UNIVERSITY OF HELSINKI

https://helda.helsinki.fi

What Makes Us Human : Insights from the Evolution and Development of the Human Neocortex

Namba, Takashi; Huttner, Wieland B.

2024-10-01
Annual Reviews Inc.
http://hdl.handle.net/10138/587005
Namba, T & Huttner, W B 2024, 'What Makes Us Human : Insights from the Evolution and Development of the Human Neocortex', Annual Review of Cell and Developmental Biology, vol. 40, no. 1, pp. 427-452. https://doi.org/10.1146/annurev-cellbio-112122-032521

Downloaded from Helda, University of Helsinki institutional repository. https://helda.helsinki.fi
This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.
Please cite the original version.

ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

What Makes Us Human: Insights from the Evolution and Development of the Human Neocortex

Takashi Namba[1] and Wieland B. Huttner[2]

[1]Neuroscience Center, Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland

[2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; email: huttner@mpi-cbg.de

ANNUAL REVIEWS CONNECT

www.annualreviews.org

- Download figures
- Navigate cited references
- Keyword search
- Explore related articles
- Share via email or social media

Annu. Rev. Cell Dev. Biol. 2024. 40:427–52

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-112122-032521

Copyright © 2024 by the author(s). This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. See credit lines of images or other third-party material in this article for license information.

OPEN ACCESS CC BY

Keywords

cortical stem and progenitor cells, human-specific genes, *ARHGAP11B*, *NOTCH2NL*, Neanderthals, metaphase length, lagging chromosomes, transketolase-like 1, neuronal circuitry, *SRGAP2C*

Abstract

"What makes us human?" is a central question of many research fields, notably anthropology. In this review, we focus on the development of the human neocortex, the part of the brain with a key role in cognition, to gain neurobiological insight toward answering this question. We first discuss cortical stem and progenitor cells and human-specific genes that affect their behavior. We thus aim to understand the molecular foundation of the expansion of the neocortex that occurred in the course of human evolution, as this expansion is generally thought to provide a basis for our unique cognitive abilities. We then review the emerging evidence pointing to differences in the development of the neocortex between present-day humans and Neanderthals, our closest relatives. Finally, we discuss human-specific genes that have been implicated in neuronal circuitry and offer a perspective for future studies addressing the question of what makes us human.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

Contents

INTRODUCTION

Neocortex Expansion and Neuron Numbers

There are various possible answers to the question "What makes us human?" This reflects the diverse features that characterize us as a species, such as the lack of fur among primates, the increased mobility of the thumb (Karakostis et al. 2021), or the upright walk. Yet, there is general consensus that the most distinguishing abilities of modern humans have their foundation in the brain and include, in particular, our cognitive capacities and language. A prerequisite for these crucial abilities is the expansion of the brain, specifically of the cerebral cortex, and the underlying increase in the number of neural cells, notably neurons, that occurred during the evolution of *Homo sapiens*. The increase in the number of neurons, and the accompanying expansion of certain relevant cortical areas that accommodate them, allowed the development of a neuronal circuitry that is thought to be unique to modern humans.

The evolutionary expansion of the human cerebral cortex pertains in particular to the neocortex, which comprises around 90% of the human cerebral cortex. The neocortex is involved in higher-order brain functions such as sensory perception; the generation of motor commands; and, particularly in humans, cognition, consciousness, spatial reasoning, and language (Galakhova et al. 2022; Rakic 1999, 2009). Seminal work by Suzana Herculano-Houzel and colleagues (Azevedo et al. 2009; Herculano-Houzel 2009, 2012) has established that the human cerebral cortex contains approximately 16 billion neurons, the highest cortical neuron number of any mammal. For comparison, our closest living relative, the chimpanzee, has an approximately 2.7-fold-lower number of cortical neurons, in line with its brain volume being around one-third that of humans (Stephan et al. 1981).

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

What, then, are the data in support of the notion that the increase in the number of neurons and the accompanying expansion of the neocortex during human evolution underlie our abilities to perform the abovementioned higher-order brain functions? The primary lines of evidence are the lessons from brain developmental disorders, notably microcephaly.

Lessons from Microcephaly

Microcephaly is a human developmental disorder that is associated with impaired brain development and results in a reduction of head size (Alcantara & O'Driscoll 2014, Gilmore & Walsh 2013, Jayaraman et al. 2018, Kaindl et al. 2010, Phan & Holland 2021, Zaqout & Kaindl 2022). Humans born with microcephaly, whose brain may be as small as that of a chimpanzee, typically exhibit intellectual disability. This supports the notion that the expansion of the brain, notably of the neocortex, in the course of human evolution is one basis—though clearly not the only one—for the cognitive abilities that make us human.

Microcephaly has various causes, including mutations in key genes as well as environmental factors such as alcohol exposure or virus infection. Most of these causes affect the proliferation or survival of cortical stem and progenitor cells (CSPCs), resulting in their reduced abundance and consequently the greatly diminished generation of the neural cells that make up the cortical parenchyma, that is, neurons and macroglial cells (i.e., astrocytes, oligodendrocytes).

Objectives of This Review

In light of the above considerations, this review focuses on cortical development, specifically on cellular and molecular features of the neocortex that are crucial for our ability to perform higher-order brain functions. We see two main classes of such features: (*a*) those that pertain to CSPCs and (*b*) those that pertain to neurons and the circuitry they form. These topics are related to the broad field of neocortical neurogenesis in development and evolution. For a near-comprehensive treatise of the latter field, readers are referred to the recent book edited by Huttner (2023).

CORTICAL STEM AND PROGENITOR CELLS

Germinal Zones, Classes, and Types of Cortical Stem and Progenitor Cells

A hallmark of the developing neocortical wall (for details, see **Figure 1**) is its apical-basal polarity (for reviews, see Andrews et al. 2022, Kalebic & Namba 2021, Kriegstein & Götz 2003, Taverna et al. 2014). The apical side corresponds to the ventricular surface of the cortical wall, which contacts the lumen of the lateral ventricle, whereas the basal side corresponds to the pial surface of the cortical wall, which contacts the basal lamina. In the developing human neocortex, three germinal zones can be distinguished: one primary germinal zone and two secondary germinal zones. The primary germinal zone is the ventricular zone (VZ), the apical-most zone of the developing cortical wall, which contacts the ventricle. The two secondary germinal zones are the inner subventricular zone (iSVZ), a zone adjacent to the basal boundary of the VZ, and the outer subventricular zone (oSVZ) (Dehay et al. 2015, Smart et al. 2002), a zone located basally to the iSVZ (for reviews, see Kriegstein & Götz 2003, Taverna et al. 2014). The VZ is the primary germinal zone because it is originally formed by the primary stem cells of the developing brain, the neuroepithelial cells (NECs). With the onset of cortical neurogenesis, NECs transform into apical radial glia (aRG), also called ventricular radial glia (Kriegstein & Götz 2003, Namba & Huttner 2017, Taverna et al. 2014). Later in cortical development, aRG become truncated radial glia (Bilgic et al. 2023, Nowakowski et al. 2016). Another type of CSPC whose cell bodies also reside in the VZ is the apical intermediate progenitor, previously called the short neural

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

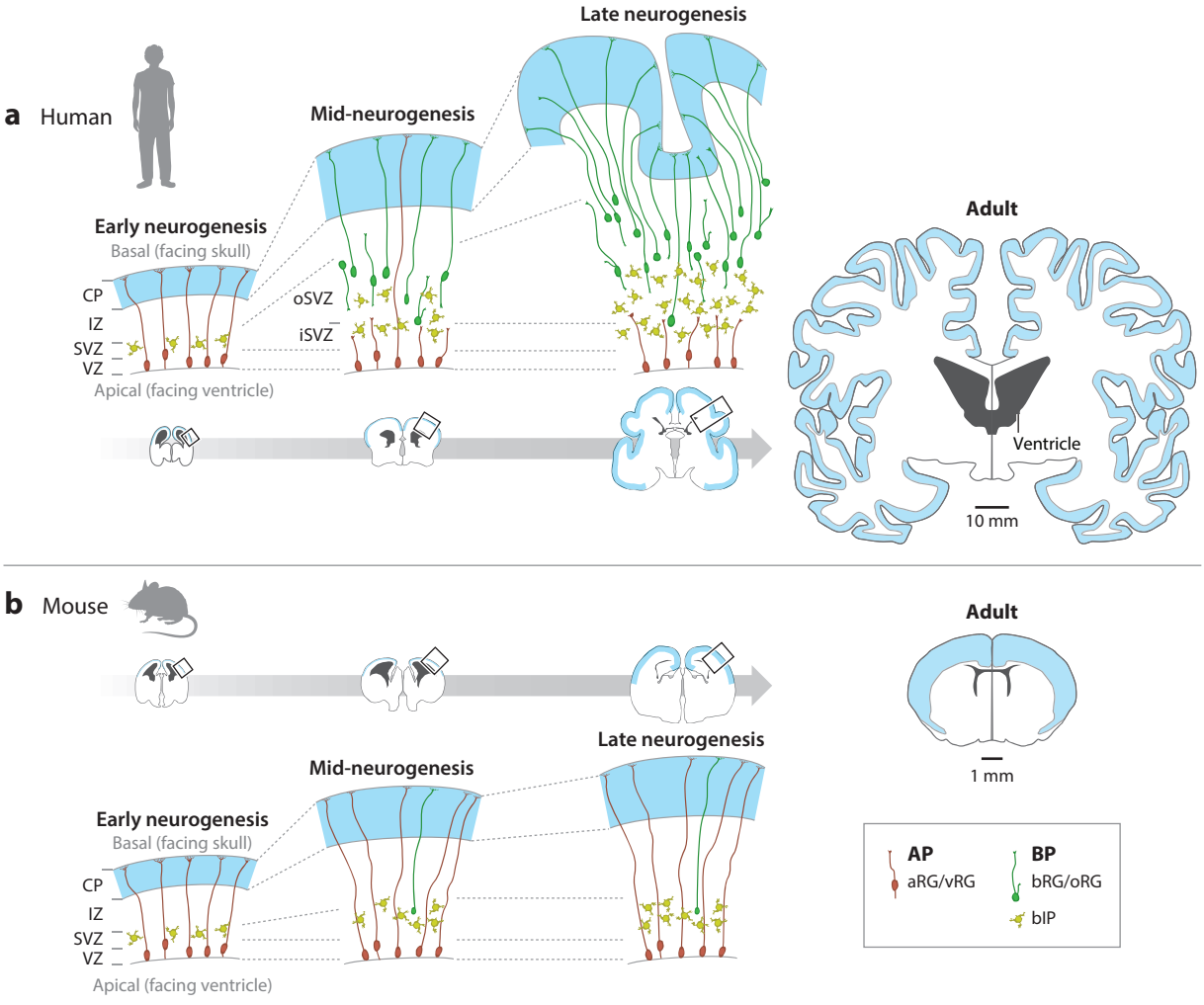

Figure 1

Neocortical development in mice and humans. (*a*) Coronal sections of the human neocortex at early (GW11), mid- (GW17), and late (GW24) neurogenesis. (*b*) Coronal sections of the mouse neocortex at early (E12.5), mid- (E14.5), and late (E16.5) neurogenesis. Enlarged images of the boxed areas in the coronal brain sections are shown at the top of panel *a* and at the bottom of panel *b*. In both panels, the rightmost coronal section is the adult brain. Blue and black areas in the coronal sections indicate the gray matter and the lateral ventricle, respectively. Scale bars of 10 mm and 1 mm apply to human and mouse coronal brain sections, respectively. Abbreviations: AP, apical progenitor; aRG, apical radial glia; bIP, basal intermediate progenitor; BP, basal progenitor; bRG, basal radial glia; CP, cortical plate; E, embryonic day; GW, gestational week; iSVZ, inner SVZ; IZ, intermediate zone; oRG, outer radial glia; oSVZ, outer SVZ; SVZ, subventricular zone; vRG, ventricular radial glia; VZ, ventricular zone. Figure adapted from Gkini & Namba (2023) (CC BY 4.0).

precursor. These various CSPC types in the VZ are collectively called apical progenitors (APs) (for a review, see Taverna et al. 2014).

The CSPCs whose cell bodies reside in the iSVZ or in the oSVZ are collectively called basal progenitors (BPs) (for reviews, see Namba & Huttner 2017, Taverna et al. 2014). BPs comprise two distinct types of CSPCs: (*a*) basal radial glia (bRG), also called outer radial glia, and (*b*) basal intermediate progenitor cells (bIPs) (Taverna et al. 2014). While APs, notably NECs, may contribute to neocortex expansion (Benito-Kwiecinski et al. 2021), BPs are thought, and in fact have

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

been shown, to play the major role in this expansion during development and human evolution. This is because AP mitoses in the VZ are confined to the ventricular surface, the apical-most region of this germinal layer. This spatial restriction limits the number of AP mitoses and hence the production of progeny. In contrast, BP mitoses in the iSVZ and oSVZ can occur anywhere in these germinal layers, notably in the oSVZ, which is greatly expanded in the fetal human neocortex. This allows for a massive increase in the production of progeny (for reviews, see Andrews et al. 2022, Namba & Huttner 2017, Taverna et al. 2014).

The reason why AP mitoses are confined to the ventricular surface lies in an epithelial feature of these cells, specifically the presence of an apical primary cilium, which results in the tethering of the centrosomes—the organizers of the mitotic spindle—to the apical cell cortex. Such apical tethering no longer applies to BPs, as these cells have delaminated from the ventricular surface and hence no longer exhibit apical primary cilia but rather carry (baso)lateral primary cilia that usually emerge from the plasma membrane in the vicinity of the nucleus (Taverna et al. 2014, Wilsch-Bräuninger et al. 2012).

In the following sections, we first review recent data suggesting that a human-specific delay in the transition from NECs to aRG may contribute to human neocortex expansion. We then summarize data on genes that amplify BPs and that are thought, or have been shown, to be able to expand the neocortex. In line with the theme of this review, a major focus in this context is on human-specific genes. The first such gene we discuss is *ARHGAP11B*, followed by a discussion of the gene *NOTCH2NL*. In addition, we review genes enriched in copy number in the human genome, such as *TBC1D3*, and primate-specific genes, such as *TMEM14B*.

Neuroepithelial Cell–Based Foundations of Human Neocortex Expansion

Irrespective of the limitations of APs with regard to maximizing the number of their mitoses, which (as explained above) is due to their cell biology, NECs as the primary neural stem cells have a role in the evolutionary expansion of the human neocortex. Specifically, the longer the phase of symmetric proliferative divisions of NECs (1 NEC → 2 NECs) lasts (i.e., the later NECs transform into aRG), the greater will be the number of the founder cells of the radial units of the developing neocortex (Rakic 1988, 2000, 2009). Interestingly, a recent study (Benito-Kwiecinski et al. 2021) comparing human, chimpanzee, and gorilla cerebral organoids showed that the transition of NECs to aRG is delayed in humans compared with the other two great apes. This delay is thought to underlie the greater NEC founder pool size in the developing forebrain of humans in comparison to other great apes, and hence constitutes a first step toward the evolutionary expansion of the human neocortex.

At the molecular level, the transcription factor ZEB2 is a driver of the NEC-to-aRG transition (Benito-Kwiecinski et al. 2021). Accordingly, *ZEB2* expression peaks earlier in gorilla than in human cerebral organoids (Benito-Kwiecinski et al. 2021). These data provide cell biological and molecular support for the notion that the first step in the evolutionary expansion of the human neocortex, the increase in the number of founder cells of the radial units (Rakic 1988, 2000, 2009), is due to a delay in this transition.

ARHGAP11B, a Human-Specific Gene That Amplifies Basal Progenitors and Increased the Size of the Neocortex During Hominin Evolution

In this section, we review key features of *ARHGAP11B*. These include (*a*) the origin of the gene, (*b*) the ability of *ARHGAP11B* to amplify BPs and expand the neocortex, (*c*) the increase in cognitive abilities resulting from the *ARHGAP11B*-induced neocortex expansion, (*d*) the crucial functional role of the single C→G nucleotide substitution in the *ARHGAP11B* gene, (*e*) the

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

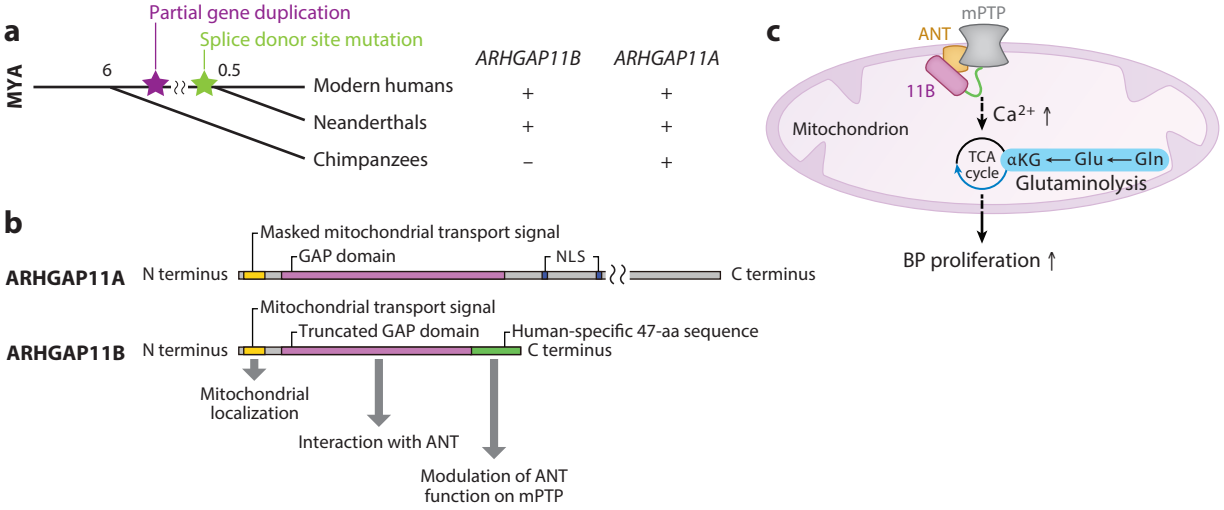

Figure 2

Evolution and molecular function of ARHGAP11B. (*a*) Evolution of *ARHGAP11B*. Partial gene duplication of *ARHGAP11A* (~5 MYA) and a splice donor site mutation (~2–0.5 MYA) happened after the split of chimpanzee and *Homo* lineages but before the split of the modern human lineage from that of Neanderthals and Denisovans. (*b*) Protein structure of ARHGAP11A and ARHGAP11B. (*c*) The role of ARHGAP11B in mitochondria. Abbreviations: aa, amino acid; αKG, α-ketoglutarate; ANT, adenine nucleotide translocator; BP, basal progenitor; GAP, GTPase-activating protein; Gln, glutamine; Glu, glutamate; mPTP, mitochondrial permeability transition pore; NLS, nuclear localization signal; MYA, million years ago; TCA, tricarboxylic acid.

mechanism of action of the ARHGAP11B protein, and (*f*) the functional synergy of this human-specific protein with the ape-specific GLUD2 protein.

Origin of *ARHGAP11B*. Pioneering research by Eichler and colleagues (Antonacci et al. 2014, Dennis & Eichler 2016, Sudmant et al. 2010) demonstrated that *ARHGAP11B* arose by a partial segmental duplication of the widespread gene *ARHGAP11A* (**Figure 2*a***), which encodes a Rho GTPase–activating protein (Rho GAP) (Kagawa et al. 2013, Xu et al. 2013, Zanin et al. 2013). This partial gene duplication comprises the N-terminal ~30% of the protein-coding part of *ARHGAP11A*, which contains the entire GAP domain (Antonacci et al. 2014, Dennis & Eichler 2016). The partial gene duplication occurred approximately 5 million years ago (MYA), which is after the lineage leading to our closest great ape relatives, the chimpanzee and bonobo, had segregated around 7 MYA from the lineage leading to us. However, the origin of *ARHGAP11B* approximately 5 MYA is, of course, much earlier than the time point of the segregation of the lineage leading to the Neanderthals and Denisovans from the lineage leading to modern humans, which occurred around 0.5 MYA. These data raised the possibility that *ARHGAP11B* may occur not only in the genome of modern humans but also in the genomes of Neanderthals and Denisovans. In fact, analyses of the latter two genomes showed that this was indeed the case (Florio et al. 2016, Meyer et al. 2012, Prüfer et al. 2014, Sudmant et al. 2010). Thus, *ARHGAP11B*, usually referred to as a human-specific gene, is actually a hominin-specific gene.

***ARHGAP11B* amplifies mouse and ferret basal progenitors and induces hallmarks of neocortex expansion.** An analysis of the transcriptomes of distinct cell populations isolated from the fetal human neocortex and greatly enriched in aRG, bRG, or neurons revealed that *ARHGAP11B* messenger RNA (mRNA) is specifically expressed in aRG and bRG but not in neurons (Florio et al. 2015). This finding raised the possibility that ARHGAP11B may promote the generation

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

of BPs, stimulate BP proliferation, and thus increase BP abundance. Expression of *ARHGAP11B* under the control of a strong constitutive promoter in the embryonic mouse neocortex, used as a test system, demonstrated that ARHGAP11B exerts all three effects (Florio et al. 2015). As the overwhelming majority (>90%) of the BPs in the embryonic mouse neocortex are bIPs (Kelava et al. 2012, Vaid et al. 2018, Wang et al. 2011), these data imply that ARHGAP11B can amplify bIPs.

Expression of *ARHGAP11B* in the developing neocortex of the ferret, which in contrast to the lissencephalic mouse is a gyrencephalic mammal in which approximately half of the BPs are bRG, demonstrated that ARHGAP11B can also amplify bRG, especially in the oSVZ (Kalebic et al. 2018). Moreover, *ARHGAP11B* expression in the developing ferret neocortex results in an extension of the neurogenic period and an increased abundance of upper-layer neurons, both hallmarks of the evolutionary expansion of the neocortex (Kalebic et al. 2018). Accordingly, the postnatal ferret neocortex was found to be expanded in both the tangential and radial dimensions (Kalebic et al. 2018).

***ARHGAP11B* can amplify basal progenitors in chimpanzee cerebral organoids and expand a fetal monkey neocortex.** How are these findings in the developing mouse and ferret neocortex relevant to the evolutionary expansion of the primate neocortex during the evolution toward *H. sapiens*? The expression of *ARHGAP11B* in ventricle-like structures of chimpanzee cerebral organoids demonstrated that this human-specific gene increased BP abundance to a human-like level (Fischer et al. 2022). Conversely, the blockade of ARHGAP11B function in human cerebral organoids reduced BP abundance to that observed in chimpanzee cerebral organoids (Fischer et al. 2022). These data establish that in the great apes, ARHGAP11B is necessary and sufficient to ensure the elevated BP levels that characterize the fetal human neocortex. This in turn implies that the human-specific gene *ARHGAP11B* had a major role in the tripling of brain size, in comparison to the chimpanzee, that occurred during human evolution.

Direct evidence that *ARHGAP11B* can expand the primate neocortex was provided by its expression, under the control of its own human promoter, in fetuses of the common marmoset, a New World monkey (Heide et al. 2020). This physiological expression demonstrated that *ARHGAP11B* not only increases the size of the developing neocortex but also induces its folding in this normally near-lissencephalic primate (Heide et al. 2020). Remarkably, this *ARHGAP11B*-induced neocortex expansion was associated with a massive increase in BP abundance, notably in bRG abundance and particularly in the oSVZ (Heide et al. 2020). As there was no increase in AP abundance, these data underscore the central role of BPs, especially of those in the oSVZ, in neocortex expansion. Moreover, *ARHGAP11B* expression resulted in an increase in upper-layer neurons, but not deep-layer neurons, in the cortical plate, which is a hallmark of evolutionary neocortex expansion (Heide et al. 2020). Taken together, these findings point to a crucial role for this hominin-specific gene in the expansion of the neocortex during human evolution.

The ability of *ARHGAP11B* to amplify basal progenitors and expand the neocortex is based on a single C→G nucleotide substitution. The origin of *ARHGAP11B* from the partial gene duplication of *ARHGAP11A* approximately 5 MYA seemingly poses a problem regarding *ARHGAP11B*'s presumptive role in neocortex expansion during human evolution, because the big surge in this expansion started only around 2 MYA, not 5 MYA. Thus, is *ARHGAP11B* simply too old to have a major role in human neocortex expansion? A solution to this conundrum emerged when it was realized that the *ARHGAP11B* gene as it arose approximately 5 MYA probably lacked the ability to amplify BPs (Florio et al. 2016). The 47-amino-acid-long C-terminal sequence of ARHGAP11B is essential for the protein's ability to amplify BPs (Namba et al. 2020) (**Figure 2*b***). This C-terminal sequence is human-specific and distinguishes ARHGAP11B from

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

the corresponding part of the ARHGAP11A protein (Florio et al. 2016). This C-terminal sequence is the result of a single C→G nucleotide mutation in the *ARHGAP11B* genomic DNA at a position corresponding to c.661 in exon 5 of the *ARHGAP11A* genomic DNA (Florio et al. 2016). This C→G mutation creates a new splice donor site, which in turn leads to the removal of 55 nucleotides from the *ARHGAP11B* mRNA upon splicing, causing a reading frame shift that leads to the human-specific 47-amino-acid-long C-terminal sequence of ARHGAP11B. Reconstruction of an *ARHGAP11B* complementary DNA lacking this C→G mutation, and hence not encoding the human-specific 47-amino-acid-long C-terminal sequence, is referred to as ancestral *ARHGAP11B*, and its expression in the embryonic mouse neocortex showed that ancestral *ARHGAP11B*, in contrast to present-day *ARHGAP11B*, is unable to amplify BPs (Florio et al. 2016).

Notably, the *ARHGAP11B* gene present in the genomes of Neanderthals and Denisovans also contains the C→G mutation (Florio et al. 2016), which has two significant implications. First, this finding is in line with the fact that the brain of Neanderthals was at least as great as that of present-day humans (Neubauer et al. 2018, Ruff et al. 1997) and is consistent with the notion that *ARHGAP11B* also contributed to the evolutionary expansion of the Neanderthal brain. Second, it strongly suggests that the crucial C→G mutation that endowed *ARHGAP11B* with its ability to amplify BPs, and hence to expand the neocortex, occurred before the split of the lineages leading either to Neanderthals or to modern humans, which (as mentioned above) occurred around 500,000 years ago. We do not know when exactly this C→G mutation in the *ARHGAP11B* gene occurred, but it is an attractive thought to assume that it happened around 2 MYA, when the big surge in hominin brain size expansion started (DeSilva et al. 2021).

Mechanism of action of ARHGAP11B. How, then, does present-day ARHGAP11B amplify BPs? An unexpected finding was that the ARHGAP11B protein operates within mitochondria, being imported into the mitochondrial matrix (Namba et al. 2020). This subcellular localization of ARHGAP11B is in striking contrast to that of ARHGAP11A, which is localized mainly in the nucleoplasm (Kagawa et al. 2013, Namba et al. 2020, Pilaz et al. 2023, Xing et al. 2021, Xu et al. 2013). Interestingly, both ARHGAP11B and ARHGAP11A contain an N-terminal mitochondrial import sequence. In ARHGAP11A, however, its function appears to be blocked by the large C-terminal portion of the protein, which contains nuclear localization signals and is lacking in ARHGAP11B. In contrast, in ARHGAP11B, this N-terminal mitochondrial import sequence is functional, as its deletion abrogates the mitochondrial localization of ARHGAP11B, and this sequence alone suffices to direct enhanced green fluorescent protein to the mitochondrial matrix (Namba et al. 2020). The localization of ARHGAP11B within the mitochondria is essential for its ability to amplify BPs (Namba et al. 2020).

Within the mitochondrial matrix, ARHGAP11B interacts, via its truncated GAP domain, with the adenine nucleotide translocase in the inner mitochondrial membrane (Namba et al. 2020), a known regulator of the mitochondrial permeability transition pore (mPTP) (**Figure 2*c***). Via its human-specific 47-amino-acid-long C-terminal sequence, ARHGAP11B inhibits the mPTP (Namba et al. 2020). This inhibition ultimately results in an increase in the metabolic pathway called glutaminolysis (Namba et al. 2020), that is, the conversion of glutamine to glutamate and then to the tricarboxylic acid (TCA) cycle intermediate α-ketoglutarate (αKG) (Yang et al. 2017). Interestingly, glutaminolysis is known to be a hallmark of cancer metabolism (Yang et al. 2017). Accordingly, the increase in αKG due to the ARHGAP11B-induced stimulation of glutaminolysis is thought to fuel a so-called three-quarter TCA cycle, which starts at αKG and ends at oxaloacetate (Namba et al. 2021). This in turn would allow an increased synthesis of aspartate, an essential metabolite for cell proliferation (Namba et al. 2021). Thus, the mitochondrial action of ARHGAP11B in essence promotes an anabolic metabolism required

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

for cell proliferation. Consistent with these data, inhibition of the ARHGAP11B-induced stimulation of glutaminolysis completely blocks ARHGAP11B's ability to amplify BPs (Namba et al. 2020).

Functional synergy of the human-specific ARHGAP11B and ape-specific GLUD2 proteins. Glutamate dehydrogenase 2 (GLUD2) is an ape-specific protein that also operates in mitochondria and converts glutamate to αKG (Shashidharan & Plaitakis 2014, Spanaki et al. 2010). GLUD2 alone does not increase the abundance of BPs, neither bIPs nor bRG (Xing et al. 2024). Interestingly, however, the ability of ARHGAP11B to increase bRG abundance is strongly enhanced by GLUD2 (Xing et al. 2024). Double-transgenic bRG expressing both *ARHGAP11B* and *GLUD2* show increased production of the proproliferative metabolite aspartate (Xing et al. 2024). As hypothesized (Namba et al. 2021), this increase in aspartate production from glutamate occurs via αKG and the TCA cycle. Taken together, these findings imply that, during human evolution, the human-specific gene *ARHGAP11B* exploited the existence of *GLUD2*, a gene that emerged earlier in evolution (i.e., in apes) to increase bRG abundance via a concerted increase in the key metabolite αKG. This functional synergy likely contributed to the expansion of the neocortex during human evolution.

***ARHGAP11B*-induced neocortex expansion increases cognitive abilities in mice.** Does expansion of the neocortex due to *ARHGAP11B* result in improved cognitive abilities? Behavioral analyses of genetically modified mice that express the ARHGAP11B protein during embryonic brain development indicate that this is the case (Xing et al. 2021). Specifically, in these mice, the neocortex expansion and increase in upper-layer neurons caused by ARHGAP11B during embryonic development persist into adulthood (Xing et al. 2021). Moreover, these adult mice exhibit increased memory flexibility, a neocortex-associated trait (Xing et al. 2021). These findings suggest that an *ARHGAP11B*-induced neocortex expansion indeed results in improved cognitive abilities, which has significant implications for the increase in cognitive abilities during human evolution and the role of *ARHGAP11B* therein.

NOTCH2NL

In this section, we review key features of *NOTCH2NL*. These include (*a*) the origin of the gene, (*b*) the ability of NOTCH2NL to maintain the CSPC pool size, and (*c*) the mechanism of action of the NOTCH2NL proteins.

Origin of *NOTCH2NL*. *NOTCH2NL* is a human-specific gene consisting of four paralogs, namely *NOTCH2NLA*, *NOTCH2NLB*, *NOTCH2NLC*, and *NOTCH2NLR* (Fiddes et al. 2018, 2019) (**Figure 3*a***). *NOTCH2NL* initially arose by a partial segmental duplication of an evolutionarily conserved gene, *NOTCH2*, which is localized in chromosome 1. The partial segmental duplication of *NOTCH2*, which happened after the lineage split of orangutans from the other great apes including humans, generated a pseudogene that lacks the promoter region and start codon of *NOTCH2*. After the split of the lineages leading to either chimpanzees or humans, the *NOTCH2NL* pseudogene acquired the promoter region and the exon 1 containing the start codon to become a functional gene, namely *NOTCH2NLR*, by a gene conversion from *NOTCH2*. The functional *NOTCH2NLR* gene then underwent multiple gene duplications; thus, there are four paralogs of *NOTCH2NL* (Fiddes et al. 2018, 2019).

NOTCH2NL maintains cortical stem and progenitor cell pool size. The expression of *NOTCH2NL*, in particular of *NOTCH2NLA* and *NOTCH2NLB*, is observed mainly in aRG and bRG (Fiddes et al. 2018, Florio et al. 2018, Suzuki et al. 2018). The ectopic expression of NOTCH2NLA and NOTCH2NLB in the mouse embryonic neocortex increases the abundance

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

of progenitors in the SVZ (i.e., BPs) and in the VZ (i.e., APs), respectively, suggesting that the expression of NOTCH2NL maintains CSPC abundance in humans (Florio et al. 2018, Suzuki et al. 2018). Consistent with these results, cortical organoids derived from NOTCH2NLA-expressing mouse embryonic stem (ES) cells exhibit a delay in neurogenesis (Fiddes et al. 2018). In contrast, cortical organoids derived from *NOTCH2NLA* and *NOTCH2NLB* homozygous and *NOTCH2NLC* heterozygous knockout human ES cells show accelerated neurogenesis (Fiddes et al. 2018).

Patients with a deletion or duplication of chromosome 1q21.1, where *NOTCH2NLA* and *NOTCH2NLB* are located, exhibit various psychiatric abnormalities such as attention-deficit/hyperactivity disorder, along with microcephaly or macrocephaly (Fiddes et al. 2018, Mefford et al. 2008, Rosenfeld et al. 2012). These studies suggest that gene deletion or duplication of *NOTCH2NL* is associated with brain size abnormality, corroborating the idea that NOTCH2NL is important for the maintenance of the CSPC pool size.

Mechanism of action of NOTCH2NL. Although NOTCH2NLB has a signal peptide for entry into the secretory pathway, whereas NOTCH2NLA and NOTCH2NLC lack a signal peptide, all three proteins are transported to the extracellular space (Fiddes et al. 2018, Suzuki et al. 2018) (**Figure 3*b*,*c***). The NOTCH2NL paralogs, irrespective of the presence or absence of a signal peptide, activate the NOTCH2 signaling pathway. On the one hand, NOTCH2NLB binds to the Notch ligand Delta-like 1 (DLL1) and activates the main target of the Notch signaling pathway, the transcription factor HES1 (Suzuki et al. 2018). Since interaction between DLL1 and the Notch receptor on the same membrane inhibits Notch receptor activity (del Alamo et al. 2011), NOTCH2NLB might interrupt the *cis* interaction between DLL1 and the Notch receptor. On the other hand, NOTCH2NLA binds to NOTCH2 and activates it (Fiddes et al. 2018). In either case, NOTCH2NL promotes CSPC proliferation and maintains their stemness by activating Notch signaling.

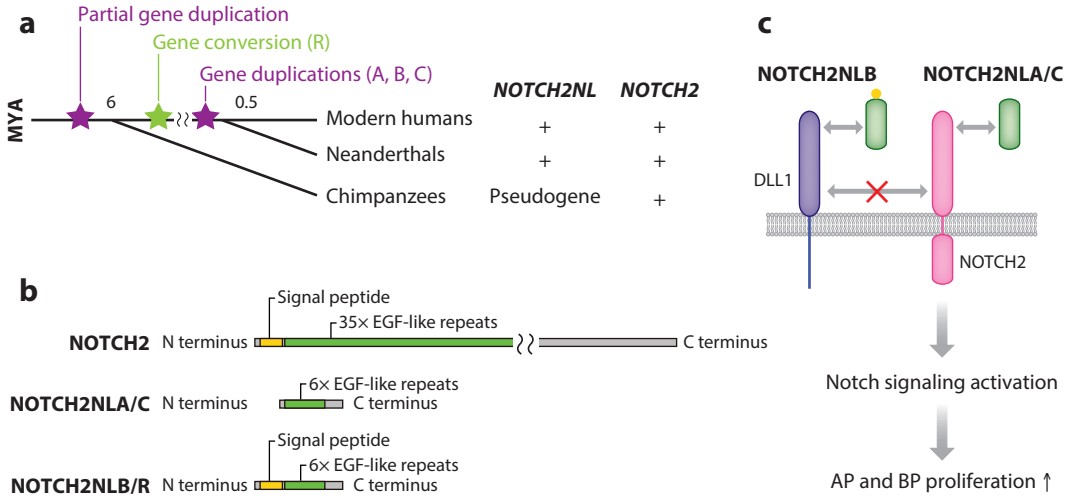

Figure 3

Evolution and molecular function of NOTCH2NL. (*a*) Evolution of *NOTCH2NL*. Partial gene duplication of *NOTCH2* (~8 MYA) and gene conversion and multiple gene duplications (~4–0.5 MYA). (*b*) Protein structure of NOTCH2, NOTCH2NLA and NOTCH2NLC, and NOTCH2NLB and NOTCH2NLR. (*c*) The role of NOTCH2NL in NOTCH2 signaling. Abbreviations: AP, apical progenitor; BP, basal progenitor; DLL1, Delta-like 1; EGF, epidermal growth factor; MYA, million years ago.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

TBC1D3 and *TMEM14B*

In this section, we review key features of *TBC1D3* and *TMEM14B*. These include (*a*) the origin of these two genes, (*b*) the ability of TBC1D3 to promote the generation and expansion of bRG, (*c*) the ability of TMEM14B to promote the expansion of both bIPs and bRG and to induce neocortex enlargement and folding, and (*d*) the mechanisms of action of the TBC1D3 and TMEM14B proteins.

Origin of *TBC1D3*. *TBC1D3* (TBC1 domain family member 3) is located on chromosome 17q12, which is located within one of the core duplicons in the human genome (Jiang et al. 2007). *TBC1D3* arose by a segmental duplication of *USP6NL*, which may have happened sometime after the split of lineages leading to New World monkeys (which lack *TBC1D3*) and to Old World monkeys (which contain *TBC1D3*). The *TBC1D3* copy number (number of paralogs) is expanded in humans but not in chimpanzees (**Figure 4*a***). As a result of copy number expansion in humans, there are at least eight *TBC1D3* paralogs that produce protein (Hodzic et al. 2006).

TBC1D3 promotes generation and expansion of basal radial glia. TBC1D3 is expressed in aRG, bIPs, and bRG (Ju et al. 2016). While TBC1D3 is mainly cytoplasmic in aRG, TBC1D3

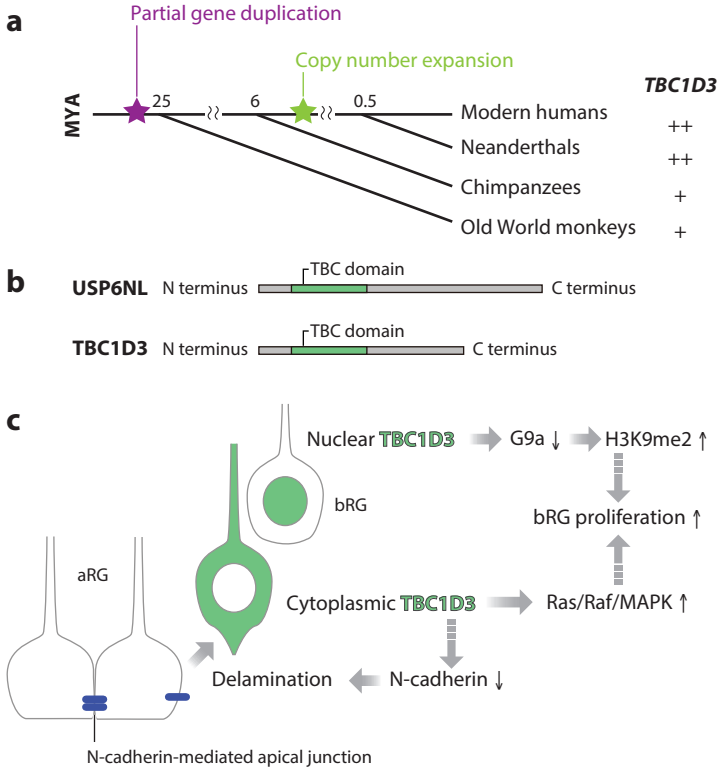

Figure 4

Evolution and molecular function of TBC1D3. (*a*) Evolution of *TBC1D3*, including partial gene duplication of *USP6NL* (~40–25 MYA) and copy number expansion (~6–0.5 MYA). (*b*) Protein structure of USP6NL and TBC1D3. (*c*) The role of TBC1D3 in aRG delamination and bRG proliferation. Abbreviations: aRG, apical radial glia; bRG, basal radial glia; H3K9me2, dimethylation of lysine 9 in histone 3; MAPK, mitogen-activated protein kinase; MYA, million years ago; TBC1D3, TBC1 domain family member 3.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

expression in bRG is mostly nuclear. The roles of TBC1D3 are distinct between aRG and bRG. The ectopic expression of TBC1D3 in the mouse embryonic neocortex by in utero electroporation or in transgenic mice, in which *TBC1D3* is expressed under the *Nestin* promoter, induces delamination of aRG, which in turn transform into bRG. TBC1D3 then promotes bRG proliferation (Ju et al. 2016). As a consequence of the bRG expansion upon TBC1D3 expression, the neocortex exhibits folding, mainly in the motor cortex (Ju et al. 2016). In contrast, knockdown of TBC1D3 in the human fetal neocortex reduces the abundance of SOX2-expressing BPs, which presumably are bRG (Ju et al. 2016).

Mechanism of action of TBC1D3. The molecular functions of TBC1D3 appear to differ depending on its subcellular localization (**Figure 4*b*,*c***). In the cytoplasm, TBC1D3 activates the small GTPase Ras, and hence the MAP kinase, leading to a promotion of CSPC proliferation (Ju et al. 2016). In contrast, nuclear TBC1D3 binds to histone methyltransferase G9a through the region close to the C terminus of TBC1D3 and inhibits the function of G9a (Hou et al. 2021). Since G9a catalyzes the dimethylation of lysine 9 in histone 3 (H3K9me2) (Tachibana et al. 2005), which is a suppressive mark for gene expression, the inhibition of G9a reduces the H3K9me2 mark. Consequently, the expression of genes associated with cell proliferation is upregulated (Hou et al. 2021). In aRG, TBC1D3 reduces the expression of *Cdh2* (N-cadherin), thereby inducing aRG delamination (Ju et al. 2016). This reduction does not seem to be caused by the G9a inhibition by TBC1D3, and its molecular mechanism needs to be further studied.

Origin of *TMEM14B*. *TMEM14B* might be a paralog of the evolutionarily conserved gene *TMEM14C*. Although *TMEM14B* is found in the genome of apes, including humans, Old World monkeys, and New World monkeys, the homology of the protein varies among species (Liu et al. 2017). Great apes' TMEM14B shares more than 95% of its amino acids with human TMEM14B; however, the homology drops to 85–95% in Old World monkeys and to only 76–80% in New World monkeys. Given that the homology between human TMEM14B and its presumably ancestral protein TMEM14C is 73%, TMEM14B has evolved toward Catarrhini (apes and Old World monkeys) (**Figure 5*a***).

TMEM14B promotes expansion of both basal intermediate progenitors and basal radial glia, thereby inducing neocortex enlargement and folding. The ectopic expression of TMEM14B in the mouse embryonic neocortex by in utero electroporation or in transgenic mice, in which *TMEM14B* is expressed under the control of the *CAG* promoter, promotes cell proliferation of both bIPs and bRG (Liu et al. 2017). Moreover, TMEM14B expression alters the mitotic cleavage plane orientation of aRG (Liu et al. 2017). Upon its expression, more aRG exhibit oblique mitotic cleavage planes, by which aRG generate bRG, suggesting that TMEM14B also induces delamination of aRG or the generation of bRG. As a consequence of the BP expansion upon TMEM14B expression, the neocortex contains more neurons, is enlarged, and exhibits folding (Liu et al. 2017).

Mechanism of action of TMEM14B. While the subcellular localization of TMEM14B has not been examined, its protein structure indicates that TMEM14B is a plasma membrane protein (**Figure 5*b*,*c***). TMEM14B interacts with IQGAP1 (Liu et al. 2017), a multifunctional scaffold protein (Noritake et al. 2005). Although IQGAP1 contains a Ras GAP domain in its C-terminal part, it does not act as a GAP for either Rac1 or Cdc42 due to the lack of the arginine finger that is critical for the GAP activity (Kurella et al. 2009); rather, IQGAP1 is an effector of Rac1 and Cdc42 (Watanabe et al. 2015). TMEM14B expression results in the phosphorylation of IQGAP1 (Liu et al. 2017), presumably by protein kinase Cε (PKCε) at its C-terminal region (Grohmanova et al. 2004). PKCε-mediated phosphorylation alters the conformation of IQGAP1 and thereby

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

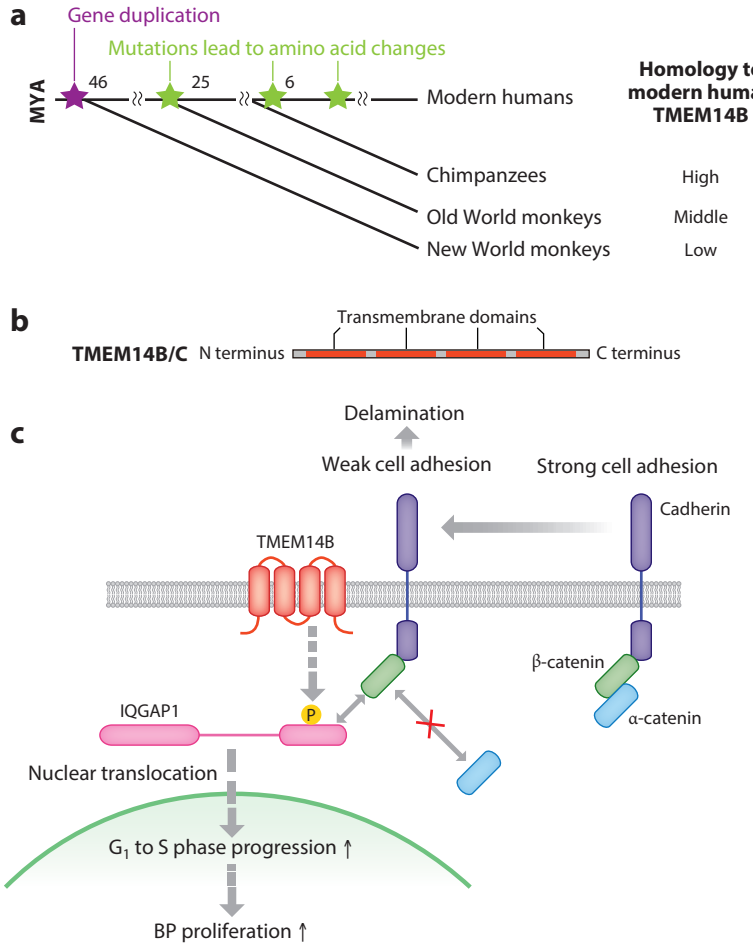

Figure 5

Evolution and molecular function of TMEM14B. (*a*) Evolution of TMEM14B. Gene duplication happened before the generation of the Simiiformes (e.g., apes, Old World monkeys, New World monkeys). Thereafter, several amino acid substitutions happened. As a result, the homology to the modern human TMEM14B is highest in apes, second highest in Old World monkeys, and lowest in New World monkeys. (*b*) Protein structure of TMEM14B and TMEM14C. (*c*) The role of TMEM14B in BP proliferation and AP delamination. TMEM14B induces the phosphorylation of IQGAP1, thereby attenuating cell adhesion, and induces G_1-to-S progression. Abbreviations: AP, apical progenitor; BP, basal progenitor; MYA, million years ago; P, phosphate.

attenuates its interaction with these small GTPases. The reduced interaction of IQGAP1 with Rac1 reciprocally strengthens the binding between IQGAP1 and β-catenin. The IQGAP1–β-catenin interaction results in weakened cadherin-mediated cell adhesion (Kuroda et al. 1998). Therefore, it is plausible that TMEM14B induces aRG delamination by attenuating cadherin-mediated cell adhesion through phosphorylation of IQGAP1.

Although IQGAP1 is localized mainly in the cytoplasm, it can be translocated into nuclei in the S phase of the cell cycle (Johnson et al. 2011). In the nuclei, IQGAP1 interacts with S-phase marker proteins, that is, proliferating cell nuclear antigen (PCNA) and replication protein A2 (RPA2), and thereby promotes G_1-to-S progression (Johnson et al. 2011). TMEM14B induces

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

nuclear translocation of IQGAP1 and thus enhances the effect of IQGAP1 in G_1-to-S progression, which leads to BP expansion (Liu et al. 2017).

DIFFERENCES IN NEOCORTEX DEVELOPMENT BETWEEN MODERN HUMANS AND NEANDERTHALS

The previous section discusses the expansion of the neocortex and the underlying increase in neuron generation from CSPCs as a basis of what makes us human. Genes that amplify CSPCs are a focus of that discussion. In the following subsections, we move beyond neocortex size to discuss CSPC-based differences in neocortex development between modern humans and Neanderthals that may contribute to modern human–specific features of the neocortex. We focus on two lines of research: (*a*) differences in the behavior of CSPCs between modern humans and Neanderthals and (*b*) differences in neocortex development between modern humans and Neanderthals due to amino acid substitutions in key proteins.

Apical Progenitor Mitosis and the Fidelity of Chromosome Segregation[1]

In this section, we discuss the fidelity of chromosome segregation for AP mitosis. We first review the data showing a longer metaphase in human than chimpanzee APs. We then address the issue of lagging chromosomes and the kinetochore proteins involved in the fidelity of chromosome segregation. Finally, we discuss the significance of the amino acid differences in these kinetochore proteins between modern humans and Neanderthals with regard to AP metaphase length and the proportion of lagging chromosomes upon AP mitosis.

Apical progenitor mitosis: metaphase length. A comparative analysis of AP mitosis by live imaging of slice cultures of human, chimpanzee, and orangutan cerebral organoids, each grown from induced pluripotent stem cells (iPSCs), revealed a longer metaphase of mitotic human APs than mitotic chimpanzee and orangutan APs (Mora-Bermúdez et al. 2016). The metaphase length of mitotic human APs in cerebral organoids is essentially the same as that observed in fetal human neocortical tissue ex vivo (Mora-Bermúdez et al. 2016), corroborating the validity of the cerebral organoid model. As all other phases of AP mitosis are the same for humans, chimpanzees, and orangutans, the selective lengthening of the metaphase in human APs points to a specific role in their mitosis.

Apical progenitor mitosis: spindle assembly checkpoint and lagging chromosomes. During metaphase, the correct (i.e., symmetric) alignment of the mitotic spindle with the chromosomes is checked. If this checking works fully, the chromosomes will be correctly (i.e., at equal number) segregated to the two daughter cells during anaphase. However, if this checking is incomplete, so-called lagging chromosomes may arise that can result in aneuploidy of the daughter cells (Holland & Cleveland 2012). The increase in the metaphase duration of mitotic human APs in comparison to mitotic chimpanzee and orangutan APs therefore raised the possibility that the checking of the mitotic spindle–chromosome alignment may be more rigorous in human APs, resulting in fewer lagging chromosomes. Intriguingly, this was found to be the case: The percentage of AP mitoses with lagging chromosomes in humans is half of that observed in chimpanzees (Mora-Bermúdez et al. 2022).

Kinetochore proteins KNL1 and KIF18a. The key structure involved in the contact between the mitotic spindle and the chromosomes is the kinetochore (Foley & Kapoor 2013, Musacchio & Desai 2017, Musacchio & Salmon 2007). Two kinetochore proteins, KIF18a (kinesin family

[1]The studies described in this section were a collaboration between the Huttner and Pääbo laboratories.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

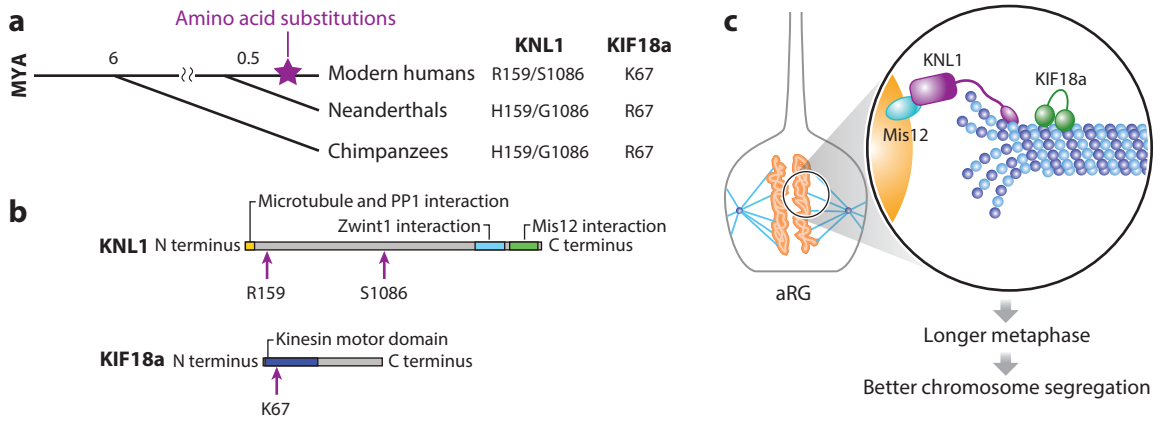

Figure 6

Evolution and molecular function of KNL1 and KIF18a. (*a*) Evolution of KNL1 and KIF18a. Amino acid substitutions happened after the split of the modern human lineage from the Neanderthal and Denisovan lineages. (*b*) Protein structure of KNL1 and KIF18a. Amino acid residues with modern human–specific substitution are indicated. (*c*) The role of KNL1 and KIF18a in metaphase in preparation for chromosome segregation. Abbreviations: aRG, apical radial glia; MYA, million years ago.

18a) and KNL1 (kinetochore null 1), also called CASC5 (cancer susceptibility candidate 5), are known to be highly expressed in the germinal zones of the developing neocortex (Fietz et al. 2012). Interestingly, there are only three amino acid substitutions in these two proteins in humans in comparison to chimpanzees (two in KNL1 and one in KIF18a) (Mora-Bermúdez et al. 2022) (**Figure 6**). As the three amino acids in question in chimpanzee KNL1 and KIF18a are the same as in the respective mouse proteins, they can be regarded as the ancestral ones. Strikingly, Neanderthal KNL1 and KIF18a contain the three ancestral amino acids at the corresponding positions (Mora-Bermúdez et al. 2022).

More lagging chromosomes in Neanderthal than modern human apical progenitor mitoses. The functional relevance of the three modern human–specific amino acid substitutions in KNL1 and KIF18a versus the three ancestral amino acids present in the two Neanderthal, chimpanzee, and mouse proteins was determined by two approaches. First, mouse Knl1 and Kif18a were humanized by changing the three amino acids in question to the modern human–specific variants, and metaphase length and lagging chromosomes were determined in mitotic APs in the embryonic mouse neocortex (Mora-Bermúdez et al. 2022). Second, modern human KNL1 and KIF18a were Neanderthalized (ancestralized) by changing the three modern human–specific amino acids to the ancestral variants, and metaphase length and lagging chromosomes were determined in mitotic APs in human cerebral organoids (Mora-Bermúdez et al. 2022). The first approach revealed that upon the humanization of mouse Knl1 and Kif18a, mitotic AP metaphase was lengthened and the percentage of AP mitoses with lagging chromosomes was reduced to half, that is, to a modern human value (Mora-Bermúdez et al. 2022). Conversely, the second approach revealed that, upon Neanderthalization of modern human KNL1 and KIF18a, mitotic AP metaphase was shortened and the percentage of AP mitoses with lagging chromosomes was increased approximately twofold, that is, to the level observed for chimpanzee and mouse APs (Mora-Bermúdez et al. 2022).

Taken together, these findings demonstrate that only three modern human–specific amino acid substitutions in two kinetochore proteins account for the longer metaphase and fewer chromosome segregation errors that characterize the mitoses of APs in the developing human neocortex

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

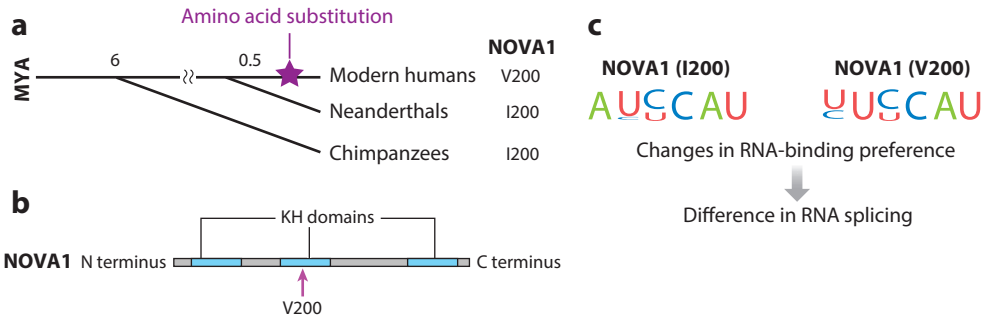

Figure 7

Evolution and molecular function of NOVA1. (*a*) Evolution of NOVA1. Amino acid substitution happened after the split of the modern human lineage from the Neanderthal and Denisovan lineages. (*b*) Protein structure of NOVA1. An amino acid residue with modern human–specific substitution is indicated. (*c*) Changes in RNA-binding preference between modern and archaic NOVA1. Abbreviations: MYA, million years ago; NOVA1, neuro-oncological ventral antigen 1.

versus those in chimpanzee. Moreover, these data imply that the mitoses of APs in the developing neocortex of Neanderthals were similar to those of chimpanzees and distinct in quality to those of modern humans. The consequences of these differences in neocortex development between modern humans and Neanderthals for the structure, functioning, and performance of the adult human neocortex promise to be an exciting topic of future research.

NOVA1

Approximately 100 proteins exhibit amino acid differences between modern humans and Neanderthals, usually showing only one such substitution (Prüfer et al. 2014). The splicing regulator neuro-oncological ventral antigen 1 (NOVA1) is one such protein (**Figure 7**). NOVA1 contains an isoleucine-to-valine substitution in modern humans in comparison to Neanderthals. The role of this single modern human–specific amino acid substitution for brain development was explored by Muotri and colleagues (Trujillo et al. 2021). These investigators used cortical organoids as a test system and reported that when such organoids express the archaic, isoleucine-containing NOVA1 variant, they exhibit slower development and changes in synaptic gene expression in comparison to organoids that express the modern human, valine-containing NOVA1 variant (Trujillo et al. 2021). However, Pääbo and colleagues (Maricic et al. 2021) commented that the iPSCs used to generate these cortical organoids exhibit heterozygous deletions of target DNA. Muotri and colleagues (Herai et al. 2021) responded to this comment by stating that upon analysis of *NOVA1* polymerase chain reaction products generated from the genomic DNA of the various genome-edited iPSC clones, only one clone exhibited a deletion. In conclusion, it will be important to exclude, in future research, the possibility that the organoid phenotypes observed upon *NOVA1* genome editing of the iPSCs used to generate the organoids (Trujillo et al. 2021) are due to deletions of target DNA and to demonstrate that these phenotypes are in fact due to the ancestralization of NOVA1.

TKTL1 and Basal Radial Glia[2]

In this section, we discuss the role of transketolase-like 1 (TKTL1) in human neocortex development. Modern human TKTL1 differs by only one amino acid substitution from Neanderthal

[2]The studies described in this section were a collaboration between the Huttner and Pääbo laboratories.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

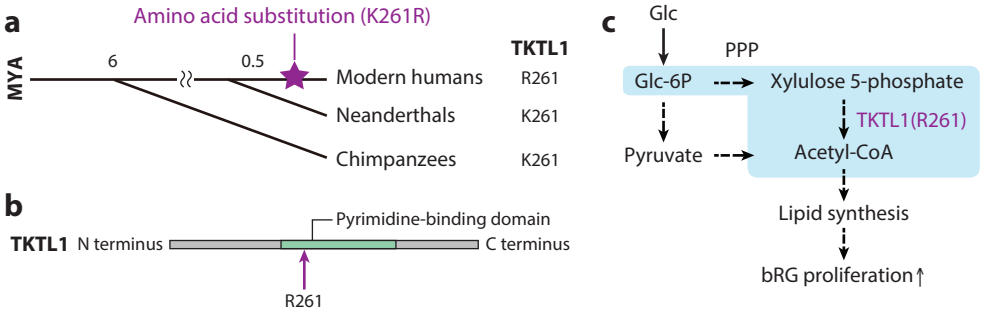

Figure 8

Evolution and molecular function of TKTL1. (*a*) Evolution of TKTL1. Amino acid substitution happened in the modern human lineage after its split from the Neanderthal and Denisovan lineage. (*b*) Protein structure of TKTL1. An amino acid residue with modern human–enriched substitution is indicated. (*c*) The role of modern TKTL1 in cell metabolism. Abbreviations: bRG, basal radial glia; MYA, million years ago; PPP, pentose phosphate pathway; TKTL1, transketolase-like 1.

TKTL1. We first review the data showing that modern human TKTL1, but not Neanderthal TKTL1, increases bRG and cortical neuron abundance, an effect essential for the maintenance of the physiological level of bRG and neuron generation in the fetal human neocortex. We then address the metabolic pathways involved in TKTL1 action. Finally, we discuss the implications for human neocortex development of the fact that TKTL1 expression in the fetal human neocortex is highest in the frontal lobe.

Modern human, but not Neanderthal, TKTL1 increases basal radial glia and upper-layer neuron abundance. TKTL1 is another of the approximately 100 proteins that exhibit amino acid differences between modern humans and Neanderthals (Pinson et al. 2022) (**Figure 8*a*,*b***). Specifically, the TKTL1 typically found in modern humans contains an arginine residue in the middle of the polypeptide chain, whereas the TKTL1 of Neanderthals contains a lysine residue at this position (Pinson et al. 2022). Although these two amino acids are related in that both carry basic side chains, this single-amino-acid substitution in TKTL1 is of great functional significance, as described in the following subsection. Relevant for this analysis of TKTL1 function is the finding that human TKTL1 is expressed in all three germinal zones of the fetal human neocortex but not in the cortical plate (Fietz et al. 2012).

Expression of either the arginine-containing modern human TKTL1 (hTKTL1) or the lysine-containing Neanderthal TKTL1 (aTKTL1, for ancestral, as the lysine residue is the ancestral one) in the embryonic mouse neocortex revealed that neither TKTL1 variant affects the abundance of APs in the VZ or of bIPs in the SVZ (Pinson et al. 2022). Remarkably, however, hTKTL1, but not aTKTL1, massively increases the abundance of bRG in the SVZ (Pinson et al. 2022). This specific increase in bRG abundance has important implications for cortical neurogenesis. As neurogenic divisions of bRG are asymmetric self-renewing—in contrast to neurogenic divisions of bIPs, which are symmetric consumptive—neurogenic bRG divisions generate more neurons over time than do neurogenic bIP divisions (Fietz et al. 2010). Accordingly, hTKTL1 expression in the embryonic mouse neocortex leads to an increase in upper-layer neuron generation (Pinson et al. 2022).

How relevant is this finding from the embryonic mouse neocortex, used as a test system, for the fetal human neocortex? CRISPR/Cas9-mediated gene disruption of *hTKTL1* in fetal human neocortical tissue ex vivo causes a significant reduction in bRG abundance (Pinson et al. 2022). Moreover, Neanderthalization of TKTL1 in human cerebral organoids by changing the relevant

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

arginine residue back to lysine using genome editing lowers bRG abundance to about half of the normal level and significantly decreases the level of neurons (Pinson et al. 2022). Taken together, these findings demonstrate that hTKTL1 makes an essential contribution to the physiological levels of bRG, and the neurons generated therefrom, in the developing human neocortex.

The relevance of cell processes for cortical stem and progenitor cell proliferative capacity. The observation that the hTKTL1-induced increase in BP abundance is specific for bRG (which retain radial processes through mitosis) and does not pertain to bIPs (which lack any cell processes at mitosis) leads us to the issue of the relevance of cell processes for the proliferative capacity of CSPCs. Indeed, an in-depth study of this topic (Kalebic et al. 2019) revealed that an increase in the proliferative capacity of BPs is linked to an increase in BP process number.

Furthermore, the morphoregulatory protein Palmdelphin has been identified as a driver of BP process growth and, consequently, of increasing their proliferative capacity (Kalebic et al. 2019). The concept emerging from this research is that the increase in BP cell processes allows for an increase in receiving proproliferative signals from the microenvironment; these signals are extracellular matrix based and are transduced into the BP via integrin receptors (Kalebic et al. 2019).

Metabolic action of hTKTL1. How does hTKTL1 achieve its effects on bRG abundance? The enzyme closely related to the hTKTL1 protein, transketolase (TKT), is known to act in the pentose phosphate pathway (PPP) (Schenk et al. 1998). Experiments with inhibitors of this metabolic pathway in *hTKTL1*-transfected embryonic mouse neocortical tissue ex vivo indicate that hTKTL1 acts in the PPP, as inhibition of the PPP blocks the ability of hTKTL1 to increase bRG abundance (Pinson et al. 2022) (**Figure 8*c***). Specifically, hTKTL1 has been proposed to promote the conversion of xylulose 5-phosphate, a metabolite in the PPP, into glyceraldehyde 3-phosphate and acetate, the latter of which is then activated to become acetyl-CoA (Diaz-Moralli et al. 2016). One role of acetyl-CoA, the level of which in bRG increases upon *hTKTL1* expression (Pinson et al. 2022), is to serve as a substrate in the first step of fatty acid synthesis. Indeed, experiments with inhibitors of fatty acid synthesis in *hTKTL1*-transfected embryonic mouse neocortical tissue ex vivo indicate that fatty acid synthesis is required to allow hTKTL1 to increase bRG abundance (Pinson et al. 2022). The precise reason for the need for fatty acid synthesis remains to be elucidated, but an attractive possibility is that it reflects the requirement to synthesize certain membrane lipids that are involved in the generation of the radial processes of bRG, which in turn promote bRG proliferation (Kalebic et al. 2019, Pinson et al. 2022).

TKTL1 expression in the fetal human neocortex and its implications for modern humans. *TKTL1* mRNA levels in the fetal human neocortex rise concomitantly with the increase in cortical neurogenesis (Pinson et al. 2022). Remarkably, *TKTL1* mRNA levels in the fetal human neocortex are highest in the developing frontal lobe (Pinson et al. 2022). Thus, taken together, the TKTL1 findings imply greater neurogenesis during fetal development in the frontal lobe of the neocortex in modern humans than in Neanderthals. It remains to be investigated whether—and, if so, to what extent—this greater neurogenesis during the fetal development of the frontal lobe has consequences for the adult human brain.

The functional relevance of the hTKTL1-mediated greater neurogenesis in the developing frontal lobe in modern humans compared with Neanderthals has been questioned by Muotri and colleagues (Herai et al. 2023). They emphasized the known facts that (*a*) a minority of present-day humans, who apparently lack any overt phenotypes, carry the ancestral *TKTL1* allele (Prüfer et al. 2014, 2017; Reardon 2022), and (*b*) because *TKTL1* is on the X chromosome (Coy et al. 2005), males carrying the ancestral *TKTL1* allele would only be able to express the lysine-containing TKTL1 variant. As stated above and published in response to the comment by Muotri and

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

colleagues (Pinson et al. 2023), it remains to be investigated whether (and, if so, to what extent) the hTKTL1-mediated greater neurogenesis in the developing frontal lobe in modern humans compared with Neanderthals has consequences for the adult human brain. In this context, in light of the complexity of human brain development, it should be realized that certain genetic effects during the development of the neocortex may be influenced, modified, or perhaps compensated for by the action of other genes (Pinson et al. 2023). Also, an in-depth analysis of specific aspects of brain performance in human individuals expressing only the lysine-containing TKTL1 variant, to the best of our knowledge, has not yet been done.

HUMAN-SPECIFIC GENE DUPLICATIONS THAT AFFECT NEURONAL CIRCUITRY

In the preceding sections, we have focused on human-specific genomic events that affect primarily CSPCs and the cortical neurons they generate. The increase in the number of neurons resulting from these genomic events and the accompanying expansion of neocortical areas constitute one line of contributions to what makes us human. Another line pertains to specific aspects of the neuronal circuitry that likely is unique to modern humans. A comprehensive discussion of the latter topic would exceed the scope of this review. However, we highlight one set of key findings that pertain to neurons and influence their ability to form neuronal networks—the seminal research of Franck Polleux and colleagues (Charrier et al. 2012, Fossati et al. 2016, Guerrier et al. 2009) on the human-specific gene *SRGAP2C*.

SRGAP2

In this section, we discuss SRGAP2. We first review the origin of the *SRGAP2A* paralogs *SRGAP2B*, *SRGAP2C*, and *SRGAP2D*. We then address the role of the SRGAP2B and SRGAP2C proteins in regulating neuronal maturation by inhibiting SRGAP2A. Finally, we discuss the mechanism of action of SRGAP2B and SRGAP2C.

Origin of *SRGAP2B*, *SRGAP2C*, and *SRGAP2D*. *SRGAP2B* arose by a partial gene duplication of *SRGAP2A* (Slit-Robo GTPase–activating protein 2A) approximately 3.4 MYA. *SRGAP2B* underwent further gene duplications around 2.4–1 MYA, resulting in *SRGAP2C* and *SRGAP2D* (Dennis et al. 2012) (**Figure 9**). Due to the lack of a large portion of the 5′ sequence, including the start codon, *SRGAP2D* is a pseudogene. Given the times when these gene duplications occurred, the implication is that all of them happened after the split of the lineages leading either to humans or to chimpanzees but before the split of the lineages leading either to modern humans or to Neanderthals and Denisovans (**Figure 9*a***). Therefore, *SRGAP2B/C/D*, often referred to as human-specific genes, are—like *ARHGAP11B*—hominin-specific genes.

SRGAP2B and SRGAP2C regulate neuronal maturation by inhibiting SRGAP2A. A key role of SRGAP2B and SRGAP2C is to inhibit SRGAP2A function during neuronal maturation (Charrier et al. 2012). After their generation by CSPCs, newly generated neurons initially exhibit a multipolar morphology and then become bipolar neurons with leading processes (future dendrites) versus a trailing process (future axon) in order to migrate toward the cortical plate (Namba et al. 2015, Vanderhaeghen & Polleux 2023). After settling in the cortical plate, neurons develop spines to form synaptic connections (Namba et al. 2015, Vanderhaeghen & Polleux 2023). SRGAP2A is known to be involved in all of the abovementioned events of neuronal maturation (Gonda et al. 2020, Guerrier et al. 2009, Martins et al. 2018). Overexpression of SRGAP2A by the in utero electroporation of the mouse embryonic neocortex increases the number of neurons with multipolar morphology in the intermediate zone, suggesting that SRGAP2A inhibits

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

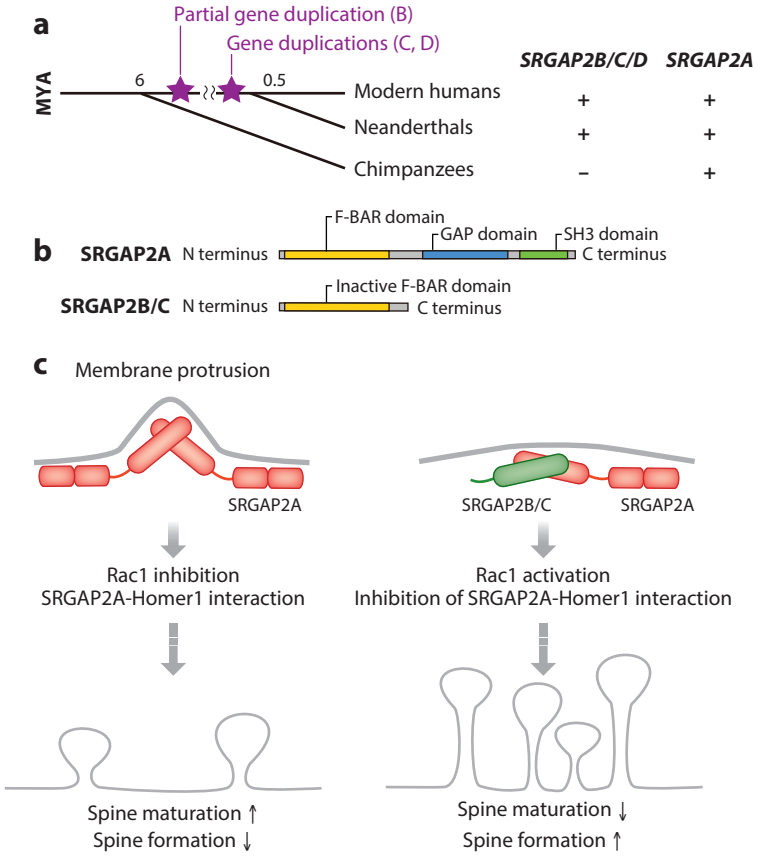

Figure 9

Evolution and molecular function of SRGAP2. (*a*) Evolution of *SRGAP2B*, *SRGAP2C*, and *SRGAP2D*. Partial gene duplication of *SRGAP2* (~3.4 MYA) generated *SRGAP2B*, and multiple gene duplications (~2.4–1 MYA) generated *SRGAP2C* and *SRGAP2D*. (*b*) Protein structure of SRGAP2A, SRGAP2B, and SRGAP2C. (*c*) The role of SRGAP2A and SRGAP2B/C in spine maturation. SRGAP2B/C inhibit the functions of SRGAP2A on spine maturation. Abbreviation: MYA, million years ago.

the multipolar to bipolar transition (Guerrier et al. 2009). After establishing bipolarity, neurons overexpressing SRGAP2A extend many branches on their leading processes; therefore, neuronal migration is inhibited (Guerrier et al. 2009). In contrast, the suppression of SRGAP2A reduces the number of processes and accelerates their migration. SRGAP2A also regulates spine development. Overexpression of SRGAP2A induces spine maturation, and in contrast, suppression of SRGAP2A delays spine maturation and immature spine formation.

Since the expression of SRGAP2B or SRGAP2C results in delayed spine maturation and an increased density of immature spines, which are similar to the phenotype upon SRGAP2A suppression, the function of SRGAP2B or SRGAP2C is thought to be an inhibition of SRGAP2A (Charrier et al. 2012). Notably, the effects are significantly stronger with SRGAP2C than with SRGAP2B, suggesting that SRGAP2C might dominantly influence the evolution of human neuronal network formation (Schmidt et al. 2019).

Interestingly, delayed neuronal maturation is a characteristic feature in humans compared with other great apes such as chimpanzees (Marchetto et al. 2019, Schörnig et al. 2021, Somel et al.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

2009). Since human iPSC-derived neurons exhibit delayed synaptic maturation compared with chimpanzee iPSC-derived neurons under 2D culture conditions (Marchetto et al. 2019, Schörnig et al. 2021), this neoteny is intrinsically encoded in the human genome. The human-specific genes *SRGAP2B* and *SRGAP2C* may provide a molecular basis of such neoteny. Further studies are needed to provide a holistic mechanistic insight into human-specific features, such as neoteny. In this context, recent studies using cerebral organoids from human and ape iPSCs with comprehensive multiomic approaches will likely provide cues for further understanding human neuron neoteny (Kanton et al. 2019, Wang et al. 2023).

Mechanism of action of SRGAP2B and SRGAP2C. SRGAP2A is an effector of the Robo receptor and has two main functions in this regard (Guerrier et al. 2009). One function is plasma membrane bending through its F-BAR domain. Notably, although the SRGAP2A's BAR domain is highly homologous to the F-BAR domain, its function resembles the I-BAR domain, in that it bends plasma membrane into filopodia (Guerrier et al. 2009). The other function is to inhibit Rac1 through its Rho GAP domain (Guerrier et al. 2009). Upon the activation of the Robo receptor by its ligand Slit, the Rho GAP is activated and thereby inhibits Rac1. The phenotype in neuronal polarity observed upon SRGAP2A overexpression might be partially explained by its Rho GAP activity toward Rac1 because Rac1 plays a critical role in neuronal polarization (i.e., the multipolar-to-bipolar transition) (Namba et al. 2014, Tahirovic et al. 2010).

Since the F-BAR domain of SRGAP2A interacts with Homer1, a postsynaptic scaffold protein for excitatory synapses, SRGAP2A induces spine maturation at the postsynaptic domain (Fossati et al. 2016). In addition, SRGAP2A inhibits spine formation through the inactivation of Rac1, which finally limits spine density. SRGAP2B and SRGAP2C bind to SRGAP2A and inhibit all of the abovementioned functions of SRGAP2A, hence delaying spine maturation while inducing spine formation (Charrier et al. 2012, Fossati et al. 2016, Schmidt et al. 2019).

PERSPECTIVES

As described above, one approach toward elucidating what makes us human has been, and continues to be, the study of proteins exhibiting a very small number (usually only one) of amino acid substitutions between modern humans and Neanderthals and the analysis of the functional implications of these substitutions. An additional approach could be to compare the genomes of present-day humans and modern humans who lived several tens of thousands of years ago. There is evidence that the shape of the brain of Neanderthals was elongated, whereas that of present-day humans is more globular. Interestingly, the shape of early modern humans was elongated rather than globular (Ganapathee & Gunz 2023). These observations suggest that during the evolution from early modern humans to present-day humans, there were mutations in the human genome—which might have occurred only a few tens of thousands of years ago—that had an impact on the shape of the brain and, perhaps, on brain performance such as cognitive abilities. As the genomic differences between early modern humans and present-day humans are likely to be fewer than the number of nucleotide differences between present-day humans and Neanderthals (Kuhlwilm & Boeckx 2019; Prüfer et al. 2014, 2017), comparative functional analyses of the genomes of early modern humans and present-day humans promise to provide further insight into what makes us human.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

LITERATURE CITED

Alcantara D, O'Driscoll M. 2014. Congenital microcephaly. *Am. J. Med. Genet. C* 166(2):124–39

Andrews MG, Subramanian L, Salma J, Kriegstein AR. 2022. How mechanisms of stem cell polarity shape the human cerebral cortex. *Nat. Rev. Neurosci.* 23:711–24

Antonacci F, Dennis MY, Huddleston J, Sudmant PH, Steinberg KM, et al. 2014. Palindromic *GOLGA8* core duplicons promote chromosome 15q13.3 microdeletion and evolutionary instability. *Nat. Genet.* 46:1293–302

Azevedo FAC, Carvalho LRB, Grinberg LT, Farfel JM, Ferretti REL, et al. 2009. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J. Comp. Neurol.* 513:532–41

Benito-Kwiecinski S, Giandomenico SL, Sutcliffe M, Riis ES, Freire-Pritchett P, et al. 2021. An early cell shape transition drives evolutionary expansion of the human forebrain. *Cell* 184:2084–102.e19

Bilgic M, Wu Q, Suetsugu T, Shitamukai A, Tsunekawa Y, et al. 2023. Truncated radial glia as a common precursor in the late corticogenesis of gyrencephalic mammals. *eLife* 12:RP91406

Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, et al. 2012. Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. *Cell* 149:923–35

Coy JF, Dressler D, Wilde J, Schubert P. 2005. Mutations in the transketolase-like gene TKTL1: clinical implications for neurodegenerative diseases, diabetes and cancer. *Clin. Lab.* 51:257–73

Dehay C, Kennedy H, Kosik KS. 2015. The outer subventricular zone and primate-specific cortical complexification. *Neuron* 85:683–94

del Alamo D, Rouault H, Schweisguth F. 2011. Mechanism and significance of *cis*-inhibition in Notch signalling. *Curr. Biol.* 21:R40–47

Dennis MY, Eichler EE. 2016. Human adaptation and evolution by segmental duplication. *Curr. Opin. Genet. Dev.* 41:44–52

Dennis MY, Nuttle X, Sudmant PH, Antonacci F, Graves TA, et al. 2012. Evolution of human-specific neural *SRGAP2* genes by incomplete segmental duplication. *Cell* 149:912–22

DeSilva JM, Traniello JFA, Claxton AG, Fannin LD. 2021. When and why did human brains decrease in size? a new change-point analysis and insights from brain evolution in ants. *Front. Ecol. Evol.* 9:742639

Diaz-Moralli S, Aguilar E, Marin S, Coy JF, Dewerchin M, et al. 2016. A key role for transketolase-like 1 in tumor metabolic reprogramming. *Oncotarget* 7:51875–97

Fiddes IT, Lodewijk GA, Mooring M, Bosworth CM, Ewing AD, et al. 2018. Human-specific *NOTCH2NL* genes affect notch signaling and cortical neurogenesis. *Cell* 173:1356–69.e22

Fiddes IT, Pollen AA, Davis JM, Sikela JM. 2019. Paired involvement of human-specific Olduvai domains and *NOTCH2NL* genes in human brain evolution. *Hum. Genet.* 138:715–21

Fietz SA, Kelava I, Vogt J, Wilsch-Bräuninger M, Stenzel D, et al. 2010. OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nat. Neurosci.* 13:690–99

Fietz SA, Lachmann R, Brandl H, Kircher M, Samusik N, et al. 2012. Transcriptomes of germinal zones of human and mouse fetal neocortex suggest a role of extracellular matrix in progenitor self-renewal. *PNAS* 109:11836–41

Fischer J, Fernández Ortuño E, Marsoner F, Artioli A, Peters J, et al. 2022. Human-specific *ARHGAP11B* ensures human-like basal progenitor levels in hominid cerebral organoids. *EMBO Rep.* 23:e54728

Florio M, Albert M, Taverna E, Namba T, Brandl H, et al. 2015. Human-specific gene *ARHGAP11B* promotes basal progenitor amplification and neocortex expansion. *Science* 347:1465–70

Florio M, Heide M, Pinson A, Brandl H, Albert M, et al. 2018. Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex. *eLife* 7:e32332

Florio M, Namba T, Pääbo S, Hiller M, Huttner WB. 2016. A single splice site mutation in human-specific *ARHGAP11B* causes basal progenitor amplification. *Sci. Adv.* 2:e1601941

Foley EA, Kapoor TM. 2013. Microtubule attachment and spindle assembly checkpoint signalling at the kinetochore. *Nat. Rev. Mol. Cell Biol.* 14:25–37

Fossati M, Pizzarelli R, Schmidt ER, Kupferman JV, Stroebel D, et al. 2016. *SRGAP2* and its human-specific paralog co-regulate the development of excitatory and inhibitory synapses. *Neuron* 91:356–69

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

Galakhova AA, Hunt S, Wilbers R, Heyer DB, de Kock CPJ, et al. 2022. Evolution of cortical neurons supporting human cognition. *Trends Cogn. Sci.* 26:909–22

Ganapathee DS, Gunz P. 2023. Insights into brain evolution through the genotype-phenotype connection. *Prog. Brain Res.* 275:73–92

Gilmore EC, Walsh CA. 2013. Genetic causes of microcephaly and lessons for neuronal development. *Wiley Interdiscip. Rev. Dev. Biol.* 2:461–78

Gkini V, Namba T. 2023. Glutaminolysis and the control of neural progenitors in neocortical development and evolution. *Neuroscientist* 29:177–89

Gonda Y, Namba T, Hanashima C. 2020. Beyond axon guidance: roles of Slit-Robo signaling in neocortical formation. *Front. Cell Dev. Biol.* 8:607415

Grohmanova K, Schlaepfer D, Hess D, Gutierrez P, Beck M, Kroschewski R. 2004. Phosphorylation of IQGAP1 modulates its binding to Cdc42, revealing a new type of Rho-GTPase regulator. *J. Biol. Chem.* 279:48495–504

Guerrier S, Coutinho-Budd J, Sassa T, Gresset A, Jordan NV, et al. 2009. The F-BAR domain of srGAP2 induces membrane protrusions required for neuronal migration and morphogenesis. *Cell* 138:990–1004

Heide M, Haffner C, Murayama A, Kurotaki Y, Shinohara H, et al. 2020. Human-specific *ARHGAP11B* increases size and folding of primate neocortex in the fetal marmoset. *Science* 369:546–50

Herai R, Semendeferi K, Muotri AR. 2023. Comment on "Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals." *Science* 379:eadf0602

Herai RH, Szeto RA, Trujillo CA, Muotri AR. 2021. Response to comment on "Reintroduction of the archaic variant of *NOVA1* in cortical organoids alters neurodevelopment." *Science* 374:eabi9881

Herculano-Houzel S. 2009. The human brain in numbers: a linearly scaled-up primate brain. *Front. Hum. Neurosci.* 3:31

Herculano-Houzel S. 2012. The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. *PNAS* 109(Suppl. 1):10661–68

Hodzic D, Kong C, Wainszelbaum MJ, Charron AJ, Su X, Stahl PD. 2006. TBC1D3, a hominoid oncoprotein, is encoded by a cluster of paralogues located on chromosome 17q12. *Genomics* 88:731–36

Holland AJ, Cleveland DW. 2012. Losing balance: the origin and impact of aneuploidy in cancer. *EMBO Rep.* 13:501–14

Hou QQ, Xiao Q, Sun XY, Ju XC, Luo ZG. 2021. TBC1D3 promotes neural progenitor proliferation by suppressing the histone methyltransferase G9a. *Sci. Adv.* 7:eaba8053

Huttner WB, ed. 2023. *Neocortical Neurogenesis in Development and Evolution*. Hoboken, NJ: Wiley

Jayaraman D, Bae BI, Walsh CA. 2018. The genetics of primary microcephaly. *Annu. Rev. Genom. Hum. Genet.* 19:177–200

Jiang Z, Tang H, Ventura M, Cardone MF, Marques-Bonet T, et al. 2007. Ancestral reconstruction of segmental duplications reveals punctuated cores of human genome evolution. *Nat. Genet.* 39:1361–68

Johnson M, Sharma M, Brocardo MG, Henderson BR. 2011. IQGAP1 translocates to the nucleus in early S-phase and contributes to cell cycle progression after DNA replication arrest. *Int. J. Biochem. Cell Biol.* 43:65–73

Ju XC, Hou QQ, Sheng AL, Wu KY, Zhou Y, et al. 2016. The hominoid-specific gene *TBC1D3* promotes generation of basal neural progenitors and induces cortical folding in mice. *eLife* 5:e18197

Kagawa Y, Matsumoto S, Kamioka Y, Mimori K, Naito Y, et al. 2013. Cell cycle–dependent Rho GTPase activity dynamically regulates cancer cell motility and invasion *in vivo*. *PLOS ONE* 8:e83629

Kaindl AM, Passemard S, Kumar P, Kraemer N, Issa L, et al. 2010. Many roads lead to primary autosomal recessive microcephaly. *Prog. Neurobiol.* 90:363–83

Kalebic N, Gilardi C, Albert M, Namba T, Long KR, et al. 2018. Human-specific *ARHGAP11B* induces hallmarks of neocortical expansion in developing ferret neocortex. *eLife* 7:e41241

Kalebic N, Gilardi C, Stepien B, Wilsch-Bräuninger M, Long KR, et al. 2019. Neocortical expansion due to increased proliferation of basal progenitors is linked to changes in their morphology. *Cell Stem Cell* 24:535–50.e9

Kalebic N, Namba T. 2021. Inheritance and flexibility of cell polarity: a clue for understanding human brain development and evolution. *Development* 148:dev199417

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

Kanton S, Boyle MJ, He ZS, Santel M, Weigert A, et al. 2019. Organoid single-cell genomic atlas uncovers human-specific features of brain development. *Nature* 574:418–22
Karakostis FA, Haeufle D, Anastopoulou I, Moraitis K, Hotz G, et al. 2021. Biomechanics of the human thumb and the evolution of dexterity. *Curr. Biol.* 31:1317–25.e8
Kelava I, Reillo I, Murayama AY, Kalinka AT, Stenzel D, et al. 2012. Abundant occurrence of basal radial glia in the subventricular zone of embryonic neocortex of a lissencephalic primate, the common marmoset *Callithrix jacchus*. *Cereb. Cortex* 22:469–81
Kriegstein AR, Götz M. 2003. Radial glia diversity: a matter of cell fate. *Glia* 43:37–43
Kuhlwilm M, Boeckx C. 2019. A catalog of single nucleotide changes distinguishing modern humans from archaic hominins. *Sci. Rep.* 9:8463
Kurella VB, Richard JM, Parke CL, Lecour LF Jr., Bellamy HD, Worthylake DK. 2009. Crystal structure of the GTPase-activating protein–related domain from IQGAP1. *J. Biol. Chem.* 284:14857–65
Kuroda S, Fukata M, Nakagawa M, Fujii K, Nakamura T, et al. 1998. Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell-cell adhesion. *Science* 281:832–35
Liu J, Liu W, Yang L, Wu Q, Zhang H, et al. 2017. The primate-specific gene *TMEM14B* marks outer radial glia cells and promotes cortical expansion and folding. *Cell Stem Cell* 21:635–49.e8
Marchetto MC, Hrvoj-Mihic B, Kerman BE, Yu DX, Vadodaria KC, et al. 2019. Species-specific maturation profiles of human, chimpanzee and bonobo neural cells. *eLife* 8:e37527
Maricic T, Helmbrecht N, Riesenberg S, Macak D, Kanis P, et al. 2021. Comment on "Reintroduction of the archaic variant of *NOVA1* in cortical organoids alters neurodevelopment." *Science* 374:eabi6060
Martins PT, Marí M, Boeckx C. 2018. *SRGAP2* and the gradual evolution of the modern human language faculty. *J. Lang. Evol.* 3:67–78
Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, et al. 2008. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N. Engl. J. Med.* 359:1685–99
Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, et al. 2012. A high-coverage genome sequence from an archaic Denisovan individual. *Science* 338:222–26
Mora-Bermúdez F, Badsha F, Kanton S, Camp JG, Vernot B, et al. 2016. Differences and similarities between human and chimpanzee neural progenitors during cerebral cortex development. *eLife* 5:e18683
Mora-Bermúdez F, Kanis P, Macak D, Peters J, Naumann R, et al. 2022. Longer metaphase and fewer chromosome segregation errors in modern human than Neanderthal brain development. *Sci. Adv.* 8:eabn7702
Musacchio A, Desai A. 2017. A molecular view of kinetochore assembly and function. *Biology* 6:5
Musacchio A, Salmon ED. 2007. The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* 8:379–93
Namba T, Doczi J, Pinson A, Xing L, Kalebic N, et al. 2020. Human-specific ARHGAP11B acts in mitochondria to expand neocortical progenitors by glutaminolysis. *Neuron* 105:867–81
Namba T, Funahashi Y, Nakamuta S, Xu C, Takano T, Kaibuchi K. 2015. Extracellular and intracellular signaling for neuronal polarity. *Physiol. Rev.* 95:995–1024
Namba T, Huttner WB. 2017. Neural progenitor cells and their role in the development and evolutionary expansion of the neocortex. *Wiley Interdiscip. Rev. Dev. Biol.* 6:e256
Namba T, Kibe Y, Funahashi Y, Nakamuta S, Takano T, et al. 2014. Pioneering axons regulate neuronal polarization in the developing cerebral cortex. *Neuron* 81:814–29
Namba T, Nardelli J, Gressens P, Huttner WB. 2021. Metabolic regulation of neocortical expansion in development and evolution. *Neuron* 109:408–19
Neubauer S, Hublin JJ, Gunz P. 2018. The evolution of modern human brain shape. *Sci. Adv.* 4:eaao5961
Noritake J, Watanabe T, Sato K, Wang S, Kaibuchi K. 2005. IQGAP1: a key regulator of adhesion and migration. *J. Cell Sci.* 118:2085–92
Nowakowski TJ, Pollen AA, Sandoval-Espinosa C, Kriegstein AR. 2016. Transformation of the radial glia scaffold demarcates two stages of human cerebral cortex development. *Neuron* 91:1219–27
Phan TP, Holland AJ. 2021. Time is of the essence: the molecular mechanisms of primary microcephaly. *Genes Dev.* 35:1551–78
Pilaz LJ, Liu J, Joshi K, Tsunekawa Y, Musso CM, et al. 2023. Subcellular mRNA localization and local translation of *Arhgap11a* in radial glial progenitors regulates cortical development. *Neuron* 111:839–56.e5

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

Pinson A, Maricic T, Zeberg H, Pääbo S, Huttner WB. 2023. Response to comment on "Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals." *Science* 379:eadf2212

Pinson A, Xing L, Namba T, Kalebic N, Peters J, et al. 2022. Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals. *Science* 377:eabl6422

Prüfer K, de Filippo C, Grote S, Mafessoni F, Korlevic P, et al. 2017. A high-coverage Neandertal genome from Vindija Cave in Croatia. *Science* 358:655–58

Prüfer K, Racimo F, Patterson N, Jay F, Sankararaman S, et al. 2014. The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* 505:43–49

Rakic P. 1988. Specification of cerebral cortical areas. *Science* 241:170–76

Rakic P. 1999. Introduction to the session. *Ann. N. Y. Acad. Sci.* 882:66–67

Rakic P. 2000. Radial unit hypothesis of neocortical expansion. *Novartis Found. Symp.* 228:30–42

Rakic P. 2009. Evolution of the neocortex: a perspective from developmental biology. *Nat. Rev. Neurosci.* 10:724–35

Reardon S. 2022. Did this gene give modern human brains their edge? *Nature* 609:665–66

Rosenfeld JA, Traylor RN, Schaefer GB, McPherson EW, Ballif BC, et al. 2012. Proximal microdeletions and microduplications of 1q21.1 contribute to variable abnormal phenotypes. *Eur. J. Hum. Genet.* 20:754–61

Ruff CB, Trinkaus E, Holliday TW. 1997. Body mass and encephalization in Pleistocene *Homo*. *Nature* 387:173–76

Schenk G, Duggleby RG, Nixon PF. 1998. Properties and functions of the thiamin diphosphate dependent enzyme transketolase. *Int. J. Biochem. Cell Biol.* 30:1297–318

Schmidt ERE, Kupferman JV, Stackmann M, Polleux F. 2019. The human-specific paralogs SRGAP2B and SRGAP2C differentially modulate SRGAP2A-dependent synaptic development. *Sci. Rep.* 9:18692

Schörnig M, Ju X, Fast L, Ebert S, Weigert A, et al. 2021. Comparison of induced neurons reveals slower structural and functional maturation in humans than in apes. *eLife* 10:e59323

Shashidharan P, Plaitakis A. 2014. The discovery of human of *GLUD2* glutamate dehydrogenase and its implications for cell function in health and disease. *Neurochem. Res.* 39:460–70

Smart IH, Dehay C, Giroud P, Berland M, Kennedy H. 2002. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* 12:37–53

Somel M, Franz H, Yan Z, Lorenc A, Guo S, et al. 2009. Transcriptional neoteny in the human brain. *PNAS* 106:5743–48

Spanaki C, Zaganas I, Kleopa KA, Plaitakis A. 2010. Human *GLUD2* glutamate dehydrogenase is expressed in neural and testicular supporting cells. *J. Biol. Chem.* 285:16748–56

Stephan H, Frahm H, Baron G. 1981. New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol.* 35:1–29

Sudmant PH, Kitzman JO, Antonacci F, Alkan C, Malig M, et al. 2010. Diversity of human copy number variation and multicopy genes. *Science* 330:641–46

Suzuki IK, Gacquer D, Van Heurck R, Kumar D, Wojno M, et al. 2018. Human-specific *NOTCH2NL* genes expand cortical neurogenesis through Delta/Notch regulation. *Cell* 173:1370–84

Tachibana M, Ueda J, Fukuda M, Takeda N, Ohta T, et al. 2005. Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9. *Genes Dev.* 19:815–26

Tahirovic S, Hellal F, Neukirchen D, Hindges R, Garvalov BK, et al. 2010. Rac1 regulates neuronal polarization through the WAVE complex. *J. Neurosci.* 30:6930–43

Taverna E, Götz M, Huttner WB. 2014. The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. *Annu. Rev. Cell Dev. Biol.* 30:465–502

Trujillo CA, Rice ES, Schaefer NK, Chaim IA, Wheeler EC, et al. 2021. Reintroduction of the archaic variant of *NOVA1* in cortical organoids alters neurodevelopment. *Science* 371:eaax2537

Vaid S, Camp JG, Hersemann L, Oegema CE, Heninger AK, et al. 2018. A novel population of Hopx-dependent basal radial glial cells in the developing mouse neocortex. *Development* 145:dev169276

Vanderhaeghen P, Polleux F. 2023. Developmental mechanisms underlying the evolution of human cortical circuits. *Nat. Rev. Neurosci.* 24:213–32

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45

Wang L, Pang K, Zhou L, Cebrian-Silla A, Gonzalez-Granero S, et al. 2023. A cross-species proteomic map reveals neoteny of human synapse development. *Nature* 622:112–19

Wang X, Tsai JW, LaMonica B, Kriegstein AR. 2011. A new subtype of progenitor cell in the mouse embryonic neocortex. *Nat. Neurosci.* 14:555–61

Watanabe T, Wang S, Kaibuchi K. 2015. IQGAPs as key regulators of actin-cytoskeleton dynamics. *Cell Struct. Funct.* 40:69–77

Wilsch-Bräuninger M, Peters J, Paridaen JTML, Huttner WB. 2012. Basolateral rather than apical primary cilia on neuroepithelial cells committed to delamination. *Development* 139:95–105

Xing L, Gkini V, Nieminen AI, Zhou H-C, Aquilino M, et al. 2024. Functional synergy of a human-specific and an ape-specific metabolic regulator in human neocortex development. *Nat. Commun.* 15:3468

Xing L, Kubik-Zahorodna A, Namba T, Pinson A, Florio M, et al. 2021. Expression of human-specific *ARHGAP11B* in mice leads to neocortex expansion and increased memory flexibility. *EMBO J.* 40:e107093

Xu J, Zhou X, Wang J, Li Z, Kong X, et al. 2013. RhoGAPs attenuate cell proliferation by direct interaction with p53 tetramerization domain. *Cell Rep.* 3:1526–38

Yang L, Venneti S, Nagrath D. 2017. Glutaminolysis: a hallmark of cancer metabolism. *Annu. Rev. Biomed. Eng.* 19:163–94

Zanin E, Desai A, Poser I, Toyoda Y, Andree C, et al. 2013. A conserved RhoGAP limits M phase contractility and coordinates with microtubule asters to confine RhoA during cytokinesis. *Dev. Cell* 26:496–510

Zaqout S, Kaindl AM. 2022. Autosomal recessive primary microcephaly: not just a small brain. *Front. Cell Dev. Biol.* 9:784700

Downloaded from www.annualreviews.org. Helsinki University (ar-000472) IP: 128.214.129.87 On: Tue, 15 Oct 2024 09:09:45