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Developmental mechanisms of gyrification

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Abstract
Folding of the cerebral cortex is a fundamental milestone of mammalian brain evolution associated with dramatic increases in size and complexity. Cortex folding takes place during embryonic and perinatal development and is important to optimize the functional organization and wiring of the brain, while allowing fitting a large cortex in a limited cranial volume. Cortex growth and folding are the result of complex cellular and mechanical processes that involve neural stem progenitor cells and their lineages, the migration and differentiation of neurons, and the genetic programs that regulate and fine-tune these processes. Here, we provide an updated overview of the most significant and recent advances in our understanding of developmental mechanisms regulating cortical gyrification.

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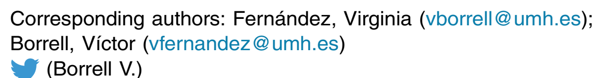
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Introduction

One of the most salient features of the human brain is its external appearance of wrinkled tissue, which corresponds to the folded cerebral cortex. The cerebral cortex is a sheet of tissue composed of six main layers of neurons in its outer part (gray matter), and an inner part (white matter) containing mostly axons, which connect cortical neurons with other distantly located cortical areas or brain regions. In mammals with big brains, like humans, the cerebral cortex grows disproportionately larger compared to the underlying deep brain regions during embryonic development [1]. This expansion of the cerebral cortex occurs mostly in surface area rather than in thickness and is accompanied by the formation of folds and fissures. Cortex folding is typical of mammals with a large brain, whereas those with a small brain like mouse have a smooth cortex, without folds [2]. Naïvely, folding may be viewed simply as a mechanism to pack a very large cortical surface area within a limited cranial volume. In other words, folding is an epiphenomenon of cortical expansion, where dramatic growth is contained within, and adapts to, the limits of the cranial bone cavity. Far from that, cortex folding is fundamental for the optimization of brain wiring and the functional organization of cortical areas [3], such that alterations in cortex size and/or folding lead to severe intellectual disability in humans [4,5]. Cortex folding takes place during embryonic and perinatal brain development [2], and work in the last decade has unraveled key determinants of this process, including cellular, molecular, and mechanical factors [6]. Here we present an updated overview of recent findings uncovering novel regulators of cerebral cortex expansion and folding, which have mostly focused on factors that control the behavior of neural stem and progenitor cells (Table 1).

Cellular mechanisms

The cerebral cortex derives from the lateral telencephalic vesicles of the early embryo. At such early stage, the cortical primordium is essentially formed by a single layer of neural stem cells with epithelial features, called neuroepithelial cells (NECs) [7]. NECs undergo several rounds of self-amplifying divisions before they transform into apical radial glia Cells (aRGCs), the primary type of progenitor cell during cortical neurogenesis. NECs and aRGCs undergo mitotic cell division at the apical surface of the cortical primordium (limiting with the ventricular cavity), and thus are called apical progenitor cells [8]. Recent work highlights the relevance of events involving NECs and aRGCs in the expansion and folding of the cerebral cortex.

NEC transition

NECs are highly elongated cells that span the entire thickness of the early telencephalic vesicles [9]. They are attached to each other by the apical (inner) domain via tight and adherens junctions, and to the basal (outer) surface of the early telencephalic vesicle via the basal lamina [8]. The nucleus of NECs is usually located at an intermediate position between the apical and basal surface, thus defining an apical and a basal process

Table 1

Novel genes regulating cortical development and gyrification.

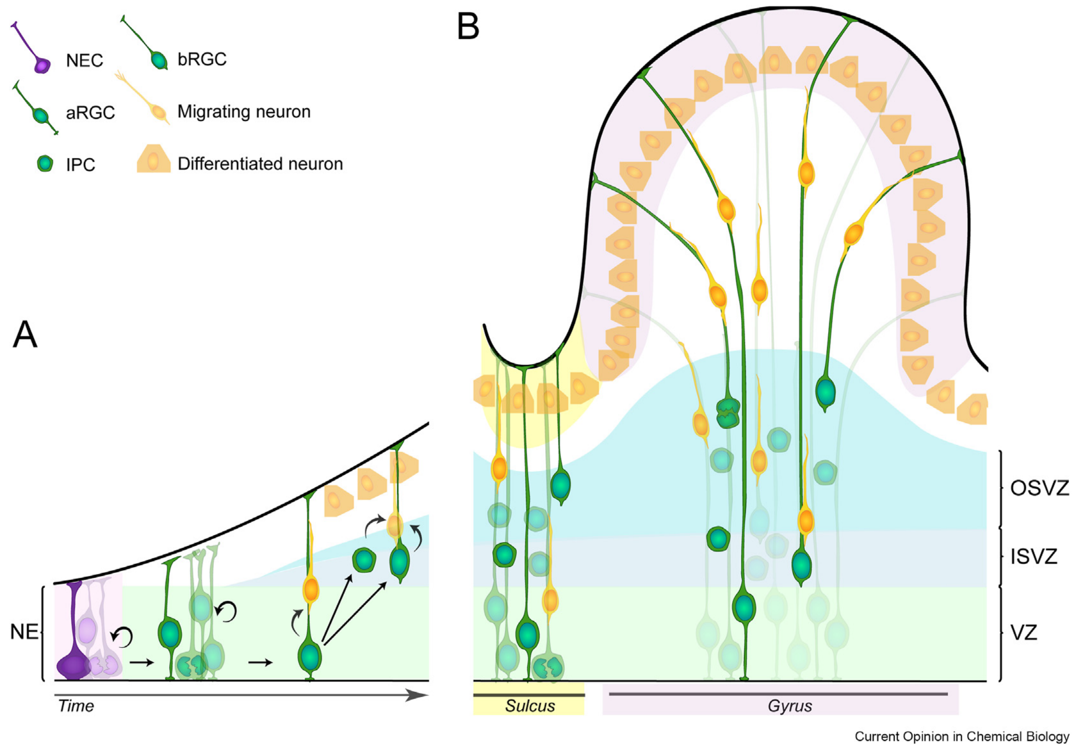
Cell type affected	Effect	Gene	Mechanism affected	Reference
NEC	Cell shape	*ZEB2*	Differences in interkinetic nuclear migration and cell cycle length.	[12]
aRGC	Cortical enlargement and folding	*CEP83*	Microtubule disorganization and apical membrane stretching and stiffening.	[19]
aRGC	VZ expansion	*miR-3607*	Amplification of aRGCs, via blocking the β-catenin inhibitor APC.	[44]
aRGC	Kinetochore function and chromosome segregation	Neanderthal *KIF18a*, *KNL1*	Metaphase shortening and increase in chromosome segregation errors in apical progenitors.	
IPC	Cell proliferation	*H3K9ac*	IPC amplification by increasing expression of Trnp1.	[25]
bRGC	Expressed in a subtype of bRGCs (among other cells)	*HOPX*	Suppressing Shh signaling reduced HOPX-positive bRGCs and cortical folding, while enhancing it had opposing effects.	[34]
bRGC	bRGCs proliferation	*ARHGAP11B*	Promotes the proliferation of basal progenitors, which are implicated in neocortical expansion through glutaminolysis.	[50,53,54]
bRGC	Increases the abundance of bRGCs	*TKTL1*	Modern human variant, hTKTL1, but not the Neanderthal variant, increases the abundance of bRGCs. The hTKTL1 effect requires the pentose phosphate pathway and fatty acid synthesis.	[58]
Astrocyte	Positive FGF feedback loop	*FGF*	Localized astrogenesis by a positive feedback loop of FGF signaling is an important mechanism underlying cortical growth and therefore cortical folding.	[37]

(Figure 1). NECs solely undergo symmetric self-amplificative divisions, by which they exponentially increase in number over time as they proliferate. After a few rounds of self-amplification, NECs undergo a rapid change in molecular features and cell identity, down-regulating tight junctions and becoming aRGCs [10]. The emergence of aRGCs triggers the onset of neurogenesis, thereby limiting the self-amplification of progenitor cells. Accordingly, aRGCs typically undergo asymmetric neurogenic divisions, which result in one aRGC plus one more differentiated cell, either a neuron or an intermediate Progenitor Cell (IPC) [8]. Importantly, because most cortical cells ultimately derive from the early NECs, their population size prior to becoming aRGCs has a critical impact on the eventual final size of the cerebral cortex [11] (see Ref. [4] for a detailed review). In the small cerebral cortex of mouse, the transition from NECs to aRGCs is fast and occurs only in a matter of hours. In contrast, a recent study by Lancaster and colleagues shows that this process is much slower and gradual in human and great apes, occurring over several days and involving an intermediate NEC status, the transitioning NEC (tNE) [12]. During this transition, the thickness of the apical process of NECs decreases dramatically, a change in shape that precedes their switch to aRGC identity. Intriguingly, this transition is protracted specifically in human NECs compared with other great apes, namely gorilla and chimpanzee, as observed in cerebral organoids [12]. ZEB2, a known epithelial–mesenchymal transition regulator, plays a central role in accelerating this process in non-human apes, promoting neuroepithelial transition. Most importantly, the lengthening of this transition in humans allows NECs to continue self-amplifying and increasing in numbers even further, before becoming aRGCs with limited amplification capacity. This study reports for the first time human-specific features of cortical development prior to the onset of neurogenesis that may have a direct and very relevant impact on cortex size [12].

Cell mechanics of aRGCs

Following NEC transition, aRGCs retain several characteristics from their parent NECs. These include a highly polarized morphology with an apical and a basal process, and anchoring of their apical end-foot to neighboring aRGCs at the apical surface of the cortical primordium, forming an apical junction belt (Figure 1) [10]. Initially, aRGCs undergo mostly self-amplificative divisions, producing two daughter aRGCs. But this shifts gradually to asymmetric divisions, giving rise to a self-renewed aRGC plus one neuron or one IPC [13–15]. Given that all cortical excitatory neurons and astrocytes derive directly or indirectly from aRGCs

Figure 1



Stem cells in the developing cerebral cortex, and mechanism of tangential expansion that generates gyri and sulci. (**a**) Schema of the most abundant types of progenitor cells and their lineage in the developing cerebral cortex. Before neurogenesis, NECs undergo self-amplifying symmetric divisions, and then they generate aRGCs that first self-amplify and then divide asymmetrically to generate neurons and secondary progenitors (IPCs and bRGCs). (**b**) Arrangement of the radial fiber scaffold in a sulcus (left) and in a gyrus (right). In sulci, the radial glia scaffold is parallel, avoiding lateral dispersion of neurons. In gyri, the radial fiber scaffold acquires a fan-like organization due to the abundance of bRGCs, allowing tangential dispersion of neurons and promoting cortical folding. NE, Neuroepithelium; VZ, ventricular zone; ISVZ, inner subventricular zone; OSVZ, outer subventricular zone.

[16–18], significant alterations on their lineage or proliferative dynamics cause dramatic changes in the final cellular composition and size of the cerebral cortex. Hence, the lineage dynamics of aRGCs must be subject to tight regulation. Novel findings highlight the importance of cell mechanics in this process [19]. In aRGCs, the centrosome is located at the apical end-foot during interphase (when not in division), where it supports the formation of the primary cilium and regulates aRGC division and neurogenesis [20–22]. A study by Shi and colleagues now demonstrates that anchoring the centrosome at the apical membrane of aRGCs controls their mechanical properties, which has a critical influence on their mitotic behavior, with consequences on cortex size and folding [19]. The authors of this study show that the removal of the centrosomal protein 83 (CEP83) eliminates the distal appendages of the mother centriole and disrupts the anchorage of the centrosome to the apical membrane. This causes the disorganization of microtubules and increases the stiffness of the apical membrane of aRGCs. This change in cell mechanical properties activates the mechanically sensitive protein YAP and promotes the overproliferation of aRGCs and the generation of IPCs, ultimately leading to the enlargement and folding of the mouse cerebral cortex [19]. Previous work demonstrated that YAP signaling drives accelerated and excessive cortical neurogenesis [23]. This study links YAP with apical stiffness and centrosome, and shows that the abnormalities caused by the loss of CEP83 are fully rescued by the simultaneous elimination of YAP. This leads to restoring the small size and absence of folds of the mouse cerebral cortex [19]. Moreover, YAP activity increases progenitor proliferation and the production of upper layer neurons [24]. Together, these findings uncover the relevance of the centrosome in regulating the mechanical properties of neural progenitor cells, and the critical impact of these on the final size and shape of the mammalian cerebral cortex.

Basal progenitor cells

Starting at the onset of neurogenesis, aRGCs undergo asymmetric divisions to produce one aRGC plus one neuron, or a basal progenitor cell. Basal progenitors accumulate outside of the ventricular zone (VZ), where aRGCs reside, to form a secondary, basal germinal zone:

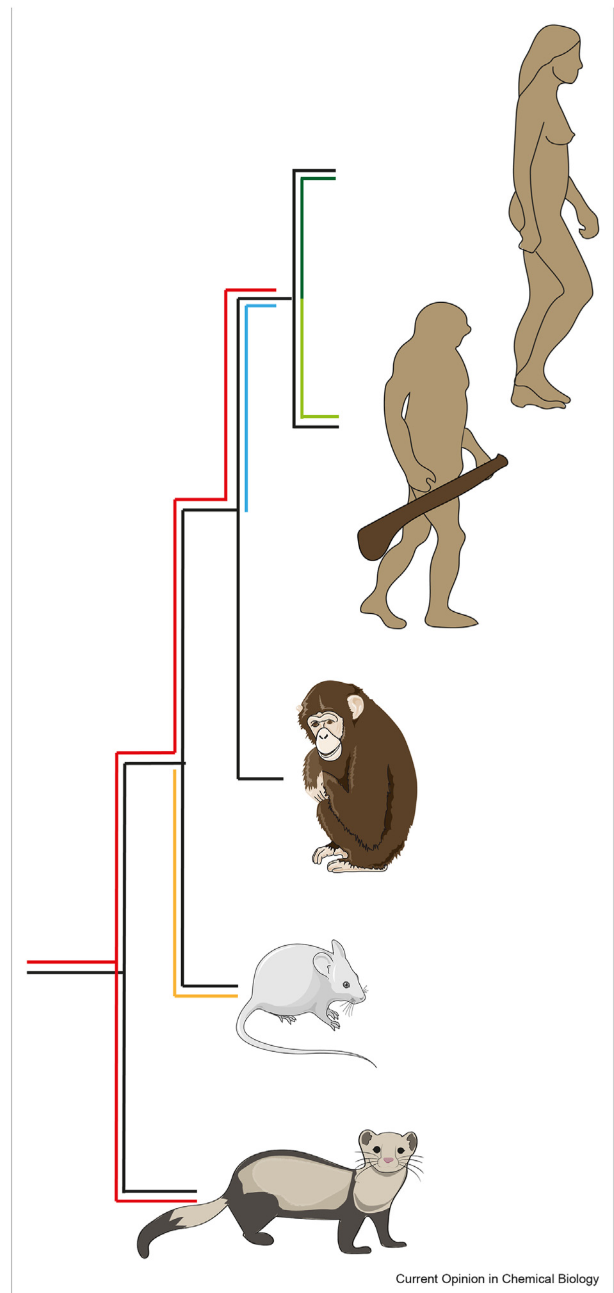
the subventricular zone (SVZ). The most abundant types of basal progenitors are Intermediate Progenitor Cells (IPCs) and basal radial glia Cells (bRGCs). In lissencephalic species, most basal progenitors are IPCs, which undergo one or more neurogenic divisions and thus amplify the final output of neurogenesis. Accordingly, factors controlling IPC proliferation and lineage directly impact cortex size. A recent study by Tuoc and colleagues demonstrates that Histone H3 lysine 9 acetylation (H3K9ac) is a critical epigenetic regulator during neurogenesis, which increases the expression levels of *Trnp1* and, consequently, promotes the amplification of IPCs and cortical expansion [25].

In gyrencephalic species, basal progenitors are extraordinarily abundant and the SVZ is much thicker than in lissencephalic species; hence split in two distinct layers: Inner (ISVZ) and Outer (OSVZ) SVZ (Figure 1), as first described in macaque monkey [26]. Contrary to the mouse SVZ, the most abundant type of basal progenitor in ISVZ and OSVZ of gyrencephalic species are bRGCs [27,28]. bRGCs are remarkably similar to aRGCs, both at the morphological and transcriptomic levels [29–32]. A study of the developing human cortex showed that at mid-gestation bRGCs may be identified by the expression of HOPX [30]. This gene has been commonly used thereafter as a universal maker of bRGCs [29,33]. However, a recent study by Kawasaki and colleagues in the gyrencephalic ferret shows that bRGCs are heterogeneous, with HOPX expressed only in one subpopulation but not the other. HOPX+ bRGCs have higher self-renewal activity than HOPX-, and specifically accumulate in cortical regions that will form gyri, suggesting that HOPX+ bRGCs are a subtype of basal progenitors important for cortex folding [34]. Indeed, HOPX expression is regulated by sonic hedgehog (Shh) signaling, as its suppression reduces both HOPX+ bRGCs and cortex folding. Is this a ferret-unique feature? In the early human embryo, HOPX expression is also not exclusive to bRGCs, nor the OSVZ, but it is highly expressed in both OSVZ and VZ, as reported by Kriegstein, Pollen and colleagues [30]. This indicates that HOPX is not a universal marker of bRGCs and; hence, it also opens the possibility that human bRGCs may be more heterogeneous than currently suspected, as in ferret.

Astrocytes

As neurogenesis reaches completion, aRGCs undergo a fate switch and enter gliogenesis, a period when they generate astrocytes [35]. Contrary to bRGCs, astrocytes have received little attention as potentially relevant in cortex folding. In ferret, astrocytes start to be generated coincident with the onset of cortex folding [36], suggesting that their role in this process, if any, may be not instructive but only secondary or supportive. A recent study addresses this possibility and finds that the rate of astrocytogenesis varies significantly across the developing ferret cerebral cortex, via a mechanism involving a positive feedback loop of FGF signaling [37]. Through experimental manipulation of developing ferrets, the study suggests that the formation of folds and fissures is the

Figure 2



Evolutionary changes in cortical development-related genes. Phylogenic relationship between human, Australopithecus, macaque, mouse, and ferret and external appearance (not at scale). In red, expression of MIR3607, in orange, secondary loss of this gene in mouse. In dark blue, expression of ARHGAP11B in humans and not in nonhuman mammals. In dark green, Human specific variant of TKTL1, which differs in one single amino acid from Neanderthal's variant (in light green).

result of these local differences in production of astrocytes, opening a fascinating new avenue of investigation.

Brain evolution and folding

Secondary loss of cortex folding

Work in the last decade shows that the evolution of gyrencephaly was a bidirectional process. Cortex folding first emerged in mammals after their last common ancestor with the other amniotes, possibly concomitant with a significant expansion in brain size, and was preserved along mammalian speciation across monotremes, marsupials, and all placental mammals [38,39]. In some clades and species, particularly in small mammals including rodents and new world monkeys, this process was reversed later on, thus undergoing a secondary loss of cortex size and folding in their evolution towards a small and smooth brain (lissencephaly) [40–43]. A recent study from our laboratory uncovers for the first time a molecular mechanism regulating this secondary reduction of cortex size and folding, focused on the particular case of mouse [44]. About 90 million years ago, carnivores (i.e., ferret) split from the common ancestor of primates and rodents, and only a few million years later this split in two separate lineages. Accordingly, the common ancestor of carnivores, primates and rodents was gyrencephalic, and mice evolved towards lissencephaly after splitting from primates (Figure 2). Given that the expansion and folding of the mammalian cerebral cortex result from the amplification of progenitor cells during embryonic development, its secondary reduction might result from the contrary mechanism. Our recent work shows that *MIR3607* is a central player in this process. *MIR3607* is a small nucleolar RNA (previously identified as micro-RNA) expressed in progenitor cells of the embryonic cerebral cortex in ferret, macaque, and human, but not in mouse [44]. *MIR3607* directly targets the 3′UTR of APC mRNA and reduces its expression. APC is a component of the protein complex that drives β-Catenin for degradation, thereby blocking Wnt signaling. Hence, by blocking APC expression, *MIR3607* promotes Wnt/β-Catenin signaling [45]. Following *MIR3607* expression in aRGCs, increased β-Catenin signaling promotes their proliferation and self-amplification while maintaining their polarity, a basis for cortical expansion and folding. This effect is evolutionarily conserved, as shown in human cerebral organoids. Conversely, the experimental loss of endogenous *MIR3607* in ferret aRGCs increases APC levels and reduces their proliferation. Thus, the loss of *MIR3607* expression in cortical aRGCs was selected during evolution as an epigenetic mechanism to decrease aRGC proliferation and cortex size in small rodents [44]. Whether this loss was specifically and selectively prevented in the lineage of the Capybara (a dog-sized rodent with a large and folded cortex), or whether this species had a secondary gain of cortex folding during evolution, remains a fascinating question to be studied.

Human-specific genes in basal progenitors

Novel genes emerged during recent human evolution (human-specific genes), encoding for new proteins with important roles in brain development [46–49]. Among these, ARHGAP11B was first recognized for its role in promoting the proliferation of basal progenitors, via the modification of mitochondrial metabolism in cortical progenitors [50,51]. Strikingly, this novel, evolutionarily recent and human-unique protein has a highly conserved function across mammals. Experimental expression of ARHGAP11B in mouse, ferret, marmoset and chimpanzee consistently drives aRGC overproliferation as well as increased production of basal progenitors and neurogenesis [52–56]. In particular, ARHGAP11B is necessary and sufficient to ensure the high number of bRGCs that characterize the fetal human neocortex, pointing to its key role in human specific cortical features. Remarkably, induced expression of ARHGAP11B in the embryonic cerebral cortex of marmoset (a lissencephaly primate) increases the amount of bRGCs, the numbers of upper-layer cortical neurons, and promotes its enlargement and folding [56]. Recent evaluation of the potential impact of ARHGAP11B expression on mouse cognitive abilities reveals an increase in memory flexibility, a trait associated to cerebral cortex function. Thus, the emergence of ARHGAP11B may have contributed particularly to the evolutionary increase in cognitive abilities during recent human evolution [53].

Neanderthal versus modern human

The genome of Neanderthals and modern humans overlaps in ca. 98% of its sequence. The small number of differences between the two species correspond to approximately 100 amino acid substitutions. To investigate the functional meaning of these changes, Pääbo, Huttner and colleagues selected genes that are expressed in progenitor cells of the embryonic human cerebral cortex and expressed the two versions in transgenic mice and cerebral organoids. In a first study they focused on the kinetochore proteins KIF18a and KNL1, involved in chromosome segregation during cell division [57]. The modern human version of these proteins caused a significant lengthening of metaphase and a decrease of chromosome segregation errors (lagging chromosomes) in aRGCs. Conversely, the Neanderthal version shortened metaphase and increased the frequency of chromosome lagging in aRGCs. Strikingly, the cellular behavior of “Neanderthalized” aRGCs recapitulated that in mouse and chimpanzee, indicating that the fidelity of chromosome segregation and quality of cell division in cortical progenitor cells improved significantly in modern humans after their divergence from Neanderthals [57].

A second study focused on transketolase-like 1 (TKTL1), an enzyme involved in the fatty acid synthesis pathway and highly expressed in aRGCs of the human frontal lobe. The Neanderthal and modern human versions of TKTL1 differ in a single amino acid. Endogenous expression of the modern human variant was proven essential to maintain the production of bRGCs in human cortical slices, which was impaired upon expression of the archaic form. Moreover, its exogenous expression in animal models, including mouse and ferret, increased the production of bRGCs [58]. This supports a role for the modern human TKTL1 in the expansion of its frontal lobe, characteristically larger than in Neanderthals.

Tissue mechanics

A fundamental aspect of cerebral cortex folding is the mechanical deformation of the growing cortical mantle. An extraordinarily popular model to explain this mechanics was proposed more than two decades ago by Van Essen as the tension-based theory [59]. Based on the evidence that axons remain under tension, the theory proposed that cortex folding is largely the result of the patterned pulling of cortical axons on the cortical mantle. According to this model, cortical areas heavily interconnected are under a greater pulling force than those with less connecting axons, and this mechanical asymmetry drives the patterned deformation of tissue [59]. Despite the great appeal and the general acceptance of this theory for more than a decade, experimental testing by Taber, Bayly and colleagues in ferret eventually demonstrated that, while cortical axons are under considerable tension, the axonal tension patterns in the cerebral cortex are not consistent with driving its folding [60]. Alternatively, more recent modeling highlights the key importance of the geometry and physical properties of the growing brain [61,62]. Among the multiple factors that may regulate cortex folding, the initial brain geometry prior to folding seems to be a very strong determinant. Iterations and variations of an elementary mechanical instability laid-on by this initial geometry appear to be sufficient to explain natural cortex folding patterns to a large extent [63]. This concept has been recently revisited and extended through a comprehensive analysis of how different early biophysical parameters of the developing brain affect cortex folding. This analysis and computational model conclude that the mode of cortical growth has almost no effect on the complexity degree of its surface morphology. In contrast, variations in the initial brain geometry change the orientation and depth of folds, and cortex thickness critically influences the depth of fissures and the spatial frequency of folds [64].

Conclusions

Recent studies have significantly advanced our understanding of the mechanisms involved in cerebral cortex expansion and folding, unraveling novel and unexpected roles for otherwise highly conserved cellular and genetic mechanisms. Together, these studies indicate that the growth and folding of the cerebral cortex during evolution resulted from the synergistic combination of relatively subtle variations in these mechanisms. The combination of increased length of a specific part of the cell cycle, duration of the transition between developmental stages, and expression of short non-coding RNAs, together with the emergence of new genes, or gene variants with improved protein performance, contributed to augmenting the abundance, diversity and performance of cortical progenitor cells. Developmental mechanisms involved in the secondary loss of cortex size and folding during evolution have also just begun to be identified, opening a new door for exciting findings to come. Our understanding of the relevance of tissue mechanics in cortex growth and folding is still limited, but we are beginning to foresee that genetics, cell biology and mechanics are intimately interrelated and interdependent. Understanding the dynamic interplay of mechanical and molecular processes during cortex development holds the key to our understanding brain folding [6].

Conflict of interest

Nothing declared.

Data availability

No data was used for the research described in the article.

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