Cellular and Molecular Mechanisms Linking Human Cortical Development and Evolution

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ABSTRACT

The cerebral cortex is at the core of brain functions that are thought to be particularly developed in the human species. Human cortex specificities stem from divergent features of corticogenesis, leading to increased cortical size and complexity. Underlying cellular mechanisms include prolonged patterns of neuronal generation and maturation, as well as the amplification of specific types of stem/progenitor cells. While the gene regulatory networks of corticogenesis appear to be largely conserved among all mammals including humans, they have evolved in primates, particularly in the human species, through the emergence of rapidly divergent transcriptional regulatory elements, as well as recently duplicated novel genes. These human-specific molecular features together control key cellular milestones of human corticogenesis and are often affected in neurodevelopmental disorders, thus linking human neural development, evolution, and diseases.

1. INTRODUCTION

Production of tools and art, language and culture learning, and planning and foresight are some behaviors that are expanded in the human species and linked to a higher degree of cognitive functions that are thought to result from the expansion and increased complexity of the human brain (35, 161, 176). The human brain is not unique—far from it. But the human cerebral cortex displays quantitative and qualitative differences in neuronal composition, cytoarchitecture, and connectivity that are thought to sustain specific neural functions in our species (23, 75, 176). Many of these differences likely originate during development, but do they arise as an emergent property of well-conserved mechanisms of neural development, or does human corticogenesis rely on unique developmental programs? “Anything found to be true of *E. coli* must also be true of elephants” was the axiom used by Jacques Monod and François Jacob (122, p. 393). Corticogenesis has been studied using mostly animal models, in particular, the mouse. One could then, perhaps, ask, “Must anything true for the mouse cerebral cortex also be true for the human cerebral cortex?”Work in nonhuman models has revealed that many key cellular and molecular mechanisms of corticogenesis are conserved among all mammals, including the human species (reviewed in 104, 110, 155, 184, 187). But, as discussed in this review, human corticogenesis displays a number of species-specific cellular features that cannot be explained by these highly conserved mechanisms only. Recent progress on human-specific aspects of corticogenesis has been made possible thanks to the emergence of several fields that are complementary to the mouse model workhorse. First, comparative genomics and transcriptomics have revealed human- or primate-specific features of the genome as potential molecular candidates underlying human

specificities (44, 103, 159). Second, the genetics of neurodevelopmental disorders has provided insights on the conservation of the function of many genes from both mouse to human and vice versa (i.e., the same genotypes leading to same phenotypes) but also on a number of genetic defects to which human brain development displays species sensitivity (78,96).Third,new experimental tools have been developed to study the cellular and molecular biology of human corticogenesis using either human fetal tissue (110) or pluripotent stem cell (PSC)-based models of cortical development (5, 191).

The recent findings from these combined efforts to understand species-specific mechanisms of human corticogenesis are the focus of this review. We first summarize the main cellular features that distinguish the human neocortex from those of other species. Then we present an overview of the cellular mechanisms of corticogenesis, including those widely conserved in all mammals, followed by a focus on divergent features that are thought to be specifically important for human corticogenesis. We then discuss which molecular mechanisms could underlie these evolutionary divergences, from transcriptional regulation to duplicated genes, including those that are selectively targeted by neurodevelopmental disorders.

2. CELLULAR FEATURES OF THE HUMAN NEOCORTEX: DIVERGENCE AND COMMONALITIES

How unique is the human brain? The cerebral neocortex is among the most divergent structures in the human brain, together with the thalamic pulvinar complex, amygdala, and cerebellum (22, 142). However, the basic cytoarchitecture of the neocortex, i.e., a six-layer cortex, is conserved in all mammals (127) and diverges from the simpler three-layer cerebral cortex found in some other amniotes, while all other vertebrates are devoid of any cortical structure. Investigating human corticogenesis requires the

identification of divergent features that would be human-specific (species-specific, unique to human) but also those that are hominid-specific (shared with great apes), simian-specific (shared with hominids and simians like macaques and marmosets), primatespecific (shared with simians and primates like lemurs), or shared between all mammals.

The neocortex is made of two main classes of neurons: glutamatergic (excitatory) neurons and GABAergic (inhibitory) interneurons (198). Glutamatergic cells account for 70–80% of the neurons that populate the human neocortex (7).Most of them are pyramidal neurons,characterized by their canonical morphology: a conical soma, apical and basal dendritic arbors that carry dendritic spines as main excitatory postsynaptic structures, and an axon typically projecting toward longdistance targets. Pyramidal neurons that populate each cortical layer display unique patterns of gene expression, morphology, and hodological properties, i.e., laminar-specific patterns of identity (198). Schematically, deeper (cortical layers V and VI) pyramidal neurons project mostly to subcortical structures, and upper (cortical layers II and III) pyramidal neurons mostly project to other cortical areas (cortico-cortical projections), while layer IV neurons constitute the major recipient layer of subcortical input (127). GABAergic inhibitory interneurons, which typically send only local axonal projections, constitute about 25% of neurons in the human cortex (45) and are subdivided into numerous types according to marker expression, morphology, connectivity, and physiological properties (198). Finally, the neocortex is parcellated into numerous cortical areas, each specialized in specific functions, linked to their connectivity with one another and with the rest of the brain. Neurons of different cortical areas also display distinct patterns of gene expression and morphology, as well as specific proportions of neuronal classes, such as layer-specific populations.

These basic features of cortical organization are conserved in all mammalian species examined, including the human cortex (for a detailed review, see 176). Recently, comprehensive transcriptomic analyses of adult human and mouse cortex at the single-cell level revealed a surprisingly similar set of main subclasses in both species, although each neuronal type also expresses unique marker genes in each species (76). These data indicate a high level of evolutionary conservation of neuronal type specification, although it should be noted that these comprehensive analyses were performed in a few areas and that sparse divergent cell populations could be missed. Moreover, they were performed on samples at adult stages, while regional, temporal, and cellular patterns of expression are likely to be much more complex and divergent during development, as cortical neurons assemble into local and long-range circuits.

The human cortex cellular composition thus appears to be mostly conserved qualitatively, but it diverges quantitatively in many aspects (**Figure 1**). With >80 billion neurons, the human is one of the mammalian species with the largest number of brain neurons (although it is notably outnumbered by elephants and whales), and the neocortex displays the highest number of cortical neurons (around 16 billion) relative to brain mass, together with other primates and cetacean species (75, 176). Among primates, the human cortex contains by far the highest number of neurons,with about twofold more neurons than the chimpanzee cortex and greater than tenfold more than the macaque cortex (176).Most strikingly, the human cortex also displays the highest relative and absolute number of upper-layer (layers II and III) pyramidal neurons compared with other species. Notably, this expansion of upper layers is already present in simians and further expanded in hominids (11, 36, 80) (**Figure 1**). This expansion is likely to be critical in the evolution of human cortical circuits, as upper-layer neurons are mostly involved in intracortical projections. Their higher number could therefore

contribute to higher levels of connectivity between cortical areas, in particular the association areas (that mostly connect to other cortical areas and not to the rest of the brain) that are dramatically expanded in the human cortex (23).

Finally,while the repertoire of interneurons in the human neocortex seems to be overall similar to the ones found in other species, the human neocortex displays a higher proportion of interneurons (45), which are also expanded in simian species (76, 98). The cortex also contains a large proportion of glial cells (astrocytes, oligodendrocytes, and microglia), which are more numerous in the primate and human cortex (7) and appear to be more divergent in terms of subtypes and gene expression (15, 67, 94).

In addition to differences within the cell composition of the cortex in human and other species, comparative morphological analyses have revealed interesting quantitative differences at the level of single neurons.For instance,human cortical pyramidal neurons, in particular from upper layers, display an increased dendritic arbor complexity and neuronal size compared with other species (17, 121) (**Figure 1**). Also, the density of dendritic spines is increased in human compared with mouse and macaque counterparts (13, 46) (**Figure 1**), and in human, their absolute number per neuron is higher than in chimpanzee (18). Finally, the size of dendritic spines is higher in human than in mouse pyramidal cells (35). These morphological differences likely lead to differences in the functional properties and connectivity of human cortical neurons. For instance, analyses of the functional properties of layer V pyramidal neurons in human and rat cortex revealed that human neurons display an increased electrical compartmentalization between dendrite and cell body, which could be related to increased dendritic length (12).

Overall, these data indicate that the human cortex is not unique and suggest that the global increase of human cortical neurons as well as the expansion of upper-layer populations constitutes an upscaling of processes already observed in simian and hominid evolution. The human cortex nevertheless displays species-specific cellular features, which are most likely linked to distinct developmental programs controlling the total number of cortical cells, the proportion of each subclass, and their fine-grained specification, as discussed below.

3. MECHANISMS OF CORTICAL NEUROGENESIS: FROM STEM CELLSTO DIFFERENTIATION

Hereafter, we focus on the mechanisms of neurogenesis of pyramidal neurons, as these have been the main target so far of the studies linking corticogenesis and human evolution. We first summarize what is known about conserved cellular mechanisms of cortical neurogenesis,followed by species differences that are potentially relevant to human brain evolution.

The generation of cortical excitatory neurons proceeds through a highly choreographed sequence of cell fate transitions, articulating around diverse types of neural stem/progenitor cells (NSPCs) (**Figure 2**). NSPCs can be distinguished by (*a*) the timing of their appearance (and disappearance), (*b*) their morphology and position in the cortical tissue, and (*c*) their cell division patterns and subsequent lineage (for more extensive reviews, see 69, 97, 187, 188). As we explain, each feature is crucial for proper corticogenesis, and each has undergone evolutionary changes that underlie human neocortex evolution.

3.1 Conserved Cellular Mechanisms

Corticogenesis starts in the cortical anlage located in the dorsal telencephalon, the anterior-most part of the forebrain, with the proliferation of the first neural progenitors: the neuroepithelial cells (NECs) (**Figure 2**). NECs divide by symmetric self-renewing divisions to generate a pseudostratified neuroepithelium that constitutes the anlage of the cortex and the cellular source of all cortical excitatory neurons (154, 187).

After this period of amplification, NECs gradually transform into neurogenic progenitors, which mostly correspond to radial glial cells (RGCs) (97, 113, 131). RGCs are located in the ventricular zone (VZ) and display a distinctive bipolar morphology: their cell bodies, located in the VZ, carry one apical process contacting the surface of cerebral ventricles and one basal process that extends all the way toward the surface of the cerebral cortex (**Figure 2**), thus providing a scaffold for neurons to migrate toward their final location, the cortical plate (CP) (187). Most importantly, while RGCs can divide symmetrically to expand, they undergo mostly asymmetric divisions, enabling them to self-renew as a stable pool of stem cells, while generating more differentiated cells (97).

RGCs can differentiate into neurons directly or indirectly through other types of progenitors, the intermediate progenitor cells (IPCs) (116, 132, 183) (**Figure 2**). IPCs lack a defined cell polarity and are also referred to as basal progenitors because they divide more basally than NECs or RGCs in the subventricular zone (SVZ) (**Figure 2**). IPCs typically divide at least once more before undergoing a final symmetric neurogenic division (132).IPCs thus constitute a first important cellular leverage to increase neuronal production from the initial pool of RGCs.

In addition to IPCs, a major class of basally dividing progenitors are the outer radial glial cells (oRGCs) (55, 73, 158). oRGCs typically display epithelial-like polarity, similar to RGCs, with a long process oriented basally toward the cortical surface,while they lack an

apical process reaching the ventricular surface (73) (**Figure 2**). Most importantly, oRGCs have undergone considerable amplification in primate and human corticogenesis, as we discuss in Section 3.2.2.

A key feature of basal progenitors (IPCs and oRGCs) is that they tend to expand at later stages of corticogenesis, which makes them more likely to contribute to later stages of neuronal production and thereby to specific cortical neuron subclasses, in particular upper-layer pyramidal neurons. Indeed, a cardinal feature of cortical neurogenesis is the temporal patterning of neuronal fate specification, by which seemingly similar cortical progenitors sequentially generate distinct subclasses of neurons at defined embryonic stages (19, 137, 162) (**Figure 2**). Most importantly, the neurons generated at different time points will migrate to populate distinct layers, mostly through an inside-out pattern (189) by which the first-generated pyramidal neurons will populate the deepest layers of the neocortex, while later-generated neurons will migrate beyond them to progressively more superficial positions in the upper layers (**Figure 2**). This fundamental developmental process implies that neurons sequentially born at the same location are then arranged radially across cortical layers to form so-called cortical columns (127, 154). Another important implication of temporal patterning is that variations in neurogenesis, whether in timing or types or number of cell divisions, can lead to different numbers of cortical neurons of distinct layer identity and thereby to quantitative differences in the laminar cellular composition of the cortex.

3.2 Divergent Cellular Mechanisms of Human Cortical Neurogenesis

The final number of cortical neurons and the proportions of different neuronal types, as observed in the human cortex,is determined by (*a*) the balance between cell proliferation and the differentiation of individual progenitors, (*b*) the size and diversity of each progenitor pool at any given stage of neurogenesis, and (*c*) the timing of fate

transitions, from cycling properties of single progenitors to global temporal patterning of cortical neurogenesis. Comparison of these parameters between humans, nonhuman primates, and other mammalian species reveals important differences that are directly relevant to human brain evolution.

3.2.1. Species-specific temporal patterns of neurogenesis shape the cortex. A first important mechanism contributing to the evolution of cortical neurogenesis is heterochrony, by which similar developmental processes unfold over distinct timescales in different species (83). The period of amplification of NECs is the first important step that displays species specificities (154). Indeed, the proliferation of NECs in the mouse anlage lasts for 1 to 2 days, compared with weeks in macaque and human embryos (154, 180) (**Figure 2**). As the cell cycle length of primate cortical cells is only twice as long as in mouse, the extended duration of NEC amplification in primates leads to an increased number of NEC divisions and thereby an expansion of this stem cell pool (154). The timing of NEC-to-RGC transition thus has a direct impact on the size of the initial pool of cortical progenitors at the beginning of neurogenesis and thereby could contribute to differences in overall cortical size and neuronal number across species. Notably, these changes are most likely causal to the expansion of the overall surface of the cortex and not necessarily to its increased thickness, since all populations of subsequent progenitors would be similarly expanded, at least in principle,which would fit with global expansion of the surface of the human cortex compared with that of other primate species (176). The proof of principle of this hypothesis was first provided in mouse, in which overexpression of a mutant form of β-catenin could lead to overproliferation of cortical progenitors and thereby to a striking expansion of the surface of the VZ of the embryonic cortex (32).Work in mouse also revealed that the delayed transition of NECs

to RGCs can lead to expansion of the progenitor pool and, as a result, increased surface of specific cortical areas (165).

However, the physiological mechanisms controlling the critical period of stem cell pool amplification have remained poorly known, especially since NECs are very transient in mice and early stages of corticogenesis are difficult to access in primate models or in human embryos. Models of corticogenesis from PSCs have provided a first important hint that at least part of the timing mechanisms of NEC-to-RGC transition is intrinsic to NECs. Indeed, when comparing corticogenesis from mouse and human PSCs, NECs were found to convert to RGCs in a few days in mouse cells, similar to corticogenesis in vivo, while the transition to RGCs is thought to take 2–3 weeks with human cells (49, 66). Similarly, comparison in organoid systems of human versus chimpanzee and gorilla PSC-derived corticogenesis also revealed a delayed NEC-to-RGC transition in human cells (14). Importantly, this delay was associated with an expanded surface of VZ-like structures in human organoids compared with nonhuman ones at early stages,illustrating the potential influence of NEC heterochrony on the initial size of the cortical stem cell pool.

RGC-dependent neurogenesis is also heterochronic, which was long predicted to directly influence the total number of neurons produced, i.e., the neuronal output (29, 154). The period of neurogenesis is considerably expanded in species that display a larger cortex. In mouse, cortical neurogenesis takes about a week, while it takes more than 3 months in human (**Figure2**), and intermediate durations have been described in macaque cortex, for instance (173). PSC-based models of corticogenesis, whether in two-dimensional or organoid cultures, have revealed a species-specific tempo remarkably similar to the in vivo situation, i.e., shortest in mouse, intermediate in macaque, and longest in chimpanzee and human (49,139). Another interesting

observation made in these in vitro settings is that the temporal patterning of neuronal specification, i.e., the sequential generation of neurons of distinct laminar identity, is also heterochronic so that upper layer neurons are generated much later in macaque than in mouse cultures, and even later in the human ones (49, 139). Clonal analyses performed in human and nonhuman primate corticogenesis revealed the increased self-renewing capacities of human RGCs compared with macaque RGCs and confirmed the heterochrony of neurogenesis at the single-cell level, which is even observed when human and macaque cortical cells are cocultured (139). Similarly, human cortical progenitors xenotransplanted in neonatal mouse cortex still display prolonged neurogenesis and temporal patterning (49). The influence of the duration of cortical neurogenesis on neuronal output is also exemplified by the comparison of different mouse strains characterized by longer periods of gestation and cortical neurogenesis (179): mouse embryos with longer gestation display prolonged neurogenesis and an increased number of cortical neurons, especially of late-generated upper-layer pyramidal neurons.

Collectively, these data indicate that the temporal features of cortical neurogenesis are largely encoded within the NEC and RGC lineages of each species, and can make a substantial contribution to differences in cortical number and composition across species. At the individual cell level, comparison of RGCs of different species revealed additional temporal differences. As already mentioned, the cell cycle length of human cortical progenitors is longer overall than in macaque,for instance,but increased cell cycle length would be predicted to lead to decreased selfrenewal (37,112),so it is unlikely to account for the global changes in neurogenic tempo described above. On the other hand, the duration of mitotic metaphase of RGCs appears to be longer in human compared to other hominid species (chimpanzee and orangutan) (124). This was

proposed to contribute to higher proliferative capacity, although it should be noted that, at least in rodent cortical progenitors, increased mitotic length instead leads to increased neurogenesis (145). Finally, a critical period of fate plasticity was recently uncovered in human and mouse RGCs right after mitosis, during which RGCs can be maintained in a self-renewing state by modifications of mitochondria dynamics (82). Intriguingly, this period is doubled in human RGCs compared with the mouse, which might contribute to higher self-renewing capacities, although this remains to be tested experimentally.

3.2.2. Expansion and increased diversity of cortical progenitors. Another major component of the evolution of human corticogenesis takes its roots in the amplification and diversification of specific subtypes of basally located progenitors, i.e., the IPCs and, most importantly, the oRGCs (16, 55, 73, 158).

Indeed, while all types of cortical progenitors can be found in mammalian species, including oRGCs,which are also present in mouse and marsupials (167,194),their numbers are considerably increased in higher mammals (55, 73, 158) (**Figure 2**). In primates, the highly expanded pool of oRGCs populates an additional layer,the outer subventricular zone (OSVZ),that constitutes most of the proliferative zone at later stages of corticogenesis (73, 174). Importantly, live imaging of oRGCs has revealed their high expansion capacity, mainly through asymmetric division, enabling self-renewal and differentiation into more committed precursors like IPCs or directly into neurons (16, 73).

oRGCs are thought to contribute to increased cortical size expansion in several ways. First, oRGCs are found exclusively in basal compartments of the OSVZ (**Figure 2**), so that their increased number does not lead to an increase in the surface of the VZ, thus providing a spacesaving way to increase the stem cell pool without increasing the

surface of their niche. A second key aspect is their high self-renewal capacities: human oRGCs carry particularly high proliferative abilities. For example, in vitro clonal analyses revealed that a single human oRGC can generate up to 1,000 daughter cells (149), compared to 10–100 daughter cells for one mouse RGC (153). Third, as oRGCs only appear prominently at later stages of corticogenesis (73), they constitute the main source of the latest-generated neurons, i.e., upper-layer neurons, while the neurogenic contribution of human RGCs at that stage appears to become minor (135). Finally, oRGCs contribute to the gyrification process, by which the cortical surface is folded in specific gyri and sulci, through increased neuronal output and patterning of neuronal migration (for an extensive review on this topic, see 109).

In addition to oRGCs,IPCs are also critical to human cortical size and possibly its evolutionary expansion.The amplification of IPCs is indeed a key feature of increased cortical size in amniotes, leading to neocortex expansion in all mammals (27). Moreover, although they display much less self-renewing capacity than oRGCs, they do expand in number and diversity in the basal compartments of mammals with expanded cerebral cortex, in particular in the human, and therefore contribute in an important way to the increase of upper-layer neuronal compartments (133, 141, 183).

The importance of basal progenitors in human corticogenesis is likely to be linked to speciesspecific sensitivity to neurodevelopmental disorders, such as microcephaly. For instance, while mutations in the *ASPM* gene lead to drastic reduction in the size of the cerebral cortex in affected patients (90), *Aspm* disruption in mouse,which contains very few oRGCs,leads only to mild effects (152). The *ASPM* coding sequence was also shown to display evolutionary changes in the human and primate lineage that suggest positive selection related to brain size control (123). Moreover, genetic disruption of *Aspm* in the ferret cortex, whose corticogenesis involves an expanded pool of oRGCs, leads to severe

microcephaly and altered patterns of oRGC generation, suggesting a specific sensitivity of oRGCs to ASPM disruption (86). Similarly, the importance of IPCs in human corticogenesis is also attested by the severe microcephaly observed in patients presenting a mutation in *TBR2/EOMES*, a master transcription factor of IPC generation (8).

3.2.3. Generation of interneurons and glia. Much less is known concerning species difference in the generation and specification of other cortical cell types, i.e., interneurons and glial cells.As for GABAergic interneurons (101),comparative analysis of the volume of the proliferative zones in the fetal cerebral cortex and the ganglionic eminences (located in the subpallium, where cortical GABAergic interneurons are born before their migration toward the cerebral cortex) in human, macaque, and mouse species showed concerted development (31). Indeed, the proliferative zones of the human ganglionic eminences, which generate GABAergic interneurons, are also enriched with basal progenitors and follow a protracted development, which likely underlies the coevolution of the increased number of excitatory and inhibitory neurons (72). Since GABAergic interneurons still migrate and integrate in the frontal cortical areas during the first year of human life, their development may be even more protracted (140). The relative size of the caudal ganglionic eminence, one of the specific niches of interneuron progenitors in the ventral telencephalon, is also increased in macaque and human (72, 101), which could lead to an increased proportion of specific types of interneurons.

As for glial cells,very little is known concerning the mechanisms leading to their larger number and diversity in the human brain. Gliogenesis starts at mid-gestation in the human fetal cortex and continues during the first years of life (79, 186) (**Figure 2**). The increased proportion of glial cells, in particular oligodendrocytes, in the simian and

human cerebral cortex, could thus rely on prolonged gliogenesis, but the mechanisms underlying the seemingly increased number and diversity of human glial cells remain to be explored (79, 186).

4. DIVERGENT MOLECULAR FEATURES OF HUMANCORTICAL NEUROGENESIS

4.1 Divergence of Gene Regulatory Networks

Evolution is thought to be driven mainly by changes in gene regulatory networks (GRNs) (33). Considerable efforts have been made to identify the functionally relevant differences in gene regulation and expression that can distinguish human brain development from that of other species (159). But this has proven to be a daunting task, as the brain is the organ that displays the least differences in gene expression trajectories between mammalian species (28). Comparative transcriptomic studies have revealed remarkable conservation of gene coexpression and cell type–specific expression during cortical development (51,52,57,93,94,124,148,200).For instance,most of the genes expressed in cortical RGCs or neurons in cerebral organoids derived from PSCs are largely thesame between human andchimpanzee (94%) ormouse(75%) (124). As a consequence, perhaps not surprisingly, significant species differences in corticogenesis must rely on subtle molecular differences in *cis*-regulatory mechanisms and downstream transcriptional patterns, as suggested by the very strong molecular similarities between human and chimpanzee species (95). These studies have, however, revealed interesting global mechanisms of regulatory divergence, especially in relation to timing, as well as more specific changes that may bear direct relevance to the cellular mechanisms of human corticogenesis, as detailed below.

On the one hand, comparative genomics has revealed the existence of hundreds of human genomic accelerated regions (HARs), which are ultraconserved genomic regions that display selective accelerated sequence changes in the human genome (147, 150). While the first HAR to be described (*HAR1*) corresponds to long noncoding RNAs expressed in pioneer cortical neurons during fetal development (147), most of the HARs are thought to constitute regulatory regions (26, 151). HARs are enriched in the vicinity of genes expressed in the human developing brain and cortex, suggesting their contribution to human-specific patterns of gene expression during corticogenesis (43, 85, 94, 102, 120, 195, 196).

On the other hand, the combination of comparative genomics and epigenomic profiling of regions of active transcription has uncovered enhancer regions that display activity only in human and not in other species, known as human-gained enhancers (HGEs) (160). Thousands of HGEs were found to be active in corticogenesis (160) and display enrichment close to genes selectively expressed in oRGCs (34), as well as genes of the NOTCH signaling pathway (175), a key factor of self-renewal of RGCs (89). Interestingly, some of the HGEs may have evolved very recently, as they display sequence changes present only in human and not in other hominins, such as Neanderthals (125).

Several studies have started to test directly the functional impact of HARs and HGEs. On the one hand, massively parallel enhancer assays using in vitro models of human neurogenesis have revealed hundreds of HARs and HGEs that can function as enhancers in neural cells. These show consistent changes in activity between human and chimpanzee sequences, likely due to changes in transcription factor binding sites (164, 190). On the other hand, several HARs were found to be mutated in various forms of

neurodevelopmental diseases (NDDs), such as autism spectrum disorders (ASDs), leading to the dysregulated expression of genes implicated in neuronal specification(43). Moreover, a specific HAR, HAR5, was functionally linked to species-specific changes of cortical neurogenesis(20). HAR5 acts as an transcriptional enhancer for the gene coding for FZD8, a key WNT receptor (Figure 3).

The human *HAR5* and its ancestral counterpart in chimpanzee displayed selective activity in the developing forebrain/cortex (20), but the human *HAR5* drove higher levels of expression than the ancestral enhancer. WNT signaling is well known to drive increased self-renewal and proliferation of RGCs (188), suggesting that increased expression of the receptor could lead to increased expansion of RGCs.This was tested using transgenic mice driving *Fzd8* by the chimpanzee or human *HAR5*. Remarkably, human *HAR5*-driven expression of *Fzd8* led to accelerated proliferation together with increased expansion of RGCs and cortical size, while the chimpanzee *HAR5* transgene was without effect (20). These data illustrate how specific regulatory regions that underwent rapid evolution in the human lineage may underlie species-specific aspects of human corticogenesis. Further emphasizing its importance in human corticogenesis, upregulation of the WNT pathway has been linked to some forms of human macrocephaly associated with ASDs (100, 182) and was found to be downstream of the Amyloid Precursor Protein (APP), which is required selectively for human cortical neurogenesis (170).

In parallel with these studies on *cis*-regulation, comparative transcriptome profiling, centered on human and nonhuman primate corticogenesis and using fetal material but also PSC-derived cortical organoids, has been extensively used to try to uncover human-specific developmental mechanisms of corticogenesis. This has revealed that the highest interspecies differences in gene expression take place prenatally at mid-gestation (2, 93,

146, 196, 200). Comparisons of developmental trajectories of gene expression have also revealed protracted, or prolonged, temporal patterns of human corticogenesis compared with all other primate species, even the chimpanzee (10, 93, 148). These temporal changes in gene expression reflect the heterochrony of corticogenesis across species described in Section 3.2.1. In line with this, gene expression profiling of human versus gorilla cortical organoids at early stages of NEC amplification recently revealed the potentially important role of the ZEB2 transcription factor, which displays delayed expression in human compared with gorilla organoids (14) (**Figure 3**). ZEB2, which was previously associated with epithelial-to-mesenchymal transition, triggers NEC morphology changes that may further facilitate their conversion into RGCs. *ZEB2* was found to be necessary and sufficient to regulate the timing of the NEC-to-RGC transition, suggesting that it constitutes an important heterochronic component of the GRN controlling early corticogenesis. Interestingly, *ZEB2* is located in the vicinity of several HAR elements, which could contribute to the differential timing of expression of *ZEB2* in human versus other hominids (47). A recent study also focused on transcriptomic profiles of the early human fetal cortex and identified a surprisingly high number of distinct types of NECs, some of which do not appear to be found in the mouse embryonic cortex (50), further linking NEC biology with species-specific features of human corticogenesis.

Comparative transcriptomics of human/macaque versus mouse fetal cortex revealed the expression of the growth factor PDGFD (10, 111) (**Figure 3**) in simians but not in mouse RGCs, while its receptor PDGFRβ is also enriched in human cortical germinal zones (111). PDGFD signaling was then shown to be necessary for the proliferation of human, but not mouse, cortical progenitors, and its constitutive activation could lead to RGC expansion into the basal compartments (111). Intriguingly, PDGFRβ signaling was

recently found to be required to maintain the quiescence of adult neural stem cells (NSCs) (39): It will be interesting to determine the molecular commonalities and differences between mouse adult and human embryonic NSCs that account for these cell type–specific effects.

Finally, single-cell RNA profiling of RGCs and oRGCs in human and nonhuman primates has led to the identification of gene signatures that are specifically enriched in oRGCs. Although they are largely congruent with those of RGCs, this also revealed several pathways displaying humanspecific patterns of expression (34, 134, 148, 149). Most strikingly, the mTOR signaling pathway was found to be highly upregulated in human oRGCs compared with macaque or chimpanzee cellular counterparts (134, 148) (**Figure 3**). The study of the mTOR pathway in human oRGCs has so far revealed an effect on cell morphology and migration, rather than on proliferation itself (3), although other molecular components of the mTOR pathway could also contribute to the expansion of basal progenitors (59, 192). Consistent with the importance of mTOR in human corticogenesis, mutations in this pathway have also been linked to human macrocephaly, which could thus be linked to cell hyperproliferation and/or increased cell size (42).

Overall, these data have so far identified species-specific properties of oRGCs that are largely cell extrinsic. This is consistent with the observation that cell- and extracellular matrix–derived growth factors are upregulated in the primate versus mouse fetal cortex, which could constitute a species-specific niche for the selective expansion of basal progenitors, in particular oRGCs, in primates (56). This would be also consistent with the observation that several molecular manipulations of RGCs in mouse that lead to increased generation or expansion of oRGCs do not necessarily lead to an overt increase in cortical size (63, 172), as well as the relative difficulty to sustain oRGC amplification in

in vitro systems of corticogenesis (191), suggesting the need for niche-derived extrinsic signals for human oRGCs to display their remarkable species-specific features.

While the data above illustrate gene upregulation as an evolutionary scenario, downregulation of specific genes is also involved in human specificities of corticogenesis. Comparative genomics revealed hundreds of putative regulatory elements that are highly conserved in primates but selectively deleted in the human species (119). These lost enhancers are found enriched near genes involved in neural development,including an enhancer found close to the cell cycle inhibitor gene *GADD45G* whose mouse ortholog drives selective expression in the SVZ. Similarly, the low levels of expression of genes coding for the FLRT1/FLRT3 adhesion receptors in the human cortex may contribute to changes in neuronal migration and adhesion facilitating the gyrification of the cortical surface (38). More refined changes of gene expression levels may also be important evolutionarily; for instance, the *Trnp1* gene displays uniform expression in the mouse embryonic cortex, but its local downregulation can lead to increased basal progenitor expansion. Moreover, in the human and ferret developing cortex, *TRNP1* displays regional and temporal differences in levels of expression that relate to patterns of gyrification and/or oRGC generation (117, 178).

Beyond gene expression regulation, other molecular sources of human specificity during corticogenesis likely involve posttranscriptional regulation, including alternative splicing (9, 25, 92, 105,126,193,200). For instance, the *NUDE1* gene, which encodes a key protein regulating neural cell proliferation and migration, displays a human-specific exon that might explain the high sensitivity of the loss of this gene in human, which leads to severe microcephaly, while its disruption in mouse leads to milder effects (126). MicroRNA (miRNA)-based regulation of messenger RNA (mRNA) stability and translation has also undergone evolution in primate corticogenesis, leading to the emergence of

several miRNAs uniquely expressed in primate cortex that target genes related to cell cycle and proliferation (4).Other RNA modifications such as specific methylation have also been shown to display distinct patterns in human corticogenesis (197). Finally, the human cerebral cortex expresses a number of primate-specific and hominid-specific noncoding RNAs (4, 54, 136, 166). These include the long noncoding RNA LncND, which enhances NOTCH signaling by targeting cognate miRNAs, thereby increasing human RGC amplification (156) (**Figure 3**).

Finally, evolution of *cis*-regulatory elements can lead to changes in both transcriptional and splicing regulation relevant to corticogenesis. This is exemplified by the *GPR56* gene that is involved in cortical progenitor proliferation and neuronal migration, and is mutated in neurodevelopmental defects (9) (**Figure 3**). The gene displays additional alternative transcription starts in higher mammals, leading to the emergence of species-specific exons and splicing events, which are responsible for species-specific patterns of expression (9).

4.2 Duplicated Genes: Genomic Novelties to Modulate Ancestral Processes

A less-studied source of evolutionary novelty during development is the emergence of new encoding genes (138). Novel genes typically emerge by segmental genomic duplications or retrotransposition, or, much less frequently, they emerge de novo (41, 88). Gene duplicates can constitute prime targets for further alterations due to their functional redundancy with the ancestral gene: Mutations or truncated duplications often lead to nonfunctional gene products (pseudogenes) or increased gene dosage, but they can also lead to novel functions of the gene duplicate. Human-specific genes resulting from retrotransposition have been shown to be expressed during corticogenesis (163), but their functional relevance remains undetermined. However,

novel genes resulting from segmental genomic duplication—where an ancestral gene is duplicated, wholly or only partially, leading to the emergence of paralog genes—have come up recently as a potentially rich source of novelty to drive human evolution (40). Indeed,the frequency of segmented duplications is particularly increased in the genomes of hominids and concentrated mainly in >20 hotspot genomic areas (40, 41, 61, 199). The human genome carries >50 gene families with copy-number variation. Some of the families comprise only 2 members, others consist of dozens of paralogs, and, overall, >20 are hominid-specific and about 10 are human-specific (41, 181). Several studies combining comparative transcriptomics and genomics converged to identify >30 gene duplicates that comprise hominid- and human-specific members that are dynamically expressed during human fetal corticogenesis (58, 99, 185). While in many cases the hominid-specific gene duplicates appear to be coexpressed with their ancestor,they often display distinct patterns of expression,and sometimes only the duplicate is detectable during corticogenesis. Hominid-specific genes active during corticogenesis can be found selectively in cortical progenitors (RGCs and/or oRGCs) or neurons, suggesting their implication throughout human corticogenesis.

Among hominid-specific genes expressed in cortical progenitors, the human-specific genes of the *NOTCH2NL* family stand out for their ability to promote self-renewal of RGCs following their overexpression in mouse (185). The four *NOTCH2NL* genes arose through several duplication events from the ancestral gene coding for the NOTCH2 receptor, which is present in all mammalian genomes,three of which (*NOTCH2NLA*,*NOTCH2NLB*,and *NOTCH2NLC*) are only present in the human genome. They are located within a specific interval of 1q21.1, close to an important locus where copy number variants are associated with microcephaly and macrocephaly (21) (**Figure 3**). These genes display very high sequence conservation with their ancestor, suggesting

very recent (<4 million years) evolution in the human lineage (53). *NOTCH2NL* genes are expressed in cortical progenitors, both in RGCs and oRGCs, and throughout corticogenesis, suggesting a role in multiple progenitor subtypes (53, 185) (**Figure 3**). Clonal analyses with PSCderived human RGCs revealed that overexpression of *NOTCH2NL* paralogs leads to the expansion and prolonged self-renewal of human cortical progenitors, thereby increasing their neuronal output at the clonal level (185). Conversely, the deletion of *NOTCH2NL* genes in human cerebral organoids led to precocious differentiation and decreased organoid size (53). *NOTCH2NL* duplicated genes thus appear to be necessary and sufficient to increase the self-renewal abilities of cortical progenitors, thereby leading to prolonged neurogenesis and increased neuronal production (**Figure 3**). Mechanistically, NOTCH2NL acts through direct interaction with NOTCH and its ligand DLL1, leading to increased NOTCH signaling,which promotes RGC self-renewal (65). *NOTCH2NL* paralogs thus stand out as human-specific modifiers of cortical neurogenesis through direct modulation of its ancestor gene. Human genetics further links *NOTCH2NL* genes to corticogenesis through the study of copy number variants. Two *NOTCH2NL* genes were found to be consistently localized at the breakpoints of the deletions or duplications of the 1q21.1 locus (53), which are causally linked respectively to microcephaly and macrocephaly (21). These observations provide further evidence for the implication of *NOTCH2NL* genes in the control of the size of the human cortex, although it should be noted that several other genes, including most strikingly other hominid- or human-specific gene duplicates, are also found within the pathological interval. Furthermore, recent work focused on invasive glioma has revealed that *NOTCH2NL* genes are amplified in some of these tumors, in which they contribute to their oncogenic growth (64), illustrating how recently evolved genes may constitute species-specific factors of disease sensitivity. Finally, other genomic regions containing

gene duplicates are likely to be involved in defects of corticogenesis, leading to NDD (40).

Other hominid-specific genes have been characterized functionally, and many of them are implicated in the expansion of basal progenitors (**Figure 3**). The human-specific gene *ARHGAP11B*, which is expressed in RGCs and oRGCs, was found to increase the number of basal progenitors following overexpression in mouse, ferret, and marmoset (57,74,91). *ARHGAP11B* arose through the partial duplication of the ancestral gene *Rho GTPase Activating Protein 11A* (*ARHGAP11A*) (41) but appears to act independently from it. It was found to act at the mitochondrial level (129), while the ancestral RNA and protein ARHGAP11A are found in the basal process of mouse and human RGCs, where they control their morphology (144). Other genes amplified in primate or hominid lineages were found to increase the specification and/or amplification of basal progenitors (*TBC1D3*, *TMEM14B*, *CROCCP2*) (77, 87, 107), some linked with other pathways thought to be selectively involved in oRGC biology. *TBC1D3* (87), a hominid-specific gene duplicate, increases the production of oRGCs through inhibition of the *TRNP1* gene implicated in oRGC generation (117, 178), and *CROCCP2*, another hominid-specific gene expressed only in the human fetal cortex and no other species, promotes the generation of basal progenitors through the modulation of mTOR signaling (192) (**Figure 3**).

Overall, these data indicate that human corticogenesis implicates a surprisingly high number of novel duplicated genes, some of which emerged early in hominid evolution and some quite recently in the human lineage, and which collectively have changed the neurogenic patterns and diversity of cortical progenitors.

5. BEYOND NEUROGENESIS: DIVERGENT MECHANISMSOF CORTICAL NEURONAL DEVELOPMENT

The wealth of data described above constitutes a strong framework to understand the mechanisms that underlie the evolution of cortical neuron number and diversity.But brain size alone is unlikely to explain human cortex evolution,which is also linked to specific features of the development of human neurons and their assembly into neural circuits, most of which will occur at postnatal stages. While most of these features remain to be discovered and as such constitute an exciting frontier for the developmental biology of corticogenesis, recent work has uncovered new insights on the cellular and molecular mechanisms of one striking developmental aspect of neuronaldevelopment: neoteny.

The human cerebral cortex is characterized by a prolonged developmental timing compared with other species. The resulting brain neoteny, or retention of juvenile traits in a more mature organism,is thought to reflect the protracted maturation of cortical neurons and glial cells (143,176). It involves all critical steps of maturation of cortical neurons from axonal and dendritic growth and patterning to synapse formation and refinement (taking up to several years, instead of months in macaque or weeks in mouse) (81,128,143) (Figures 2 and 4). Human brain neoteny has been long proposed to play a key role in the acquisition of cognitive features expanded in human by extending critical periods of learning and plasticity, thus strongly suggesting that it is a selected trait in the human lineage (24, 68, 171), although prolonged development of cortical neurons is also observed in chimpanzee brain (18). Interestingly, altered timing of neuronal development have been described in NDDs, such as ASDs and schizophrenia, further suggesting its functional importance (60).

A first hint of the cellular mechanisms underlying cortical neuron neoteny has come from PSCbased models of corticogenesis, in particular from xenotransplantation experiments in mouse. In this system, cortical neurons are first generated from PSCs, followed by their transplantation into neonatal mouse cortex. Comparison of the morphological development of transplanted mouse and human pyramidal neurons revealed that they develop at their own species' pace (49, 66). Specifically, transplanted mouse neurons reach mature stages of morphology and electrophysiology within a month, like native neurons, while transplanted human neurons take 6–9 months to mature at these morphological and functional levels. Even when transplanted as single cells, the human neurons develop along this extended timeline that is highly reminiscent of the protracted maturation that takes place in the human brain (106). Similarly, macaque neurons develop faster at the electrophysiological level than human and chimpanzee counterparts in vitro (139), and xenotransplanted chimpanzee neurons develop faster at the morphological and electrophysiological levels than human neurons (114). Human neurons develop along more protracted timelines even when their neuronal induction is accelerated by the forced expression of proneural factor NGN2 (169). Xenotransplanted PSC-derived cortical interneurons also develop along a prolonged timeline in vitro and following transplantation (115, 130), while noncortical neurons, which are not expected to display neoteny, do not display such dramatic species differences (157). Overall, these data indicate that the timeline of cortical neuronal maturation depends a least in a significant part on neuron-intrinsic mechanisms.

As for cortical neurogenesis, the neotenic maturation of pyramidal neurons could rely on differences in gene transcription, gene transcription trajectories, and duplicated genes. The transcriptomic trajectory of genes involved in synaptogenesis is indeed

protracted in human developing cortex compared to that of macaque and chimpanzee (108, 200). Many of these genes bear regulatory regions with binding sites for *MEF2A*, a transcription factor involved in neuronal maturation in mouse and also enriched for genes known to be involved in ASDs (108). In line with this, a screen for genes differentially expressed between mouse and human neurons following depolarization identified a subset of genes specifically upregulated by human neuronal activity, including *osteocrin* (6). Activity in human but not mouse neurons selectively induces *osteocrin*, and this gain of expression appears to result from the acquisition of MEF2 response elements.

Among the gene duplicates expressed in human developing cortex, the *SLIT-ROBO Rho GTPase-Activating Protein 2* (*SRGAP2*) family of human-specific duplicated genes have been identified as strong candidates to control human-specific features of neuronal development. SRGAP2A is a postsynaptic protein conserved in mammals, localized in excitatory and inhibitory synapses, that acts as a positive regulator of synaptogenesis (30, 62). *SRGAP2B* and *SRGAP2C* are humanspecific paralogs that encode truncated forms of SRGAP2A with a unique C-terminal region and are expressed in human corticogenesis, in particular in neurons (30). Gain-of-function of *SRGAP2C* in the mouse cortex leads to a delayed, neotenic-like maturation of dendritic spines and synaptogenesis, while at mature stages they display a stable increase in dendritic spine density, another feature characteristic of human neurons (30, 62) (**Figure 4**). Importantly, the loss-of-function of *Srgap2* in mouse led to the same phenotypes as the gain-of-function of *SRGAP2C* (30, 62, 70, 71). This suggests that *SRGAP2C* acts as a negative regulator of the ancestral gene, which it does at least in part by targeting SRGAP2A towards degradation (168, 177). These data point to the human-specific gene

SRGAP2C as a strong candidate to control some intrinsic component of human neuronal neoteny, although this remains to be tested directly in human neurons.

6. CONCLUSIONS, REMAINING QUESTIONS, AND PERSPECTIVES

In conclusion, recent work has uncovered important cellular and molecular mechanisms underlying corticogenesis and how they relate to human brain evolution. A wide variety of evolutionary genomic changes have been identified, from single-point mutations to large-scale segmental duplications, whether in regulatory or coding regions. For a few of them, a chain of causality could be identified and linked to molecular and cellular processes that are divergent in human and primate species, which could explain part of the specificity of corticogenesis. Finally, the genetics of human NDD has contributed in a decisive way to uncover the contribution of these basic mechanisms to normal cortical formation and function.

But clearly, human corticogenesis remains a terra incognita for the most part. Identifying the mechanisms underlying the heterochrony of human corticogenesis, involving most critical developmental steps but each with a different magnitude, remains an outstanding puzzle to solve. In this frame, in addition to divergence in gene regulatory networks that remain to be uncovered, global changes such as the regulation of proteostasis and metabolism could bear special importance, as recently suggested by several reports. Comparative studies of the developmental timing of motor neuron generation and somitic development have uncovered striking differences in protein turnover between mouse and human cells, which could be causally linked to the heterochrony in these systems (118, 157). Moreover, cortical neurogenesis was found to be dependent on the instructive influence of mitochondria and metabolism (84), which were linked to species differences in the timing of fate transitions (82). Overall,

these new findings suggest that the properties of organelles and metabolism, intuitively thought to be largely conserved among all species, could underlie some of the divergence of developmental mechanisms,including human corticogenesis (84).

The human-specific mechanisms of neuronal differentiation and connectivity constitute another important frontier to explore further, in particular the developmental programs that underlie neuronal specification and connectivity. A focus on human brain development at postnatal ages will be critical to making progress, despite the associated technical and ethical challenges, as will the design of more refined models, including organoids and xenotransplantation, targeted at later stages of neuronal development and function.

However, while a handful of candidate genes and regulatory elements have now been characterized functionally, with a few exceptions, most of them have been studied by gain-of-function in nonhuman models, but their requirements for normal brain development should be studied more systematically in human corticogenesis, whether using ex vivo material or PSC models. Another critical missing gap is that the evolution of corticogenesis does not rely on just one gene, but rather on the (sometimes small) effects of many genes, the combination of which may be greater (or smaller) than their individual contributions. The emergence of CRISPR-based combinatorial screens, in animal or PSC-based models (48), should make it possible to manipulate several candidate genes (and/or their regulatory regions) at the same time and to define the hierarchy and interactions between seemingly distinct evolutionary innovations.

Finally, while the mouse model has clearly proved to be most useful, the range of species currently used for the experimental study of corticogenesis remains far too narrow, especially since many of the mouse cortical features are often divergent and quite distinct from the human,and,in some features, even from other mammals. The

combined use of other models with more complex corticogenesis, such as ferret, marmoset, and macaque species, will be of paramount importance. Conversely, more studies should be aimed at dissecting the development of cortical-like structures in nonmammalian species, such as reptiles and birds, while simpler vertebrate and invertebrate models will continue to feed the field with more insights on general developmental mechanisms. Expanding the number of species could also rely on designing completely new animal models, which should be made possible by progress in genomics and genetic engineering but also with the use of PSC models for species displaying distinctive brain expansion, such as other primates and cetaceans, for instance. The fusion of PSC from different species, as recently described for neural organoids derived from chimpanzee/human fused PSC (1), could also help to dissect the relative contributions of *cis*- and *trans*-regulatory mechanisms of corticogenesis. While cortical pyramidal neuron lineage has been the main target for evo-devo studies on the human brain, the scope should be expanded to interneurons and glial cells as well as to noncortical regions, such as the thalamus or the cerebellum, all of which contribute in a key fashion to human brain specificities.

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FIGURES

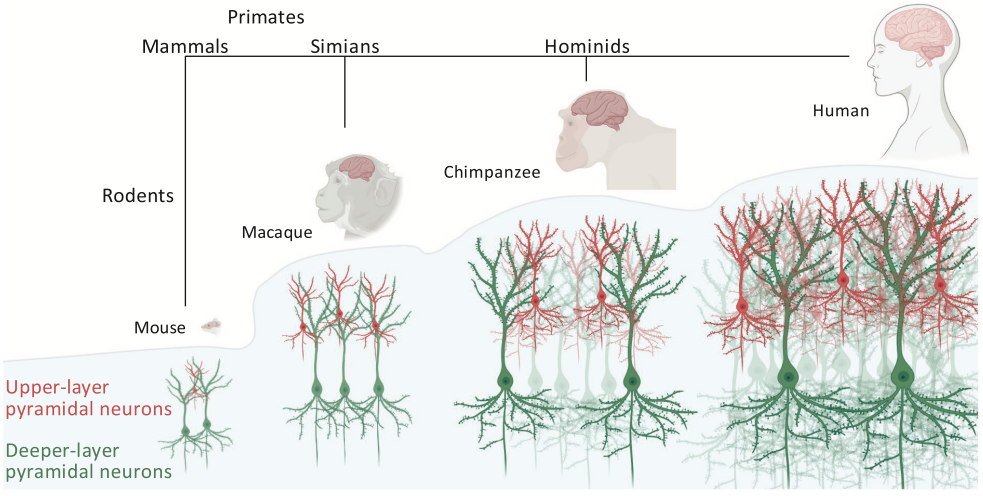


Figure 1

Evolution of the neuronal cytoarchitecture of the cerebral neocortex. The phylogenetic tree indicates representative mammalian (mouse), primate/simian (macaque), and hominid (chimpanzee) species, including human. Humans have the largest brain among primates. Primates have more cortical neurons (relative to brain mass) than most other mammalian species and present a higher proportion of upper-layer pyramidal neurons. Among primates, the human cortex has the highest number of neurons and upper-layer pyramidal neurons. At the single-neuron level, dendritic arbor complexity and dendritic spine density are increased in hominids. Among primates, the human cortex has the largest neurons and the highest number of dendritic spines per neuron. Figure adapted from images created with BioRender.com.

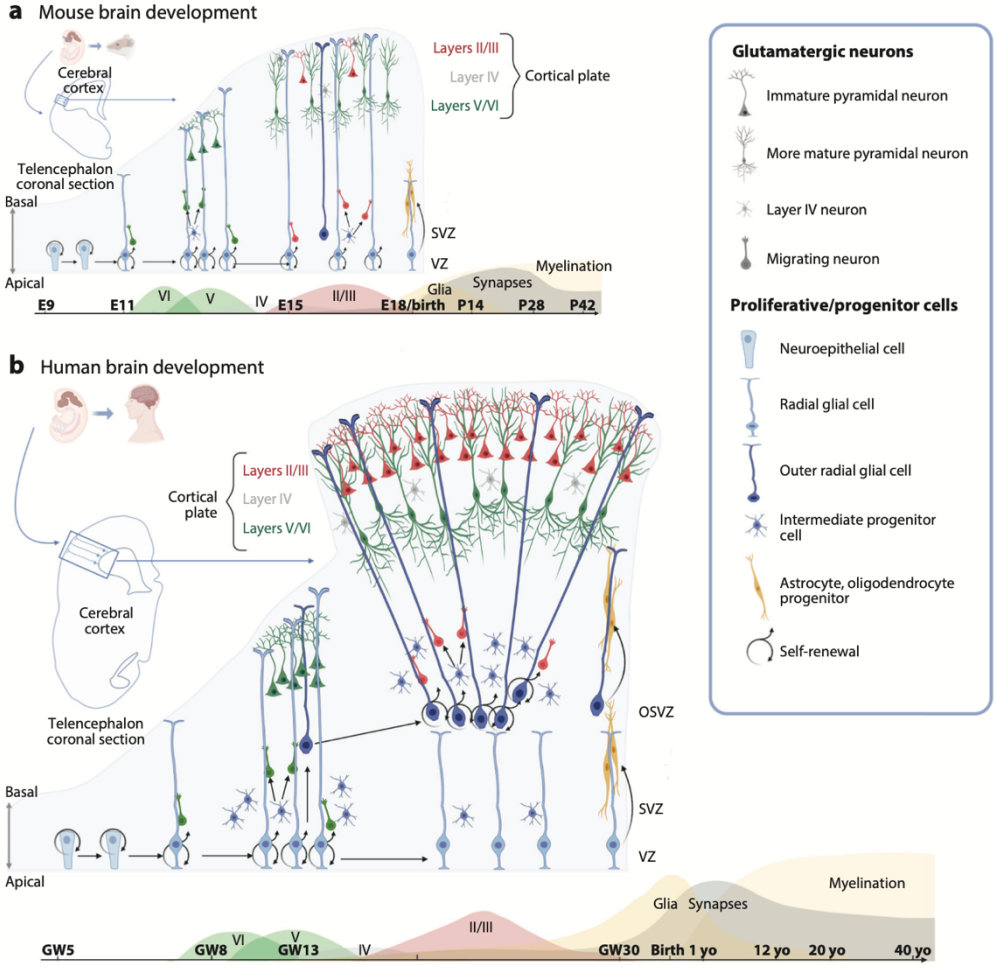


Figure 2

Comparative representations of corticogenesis in (a) mouse [from embryonic day 9 (E9) to postnatal day 42 (P42)] and (b) human [from gestation week 5 (GW5) to 40 years old (40 yo)].Human cortical neurogenesis is protracted in time and involves a higher

diversity of neural progenitors,which accounts for a higher number of cortical neurons and a higher proportion of upper pyramidal neurons. Corticogenesis begins in mammals in the ventricular zone (VZ) with the amplification of neuroepithelial cells by symmetric divisions and then asymmetric divisions of radial glial cells (RGCs) giving rise to intermediate progenitor cells (IPCs) and cortical neurons.IPCs divide symmetrically in the subventricular zone (SVZ) and in the outer subventricular zone (OSVZ) to generate cortical neurons.

Deeper (in cortical layers V and VI) pyramidal neurons are generated first,followed by upper (in cortical layers II and III) pyramidal neurons; they migrate radially toward their final location,the cortical plate,using fibers of RGCs as a scaffold.In primates,outer radial glial cells are amplified in the OSVZ and constitute an additional pool of progenitors,as well as a scaffold for neuronal migration. Figure adapted from images created with BioRender.com.

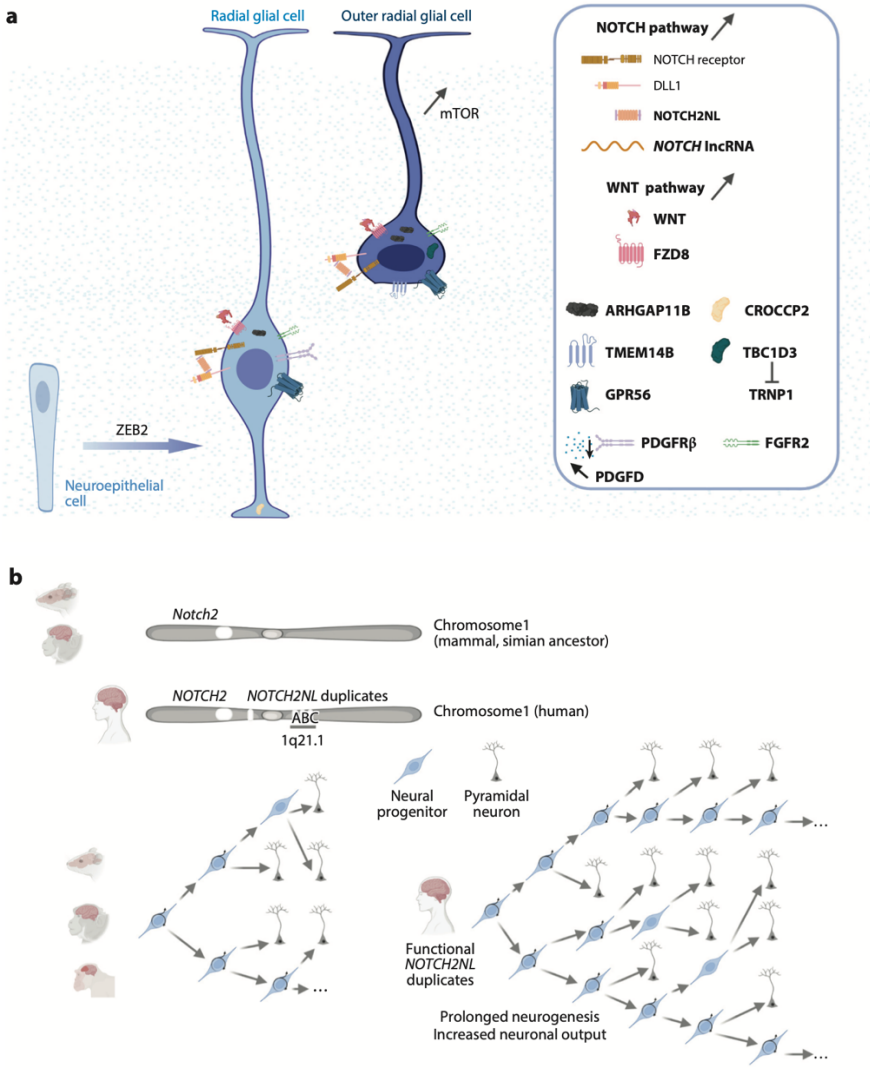


Figure 3

Divergent molecular features of human corticogenesis. (a) While most of the genes expressed in cortical progenitors are shared between mammalian species, human progenitors display molecular novelties linked to increased proliferative properties. The transcription factor ZEB2 is necessary and sufficient to regulate the timing of transition

from neuroepithelial cells to radial glia cells; its expression is protracted in human progenitors. Upregulated pathways in human progenitors include NOTCH,through the human-specific NOTCH2NLgenes (NOTCH2NLA,NOTCH2NLB,and NOTCH2NLC) and a simian-specific long noncoding RNA (lncRNA). Other upregulated pathways include WNT, notably with the upregulated expression of the WNT receptor FZD8, and the mTOR pathway. Other signaling pathways upregulated in human progenitors involve PDGFRβ and its ligand PDGFD, GPR56 (in simians), and FGFR2 (in human). Other duplicated genes are enriched in human neural progenitors and linked to increased proliferative abilities: they encode the human-specific protein ARHGAP11B (associated with mitochondria), the hominid-specific proteins TBC1D3 (through inhibition of TRNP1) and CROCCP2 (associated with ciliary dynamics and mTOR pathway), and the simian-specific protein TMEM14B. (b) The human-specific NOTCH2NL proteins,whose genes emerged through gene duplication from the ancestor gene NOTCH2, interact with NOTCH and its ligand DLL1, leading to increased NOTCH signaling and thereby prolonged cortical neurogenesis and increased neuronal output. Figure adapted from images created with BioRender.com.

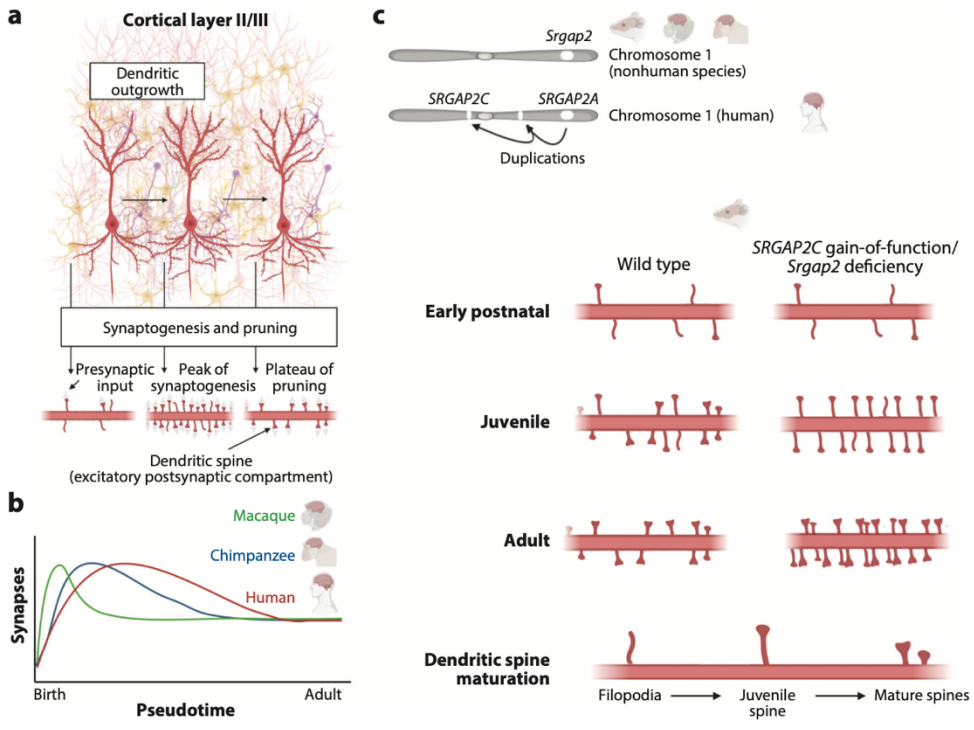


Figure 4

Neoteny of human cortical neurons.(a) The maturation of pyramidal neurons involves dendritic outgrowth, synapse formation (synaptogenesis), and refinement (pruning). (b) These processes are protracted in human; notably, synaptogenesis and pruning are protracted in hominids—and even more in human—in absolute duration and relative to life span. (c) The human-specific gene SRGAP2C emerged through gene duplication of the ancestor gene SRGAP2A. Gain-of-function of SRGAP2Cin mouse leads to a delayed maturation of dendritic spines, in a cell-autonomous manner, and an increased size and density of dendritic spines at the adult stage. Figure adapted from images created with BioRender.com.