Review

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Cerebral cortex expansion and folding: what have we learned?

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Abstract

One of the most prominent features of the human brain is the fabulous size of the cerebral cortex and its intricate folding. Cortical folding takes place during embryonic development and is important to optimize the functional organization and wiring of the brain, as well as to allow fitting a large cortex in a limited cranial volume. Pathological alterations in size or folding of the human cortex lead to severe intellectual disability and intractable epilepsy. Hence, cortical expansion and folding are viewed as key processes in mammalian brain development and evolution, ultimately leading to increased intellectual performance and, eventually, to the emergence of human cognition. Here, we provide an overview and discuss some of the most significant advances in our understanding of cortical expansion and folding over the last decades. These include discoveries in multiple and diverse disciplines, from cellular and molecular mechanisms regulating cortical development and neurogenesis, genetic mechanisms defining the patterns of cortical folds, the biomechanics of cortical growth and buckling, lessons from human disease, and how genetic evolution steered cortical size and folding during mammalian evolution.

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Introduction

In 1990, Wally Welker published a landmark book chapter updating on all that was known about the physiological, experimental, and pathological causes of cortical folding and misfolding, including comparative and phylogenetic considerations (Welker, 1990). Twenty-five years later, we have been invited to present an update of current knowledge on this matter. Welker's review was 134 pages long and cited 665 references, and much progress has been made since then. Clearly, if our review on cortical folding has to fit in this issue of *The EMBO Journal*, something has to be done. Our solution is to limit the size of this review by focusing and briefly touching on what we feel have been the most significant advances in this period leading to our current understanding of the biology of this problem.

One of the most prominent features of the human brain is the fabulous size of the cerebral cortex and its folding, visible as bulges and grooves on its external surface. Most animals with a large brain have a folded cortex, whereas most animals with a small brain have a smooth cortex, without folds. The cerebral cortex is a laminar tissue where neurons lie on the upper part, and the lower or inner part contains most of the wire connecting neurons between brain areas. In big brains, this sheet of neural tissue covering the outside of the brain is disproportionately larger than the deep brain structures it covers, and instead of adopting a balloon-like conformation it folds onto itself, minimizing total brain and cranial volume. In addition to minimizing brain volume, cortical folding is of key importance for the optimization of brain wiring and functional organization (Klyachko & Stevens, 2003), and alterations in cortical size or folding lead to severe intellectual disability and intractable epilepsy in humans (Walsh, 1999; Barkovich *et al*, 2012).

The process of cortical folding takes place during brain development, and thus, it is essentially a developmental problem. In this review, we will start describing exciting discoveries made over the last fifteen years on the central role of progenitor cell proliferation and survival in cortical size, the discovery of novel germinal zones and progenitor cell types, which are greatly overrepresented in gyrated brains, and their key function in cortical folding. This will be followed by our understanding of the genetic mechanisms regulating the patterns of cortical folding and the biomechanics of this process, what we have learned from human disease, and finally will close with a view of how genetic evolution may have steered cortical size and folding during mammalian evolution.

Cellular mechanisms leading to cerebral cortex expansion and folding

In order to understand the developmental mechanisms involved in cortical expansion and folding, we will frame these within the basic principles of cerebral cortex development as found in the most common animal model, the mouse.

Telencephalic neuroepithelium

In the early embryo (in mouse *c.* 9 days postconception, E9), the expansion of the rostral-most domain of the neural tube gives rise to the two telencephalic vesicles. The dorsal half of these vesicles is then

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molecularly specified as the primordium of the cerebral cortex. At this stage, the cortical primordium is solely composed of a monolayer of neural stem neuroepithelial cells (NECs; Bayer & Altman, 1991). NECs are highly polarized and attached to each other by adherens and tight junctions at the level of the apical domain (inner surface of the telencephalic vesicle) and which move their cell nucleus between the apical and the basal sides of the neuroepithelium in coordination with the cell cycle: basal-directed movement during G1, basal position during S-phase, apical-directed movement during G2, and mitosis at the apical surface. This cyclic movement is known as interkinetic nuclear migration and is completely asynchronous between NECs, conferring the neuroepithelium a pseudostratified appearance (Sauer, 1935; Bayer & Altman, 1991; Taverna & Huttner, 2010). NECs only undergo symmetric self-amplificative divisions, whereby each division generates two daughter NECs, hence exponentially increasing their number (Miyata *et al*, 2010; Fig 1).

Because NECs are the founder progenitor cells of the cerebral cortex, their pool size determines the numbers of their derived neurogenic progenitor cells and the final number of cortical neurons, and hence, it has a fundamental impact on the size of the mature cerebral cortex. Accordingly, already at this early stage, the size of the telencephalic vesicles is much larger in the human than in mouse embryo, reflecting significant differences in size of the neuroepithelium as a consequence of different NEC abundance (Sidman & Rakic, 1973; Rakic, 1995). NEC abundance may be increased by extending the time period of their self-amplification and delaying the onset of neurogenesis, as observed in primates compared to rodents (Rakic, 1995, 2009; Kornack & Rakic, 1998). The importance of NEC amplification on cortical size has been experimentally demonstrated in mouse, where NEC abundance in the embryonic cortex may be increased by either promoting their re-entry into cell cycle or preventing programmed cell death (Chenn & Walsh, 2002; Kingsbury *et al*, 2003). In both cases, increased NEC abundance leads to expansion in surface area and folding of the neuroepithelium.

Proliferation and neurogenesis

Immediately prior to the onset of neurogenesis, NECs start losing tight junctions and begin acquiring features typical of glial cells,

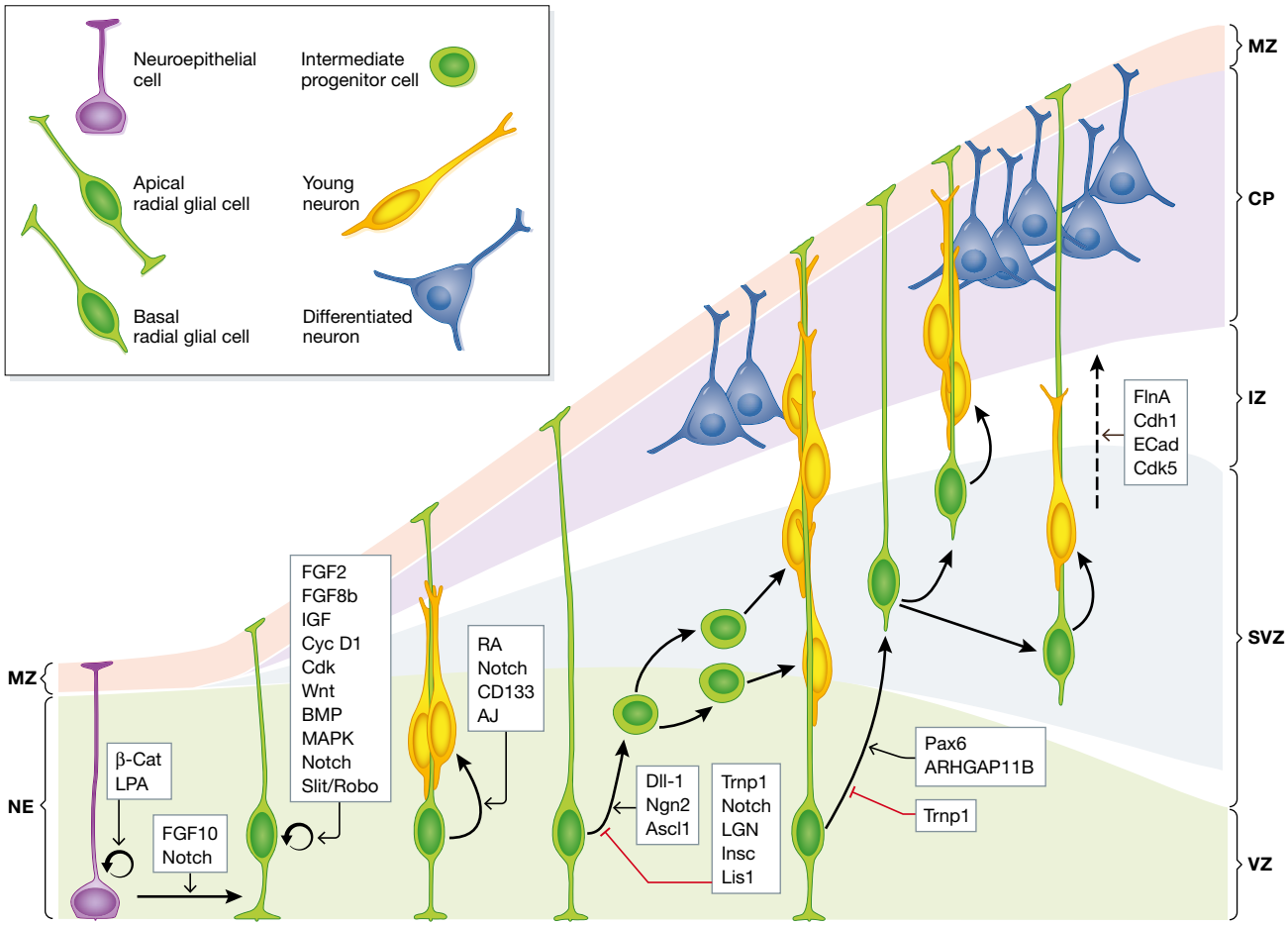


Figure 1. Stem cells in the developing cerebral cortex of gyrencephalic brains and their molecular regulation.
Schema depicting the main types of progenitor cells and their lineage relationships in the developing cerebral cortex. Arrows indicate lineage relationships demonstrated by time-lapse imaging and/or by retroviral lineage tracing. During the expansion phase, most neuroepithelial cells divide symmetrically to self-amplify to generate apical radial glial cells. During the neurogenic phase, most aRGCs divide asymmetrically to generate neurons, either directly or indirectly through intermediate progenitor cells or basal radial glial cells. Molecules or pathways regulating some of these steps are indicated. MZ, marginal zone; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone.

including the expression of brain lipid-binding protein (BLBP), vimentin, and Pax6, thus becoming apical radial glial cells (aRGCs; see Taverna *et al* (2014) for a detailed review on the cell biology of this process). Like NECs, aRGCs undergo interkinetic nuclear migration, divide at the apical surface of the developing cortex and, at this early stage (*c*. E10 in mouse), also undergo self-amplifying divisions. However, aRGCs gradually start dividing asymmetrically to generate one aRGC plus a different cell. These new cells accumulate at the basal side of the cortical primordium, while the cell bodies of aRGCs remain in the apical side, forming the ventricular zone (VZ; Fig 1). With the accumulation of cells above the VZ, the basal process of aRGCs elongates while remaining attached to the basal lamina and is now termed radial glial fiber. Asymmetric aRGC divisions generate one aRGC plus either one neuron or one intermediate progenitor cell (IPC; Malatesta *et al*, 2000; Noctor *et al*, 2001, 2004; Haubensak *et al*, 2004; Miyata *et al*, 2004; Fig 1). IPCs are secondary progenitor cells without apical–basal polarity, do not undergo interkinetic nuclear migration, reside and divide in a location immediately basal to the VZ, the subventricular zone (SVZ), and contrary to aRGCs, they all express the transcription factor Tbr2 (Englund *et al*, 2005). In mouse, the vast majority of IPCs divide once to produce 2 neurons (neurogenic, self-consuming divisions), and hence, they are viewed as a strategy to amplify the production of cortical neurons. However, because each IPC self-consumes at mitosis, their relative abundance compared to aRGCs is quite low (Kowalczyk *et al*, 2009). IPCs in the cerebral cortex generate most cortical excitatory neurons (Attardo *et al*, 2008; Kowalczyk *et al*, 2009), whereas inhibitory interneurons are generated extra-cortically (Anderson *et al*, 1997). As neurogenesis progresses, there are a lower requirement for aRGC expansion/renewal and a greater need for neuron production, so there is a gradual predominance of asymmetric aRGC divisions producing IPCs (Noctor *et al*, 2004; Kowalczyk *et al*, 2009).

In addition to aRGCs and IPCs, the embryonic mouse cortex includes other much less abundant types of progenitor cells. Populating the VZ, we find apical intermediate progenitors (aIPs), which divide at the apical surface to produce neurons (Stancik *et al*, 2010; Tyler *et al*, 2015), and subapical progenitors (SAPs), which divide within the VZ, but away from the apical surface to generate IPCs (Pilz *et al*, 2013). Populating the SVZ, we find basal radial glial cells (bRGCs), which share many similarities with aRGCs including a basal process extended radially and contacting the basal lamina of the telencephalon, and expression of the transcription factor Pax6, but whose cell body is located and divides at basal positions in the SVZ (Shitamukai *et al*, 2011; Wang *et al*, 2011; Fig 1). As opposed to aRGCs, bRGCs in mouse do not self-amplify nor produce IPCs, but are highly neurogenic (Wang *et al*, 2011).

In gyrencephalic species like humans, monkey, or ferret, the abundance of aRGCs is much greater than in species with a smooth cortex like mouse, producing a more extended VZ. This is a direct consequence of the higher abundance of founder NECs at earlier stages (see above), further promoted by an increased self-amplification of aRGCs in these species. In addition to having an extended VZ, the most remarkable distinction of gyrencephalic species is having a thickened SVZ populated by an outstanding abundance of basal progenitors, especially at later stages of neurogenesis when these greatly outnumber apical progenitors (Smart *et al*, 2002; Lukaszewicz *et al*, 2005; Reillo *et al*, 2011; Reillo & Borrell, 2012). This abundance of basal progenitors is accompanied by the splitting of the SVZ in inner (ISVZ) and outer (OSVZ) subdivisions, not found in mouse (Smart *et al*, 2002; Fietz *et al*, 2010; Hansen *et al*, 2010; Reillo *et al*, 2011; Reillo & Borrell, 2012).

The OSVZ contains a wide diversity of progenitor cell types with high amplificative potential, and the combination of these two factors is considered key for cortical expansion and folding (Lui *et al*, 2011; Betizeau *et al*, 2013; Borrell & Gotz, 2014). Contrary to the lissencephalic mouse, in gyrencephalic species few basal progenitors are IPCs, but most are bRGCs (Hansen *et al*, 2010; Reillo *et al*, 2011; Reillo & Borrell, 2012; Betizeau *et al*, 2013). A seminal videomicroscopy study demonstrated that in macaque (a gyrated primate), bRGCs come in different modalities, which frequently transition between them and with IPCs, and all of these types of progenitors may self-amplify prior to generating neurons (Betizeau *et al*, 2013). Importantly, in gyrencephalic cortices like macaque and ferret, neurogenesis takes place during a period of time much longer than in rodents (up to tenfold; Takahashi *et al*, 1993; Kornack & Rakic, 1998; Lukaszewicz *et al*, 2005; Reillo & Borrell, 2012), allowing more rounds of cell division and increasing neuronal output (Dehay & Kennedy, 2007; Florio & Huttner, 2014). On these grounds, we and others have proposed that the OSVZ, with its wealth of neurogenic basal progenitors, plays central roles in the dramatically increased neurogenesis and folding in the cerebral cortex of higher mammals (Fig 2A; Fietz & Huttner, 2011; Lui *et al*, 2011; Borrell & Reillo, 2012; Borrell & Calegari, 2014; Borrell & Gotz, 2014; Florio & Huttner, 2014). This idea is well supported by experimental work in the gyrencephalic ferret, which demonstrates that forced overproliferation of OSVZ progenitors increases cortical surface area and folding, whereas blockade of their proliferation has the opposite effect (Reillo *et al*, 2011; Nonaka-Kinoshita *et al*, 2013). The central relevance of the OSVZ in cortical expansion and folding resides not only on its prominent contribution to increase neuron production, but also specifically on its high content of bRGCs, as explained in the next section.

Radial migration

Newborn cortical excitatory neurons must travel (migrate) from their layer of birth to the vicinity of the cortical surface, where they will coalesce into nascent neuronal layers. This process is named radial migration (Rakic, 1972). The scaffold of radial glial fibers, which span perpendicular between the ventricular and the pial surface of the cortex, provides the necessary substrate and guide for these neurons during their radial migration, much like the rail tracks for a train. This cell–cell interaction is under tight molecular regulation, and its disruption leads to severe defects of neuronal positioning and layer formation in the cortex (Sidman & Rakic, 1973; Rakic *et al*, 1974; Rakic, 1978; Anton *et al*, 1997, 1999; Elias *et al*, 2007). Due to this dependence of radially migrating neurons on radial glial fibers, the trajectory of these fibers largely defines the migratory route and final location along the cortical surface of new neurons (Rakic, 1995). Consequently, sibling neurons born from one progenitor normally occupy neighboring positions in the mature cerebral cortex (Fig 2B; Soriano *et al*, 1995; Gupta *et al*, 2003; O'Leary & Borngasser, 2006; Gao *et al*, 2014).

In contrast to the mouse cerebral cortex, a characteristic feature of folded cortices during development is their much greater surface area on the pial than on the ventricular side (Sidman & Rakic, 1973; Kriegstein *et al*, 2006; Rakic, 2009). This expansion of the pial

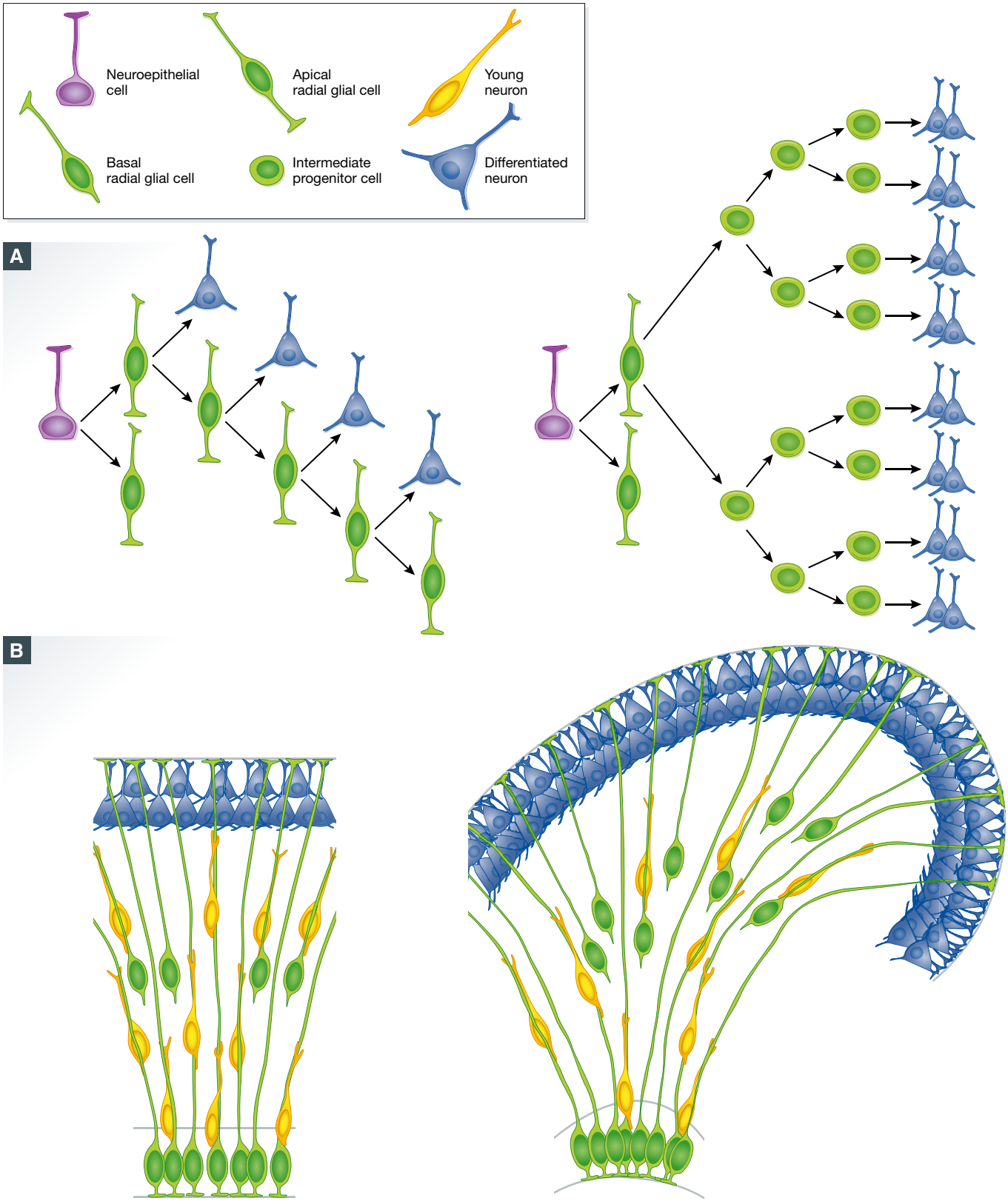


Figure 2.

Figure 2. Patterns of cell division in the embryonic cerebral cortex and mechanisms for tangential versus radial expansion.
(A) Two patterns of cortical progenitor cell division with opposite neurogenic outcome: The total number of neurons produced is proportional to the generation of intermediate progenitor cells, very small in species with a smooth cortex (left) and large in species with a folded cortex (right). (B) Difference in the general arrangement of the radial fiber scaffold in the cerebral cortex undergoing tangential (left) versus radial expansion (right). In species with a smooth cortex like mouse (left), radial glial fibers are parallel (green) and there is no net lateral dispersion of radially migrating neurons (yellow) with respect to the positions of their progenitor aRGCs (green). In gyrencephalic species, the radial fiber scaffold becomes divergent due to the intercalation of radial fibers from bRGCs. As a result, radially migrating neurons follow divergent trajectories which cause their lateral dispersion; this increases cortical surface area and ultimately promotes folding (modified from Borrell & Reillo, 2012).

surface relates to the increased numbers of cortical neurons, largely based on the expansion of basal progenitors. Several hypotheses have been proposed to explain how basal progenitors may promote this difference between pial and ventricular surface during development (Kriegstein *et al*, 2006; Fietz & Huttner, 2011). However, a critical issue overseen in early hypotheses is that whereas the addition of basal progenitors explains increased neurogenesis without expanding the ventricular surface area, the amount of radial fibers from aRGCs also does not expand, which is problematic. If the radial fiber scaffold does not increase as neurogenesis increases, then radial fibers (the rail tracks for migrating neurons) become a limiting factor to be shared by the augmented population of radially migrating neurons. As a result, neurons pile up in thickened cortical layers without a significant lateral separation, and thus, surface area does not increase and folds do not form. Intriguingly, this is reminiscent of human lissencephaly, where cortical layers are several-fold thicker than normal and folds fail to form (see section below). However, this is not the case in gyrencephalic brains, where the outer surface area of the cerebral cortex increases several orders of magnitude more than thickness (i.e., the human cortex has 1,000 times more surface area than in mouse, but is only 10 times thicker) and without a similar increase in VZ surface area.

Thus, the radial fiber scaffold must be modified so that the increased numbers of radially migrating neurons do not pile in thick layers, but are distributed along the cortex. An elegant solution to this problem is to create a divergence in the array of radial fibers with additional, intercalated fibers. Under this circumstance, neurons migrating radially and in intimate association with radial fibers find additional paths for migration, which has two advantages: (i) overcrowding of the migratory paths is released so radial migration is not delayed and (ii) radially migrating neurons are delivered to distant positions along the cortical surface (Fig 2B). The source of additional radial fibers to create this divergence is bRGCs (Reillo *et al*, 2011). Basal RGCs have a radial fiber extended to the pial surface, their cell soma is located in ISVZ or OSVZ, basal from aRGCs, and they are not anchored to the apical VZ surface. Hence, bRGCs are in the optimal position to provide additional radial fibers to create the divergence and fanning out of the radial fiber scaffold, without increasing VZ surface area, as reported in ferret (Fig 2B; Reillo *et al*, 2011). Importantly, in spite of the divergence of radial fibers, thanks to bRGCs their density remains high through the cortical thickness, and many are even intercrossed (Rakic *et al*, 1974; Reillo *et al*, 2011), so neurons can disperse laterally while maintaining radial glia-dependent migration, as recently demonstrated in ferret (Gertz & Kriegstein, 2015). In the developing ferret cortex, the fanning of the radial fiber scaffold is significant in prospective gyrus, but not in prospective sulcus regions, which further supports that this is a driving force in cortical surface area expansion and folding (Lui *et al*, 2011; Reillo *et al*, 2011; Borrell & Reillo, 2012). Importantly, this "radial divergence model" has been validated by several laboratories, where bRGC abundance and proliferation have been experimentally manipulated in ferret and mouse. Partial blockade of OSVZ progenitor proliferation in the developing ferret cortex without a significant neuronal loss leads to a reduction in size of cortical folds (Reillo *et al*, 2011) and even lissencephaly (Poluch & Juliano, 2015). Conversely, forced overproliferation of OSVZ progenitor cells in ferret significantly increases cortical surface area and folding (Nonaka-Kinoshita *et al*, 2013). Finally, even in the naturally smooth mouse cortex, genetic manipulations forcing an increased abundance of bRGCs during embryonic development lead to the formation of cortical folds (Stahl *et al*, 2013; Florio *et al*, 2015).

Differentiation

Once neurons finish radial migration and detach from the radial fiber, they begin terminal differentiation. This process has a fundamental impact in the final size of the cerebral cortex, essentially by increasing the size of cell somas and the volume of the neuropile: growth and branching of apical and basal dendrites; extension, navigation, and branching of the axon; formation of spines and boutons for synaptic connectivity. Packing density of cortical neurons, cell body size, and extent of their dendritic and axonal arbors are remarkably different between mammals, correlating with brain size (Purves, 1988) and also contributing to cortical expansion (Reillo *et al*, 2011).

Molecular regulation of cellular mechanisms

Over the last two decades, hundreds of studies have deciphered some of the key molecular mechanisms regulating cerebral cortex development in mouse. Although this invaluable knowledge is frequently extensible to humans and other species at the level of basic cellular mechanisms, much less is really known about the molecular regulation of events key for cortical expansion and folding. In this section, we provide an overview of some of this knowledge, and how it may be extensive to cortical folding.

NEC amplification versus transition to RGC

At early stages of cortical development, several signaling cascades regulate the maintenance of NEC self-amplification. One of the most potent promoters of NEC self-amplification is the β-catenin pathway, such that its constitutive activation in mouse embryos leads to the overexpansion and folding of the cortical neuroepithelium (Chenn & Walsh, 2002). Expansion and significant folding of the mouse cortical neuroepithelium is also achieved by limiting developmental apoptosis, by means of lysophosphatidic acid signaling (Kingsbury *et al*, 2003). Conversely, the fibroblast growth factor (FGF) pathway, by means of FGF10, drives NECs into expressing radial glial cell markers and thus promotes their transition toward aRGC fate, terminating the phase of NEC amplification (Sahara & O'Leary, 2009).

Intriguingly, FGF signaling promotes retention of the aRGC fate, as it also inhibits the subsequent transition of aRGCs toward basal progenitors (Kang *et al*, 2009). The transition from NECs to aRGCs is also strongly promoted by the activation of the Notch signaling pathway (Gaiano *et al*, 2000; Hatakeyama *et al*, 2004; Fig 1).

Progenitor amplification

In addition to their role in blocking the maturation of cortical progenitors, FGF ligands promote their proliferation and inhibit neurogenesis by regulating the duration of the cell cycle. This role of FGF signaling is critical for cortical growth, as its loss at early stages accelerates neuron production and loss of RGCs, resulting in reduced cortical surface area (Rash *et al*, 2011). Conversely, overactivation of FGF signaling by infusion of FGF2 or FGF8b causes the overproliferation of cortical progenitors and cortical expansion in surface area (Rash *et al*, 2013; Fig 1). FGFs influence cortical progenitors via regulation of cell cycle proteins. FGF2 and insulin-like growth factor (IGF) 1 upregulate the expression of cyclin D1 and downregulate the expression of p27(kip1), a cyclin-dependent kinase (Cdk) inhibitor, thereby shortening the G1 phase of the cell cycle and promoting self-amplificative divisions (Raballo *et al*, 2000; Lukaszewicz *et al*, 2002; Mairet-Coello *et al*, 2009). Activation of this FGF signaling cascade drives cortical expansion, which is accompanied by the incipient folding of the otherwise smooth mouse cortex (Rash *et al*, 2013), and in the already gyrencephalic ferret cortex, it causes extra folding (Masuda *et al*, 2015). Not surprisingly, cortical progenitor populations may be expanded directly by overexpressing Cdk4 and cyclin D1 (Lange *et al*, 2009). However, in the mouse cortex, this promotes cortical growth and megalencephaly, but not folding (Nonaka-Kinoshita *et al*, 2013), which highlights the molecular and cellular complexity of the process of cortical folding (Borrell & Calegari, 2014).

Other important signaling pathways regulating cortical progenitor proliferation and self-renewal include the Wnt, BMP, MAPK, and Notch pathways. Unfortunately, none of these pathways have been found able to induce *bona fide* cortical folding in mouse, and there are no experimental data in naturally gyrencephalic species. One of the best-known pathways regulating the balance between progenitor proliferation and neurogenesis is Notch. In addition to promoting the transition from NECs to aRGCs at early developmental stages (Gaiano *et al*, 2000; Hatakeyama *et al*, 2004; Martynoga *et al*, 2012), activation of the Notch pathway at later stages inhibits the generation of IPCs from aRGCs (Mizutani *et al*, 2007; Ohata *et al*, 2011; Martynoga *et al*, 2012). Conversely, the onset of *Dll1* expression (the main ligand of Notch1) coincides with the expression of the proneural proteins Ngn2 and Ascl1, which are major transcriptional regulators of neurogenesis and also directly regulate *Dll1* expression (Castro *et al*, 2006; Martynoga *et al*, 2012). The Notch pathway can be additionally activated by Slit/Robo signaling, with similar consequences: impairment of neurogenesis at early stages by favoring the self-renewal of aRGCs (Borrell *et al*, 2012). Importantly, Notch signaling seems to be required for the self-renewal of OSVZ progenitors in the human cerebral cortex (Hansen *et al*, 2010).

Regarding Wnt, activation of this pathway promotes proliferation and self-renewal of aRGCs at early developmental stages (Machon *et al*, 2003; Woodhead *et al*, 2006; Zhou *et al*, 2006), while at later stages it promotes the maturation of aRGCs into IPCs (Viti *et al*, 2003; Hirabayashi *et al*, 2004) and it may even promote neurogenesis (Munji *et al*, 2011). Thus, the effects of Wnt signaling in cortical development are complex and time-regulated during development. The BMP pathway has similarly elusive and complex inputs into the regulation of cortical neurogenesis. At early developmental stages, BMP signals induce neurogenesis (Li *et al*, 1998; Mabie *et al*, 1999), while at later stages, they block neurogenesis to promote astrocyte differentiation (Gross *et al*, 1996). The Ras-MAPK-ERK pathway controls the mitogen-stimulated proliferation of cortical progenitors and its negative regulators, thus ensuring progenitor self-renewal and preventing premature differentiation (Phoenix & Temple, 2010). Finally, IGF-2 secreted into the cerebrospinal fluid by the choroid plexus is another potent mitogen promoting proliferation of VZ cortical progenitors (Lehtinen & Walsh, 2011; Lehtinen *et al*, 2011).

Recently, retinoic acid (RA) signaling has also been identified as important in regulating the balance between cortical progenitor self-renewal and neurogenesis, where RA secreted by the meningeal membranes promotes neurogenesis while limiting aRGC amplification (Siegenthaler *et al*, 2009). Contrary to the classical signaling pathways mentioned above, the RA pathway holds promise as an important regulator of cortical expansion and folding, as its genetic blockade induces remarkable folding of the mouse cortex (Siegenthaler *et al*, 2009). The downstream transducers of RA signaling in this context are not known, but the orphan nuclear hormone receptor CoupTF1 and the pro-neural transcription factors Ngn1 and 2 may be involved (Ribes *et al*, 2008; Harrison-Uy *et al*, 2013).

Progenitor cell lineage

Whereas cortical folding is positively correlated with increased brain size and greater numbers of neurons, mounting evidence from comparative neuroanatomy and human pathology strongly supports that increased neuron numbers are not sufficient, but cortical folding requires additional mechanisms of developmental regulation (Welker, 1990; Borrell & Reillo, 2012). As explained in the previous section, the tangential dispersion of radially migrating neurons seems key in the expansion of cortical surface area that leads to folding, and this depends on the relative abundance of bRGCs (Reillo *et al*, 2011; Pilz *et al*, 2013). Hence, lineage regulation of the different types of cortical progenitors, particularly the production and maintenance of bRGCs, is critical in this process (Borrell & Gotz, 2014).

In the cerebral cortex of mouse, ferret, and humans, bRGCs are generated from aRGCs in the VZ (Reillo *et al*, 2011; Shitamukai *et al*, 2011; Wang *et al*, 2011). This process is seemingly associated with, and controlled by, the mitotic spindle orientation of aRGCs (Shitamukai *et al*, 2011; LaMonica *et al*, 2013), which is known to have a strong influence on the acquisition of asymmetric cell fates by cortical progenitors (Postiglione *et al*, 2011; Xie *et al*, 2013). Progenitors in the early neuroepithelium divide mostly in perpendicular orientations while oblique cleavage planes augment as neurogenesis becomes predominant, and disruption of these orientations at early stages leads to depletion of the progenitor pool (Mitchison & Kirschner, 1984; Chenn & McConnell, 1995; Yingling *et al*, 2008). Orientation of the mitotic spindle in the mammalian cerebral cortex is regulated by a number of factors, including LGN, Insc, and Lis1 (Yingling *et al*, 2008; Postiglione *et al*, 2011; Shitamukai *et al*, 2011; Fig 1).

In addition to the cleavage plane orientation, there are other cellular mechanisms regulating cortical progenitor cell fate,

particularly symmetric versus asymmetric fates, which include the maintenance of the apical–basal polarity by Notch signaling, Par3, Par6, prominin-1 (CD133), and other proteins related to the apical adherens junctions (Hatakeyama *et al*, 2004; Gotz & Huttner, 2005; Costa *et al*, 2008; Bultje *et al*, 2009; Kriegstein & Alvarez-Buylla, 2009; Imayoshi *et al*, 2010). A decrease in apical junction proteins like Par3 or Par6 switch the mode of aRGC division from self-renewing to neurogenic, while their overexpression promotes aRGC self-renewal (Costa *et al*, 2008).

A landmark finding on the molecular regulation of cortical folding via control of progenitor cell lineage was the identification of *Trnp1*, a key player in this process (Stahl *et al*, 2013). Trnp1 is a DNA-associated protein initially identified as being strongly expressed in self-amplifying aRGCs (Pinto *et al*, 2008). Starting at high levels in the early embryo, Trnp1 expression decreases as aRGCs gradually stop self-amplifying to produce IPCs and neurons. Overexpression of Trnp1 promotes aRGC self-renewal at the expense of IPCs and neurogenesis. Conversely, knockdown of Trnp1 function increases IPCs and, in addition, it dramatically increases the abundance of bRGCs, otherwise very scarce in the mouse embryo. Most importantly, these changes are associated with a significant expansion of the cortical surface area and the formation of structures resembling *bona fide* cortical folds, including modification of the radial fiber scaffold and the divergent distribution of radially migrating neurons (Stahl *et al*, 2013). Analyses of *Trnp1* expression in the developing cortex of ferret and human embryos are consistent with these functional results, as *Trnp1* levels are specifically low in cortical regions prospectively undergoing tangential expansion and folding (Stahl *et al*, 2013; de Juan Romero *et al*, 2015). Thus, *Trnp1* was the first gene able to induce *bona fide* folding of the mouse cerebral cortex.

A second gene causing folding of the mouse cortex has been recently found: *ARHGAP11B* (Florio *et al*, 2015). This is a truncated paralog of *ARHGAP11A* that arose on the human evolutionary lineage after the divergence from the chimpanzee (Antonacci *et al*, 2014), and hence does not exist in mouse. Similar to the loss of function of *Trnp1*, experimental expression of *ARHGAP11B* in mouse embryos drives cortical aRGCs into massively producing basal progenitors, many of which are bRGCs, and this ultimately translates into folding of the mouse cerebral cortex. The seminal relevance of this study is the discovery of a gene with a likely central role in human brain evolution and uniqueness, contributing to further promote cortical expansion and folding in the hominid lineage. High levels of the transcription factor Pax6 have also been reported to promote the generation of bRGCs in mouse, but intriguingly this does not cause cortical folding (Wong *et al*, 2015).

Progenitor cell heterogeneity: single-cell transcriptomics

One of the classical limitations of studies searching for genes important in cortical development, expansion and folding was to analyze progenitor cell pools instead of individual cells. The advent of single-cell transcriptomics has revolutionized this field by allowing uncovering an extraordinary molecular heterogeneity of progenitor cell types in the developing cerebral cortex (Pollen *et al*, 2014). Importantly, this technology has revealed that aRGCs and bRGCs are molecularly more heterogeneous in the developing folded cortex of humans and ferret (3 classes of aRGC, 2 classes of bRGC) than in the smooth cortex of mouse (2 classes of aRGC, 1 class of bRGC; Camp *et al*, 2015; Johnson *et al*, 2015; Pollen *et al*, 2015). Although we are just beginning to profit from the power of this technology, single-cell transcriptomics is already highlighting the potential relevance of specific signaling cascades and molecular programs in the formation and maintenance of bRGCs in general and the OSVZ in particular. For example, in agreement with previous population-wide transcriptomic analyses and functional manipulations, the extracellular matrix and its components are being highlighted as central in stimulating cortical progenitor proliferation and self-renewal (Fietz *et al*, 2010, 2012; Stenzel *et al*, 2014; Pollen *et al*, 2015).

Genetic patterning of cortical folds

What defines the location and shape of cortical folds and fissures as they form in the developing cortex? The traditional view has been that cortical folds form randomly, essentially based on the idea that cortical growth exceeds cranial volume, and thus, cortical folding occurs purely as a mechanical consequence of cranial constraint (Welker, 1990). However, as discussed in a later section, evidence indicates that both notions are wrong. If any portion of the cerebrum fails to develop or grow, due to either pathological or experimental causes, the skull tends to conform to the size and shape of the remaining neural tissue, which in fact still folds, thus demonstrating that limited cranial volume does not force cortical folding (Welker, 1990). Further evidence demonstrating that cortical folds do not form randomly includes that (i) the pattern of folds and fissures is highly stereotyped and well conserved between individuals of a species, particularly in those with small gyrated brains (i.e., ferret, cat); (ii) folding patterns of phylogenetically related species follow remarkably similar trends (Welker, 1990; Borrell & Reillo, 2012); (iii) even in species with large cortices and very complex folding patterns, like humans, the deepest and earliest fissures to develop do so at strikingly conserved positions, particularly in monozygotic twins where the overall folding pattern is significantly well conserved (Lohmann *et al*, 1999, 2008). Taken together, cortical folding patterns appear subject to strong genetic regulation.

In species with a simple pattern of folds, like ferret and cat, the stereotyped location of cortical folds and fissures is preceded and mirrored by regional variations in progenitor cell proliferation, in all three germinal layers, but most prominently in the OSVZ (Reillo *et al*, 2011). Local manipulations of OSVZ proliferation in ferret have a significant impact on the size and shape of cortical folds, without altering cortical area identity nor lamination (Reillo *et al*, 2011; Ghosh & Jessberger, 2013; Nonaka-Kinoshita *et al*, 2013). To identify genes whose expression covaries with the stereotypic patterning of progenitor proliferation and cortical folds, microarray technology was recently used to compare the transcriptome of progenitors in prospective folds versus fissures of the developing ferret cortex (de Juan Romero *et al*, 2015). This analysis demonstrates the existence of thousands of genes differentially expressed (DEGs) between these regions, mostly in OSVZ and VZ. Identified DEGs include genes key in cortical development and folding like *Trnp1* or *Ccnd1*, as well as genes mutated in human malformations of cortical development. Many DEGs are expressed in modules along the OSVZ and other germinal layers of the gyrencephalic ferret and also the human embryo cortex, but not in the lissencephalic mouse cortex (Sansom & Livesey, 2009; Elsen *et al*, 2013; de Juan

Romero *et al*, 2015). Most remarkably, expression modules along the OSVZ map faithfully the eventual location of cortical folds and fissures (de Juan Romero *et al*, 2015). This strongly supports a role for the OSVZ and some of those DEGs on cortical patterning, particularly in its stereotyped folding (Kriegstein *et al*, 2006; Lui *et al*, 2011; Reillo *et al*, 2011; Albert & Huttner, 2015; Fig 3).

Multiple genetic maps seem to overlap across germinal layers, as modular expression patterns also exist for DEGs in VZ and ISVZ of ferret and humans (de Juan Romero *et al*, 2015). Given that these germinal zones are major sites of neurogenesis and neural fate determination, these gene expression patterns may contribute significantly to further define cortical folds and/or functional areas of the cerebral cortex (Lui *et al*, 2011; Reillo *et al*, 2011; Taverna *et al*, 2014; Dehay *et al*, 2015). Indeed, many DEGs between the prospective gyrus and sulcus are known to regulate progenitor proliferation, neurogenesis, or fate specification, including key signaling pathways such as Notch, Shh, MAPK, and Wnt, which directly regulate cortical growth (see above). In the case of cortical folds, modular patterns of expression for a combination of genes, possibly different depending on the specific gyrus or sulcus, may impose differential tissue growth between modules, eventually leading to the evagination of the cortex and formation of folds (Smart & McSherry, 1986; Fig 3).

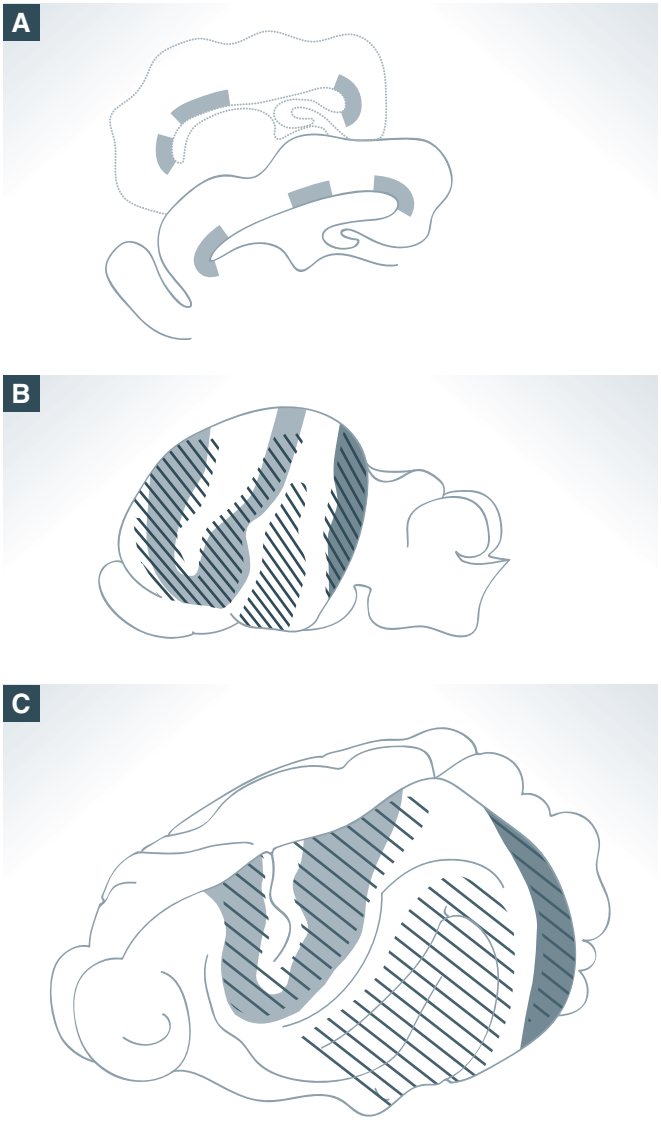


Figure 3. Patterns of gene expression map the prospective location of cortical folds in the developing ferret brain.
(A) Schema of sagittal sections of ferret brains at postnatal day P6 showing the modular pattern of mRNA expression for *Eomes* at the outer subventricular zone (shaded areas). (B, C) Representation of the ferret brain surface at postnatal day P2 (B) and adult (C) overlapped with the map of *Eomes* expression modules (shaded) and prospective gyri (striped pattern), showing the spatial correlation between *Eomes* expression and gyri (adapted from de Juan Romero *et al*, 2015).

Small enhancer elements have been recently identified to drive reporter gene expression in discrete modules, or protodomains, also in the embryonic mouse cerebral cortex (Visel *et al*, 2013; Pattabiraman *et al*, 2014). These enhancers have been proposed to integrate broad transcriptional information, including expression of several transcription factors regulating cortical patterning, to define gene expression in those protodomains (Nord *et al*, 2013; Pattabiraman *et al*, 2014). Variations in such small enhancer elements may be indeed at the core of cortical patterning (and folding) during development and evolution (Borrell & Gotz, 2014; see below). However, the definition of discrete cortical subdivisions requires the regional control over the expression of protein-coding genes or their interfering RNAs (i.e., cell cycle regulators, cell fate determinants, neuron terminal selectors) in protodomains along the embryonic germinal layers, as demonstrated in ferret and humans, but not in mouse (Dehay & Kennedy, 2007; Molyneaux *et al*, 2007; Hobert, 2011; de Juan Romero *et al*, 2015). Walsh and colleagues recently identified a key regulatory element for the expression of human *GPR56* which varies significantly across mammals and, when introduced in transgenic mouse embryos, drives different patterns of expression (Bae *et al*, 2014). Most importantly, *GPR56* expression levels regulate cortical progenitor proliferation, and mutation of this regulatory element disrupts human cortex folding around the Sylvian fissure, demonstrating the importance of the expression pattern of this gene in defining the pattern of folds. Similarly, mutations in the regulatory region of human *EOMES* lead to significant alterations of cortical size and folding (microcephaly with polymicrogyria; Baala *et al*, 2007). In agreement with the notion of a protomap of cortical folding, both *GPR56* and *EOMES* are expressed in modular patterns in the developing ferret and human cortex (de Juan Romero *et al*, 2015).

Biomechanics of cortical folding

Cranial pressure

One of the first hypotheses on the biomechanics of cortical folding proposed that cranial volume limits cortical size, so that as the cortical tissue grows in surface area the skull offers resistance to its outward expansion, forcing the neural tissue to fold onto itself (Le Gros Clark, 1945). The concept that brain morphology adapts to fit in a limited volume has been recently supported by experiments in chick embryos, where an experimentally expanded optic tectum folds to maintain cranial size (McGowan *et al*, 2012). However, studies focused specifically on the mammalian cerebral cortex have shown that cortical folding takes place even in the absence of

compressive constrain from the skull (Welker, 1990). For example, the removal of non-cortical brain tissue in sheep embryos alleviated any possible cranial pressure onto the growing cortex, and yet this did not suppress or simplify the formation of cortical folds (Barron, 1950; Muckli *et al*, 2009). On the contrary, development of the skull appears to be strongly influenced by brain growth, as cranial volume is significantly enlarged in pathologies where brain and cortical size are abnormally large, such as hydrocephalus or megalencephaly (Barkovich *et al*, 2012).

Axonal tension

The first attempt to mathematically model the biomechanical basis of cortical folding dates back four decades (Richman *et al*, 1975). This model proposed that cortical folding occurs as a result of differential growth between upper and lower cortical layers, which generates stress that is sufficient to induce cortical surface buckling. Thirty years later, Kriegstein and colleagues (Kriegstein *et al*, 2006) proposed that basal progenitors increase significantly neurogenesis at later stages, precisely when upper layers form, and that this enables the differential growth between layers and ultimately drives cortical folding. Whereas the Richman model showed that this principle is sufficient to convert a flat surface into wavy, it relies on such a difference in stiffness between neuronal layers that it seems unrealistic (Bayly *et al*, 2014).

Two decades passed before an alternative model was proposed to explain the biomechanics of cortical folding: the tension-based theory (Van Essen, 1997). This theory by Van Essen was conceptually based on D'Arcy Thompson's analysis on how tension and pressure can interact with structural asymmetries to determine the shape of biological structures (Thompson, 1917). Van Essen argued that cortico-cortical axons are under strong tension, exerting significant pulling forces capable of deforming the cortical mantle, and that these cortico-cortical connections are not symmetric or homogeneously distributed, but some areas are more strongly connected together than with others. Under this scenario, he proposed that those areas connected with a larger amount of axons withstand a greater pulling force, and thus come close together to form a fold, whereas areas poorly interconnected are relayed to the opposite banks of a sulcus. This hypothesis attracted the enthusiasm of many, not only for its simplicity but also for its coherence. Indeed, two simple but fundamental observations made this a very attractive model: (i) The cortical sheet is physically tethered in only one axis, initially by radial processes and followed by connections between cortex and subcortical nuclei (De Carlos & O'Leary, 1992). Tension along these processes may provide a cohesive force against intraventricular hydrostatic pressure, ensuring that the cortical mantle remains tightly wrapped around the subcortical interior. (ii) Specific and topographically organized cortico-cortical projections are established early in development while convolutions are forming (Coogan & Van Essen, 1996; Hilgetag & Barbas, 2006). Van Essen proposed that if developing CNS axons *in vivo* generate even a modest fraction of the specific tension measured *in vitro* (Dennerll *et al*, 1988), then populations of axons pulling together should have ample strength to cause folding of the highly pliable embryonic cortical sheet (Van Essen, 1997). Importantly, this hypothesis also provided a basis for individual variability in brain morphology.

Remarkably, the tension-based theory was widely accepted for more than a decade without rigorous experimental testing. But 14 years later, Taber, Bayly, and colleagues performed a series of very simple and elegant experiments that frontally challenged the foundations of this theory (Xu *et al*, 2010). Their idea was that if axonal tension between opposite sides of a gyrus pulls them together, then these should come apart if these axons are cut and tension is released. This was tested in living brain slices from developing ferrets of various ages throughout the period of gyrus formation. These experiments showed that axons are indeed under considerable tension in the developing brain, but most of this tension is found along axon bundles in deep white matter tracts, not within the core of individual gyri and thus too far to play a major role in initiating, sustaining, or maintaining cortical folding (Armstrong *et al*, 1991; Xu *et al*, 2010). In addition, other computational models show that cortical folding may occur in the complete absence of cortico-cortical fibers, only as an effect of buckling instability (see below; Toro & Burnod, 2005).

Tissue buckling

Folding of the cerebral cortex, like any other tissue, is limited by its physical–mechanical properties of rigidity and pliability, which define the relationship between cortical thickness and surface area. As a general principle, given a cortical surface area, the periodicity of folding is inversely correlated with gray matter thickness (Hofman, 1985; Toro & Burnod, 2005; Pillay & Manger, 2007). This applies to interspecies differences (i.e., ungulates have a thinner and more folded cortex than primates, whereas in the manatee, the cortex is thicker and rather smooth; Welker, 1990) and also to human pathology, where lissencephalic patients with reduced folding display an abnormally thick cortex, and polymicrogyric cortices are abnormally thin (Olson & Walsh, 2002; Barkovich *et al*, 2012).

A very recent study proposes a general law for this inverse correlation between thickness and folding, based on measurements from 62 different species and describing a mathematical relationship between total cortical surface area, thickness, and exposed area (Mota & Herculano-Houzel, 2015). The basic idea is that cortical folding is similar to crumpling a ball of paper, where the periodicity of individual folds will be high in a ball of thin smoking paper (small folds) and very low if using thick cardboard paper (large folds). According to this study, the combination of total cortical area and thickness grows with exposed cortical area to the power of 1.25, in a similar fashion as the area of a circle increases with its radius raised to the power of 2. This relationship is also proposed to represent the topological configuration involving the minimal energy, and therefore, cortical folding may settle into the configuration of least energy (Mota & Herculano-Houzel, 2015). But there are several fundamental problems with the paper ball analogy: (i) It describes the folding of a structure that no longer grows, whereas the cortex folds while it continues to develop and grow; (ii) it is based on physical models only valid for single-molecule thin materials; (iii) the theory considers that the whole brain folds, while folding only involves the cortical gray matter, not the rest of the brain (Mota & Herculano-Houzel, 2015). Finally, folding of the cerebral cortex does not occur randomly, as crumpling of a paper ball, but in a rather highly stereotyped process, defining patterns that are characteristic and distinct for each species. Prior to their appearance, folding patterns are delineated by regional variations of progenitor cell proliferation (Reillo *et al*, 2011), and by modules of differential gene expression along germinal layers, as recently demonstrated via

transcriptomic expression analyses (de Juan Romero *et al*, 2015). These fundamental heterogeneities of the developing cortex immediately prior to folding are completely ignored by the Mota and Herculano-Houzel model. Additional problems with this model, at the mathematical level, have been recently highlighted (De Lussanet, 2016).

Using a finite element computational model, Taber and colleagues suggested that a critical factor leading to the formation of outward folds is differential cortical growth (Xu *et al*, 2010), which precisely matches our experimental data and histogenic model of regional cortical expansion and folding in ferret (Reillo *et al*, 2011). This hypothesis where the local difference in tissue growth, particularly lateral/tangential expansion, is a key factor driving cortical folding (Reillo *et al*, 2011; Borrell & Reillo, 2012; Borrell & Gotz, 2014) is progressively gaining acceptance in the field (Ronan *et al*, 2014; Ronan & Fletcher, 2015; Striedter *et al*, 2015). Although these new findings contradict Van Essen's tension-based hypothesis of cortical folding, and axonal tension may not directly be the driving force of cortical folding, alternative computational models do propose that axonal tension may play an important role in tissue buckling (Chada *et al*, 1997). In this case, however, models propose that upon incipient folding, the mechanical stress created induces cell proliferation and differential growth, which further accentuates the folded appearance of the cortex and defines the shape of cortical folds (Toro & Burnod, 2005).

A major breakthrough in our understanding of the biomechanics underlying cortical folding came with a recent study by Tallinen and colleagues (Tallinen *et al*, 2014). Many of the ingredients of this model were already present in the mentioned models of Richman, Toro, and Taber (Richman *et al*, 1975; Toro & Burnod, 2005; Xu *et al*, 2010), but this new study is the first to propose a coherent framework with physical simulations, computational simulations, and a very compelling mathematical analysis of the problem. The key point of this model is that two different materials are sticking together while growing homogeneously, if one grows faster than the other, the system becomes unstable and will change shape by folding, which is an emergent property of any such mechanical system (Tallinen *et al*, 2014). Most interestingly, solely based on the slightly different physical properties of the two materials (i.e., upper versus lower cortical layers), homogeneous continuous growth is sufficient to lead the system to develop a dramatic change in shape, forming a heterogeneous pattern of stress that in turn can influence the biology of the tissue (i.e., cell proliferation, apoptosis, cell fate, and even axon guidance). Therefore, tissue buckling and cortical folding may ultimately result from the mutual influence between physical properties, biomechanics, and differential tissue growth.

Defects of cortical folding: human disease

The size and folding of the cerebral cortex have a fundamental impact on brain function. Significant changes (excess or defect) in these parameters are the most common cause of severe intellectual disability and intractable epilepsy (Guerrini *et al*, 2008; Andrade, 2009). Defects in human cortical development have been recognized as being originated by the disruption of some of the cellular and molecular mechanisms described above in this review (Barkovich *et al*, 2012), hence critically impacting on key developmental events. In this section, we briefly present these human malformations grouped according to the main affection: brain size, cortical folding, or the formation of ectopias (groups of cells in an abnormal location). It is important to note that cortical malformations usually appear as compound phenotypes, only very rarely as a single defect (Fig 4).

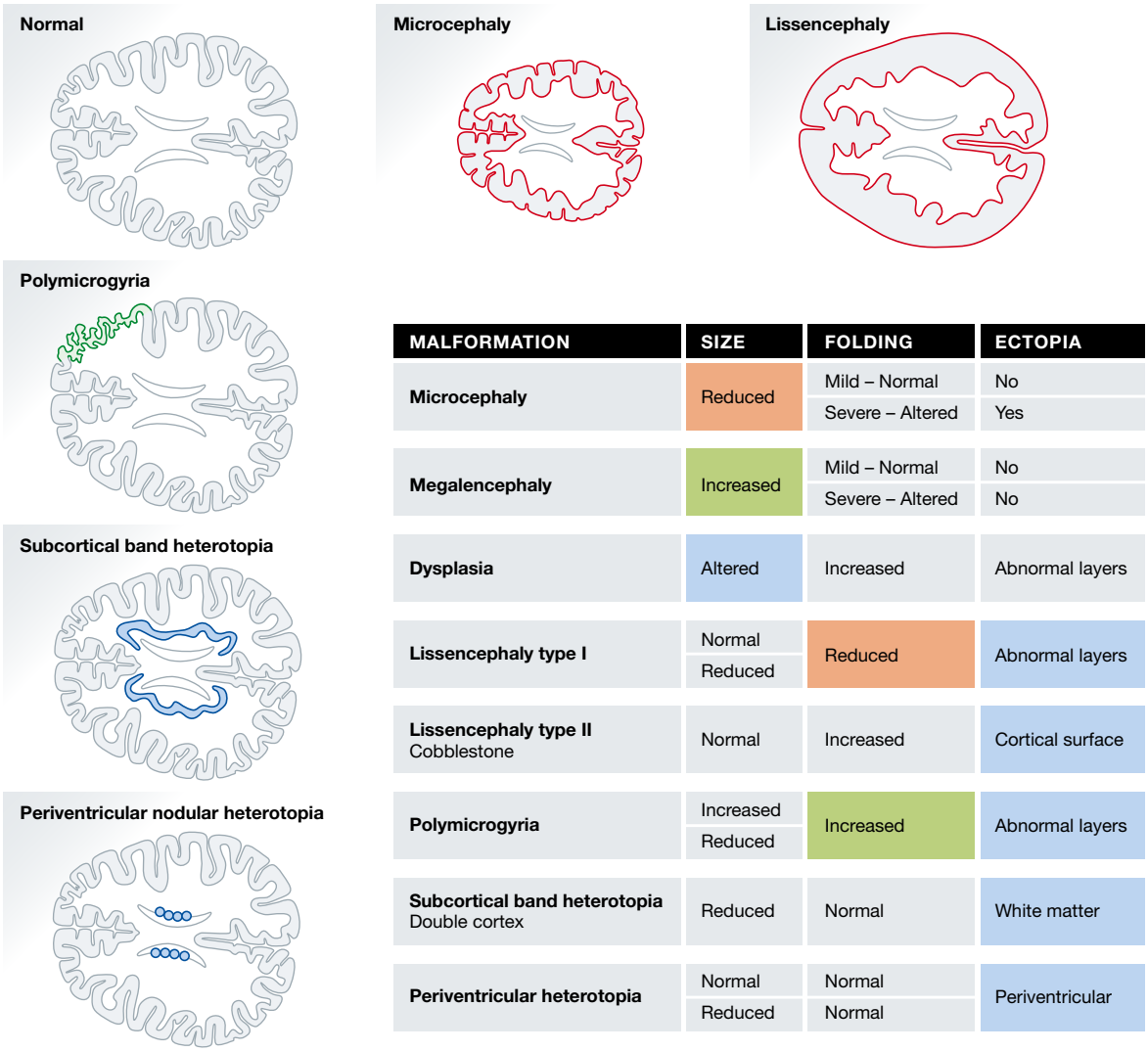
Brain size

As explained in the first section, the pool size of founder and neurogenic cortical progenitors plays a central role in defining cortical size. Consequently, alterations in proliferation and/or survival of neural progenitors lead to abnormal brain size, either excessive (megalencephaly), defective (microcephaly), or imbalanced (dysplasia; Barkovich *et al*, 2012; Fig 4).

Microcephaly Microcephaly is a rare developmental disorder in which affected individuals display a significantly reduced brain size compared to controls (Bond *et al*, 2002; Gilmore & Walsh, 2013). This condition may be mild (only brain size is affected) or severe (small brain size and altered cortical folding; Bilguvar *et al*, 2010; Yu *et al*, 2010; Adachi *et al*, 2011). Microcephaly has been associated with mutations in genes important for a wide variety of cellular processes: DNA repair efficiency; cell cycle length; mitotic spindle positioning; and centrosome maturation, duplication, and position (Table 1). For example, the most common causes of primary microcephaly are mutations in microcephalin (*MCPH1*), which lengthens the cell cycle and alters chromosome alignment during mitosis (Jackson *et al*, 2002; Woods *et al*, 2005; Gruber *et al*, 2011), and *ASPM*, which is important to maintain the orientation of the mitotic cleavage plane (Kumar *et al*, 2004; Shen *et al*, 2005; Fish *et al*, 2006; Gul *et al*, 2006; Table 1). For an extensive review on the role of different genes identified in microcephalic patients on the emergence of this condition, please refer to Bizzotto and Francis (2015).

Megalencephaly Megalencephaly is characterized by an abnormal enlargement of the brain, which has been related to an excessive production of progenitor cells and cortical neurons due to a decreased apoptosis, or a shortening of cell cycle and increased cell cycle re-entry (Dehay & Kennedy, 2007; Hansen *et al*, 2010; Wang *et al*, 2011; Barkovich *et al*, 2012). As in other malformations, severe forms of megalencephaly may occur together with altered patterns of cortical folding. Usually, the increased abundance of progenitor cells and neurons results in polymicrogyria or excessive cortical folding (Barkovich *et al*, 2005). In fact, megalencephaly normally occurs in syndromes, in combination with other alterations of development, such as MPPH (macrocephaly, polymicrogyria, polydactyly, hydrocephalus), M-CMTC (macrocephaly cutis marmorata telangiectasia congenita), and MCAP (macrocephaly capillary malformation; Mirzaa *et al*, 2004; Conway *et al*, 2007; Tore *et al*, 2009). The genetic causes of megalencephaly are only partially understood, but recent progress highlights the importance of phosphatidylinositol 3-kinase (PI3K)-Akt signaling, which seems to play a central role in controlling brain size (DiLiberti, 1998; Lee *et al*, 2012; Riviere *et al*, 2012; Mirzaa *et al*, 2013; Table 1).

Dysplasia A very common group of malformations of cortical development related to epilepsy is focal cortical dysplasia (FCD), which classically has included patients showing a variety of histologic



MALFORMATION	SIZE	FOLDING	ECTOPIA
Microcephaly	Reduced	Mild – Normal	No
		Severe – Altered	Yes
Megalencephaly	Increased	Mild – Normal	No
		Severe – Altered	No
Dysplasia	Altered	Increased	Abnormal layers
Lissencephaly type I	Normal	Reduced	Abnormal layers
	Reduced		
Lissencephaly type II Cobblestone	Normal	Increased	Cortical surface
Polymicrogyria	Increased	Increased	Abnormal layers
	Reduced		
Subcortical band heterotopia Double cortex	Reduced	Normal	White matter
Periventricular heterotopia	Normal	Normal	Periventricular
	Reduced	Normal	

Figure 4. Human cortical malformations and their phenotypic manifestations.
Schematic of horizontal sections through the cerebral cortex of a normal human brain compared to those of patients with cortical malformation: microcephaly, lissencephaly type I, polymicrogyria, subcortical band heterotopia (double cortex), and periventricular nodular heterotopia. The table summarizes the phenotypic manifestations associated with each malformation regarding brain size, cortical folding, and the formation of ectopias. The most representative effects are highlighted and color-coded: Features negatively affected by the pathology are in red, features augmented in green, and particularities are in blue. Uncolored cells indicate additional alterations that may be associated with the primary defect.

alterations such as cortical disorganization and architecture (abnormal layering, polymicrogyria) and cells with abnormal location or morphology (neuronal heterotopia, balloon cells, neuronal cytomegaly; Palmini *et al*, 2004; Barkovich *et al*, 2005). These alterations may appear in any part of the cortex and affect regions of different size, even multiple cortical lobes (Tassi *et al*, 2002), which determine the semiology of seizures. Genes in the mTOR pathway are emerging as important players on the origin of developmental cortical dysplasias (Crino *et al*, 2006; Barkovich *et al*, 2012). FCDs are distinguished in three different varieties (Blumcke *et al*, 2011; Barkovich *et al*, 2012): (i) type I (isolated), with disrupted cortical lamination that may be radial (Ia), tangential (Ib), or both (Ic); (ii) type II (isolated), presenting dysmorphic neurons with balloon cells (IIb) or without them (IIa); (iii) type III, associated with another main lesion, such as hippocampal sclerosis (IIIa), glial or glio-neuronal tumor (IIIb), vascular malformation (IIIc), and others (trauma, ischemic injury, encephalitis) (IIId) (Blumcke *et al*, 2011).

Cortical folding

Alterations of cortical folding in the human brain have been classically attributed to defects of neuronal migration (Ross & Walsh, 2001). However, disruption of neuron migration and positioning also leads to other cortical defects with a mild alteration of cortical folding, such as lissencephaly type II and subcortical band heterotopia. The latter will be discussed in the next section focused on ectopias.

Lissencephaly (smooth brain) This includes several disorders collectively characterized by the simplification of the folding pattern: agyria (complete absence of folds), pachygyria (simplified pattern of folds), and subcortical band heterotopia (gyral pattern is either

Table 1. Types of human cortical malformation, molecular mechanisms altered, and genes associated.

	Malformation	Molecular mechanism	Genes	References
Size	Microcephaly	DNA repair efficiency	MCPH1, PNKP, PNCT	Woods *et al* (2005); Griffith *et al* (2008); Sheen *et al* (2010); Gruber *et al* (2011)
		Cell cycle length	ASPM, STIL, AKT3	Boland *et al* (2007); Desir *et al* (2008); Kumar *et al* (2009); Passemard *et al* (2009)
		Mitotic spindle positioning	ASPM, STIL, WDR62, NDE1, TCOF1, DYNC1H1, TUBG1, KIF5C, KIF2A	Feng & Walsh (2004); Bilguvar *et al* (2010); Nicholas *et al* (2010); Yu *et al* (2010); Sakai *et al* (2012); Poirier *et al* (2013)
		Centrosome maturation, duplication, and position	NDE1, CDK5RAP2, CENPJ, ASPM, CMPH1, WDR62, STIL, CEP152, CEP63	Abrieu *et al* (2000); Alkuraya *et al* (2011); Bhat *et al* (2011); Bond *et al* (2005); Graser *et al* (2007); Bakircioglu *et al* (2011); Marthiens *et al* (2013); Mirzaa *et al* (2014); Nicholas *et al* (2010); Sir *et al* (2011); Thornton & Woods (2009); Yao *et al* (2000)
	Megalencephaly	Cell growth	PI3K-AKT signaling AKT3, PIK3R2, PIK3CA	DiLiberti (1998); Lee *et al* (2012) #7409; Mirzaa *et al* (2013); Poduri *et al* (2013); Riviere *et al* (2012)
	Dysplasia	Cell cycle and growth, ribosome biogenesis, mRNA translation	mTOR pathway activation (tuberous sclerosis complex 1–tuberous sclerosis complex 2)	Crino *et al* (2006); Barkovich *et al* (2012)
Folding	Lissencephaly type I	Radial migration	LIS1, DCX, TUBB3, TUBA1A, RELN	D'Arcangelo *et al* (1995); Sapir *et al* (1997); Pilz *et al* (1998); Caspi *et al* (2000); Dulabon *et al* (2000); Hong *et al* (2000); Rice & Curran (2001); Fallet-Bianco *et al* (2008); Morris-Rosendahl *et al* (2008); Kumar *et al* (2010)
		Cortical lamination	RELN	D'Arcangelo *et al* (1995); Dulabon *et al* (2000); Hong *et al* (2000); Rice & Curran (2001)
	Polymicrogyria	Cell adhesion, regulation of phosphorylation, cell motility, synaptogenesis, angiogenesis	SPRX2	Roll *et al* (2006)
		Gene regulator	GPR56	Piao *et al* (2002, 2004, 2005); Bae *et al* (2014)
		Cytoskeleton regulation	TUBB2B, TUBB3, TUBA1A, TUBA8, KBP	Abdollahi *et al* (2009); Jaglin & Chelly (2009); Jansen *et al* (2011); Tischfield *et al* (2011); Poirier *et al* (2013); Valence *et al* (2013); Squier & Jansen (2014)
		Neurite outgrowth	KBP	Valence *et al* (2013)
		DNA repair efficiency	NHEJ1	Cantagrel *et al* (2007)
			Microdeletions in 22q11	Robin *et al* (2006)
		Suggested: centrosomal role	WDR62	Yu *et al* (2010)
Ectopia	SBH/double cortex	Cytoskeleton regulation/ neuronal migration defects	DCX, LIS1, TUBA1A, TUBG1, EML1	Gleeson *et al* (1998); Francis *et al* (1999); Sicca *et al* (2003); Keays (2007); Mineyko *et al* (2010); Kielar *et al* (2014)
	Lissencephaly type II (cobblestone)	Pial surface stability	POMT1; POMT2; FKTN, FKRP, LARGE, POMGNT1, LAMB1	Brockington *et al* (2001); Yoshida *et al* (2001); Beltran-Valero de Bernabe *et al* (2002); Longman *et al* (2003); van Reeuwijk *et al* (2005b); Roscioli *et al* (2012); Willer *et al* (2012); Kariminejad *et al* (2013)
	Periventricular heterotopia	Actin cytoskeleton	FLNA	Fox *et al* (1998); Sheen *et al* (2001); Parrini *et al* (2006); Ferland *et al* (2009)
		Vesicle trafficking	ARFGEF2	Sheen *et al* (2004); Ferland *et al* (2009)
		Neuronal migration	C6orf70	Conti *et al* (2013)
		Molecular adhesion	FAT4	Cappello *et al* (2013)
		Molecular adhesion	DCHS1	Cappello *et al* (2013)
		(unknown)	Microdeletions in 22q11	Kiehl *et al* (2009)

normal or simplified with broad convolutions and a thickened cortex; Guerrini & Marini, 2006; Fig 4). Lissencephalies are classified into two main types: type I or classic, caused by mutations in genes related to the cytoskeleton and affecting cell migration; newborn neurons fail to migrate properly and, instead of forming the characteristic six layers, they accumulate below the preplate in only four distinguishable layers, resulting in a largely disorganized and thickened cortex (Golden & Harding, 2004). Type II, or

cobblestone, is caused by alterations in the interaction between radial glia and the pial surface, which result in the disruption of the cortical surface and the overflow of neurons above the meninges (Bizzotto & Francis, 2015).

Most cases of type I lissencephaly are due to mutations in *LIS1* or *DCX* (Pilz *et al*, 1998). These are proteins that interact with the tubulin cytoskeleton allowing its polymerization and stability (Sapir *et al*, 1997; Caspi *et al*, 2000). This is also the case for *TUBB3* and *TUBA1A*, mutated in 1–4% of type I lissencephalies (Morris-Rosendahl *et al*, 2008; Kumar *et al*, 2010) and 30% of lissencephalies with cerebellar hypoplasia (impaired growth). Interestingly, the loss and simplification of folds displayed by these patients are very similar to those associated with mutations in *LIS1*, suggesting a shared molecular pathway (Barkovich *et al*, 2012). A small number of patients with autosomal recessive type I lissencephaly with cerebellar hypoplasia have mutations in *RELN*, also a gene essential for radial migration and normal cortical lamination in mouse and humans (D'Arcangelo *et al*, 1995; Dulabon *et al*, 2000; Hong *et al*, 2000; Rice & Curran, 2001; Table 1).

Polymicrogyria (many small folds) This includes a group of cortical malformations characterized by the formation of abnormally abundant and small cortical folds. It usually also involves the interdigitation of white matter resulting in abnormal lamination (Barkovich *et al*, 1999; Walsh, 2001). The defects in cortical lamination may be either simplification, with four layers similar to type I lissencephaly, or complete disruption and disorganization. Most frequently polymicrogyria (PMG) phenotypes are very complex and combined with other alterations such as microcephaly (Bilguvar *et al*, 2010; Yu *et al*, 2010). Due to this phenotypic complexity, the causative genes for human PMG have been very elusive. Genetic mutations linked to PMG include alterations in *SPRX2* (Roll *et al*, 2006), microsomal deletions in 22q11 (Bassett *et al*, 2005; Robin *et al*, 2006), and mutations in a number of cytoskeleton-associated genes (Table 1). Nongenetic causes of PMG have also been identified including insults during embryogenesis such as hypoxia, hypoperfusion, and congenital infections (Jacobs *et al*, 1999; Squier & Jansen, 2014).

Ectopia

The proper position of cortical neurons depends on a complex cellular and molecular regulation of two variables: where and when. Neurons must migrate through the entire cortical thickness and stop precisely near the cortical surface, a process determined by the time and place of their generation. Altering these events leads to misplaced neurons, a malformation generically called ectopia (out of place; Fig 4).

Subcortical band heterotopia/double cortex This type of ectopia is characterized by the accumulation of neurons in the cortical white matter (Barkovich *et al*, 2001; Ross & Walsh, 2001). Typically, the cluster of ectopic neurons forms a thick band of cells below an otherwise normal cortical gray matter (Gleeson *et al*, 1998; Francis *et al*, 1999). Importantly, double cortex is accompanied by a reduction in the size of the cerebral cortex due to the loss of neurons from the normocortex. This frequently affects cortical surface area and thickness, and in some cases, it even results in microgyria (Barkovich *et al*, 2012). Genetic mutations causative of double cortex affect a variety of cytoskeleton-interacting proteins (Table 1).

Cobblestone Whereas most types of heterotopia are due to deficient neuronal migration, cobblestone (type II lissencephaly) is caused by their excessive migration. In this case, the anchoring and attachment of the radial fiber of RGCs to the pial surface is disrupted, thus altering the basement membrane (Yamamoto *et al*, 2004; Luo *et al*, 2011). Given that the cortical basement membrane and the attachment of RGCs to it are the finish line for radially migrating neurons, this disruption leads to their overmigration, which continue moving up to the meningeal space, thus resembling cobblestones on the cortical surface (van Reeuwijk *et al*, 2005a). Several complex syndromes cause cobblestone lissencephaly: Fukuyama congenital muscular dystrophy (FCMD), muscle–eye–brain disease (MEB), and Walker–Warburg syndrome (WWS). In spite of this wide spectrum of phenotypes, mutations linked to cobblestone are found in genes involved in the attachment of the radial glial fiber to the pial surface (Li *et al*, 2008; Luo *et al*, 2011), or associated with reduced glycosylation of alpha dystroglycan, which is fundamental to anchor the dystrophin complex to the extracellular matrix (van Reeuwijk *et al*, 2005a; Roscioli *et al*, 2012; Buysse *et al*, 2013). Six major genes have been identified encoding putative or confirmed glycosyltransferases (Table 1).

Periventricular heterotopia Contrary to cobblestone, in periventricular heterotopia (PH) cortical neurons are unable to undergo radial migration. Due to defective remodeling of the actin cytoskeleton, newborn neurons cannot perform the changes in cell shape and locomotion required for their migration and completely fail to leave the germinal zones, remaining in the vicinity of the ventricular surface clustered into nodules, which eventually act as epileptic foci (Sheen *et al*, 2001, 2005; Sheen & Walsh, 2005; Sarkisian *et al*, 2006, 2008; Andrade, 2009). These periventricular nodules may appear in a variety of locations and conformations: bilateral, unilateral, laminar, sub-ependymal, and subcortical white matter (Andrade, 2009; Ferland *et al*, 2009). Remarkably, most of the cortex appears completely normal and patients show no gross defects in intellectual development or performance. PH may appear alone or as part of complex syndromes, associated with other cortical malformations such as microcephaly (Parrini *et al*, 2006). The most frequent genetic alterations linked to periventricular nodular heterotopia affect *FLNA* and *ARFGEF2* (Table 1; Fox *et al*, 1998; Sheen *et al*, 2001, 2004; Parrini *et al*, 2006; Ferland *et al*, 2009). Although these two proteins have very different cellular functions (FLNA acts on the actin cytoskeleton; ARFGEF2 has a role in intracellular membrane and vesicle trafficking), they may act in a common pathway and even interact directly (Sheen *et al*, 2004; Ferland *et al*, 2009). It has been proposed that the disruption of vesicle trafficking due to alterations of the cytoskeleton may impair cell adhesion and the integrity of the apical adherens junctions, thus leading to the formation of the periventricular nodules (Ferland *et al*, 2009).

Evolution of cortical folding

Brain size varies in several orders of magnitude between mammalian species, which is mostly the result of a disproportionate difference in size of the cerebral cortex (Finlay & Darlington, 1995). Increased cortical size is largely due to increased surface area (Rakic, 1995), and this is accompanied by cortical folding and fissuring, which in part allow its effective packing within a minimal

cranial volume (Welker, 1990; Albert & Huttner, 2015). Based on our previous sections, evolution of cerebral cortex size and topology may be attributable to modifications in the abundance and behavior of cortical progenitor cells (Borrell & Reillo, 2012). Changing the duration of the cell cycle, generating IPCs, and increasing their abundance would augment exponentially the production of neurons and therefore brain size, whereas generating bRGCs would augment cortical surface area and folding (Borrell & Calegari, 2014; Lewitus *et al*, 2014).

Early gyrification and secondary loss

Classically, the remarkable expansion and folding of the mammalian cerebral cortex along evolution has been viewed as a unidirectional process, where the small and smooth cortex of a primitive ancestor (presumed similar to mouse) gradually evolved to be larger, more complex, and folded, and from there on, it further evolved to have an increasing number of folds, like the human cortex (Kriegstein *et al*, 2006; Rakic, 2009). However, this view was challenged recently with the hypothesis that gyrencephaly might be an evolutionarily ancient trait, expressed in a common ancestor to all mammals and retained during speciation (Borrell & Reillo, 2012). This hypothesis is based on two facts: a) gyrencephaly develops in species from across mammalian phylogeny, ranging from monotremes and marsupials to ungulates, carnivores, primates, and even rodents, and b) during embryonic development of the cerebral cortex, gyrencephaly differs from lissencephaly in two critical features: subdivision of SVZ into ISVZ and OSVZ and high abundance of basal progenitors (bRGCs and IPCs in ISVZ+OSVZ) that greatly outnumber apical progenitors (aRGCs in VZ; Reillo *et al*, 2011; Borrell & Reillo, 2012; Kelava *et al*, 2012; Reillo & Borrell, 2012). Based on these facts, it seems most parsimonious to propose that gyrencephaly emerged in the stem mammal ancestor upon the innovative generation of bRGCs and OSVZ, and these traits were retained along mammalian speciation. This hypothesis of early gyrification was subsequently supported by studies using a phenomic character matrix of living placental orders and fossil species, which conclude that the ancestor of placental mammals was a small gyrencephalic animal (O'Leary *et al*, 2013). In this scenario, the smooth cortex of lissencephalic mammals (namely rodents and lagomorphs) would have emerged by the simplification, or phenotypic reversal, of gyrencephaly. This reversal may have occurred by reducing the abundance and self-amplificative capacity of basal neurogenic progenitors and bRGCs, as supported by recent studies (Kelava *et al*, 2012, 2013; Martinez-Cerdeno *et al*, 2012; Borrell & Gotz, 2014; De Juan Romero & Borrell, 2015). There are various examples of phenotypic and genomic reversals documented (Teotonio & Rose, 2001), one of its attributes being the ability to acquire new evolutionary trajectories (Borowsky & Wilkens, 2002). The seeming ability of the mammalian brain to undergo significant phenotypic reversals and change in various directions during evolution may explain the remarkable adaptability of mammals along this process (Kelava *et al*, 2013).

Molecular evolution

As discussed previously, the biology of cortical progenitor cells is regulated by the coordinated action of multiple proteins, and the experimental manipulation of these proteins has a profound influence on cortical size and folding. However, it remains to be defined whether the development of folded versus smooth cortices is due to the differential regulation of these genes between species during their normal development, and if so how they became differently regulated during evolution. Only recently, we have begun to identify molecular changes occurred during evolution that provide answers to these questions.

Human accelerated regions (HARs) Our understanding of the genetic basis of cerebral cortex evolution was jump-started by the generalized interest in identifying the genetic determinants of human uniqueness (Dorus *et al*, 2004). Strategies to identify the genetic and molecular mechanisms underlying the distinction of humans focused on searching for variations between the genome of human and immediate relatives in phylogeny. In a seminal study, Lahn and colleagues compared the genomic sequence of humans, chimpanzee, rat, and mouse and found that the genome is moderately well conserved across these species, but the human genome showed sites of uniquely high divergence rate (Dorus *et al*, 2004). Subsequently, they also found evidence demonstrating that genetic evolution is still ongoing in humans, with hotspots in genes related to brain development and also relevant in pathology of human cortical development, such as *ASPM* and *MCPH1* associated with microcephaly (Evans *et al*, 2004, 2005; Mekel-Bobrov *et al*, 2005).

Whereas sequence changes in protein-coding genes might seem the most straightforward mechanism to drive brain evolution (Hill & Walsh, 2005), further analyses have revealed an even more likely role for regions regulating gene expression (Prabhakar *et al*, 2008; Bae *et al*, 2014). Improved genomic comparisons between humans and chimpanzees identified hundreds of small DNA segments, the sequence of which diverged rapidly in humans (Pollard *et al*, 2006a,b). These segments were called "human accelerated regions" (HARs), in reference to their uniquely high rate of nucleotide substitution in the immediate human lineage. This accelerated evolution was proposed to have contributed to acquiring the unmatched size and complexity of the human brain in a relatively brief period (Pollard *et al*, 2006a). Importantly, instead of being part of protein-coding genes, HARs are mainly located in introns and intergenic regions, strongly suggesting their role in gene regulation (Bejerano *et al*, 2006; Pollard *et al*, 2006b). Significantly, relevant genes nearby HARs code for transcription factors and other DNA binding proteins involved in development and morphogenesis. In fact, HAR1, the HAR with the highest level of difference, contains an RNA gene (HAR1F) expressed in Cajal–Retzius cells during development, a peculiar type of cell essential for neuronal migration and lamination of the cerebral cortex (Pollard *et al*, 2006b).

Novel regulatory sequences Recent analyses of HARs focusing on their sequence, histone modification, chromatin state, and transcription factor binding sites conclude that at least 30% of HARs are developmental enhancers (Capra *et al*, 2013). Indeed, the activity of 29 noncoding HARs was tested by generating transgenic mice, which demonstrated that the majority of them are active enhancers in humans and chimpanzee. This confirms that HARs are good candidates as human-specific regulatory regions and also that human-specific brain evolution might be particularly associated with changes in spatial–temporal gene expression, instead of changes in protein sequence (King & Wilson, 1975; Mouse Genome

Sequencing Consortium *et al*, 2002; Lunter *et al*, 2006; Pollard *et al*, 2006b; Ponting & Lunter, 2006; Prabhakar *et al*, 2008).

In order to investigate how the activity of enhancers influences the developing telencephalon, Visel and colleagues performed an *in vivo* digital atlas of transgenic mouse embryos using 145 selected enhancers to drive reporter gene expression (Visel *et al*, 2013). Using a similar strategy, Pattabirman and colleagues generated stable transgenic mouse lines to characterize the gene-promoting activity of putative enhancers and demonstrated that these exhibit sharp boundaries of reporter gene expression in the E11.5 mouse pallium (Pattabiraman *et al*, 2014). These mouse lines were also used to determine the regional fate map of the mouse telencephalon, which demonstrated the existence of distinct progenitor protodomains defined by the activity of those enhancers at various developmental stages.

More recently, the evolution of active genomic enhancers was demonstrated with the analysis of the human *GPR56* locus, mutated in malformations of cortical development including polymicrogyria (Bae *et al*, 2014). In this study, a collection of transgenic mice was generated driving GFP expression from a portion of the same *GPR56* enhancer from various species (humans, mouse, marmoset, dolphin, and cat). The enhancer from these gyrencephalic species drove a similar GFP expression pattern, but this was different from the endogenous pattern in the lissencephalic mouse. Given that mouse and primates share a phylogenic ancestor more recent than with carnivores and cetaceans (Bininda-Emonds *et al*, 2007), the similar regulation of *GPR56* expression among these gyrencephalic species suggests convergent evolution.

Novel non-protein-coding genes In addition to variations in enhancer sequences, an extra level of control over the spatial–temporal patterns of gene expression is provided by non-protein-coding RNAs. Mounting evidence demonstrates the relevance of noncoding RNAs (miRNAs, lncRNAs, etc.) on cerebral cortex development (Aprea & Calegari, 2015; Liu & Sun, 2015), and their significance on evolution is supported by their increased number at evolutionary divergence points (Heimberg *et al*, 2008). As for the regulation of cerebral cortex expansion and folding, hundreds of miRNAs have been found expressed in apical and basal progenitor cells of the developing cortex of macaque embryos, but not in mouse (Arcila *et al*, 2014). These primate miRNAs prominently target proteins regulating the cell cycle and neurogenesis, hence contributing to the differential growth, amplification, and complexification of germinal layers and cortical areas, including the OSVZ (Lukaszewicz *et al*, 2005; Dehay & Kennedy, 2007). Altogether, the coevolution of emergent miRNAs and their target genes suggests that novel miRNAs became integrated into ancient gene circuitry to exert additional control over cortical progenitors, ultimately leading to the acquisition of primate-specific cortical features and, potentially, higher brain performance (Dehay *et al*, 2015).

Novel protein-coding genes As shown by Lahn and colleagues (Dorus *et al*, 2004), biological evolution also comes from variations in the sequence of protein-coding genes (True & Carroll, 2002). Looking for gene innovations that might have been involved in cortical expansion and folding during evolution, the focus has recently turned specifically onto cortical progenitor cells. A transcriptomic search for genes differentially expressed in radial glial cells between species identified 56 genes expressed in human, but not mouse, RGCs (Florio *et al*, 2015). Among these, *ARHGAP11B* was uniquely intriguing because it had the highest degree of radial glia-specific expression, and there is no mouse ortholog. In fact, *ARHGAP11B* arose from partial duplication of the *ARHGAP11A* gene on the human lineage after separation from the chimpanzees, and thus, it is a hominid-specific gene (Antonacci *et al*, 2014). Most impressively, experimental expression of *ARHGAP11B* in aRGCs of the embryonic mouse cortex promoted the generation of self-renewing basal progenitors (particularly bRGCs) and, in turn, increased cortical surface area and induced the formation of cortical folds in mouse (Florio *et al*, 2015). Therefore, the emergence of new protein-coding genes along mammalian phylogeny, such as *ARHGAP11B* in the hominid lineage, may have contributed significantly to the evolutionary expansion and folding of the mammalian cerebral cortex.

Concluding remarks and open questions

The expansion and folding of the mammalian cerebral cortex during embryonic development is a rather complex process regulated by multiple factors, where the abundance, type, and lineage of cortical progenitor cells play central roles. These cellular mechanisms are subject to molecular regulation by multiple proteins and signaling pathways, the expression of which is tightly controlled by a variety of enhancer elements and non-protein-coding genes. The specific spatial–temporal expression patterns of some of these proteins on the embryonic cortex faithfully map the prospective pattern of folds and fissures, and their mutation frequently leads to malformations of cortical size and folding in human patients. Yet, we are still far from identifying the specific role of these genes and their spatial–temporal regulation on the normal development of the human cerebral cortex.

Studying cortical development and folding across species helps us to understand the evolution of this complex process, and in return, we hope that this helps us to understand the aspects of cortical development critical to its expansion and folding during embryogenesis. Again, more and more refined molecular and genomic analyses are shedding some light on this problem, and emerging experimental animal models in this field like the ferret are of great help, but the truth is that we remain far from having a significant level of understanding.

Given the complexity of the developmental mechanisms involved in the expansion and folding of the cerebral cortex, and thus its tremendous cost in terms of genetic, cellular, and histogenic evolution, the ecological advantages must be more than remarkable. But what are the advantages of cortical folding? Clearly, a bigger cortex contains more neurons and more neuropile, and thus in principle, it provides greater computational power. Folding brings together highly interconnected cortical areas, thus minimizing cortical wiring and favoring high speed associational communication, ultimately optimizing brain circuitry (Klyachko & Stevens, 2003). However, the human cortex is neither the largest nor the most folded among mammals, as compared with elephants or dolphins, for example, and yet it is assumed that humans have a higher intelligence over any other earthly species. Are we really more intelligent than these other species? (Roth & Dicke, 2005) Or it is only that we have the

advantage of speech and manual ability to express our intellectual abilities? Is there a trade-off between cortical size, folding, and intellectual performance? Modern humans and Neandertals co-existed until 30,000 years ago, but we persisted and the latter perished. Was there some critical (albeit small) disadvantage in the organization or folding of the Neandertal brain? Was their extinction related to a less competitive intelligence? Were their cortical circuits or synapses less efficient? With the genomes of Neandertal and modern humans at hand, the specific differences in their DNA sequence identified (Green *et al*, 2010; Prufer *et al*, 2014), in combination with novel *in vitro* models of human brain development like cerebral organoids (Lancaster *et al*, 2013), these fascinating questions may soon be approachable. Hopefully, our understanding of the mechanisms and consequences of cortical expansion and folding will be much deeper 25 years from now.

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Conflict of interest

The authors declare that they have no conflict of interest.

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