that ectopic egr2 expression in r4 was strongly decreased by a retinoic acid receptor (RAR) inhibitor, suggesting that high retinoid levels may be required for egr2 maintenance. Lastly, Addison et al. (2018) showed that cyp26 genes and hoxb1 may contribute to RA-dependent cell identity switching, because cyp26b1 plus cyp26c1 or hoxb1 knockdowns in the egr2b::H2B-citrine transgenic line both result in maintenance of ectopic expression of endogenous egr2b in isolated citrine-expressing cells intermingled in r4.

In summary, Addison et al. (2018) provide strong evidence that single-cell identity switching of neuroepithelial cells could be triggered by a discontinuity in retinoid signaling between cells and their neighbors. During normal hindbrain development, when low cyp26b1/c1-expressing, high RA, single r3/r5 cells intermingle with groups of high cyp26b1/c1-expressing, low RA, r4 cells (Figure 1B), community feedback regulation between segment identity and retinoid signaling leads to lowering RA levels in ectopic isolated r3/r5 cells (Figure 1D), in turn allowing hoxb1 induction and egr2 down-regulation (Figure 1E), and thus the maintenance of homogeneous segmental identities by cell identity switching.

Community effects may be generally required during development to create homogeneity in a cell population; for example, to maintain homogeneous regional identity within streams of migratory cells, or when one tissue arises from different lineages at different times, or to correct the identity of randomly errant single cells during early morphogenesis. Indeed, Addison et al. (2018) discovered a truly remarkable mechanism that allows cells to blend into a new community by taking on a new identity. In the context of recent progress in the analysis of cell fate specification at the single-cell resolution, it will be interesting, though challenging, to extend this work and further explore the genome-wide transcriptional and epigenetic dynamic changes occurring during cell identity switching in isolated cells.

REFERENCES


Figure 1. NOTCH2NL Paralogs Differentiate Cortical Expansion in Human Compared to its Closest Relatives

Four paralogs of the NOTCH2 gene (NOTCH2NLs) are found in human, compared to chimpanzee, which only has NOTCH2NL-like pseudogenes. Three of the human-specific paralogs form paratypes that further contribute to functional variation. NOTCH2 regulates cortical development by promoting progenitor self-renewal and inhibiting neuronal differentiation. NOTCH2NL paralogs contribute to the expansion of the human cortex by further enhancing NOTCH2 action through direct binding to NOTCH2 and/or inhibition of DLL1, which promotes neuronal differentiation.

structure. The identification of such characteristic genetic features is challenging because it is often the result of small sequence substitutions or structural rearrangements that can be difficult to resolve. However, in the last decade, next-generation sequencing (NGS) has allowed unprecedented resolution and revealed new levels of complexity and new mechanisms of genome regulation and variation.

Comparative genomic studies have led to the discovery of human-specific genomic sequences, such as novel genes, gene paralogs, human accelerated regions (HARs), and hominin-specific duplicated genes (HSS), that all are likely to contribute to differentiating the human brain from that of other species (Boyd et al., 2015; Doan et al., 2016; Reilly et al., 2015; Dennis et al., 2017). Of these sequences, some suggest straightforward mechanisms, because they code for proteins that can be functionally related to processes of cortical development (Florio et al., 2015; Ju et al., 2016).

Somewhat more surprising is the extent to which pathological mutations in some of these same genes have been associated with developmental and psychiatric disorders. Now, in a recent issue of Cell, Fiddes et al. (2018) and Suzuki et al. (2018) unravel how the Notch pathway, a signaling pathway that is highly conserved from Drosophila to humans, has nonetheless been evolving to create human-specific paralogs of the NOTCH2 gene that regulate neuronal progenitor proliferation and neuronal differentiation. Furthermore, these two studies reveal how these evolutionarily duplicated new genes contribute to 1q21.1 distal deletion/duplication syndrome, associated with macrocephaly (large brain), microcephaly (small brain), autism, and schizophrenia.

Applying two different approaches, Fiddes et al. (2018) and Suzuki et al. (2018) independently found the same paralogs of the NOTCH2 gene, NOTCH2NL, NOTCH2NLB, NOTCH2NL, and NOTCH2NL, which formed as a result of segmental duplications of the ancestral gene. Of these four paralogs, three are specific to the hominid lineage and one is also found in chimpanzee. Interestingly, this suggests a very recent emergence of these paralogs during the hominid evolution. By comparative analysis in primates and archaic humans, Fiddes et al. managed to further reconstruct the evolutionary history of the NOTCH2 locus made of gene duplications and conversions, estimating the emergence of the NOTCH2NL paralogs between 4 and 3 million years ago, after the separation of hominids from the chimpanzee and during the early stages of the expansion of the human cortex. Furthermore, they described a remarkable new mechanism of inter-individual genetic diversity by identifying eight NOTCH2NL alleles of the three human-specific NOTCH2NL genes in 15 genomes analyzed, which they called paratypes. Paratypes are the product of ongoing ectopic gene conversions that produce distinct protein or protein abundance variants.

The human brain is distinguishable for the increased thickness and unique pattern of gyriification of the cerebral cortex, and a general consensus suggests that differential modulation of proliferative capacities, and the appearance of new types of neural progenitors, may underlie these changes (Borrell and Reillo, 2012). However, how exactly these changes in stem cell properties are regulated, together with many other aspects concerning neuronal migration and positioning in the developing cortex, remains to be elucidated. The Notch signaling pathway has been studied in the context of cortical development and is known to regulate progenitor proliferation and neuronal differentiation (Kageyama et al., 2009). Fiddes et al. and Suzuki et al. now link the appearance of human-specific NOTCH2 paralogs in the course of evolution to the increase in progenitor proliferation and delayed neurogenesis in the human brain (Figure 1).

Thus, these two studies describe genetic variations that confer upon this pathway a differential role in human corticogenesis. Fiddes et al. show that in both mouse and human cortical organoids, NOTCH2NL expression downregulates neuronal differentiation genes and delays the differentiation of neuronal progenitors, which in turn leads to an overall final increase in neuronal production. Similarly, Suzuki et al. show that the presence of NOTCH2NL increases neural progenitor self-renewal through symmetric proliferative divisions in human embryonic stem cells. Furthermore, both studies explore the structural characteristics and the molecular mechanisms that underlie the function of the new NOTCH2 isoforms.
Fiddes et al. show that NOTCH2NL can physically interact with NOTCH2 and enhance its activity in a non-cell-autonomous manner. Suzuki et al. suggest the presence of a cell-autonomous mechanism through which NOTCH2NL increases progenitor self-renewal by blocking the membrane expression of DLL1 Notch receptors, which promote neuronal differentiation. Both mechanisms likely work in parallel to reach the same effect. These functional explanations are consistent with the finding by Fiddes et al. that duplications of NOTCH2NL are present in patients affected by macrocephaly, whereas microcephaly is seen in patients with NOTCH2NL deletions.

Different domains of the NOTCH2NL protein have different abilities to increase Notch signaling, and small amino acid changes across paratypes appear to cause differential enhancement. These studies interestingly show how a highly conserved, well-studied signaling pathway can experience evolutionary changes that result in functional and cellular mechanisms that drive cortical expansion. This suggests that evolutionary forces can represent subtle changes to fundamental developmental pathways to shape the course of organ formation across species. Even if there is increasing evidence that mutations in human-specific genomic regions may be linked to neurodevelopmental psychiatric disorders, these studies provide a clear example of identification of the causes of a disease whose bases were previously unclear. These provocative studies raise many exciting questions. For example, it is not clear how many developmental genes show evidence of such very recent or even ongoing evolution. Similarly, there may be many pathways with paratypes that could only be discerned with similarly intensive analysis, and the frequency of these occurrences is not clear. Additionally, how often do such duplications/deletions contribute to disease? We clearly are only at the beginning of understanding the many mechanisms of genetic variation that shape our genome, which also involves non-coding regions that are, for the moment, even more difficult to study, especially at the functional level. The challenge for future research is to determine how these diverse coding and non-coding human-specific genetic changes shape the development of the brain and how they contribute to disease.

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