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News & views

Neuroscience

The genetic symphony of human brain evolution

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The prefrontal cortex of the human brain is larger than that of other species. Comparisons of mouse, macaque and human brains uncover some of the genetic and molecular factors behind these differences.

A symphony arises from the careful coordination of many musical instruments in an orchestra. Similarly, gene expression is controlled by proteins and other molecules known as *trans*-acting regulators that behave like conductors to drive the expression of multiple genes at distant sites. Further control is exerted by stretches of DNA called *cis*-regulatory elements (CREs) that act like musicians to control the expression of individual genes nearby on the chromosome. Changes in CREs have been proposed to underlie evolutionary changes across populations, because these elements have highly specific effects on an organism's characteristics. By contrast, changes in *trans*-regulatory factors coordinate the activity of large suites of genes to influence multiple traits[1]. In two studies in *Nature*, Shibata *et al.*[2,3] explore the interplay between *cis*- and *trans*-regulatory changes in a hallmark of human evolution — the large size and highly connected nature of a brain region called the prefrontal cortex (PFC).

The cerebral cortex of the brain has undergone dramatic expansion as primates have evolved, with cortical areas such as the PFC, that are thought to serve complex brain functions, having expanded most strikingly, especially in humans[4,5]. Neuronal cells in the human PFC also make more synaptic connections with other neurons than do neurons in other cortical areas[6]. However, it has been difficult to link these properties to specific changes in gene expression and regulation.

Reasoning that the mechanisms that bring about distinctions between the PFC and other cortical areas during development[7] might also be involved in creating differences in the PFC across different species, Shibata *et al.*[2] examined genes that are expressed at higher levels in the human PFC than in other human cortical regions[8]. The expression of many of these genes seemed to be regulated by retinoic acid, a molecule produced from the metabolism of vitamin A. Retinoic acid acts as a signalling molecule and has many roles in development, leading to the hypothesis that it might be a master *trans*-acting regulator of features of the PFC.

Indeed, the authors discovered much higher levels of retinoic acid in the PFC than in other cortical areas in human, macaque and mouse brains, with the highest levels in humans (Fig. 1a). These observations suggest that retinoic acid could be acting as a conductor to shape the expression of genes with high expression in the PFC.

Because gradients in retinoic acid levels were present across cortical areas in all three species studied (despite there being lower overall levels in mice), the authors used mouse models to investigate the role of retinoic acid signalling in the development of features associated with the human PFC. They generated mice lacking the genes that encode the retinoic acid receptor proteins RXRG and RARB to test the effects of reduced signalling through this pathway. Compared with unmodified mice, these animals showed lower expression of genes that are normally highly expressed in the PFC, fewer synaptic connections in this brain region and a specific reduction in reciprocal neuronal connections between the medial part of the PFC (mPFC) and another part of the brain called the medial thalamus.

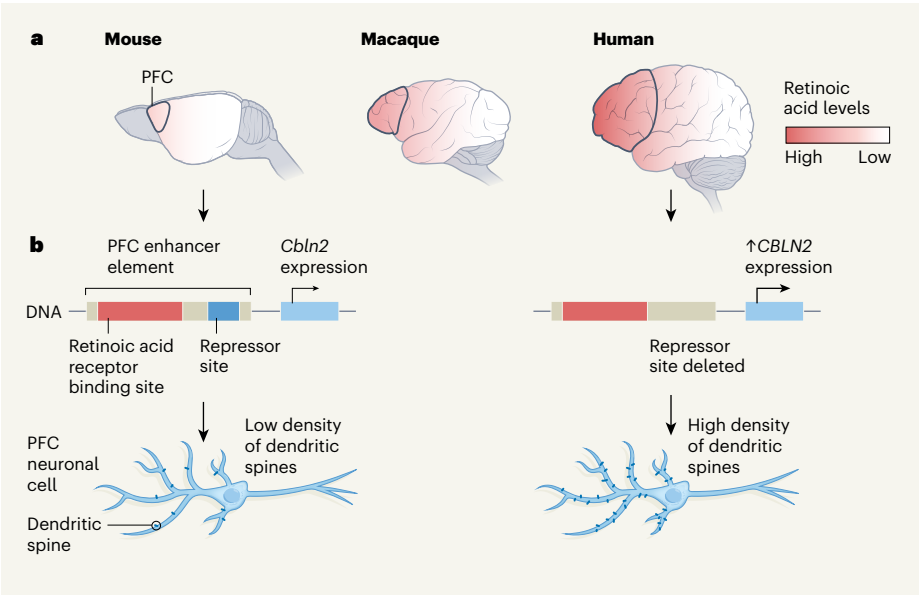

Figure 1 | Differences in the cortex of mouse, macaque and human brains. a, Shibata *et al.*[2] compared the brains of mice, macaques and humans (not to scale). They found that levels of the molecule retinoic acid show a gradient from the front to the back of the brain's cortex in all three species during development, with overall levels being highest in humans and lowest in mice. The human prefrontal cortex (PFC) is expanded compared with that of macaques and mice, and has greater numbers of reciprocal connections with another brain structure, the thalamus, than does the mouse PFC (connections not shown). **b**, Shibata *et al.*[3] identified a DNA sequence that regulates gene expression, known as an enhancer, that is active during PFC development. The enhancer contains sites to which retinoic acid receptors or SOX5 proteins can bind to promote or suppress gene expression, respectively (one of each shown). In humans, some repressive sites are deleted, increasing expression of the gene *CBLN2*. Neuronal cells in the mouse PFC have a lower density of structures called dendritic spines, which form synaptic connections, than do human PFC neurons.

By contrast, in mice genetically engineered to lack the enzyme that normally degrades retinoic acid, the resulting increase in retinoic acid signalling led to the development of more projections between the medial thalamus and the mPFC. It also increased expression of *Rorb*, a gene that is expressed by a population of cells in layer 4 of the cortex that receive inputs from the thalamus and that are present in the PFC of humans and other primates, but mainly absent in the mouse PFC[9].

It is still unclear how the reciprocal connections between the mPFC and medial thalamus contribute to human brain function, but they could be involved in coordinating computations in the cortex, facilitating cognition and flexible decision-making. Shibata and colleagues' discovery of mechanisms that control the development of the medial thalamus–mPFC pathway might lay the foundation for detailed investigations of the development and function of these connections in non-primate model organisms.

In their second article[3], Shibata *et al.* focused on *CBLN2*, a gene that is enriched in the PFC and that encodes the synapse-organizing protein cerebellin 2. *CBLN2* is expressed by more types of PFC neuron in humans than in macaques or mice. Building on their observation that *CBLN2* expression is increased by retinoic acid signalling, the authors examined putative CREs near this gene, and discovered one such DNA sequence that promotes gene expression — called an enhancer — and that is active during early PFC development. This enhancer contains several sites to which retinoic acid receptors can bind, leading to increased *CBLN2* expression in response to retinoic acid (Fig. 1b).

The authors found that the enhancer also contains several sites to which a protein called SOX5 can bind to suppress gene expression. Comparisons of the enhancer sequence in different species revealed that two deletions that probably occurred between about 7 million and 12 million years ago removed some of the SOX5 binding sites from the genome of the common ancestor of humans and chimpanzees. In cultured cells, the human and chimpanzee *CBLN2* enhancers were not suppressed by SOX5, whereas the gorilla and macaque versions of the enhancer were moderately suppressed, and the mouse version, which has more SOX5 binding sites than the primate versions, was most strongly suppressed.

The authors then generated mice in which the *Cbln2* enhancer was replaced by the human version to determine which features of the brain were specifically related to this single CRE change. Remarkably, compared with unmodified animals, mice with the human enhancer had more neuronal structures called dendritic spines (Fig. 1b), which are involved in synapse formation, as well as higher expression of *Cbln2* during PFC development and more synaptic structures in the PFC both during development and in adulthood. These results suggest that the symphony of human PFC evolution involves a coordinated increase in the expression of an ensemble of genes through retinoic acid receptor signalling that acts in *trans*, and a further 'crescendo' of increased expression of at least one response gene, *CBLN2*, through the loss of SOX5 binding sites in an enhancer element acting in *cis*.

These studies leave several questions unanswered. First, what is the source of the relatively high levels of retinoic acid in the primate brain? The authors analysed the expression of several retinoic acid-synthesizing enzymes, but, given that these enzymes are expressed in various types of neuronal and non-neuronal brain cell and in the meningeal layers that envelop the brain, it is difficult to narrow down which cell type or types and evolutionary changes are responsible.

Second, at which points during primate evolution did these changes in *cis*- and *trans*-acting regulators occur? Shibata and colleagues found a graded increase in retinoic acid signalling from mouse to macaque to human[2], as well as a previously unrecognized area of this signalling in the inferior temporal cortex (another area of the cortex that is important for high-level cognition) in humans, but not in mice or macaques. However, whether these differences are truly human-specific and are absent from our closest living relatives, chimpanzees, remains untested. Similarly, although the CRE changes near the *CBLN2* gene are shared by humans and chimpanzees, other molecular and genetic changes that have arisen exclusively in humans, concomitant with the evolution of key traits such as language and syntactical grammar, remain to be investigated.

More broadly, these studies provide an intriguing example of *cis*- and *trans*-acting mechanisms that act in concert to change key properties of the brain. Only further studies of primate brain evolution can reveal whether this symphony of mechanisms is the exception or the rule in the evolution of other structural and behavioural specializations of the human brain.

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