Clever space saving—how the cerebral cortex folds

Mareike Albert & Wieland B Huttner

The human cerebral cortex controls complex cognitive behaviors. During mammalian evolution, the number of neurons increased in many lineages, requiring a larger cortical surface area to fit into a skull that did not scale proportionally. This space problem was solved by cortical folding, resulting in gyrencephalic (folded) brains. While several hypotheses have been proposed to explain cortical gyrification, we lack mechanistic insights to understand the process itself and in particular its underlying genomic changes, that lead to the appearance of cortical folds. In this issue of *The EMBO Journal*, de Juan Romero *et al* (2015) tackle the question using a transcriptomics approach to identify gene expression changes in the developing ferret brain prior to the onset of gyrification.

See also: **C de Juan Romero *et al*** (July 2015)

Brain size changes remarkably across mammalian species, and this is mostly due to a disproportionate increase in size of the cerebral cortex. While the thickness of the cerebral cortex varies little (by less than one order of magnitude) during evolution, the cortical surface increased dramatically (> 1,000-fold) between certain species, requiring effective packaging into limited skull volume. Folding solves this problem, creating convolutions of the cortical surface called *gyri* and fissures called *sulci*. Although folded brains may erroneously be assumed to be a trait distinctive of primates, gyrencephaly is actually present in essentially all mammalian orders. In fact, it is thought that gyrencephaly already existed in the ancestor of all mammals and that the smooth (lissencephalic) cortex seen in some mammalian species evolved secondarily (Kelava *et al*, 2013; Lewitus *et al*, 2014).

Even though the folds and fissures are easy to observe, their appearance is not so easy to explain. The pattern of the major gyri and sulci is largely conserved among members of the same order of mammals, suggesting a genetic component in their specification (Zilles *et al*, 2013). Several theories of gyrification have been proposed (discussed in Kelava *et al*, 2013). The neural tension hypothesis proposes that axons of the underlying white matter may influence gyrification by pulling together strongly interconnected regions of the cortex. Kriegstein *et al* (2006) suggest that differential growth rates of upper versus lower neuronal layers result in cortical folding. Moreover, the orientation of neurons, their arborization, and incoming fibers might contribute to gyrification. Novel hypotheses have been inspired recently by progress in the characterization of cortical progenitors and the appreciation of their diversity.

The generation of cortical neurons during development is the result of proliferative and differentiative divisions of neural stem and progenitor cells that form two germinal layers: the ventricular zone (VZ) and the subventricular zone (SVZ) (Borrell & Götz, 2014; Florio & Huttner, 2014; Taverna *et al*, 2014). Apical radial glia (aRG) are progenitors, the nuclei of which reside in the VZ and that have the ability to self-renew and to generate neurons and basal progenitors, the latter of which migrate to, and form, the SVZ. In the mouse, basal intermediate progenitors (bIPs) usually divide symmetrically to produce two postmitotic neurons, whereas in primates, bIPs also frequently undergo symmetric proliferative divisions, an essential mechanistic determinant of neocortical expansion (Lewitus *et al*, 2014). Recently, a new progenitor type has been identified that is particularly abundant in gyrencephalic mammals with an expanded SVZ that is subdivided into an inner (iSVZ) and outer (oSVZ) SVZ, the basal radial glia (bRG) (Borrell & Götz, 2014; Florio & Huttner, 2014; Taverna *et al*, 2014). In addition to their ability to directly generate neurons, bRG exhibit extensive self-renewal and proliferative capacities (Betizeau *et al*, 2013), thus providing a basis for cortical expansion. The evolutionary expansion of the neocortex is thought to be based on differences in the abundance of these neural progenitor cell types and in their lineages (Lewitus *et al*, 2014). Moreover, it is reasonable to assume that the balance of proliferation versus differentiation of progenitor types, which is a determinant of germinal layer thickness, contributes to cortical folding. Indeed, in the fetal monkey cortex, the SVZ is relatively large in areas where gyri subsequently develop and relatively thin in areas of prospective sulci (Kriegstein *et al*, 2006).

In this issue of *The EMBO Journal*, Victor Borrell and colleagues (de Juan Romero *et al*, 2015) present an elegant approach to understand the mechanism underlying the folding process. They used the ferret, a gyrencephalic carnivore, to analyze transcriptional differences within a given germinal zone, comparing regions prospective of the splenial gyrus and the lateral sulcus. Specifically, they microdissected the VZ, iSVZ, and oSVZ at postnatal day 2 (P2), that is, one week prior to the first morphological distinction of these folds, and analyzed the transcriptomes using a ferret-specific microarray. Remarkably, although the two regions

are nearly adjacent and have a similar cyto-architecture and cellular composition, de Juan Romero *et al* (2015) identified 2,218 differentially expressed genes (DEGs) of which the majority was differentially expressed in only one of the germinal zones. The oSVZ contained the largest number of DEGs, which is interesting in light of the notion that bRG located in this germinal layer play central roles in the expansion of the cerebral cortex (Borrell & Götz, 2014; Florio & Huttner, 2014; Taverna *et al*, 2014).

These findings prompted the idea that in the developing ferret cerebral cortex, gene expression in the germinal layers may occur in modular patterns corresponding to gyri and sulci. The authors set out to test this hypothesis using *in situ* hybridization of DEGs with known roles in progenitor proliferation/differentiation or cortical patterning. As predicted, changes in gene expression levels often occurred quite abruptly, rather than in smooth gradients, delineating distinct, gyral or sulcal, gene expression domains. Some genes, including *Fgfr2*, *Lhx2*, *Eomes,* and *Cdk6*, showed obvious domains within the oSVZ, while others, like *Cdh8*, were specific for the VZ. In contrast, the same genes were expressed homogeneously or in long-range shallow gradients in the mouse. Interestingly, one of the genes that was expressed in specific domains in ferret was *Trnp1*, which has previously been shown to exhibit regional differences in human fetal cerebral cortex (Stahl *et al*, 2013). Moreover, knockdown of *Trnp1* in the mouse induced greater proliferation of basal progenitors, leading to radial growth and subsequent folding of the cortex, whereas forced overexpression of *Trnp1* had the opposite effect, inducing selective aRG self-amplification and decreased generation of basal progenitors (Stahl *et al*, 2013). It will be interesting to address in the future whether some of the DEGs identified by de Juan Romero *et al* (2015) have similar instructive roles.

The initial signals that induce folding, and how the differential expression patterns are generated, remain unclear. To address the age at which modular expression can first be observed, de Juan Romero *et al* (2015) analyzed expression of *Eomes*, *Fgfr2, Fgfr3,* and *Cdk6* at three different time points spanning the neurogenic period (E30, E34, and P2). All of the selected genes showed homogeneous expression at E34, and only later during early postnatal development did expression domains become distinguishable.
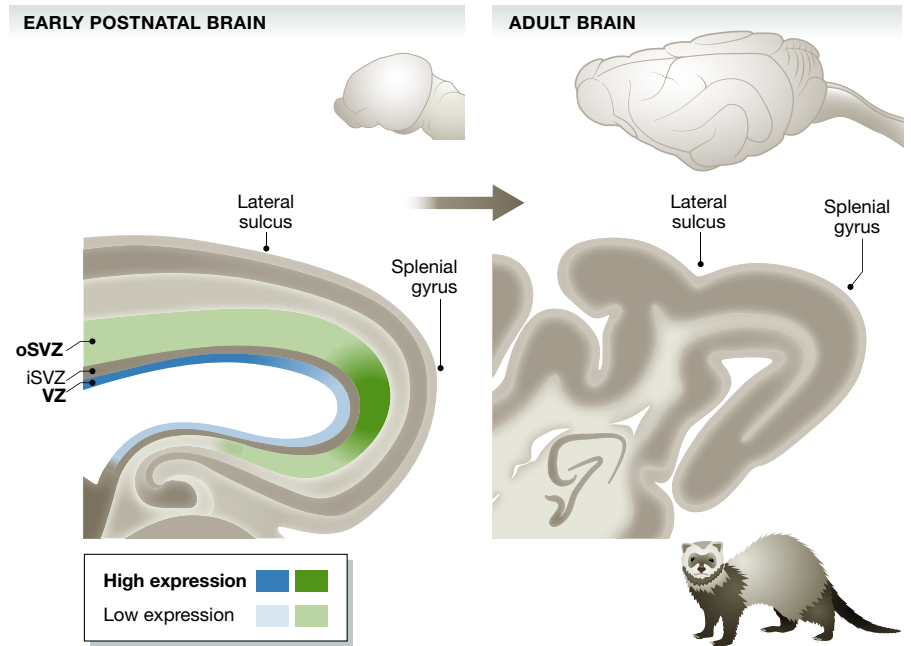

Figure 1. Gene expression occurs in modular patterns in areas prospective of folds and fissures.
Abrupt changes in gene expression levels within germinal layers delineate areas prospective of the lateral sulcus and the splenial gyrus in the early postnatal ferret brain prior to the onset of gyrification. Model based on de Juan Romero *et al* (2015).

Eomes displayed the greatest contrast between modules at P6, the age of onset of gyrus formation. Moreover, *Eomes* expression levels matched precisely to several of the emerging folds and fissures, suggesting that this could be an important gene in patterning of the cortical folds. In this regard, it is interesting to note that mutations of *EOMES* in humans cause microcephaly with polymicrogyria (Baala *et al*, 2007).

Examination of public databases revealed that 81% of genes mutated in human cortical malformation syndromes (see Sun & Hevner, 2014) are among the ferret DEGs. The authors therefore tested whether such genes might also be expressed in modular patterns in the human developing cortex. Indeed, they found variations in expression levels in brain sections from human embryos at 16 and 21 gestational weeks (the latter stage being immediately prior to the formation of the first folds and fissures) for all genes tested, including *EOMES*, *TRNP1*, and *GPR56*. In this context, it is worth mentioning that our laboratory has recently used transcriptomics to identify genes that are preferentially expressed in human apical and basal progenitors. Among these was the human-specific gene *ARHGAP11B*, which when overexpressed in mouse promotes

basal progenitor generation and self-renewal and can induce gyrification (Florio *et al*, 2015). In light of the observation by de Juan Romero *et al* (2015), it would be very interesting to examine whether *ARHGAP11B* is expressed in modular patterns during human cortical development.

The data reported by de Juan Romero *et al* (2015) (Fig 1) are consistent with the cortical protomap concept proposed by Rakic (1988) to pattern the cerebral cortex primordium into prospective anatomical and functional regions. For cortical folding, modular patterns of gene expression, with combinations of genes possibly different depending on the specific gyrus or sulcus concerned, may impose differential growth, eventually leading to the evagination of cortical tissue and formation of folds. Specific enhancer elements may serve as readout of combinatorial transcription factor expression, especially since enhancers have recently been shown to drive reporter gene expression in discrete regions, or protomaps, in the cerebral cortex (Visel *et al*, 2013). The transcriptome dataset generated by Borrell and colleagues provides a rich resource and opens novel avenues for future investigations into the genetic regulation of cortical folding. It will remain a challenge to explain

the regulation of stereotyped formation of folds and to identify the initial signals upstream of the transcriptional differences described here, so more exciting research lies ahead.

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