FGF2 induced proliferation of IPs only<sup>165</sup>; and overexpression of 4D in ferrets induced proliferation of SVZ progenitors (bIPs and bRGCs were not separately assessed<sup>168</sup>). 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<sup>15</sup>, and they are well suited for this role<sup>14</sup>. 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<sup>160</sup>.

## 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<sup>20,169,170</sup>.

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<sup>20,162,169,170</sup>. Perturbation of early subplate networks can have profound consequences for cortical development, including gyral patterns<sup>6</sup>. 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<sup>6</sup>. 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<sup>6,171</sup>. 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<sup>13,172,173</sup>. 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<sup>174</sup>. 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<sup>175,176</sup>.

It has also been proposed that axons may influence gyrogenesis by pulling together strongly interconnected regions of the cortex<sup>177</sup>. 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<sup>178,179</sup>, but little experimental support for it has been forthcoming to date<sup>4,154,180</sup>. Indeed, in mice with gyri induced by FGF2



treatment, no evidence of exuberant axon connections between gyral surfaces was detected<sup>165</sup>.

### 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<sup>11,13</sup>. 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<sup>6</sup>. 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<sup>163</sup>. 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<sup>6,181</sup>. 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 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<sup>66</sup> or increased NSC self-amplification<sup>29</sup>. 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<sup>182,183</sup>. 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<sup>182,184</sup>. Mouse models of this condition have been produced

and likewise demonstrate selective expansion of occipitotemporal cortex and ventricular surface area but no cortical folding<sup>185</sup>.

### 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<sup>186</sup>. 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<sup>187–189</sup> (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<sup>190,191</sup>.

### 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<sup>30</sup>. The FGF receptors are essential for cortical surface area and volume expansion in mice<sup>192</sup>.

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<sup>182</sup> but not in mice<sup>185</sup>. 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<sup>165</sup>. FGFs may also mediate the effects of axons on cortical growth and gyrification<sup>174</sup>.

The WNT– $\beta$ -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  $\beta$ -catenin in NE cells and RGCs promotes their self-renewal, thus driving ventricular surface expansion and folding<sup>29,193</sup>. Interestingly, WNT signalling has the opposite effect on IPs, promoting their differentiation into neurons<sup>194</sup>. WNT signalling also regulates patterning of the neocortical primordium and is necessary to induce differentiation and growth of the hippocampus and dentate gyrus<sup>195</sup>. 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<sup>196</sup>. Other growth factors and morphogens that regulate cortical development, and that might potentially regulate gyrogenesis, include SHH, bone morphogenetic proteins and IGFs.

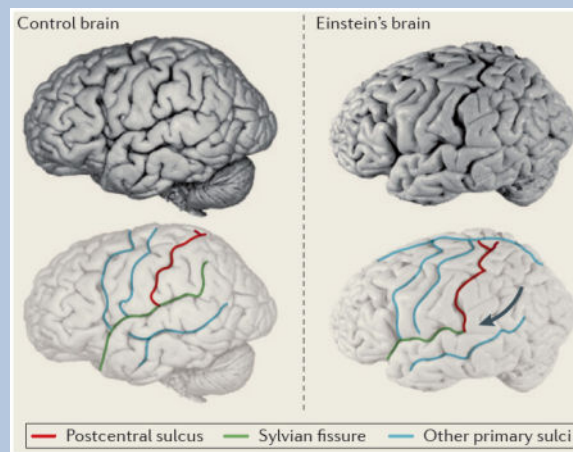
## Gyral patterns in the human brain

Diverse anomalies of gyrification have been described in the human neuropathology and neuroimaging literature<sup>197</sup>. 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<sup>198</sup>. 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<sup>200</sup>. 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<sup>170</sup>. 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 12<sup>th</sup> gestational week or earlier<sup>202</sup>.

**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<sup>208</sup>. 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<sup>209</sup>. A more recent study, including newly released photographs of Einstein's brain, identifies additional uncommon features of his gyral and sulcal morphologies<sup>210</sup>. 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.

**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<sup>11</sup>. 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<sup>163</sup>. 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<sup>203</sup>, 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.

## Acknowledgments

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-S085081 (R.F.H.) grants from the US National Institutes of Health.

## Glossary

<b>SUN-domain-containing protein</b>	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
<b>Joubert syndrome</b>	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
<b>Centriole</b>	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
<b>Hydrocephalus</b>	A medical condition in which there is an abnormal accumulation of cerebrospinal fluid in the ventricles or cavities of the brain
<b>Dicer</b>	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
<b>Myeloarchitecture</b>	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
<b>Monosomy 1p36</b>	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

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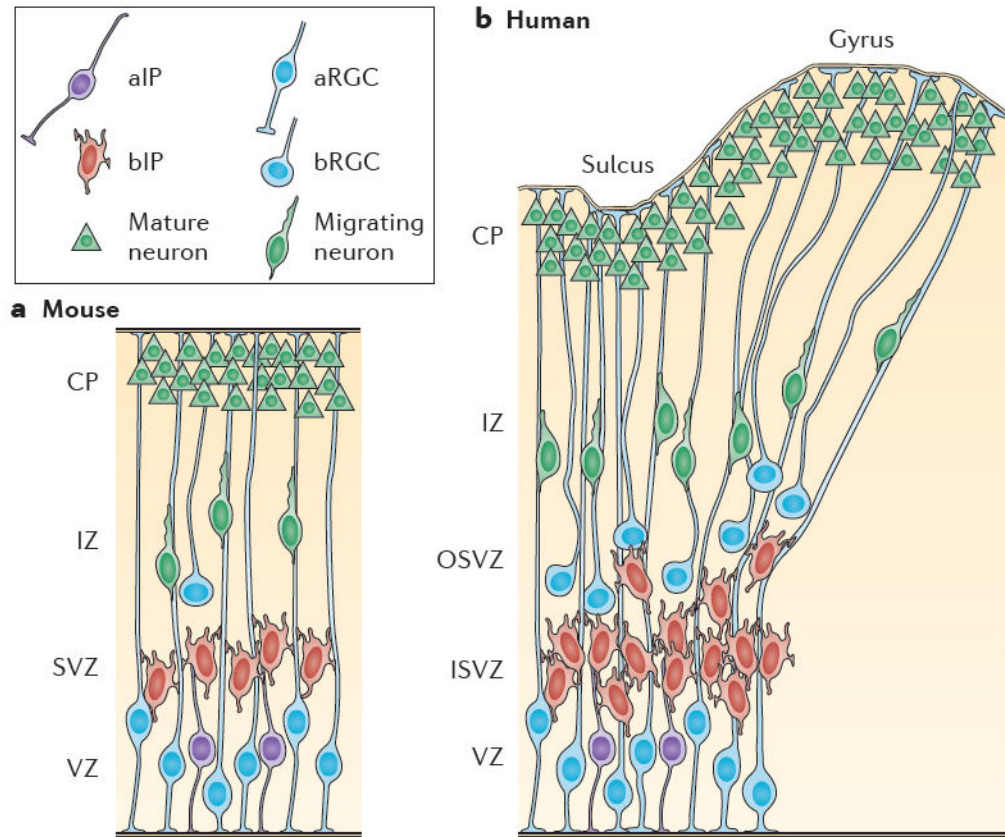


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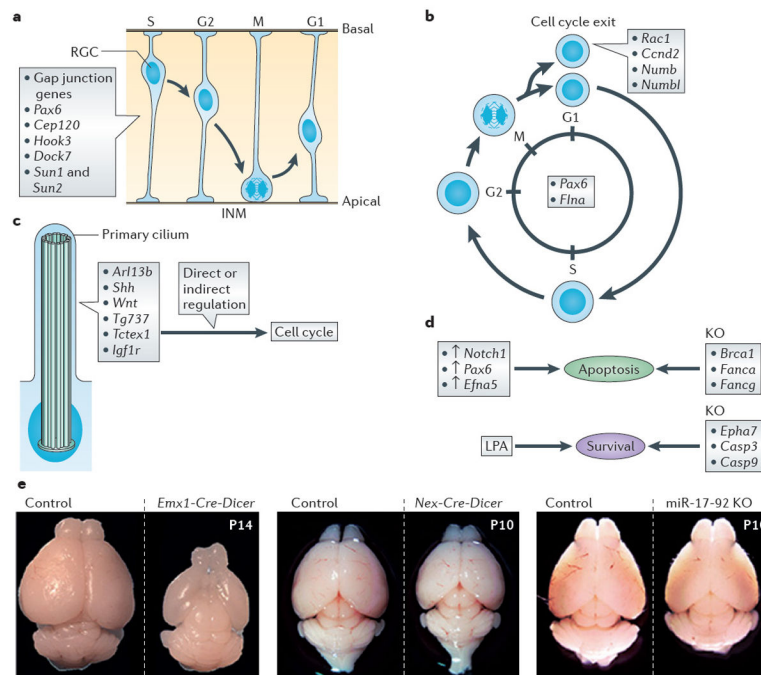
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**Figure 1. Multiple progenitors in the mouse and human developing cerebral cortex**

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<sup>28</sup>.



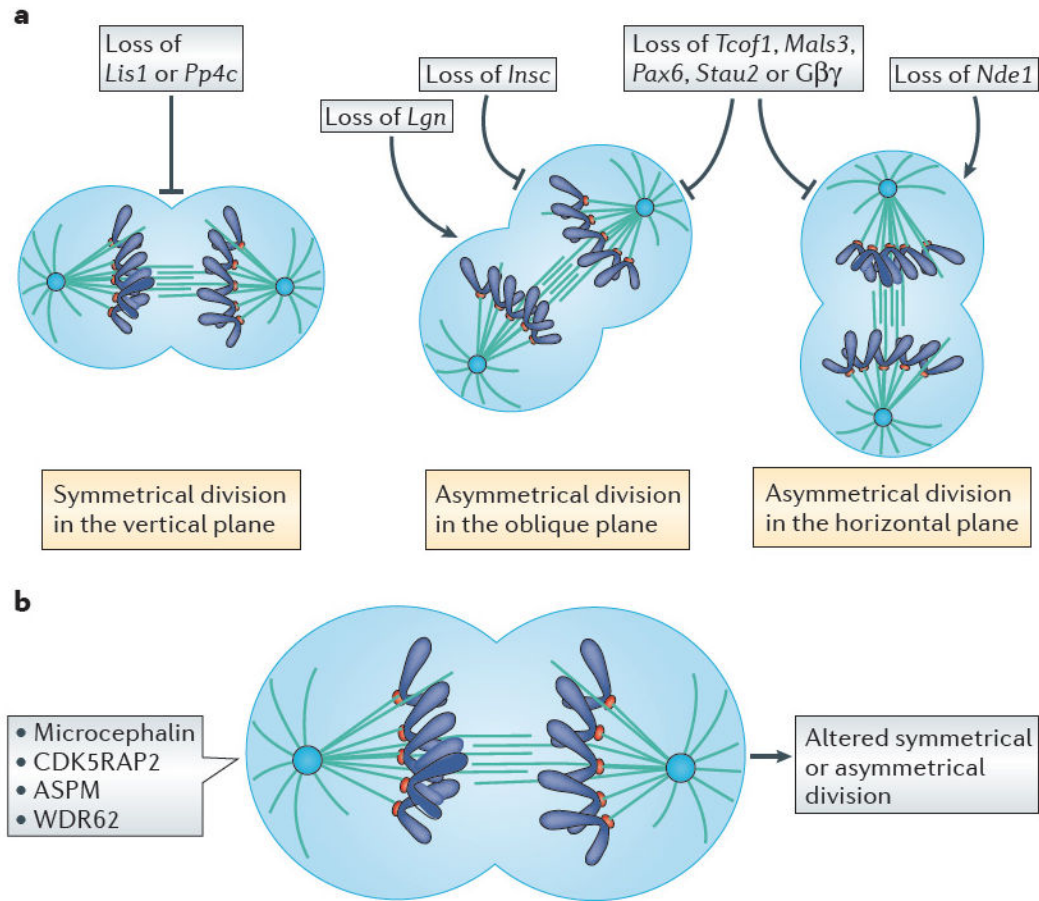


### Figure 2. Molecular mechanisms of cortical growth

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<sup>76,81</sup> (left and middle panels). Mice in which the specific miRNA

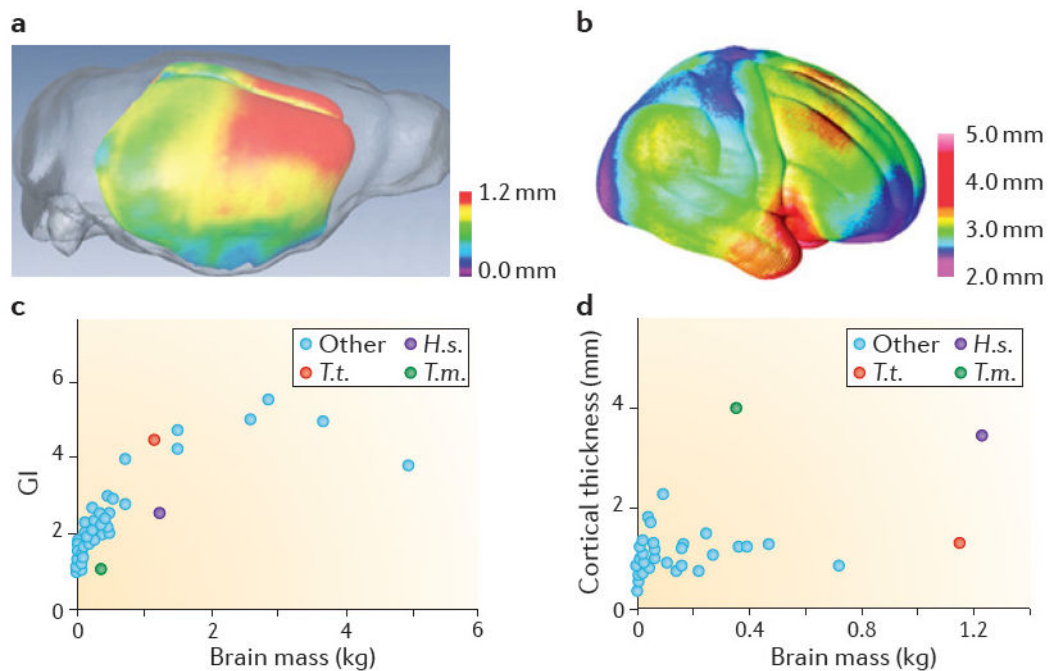


cluster miR-17-92 is knocked out also exhibit a small cortex<sup>83</sup> (right panel). *Cep120*, centrosomal protein 120; *Dock7*, dedicator of cytokinesis 7; *Hook3*; hook microtubule-tethering protein 3; P10, postnatal day 10; *Sun*, SUN-domain-containing. The middle panel of part e is reproduced from REF. 81. The right-hand panel of part e is reproduced, with permission, from REF. 83 © (2013) Elsevier.



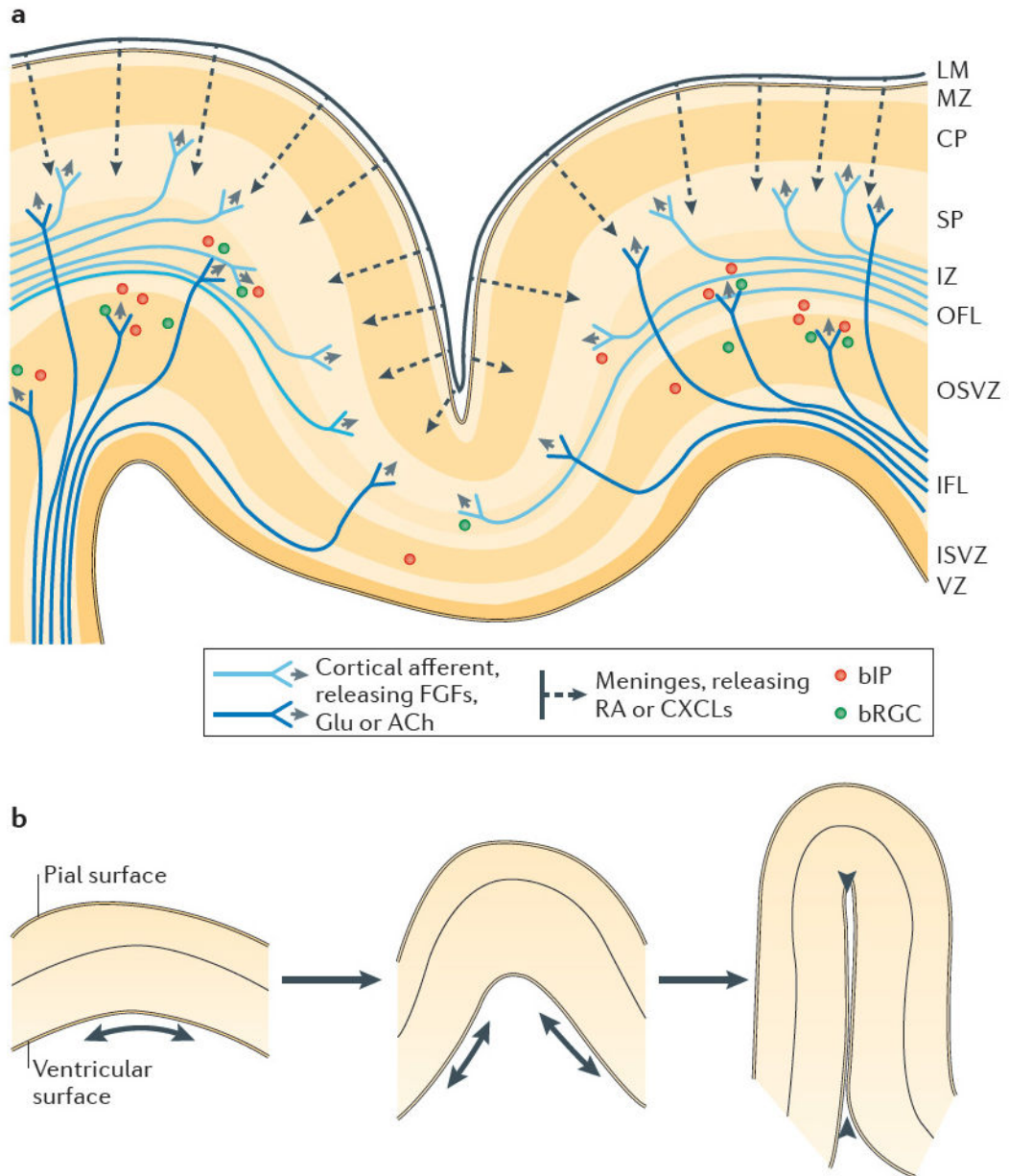
**Figure 3. Symmetrical–asymmetrical cell division, centrosome associated proteins and neural progenitors**

**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  $G\beta\gamma$  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.



**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 truncatus* (*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.



### 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.