Minireview
Functional genomics of early cortex patterning
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Abstract

Several lines of evidence have illuminated the fundamental developmental principles involved in establishing and implementing pattern formation in the mammalian neocortex. A recent study has sought to unravel the underlying genetic control of cortex patterning by elucidating the transcriptional profile of discrete neocortical regions.

Patterning the neocortex

The cerebral cortex where the higher brain functions reside is an exquisitely patterned structure composed of numerous specialized areas that subserve sensory, motor and cognitive modalities (for overview see [1]). This is achieved through the cortex being iteratively organized into discrete functional columns each of which has a laminar organization. Elucidating the mechanisms that govern the initial divisions of the cerebral cortex into these morphological and anatomically distinct areas, and determining the means by which these columns subsequently become organized into functionally specialized units represents some of the key goals in developmental neurobiology. Considerable evidence suggests that final specification of areal territories within the cortex can be explained by a composite of the two prevailing models known as the protomap [2] and protocortex hypotheses [3] (for review see [4]).

The protomap model dictates that the ventricular zone of the embryonic neural tube from which the neocortex is derived is autonomously patterned early in development such that the coarse identities and locations of the cortical areas are laid down before the cortex receives afferent innervation from the thalamus. The thalamus receives, like a relay station, information from diverse brain areas including all the senses except olfaction and is also responsible for regulating motor control. In the protocortex model, it is implied that the areal identity of the cortex is imparted later in development by the action of extrinsic cues, such as thalamo-cortical input, as they arrive into a naive, imprintable territory. It now appears that patterning of the developing cortex relies sequentially on both intrinsic and extrinsic cues [4]. Successful cortical arealization relies on a broad prepattern being initially laid down in the ventricular zone (following the protomap model), and the map is then subsequently refined and reinforced by the arrival of thalamo-cortical axons (fitting with the protocortex model).

A recent study by Sansom *et al*. [5] has sought to illuminate the underlying genetic control of the protomap model by using high-density oligonucleotide arrays to elucidate the transcriptional profile of discrete neocortical regions. The authors also attempt to integrate specific gene function into the known neocortical signaling systems. The data obtained from this study have significantly enriched the range of genes that can now be associated with cortical patterning, as well as identifying Mest, a novel potential regulator of neocortical patterning mediated by fibroblast growth factor 8 (FGF8).

Recent evidence [4] has shown that two homeobox transcription factors, Pax6 and Emx2, that are expressed in a complementary and graded rostro-caudal (anterior-posterior) manner in the ventricular zone of the developing neocortex play a pivotal role in establishing the initial patterning in the cortex (Figure 1a,b). In mice lacking either Pax6 or Emx2, there is a concomitant reduction in the size of the region of
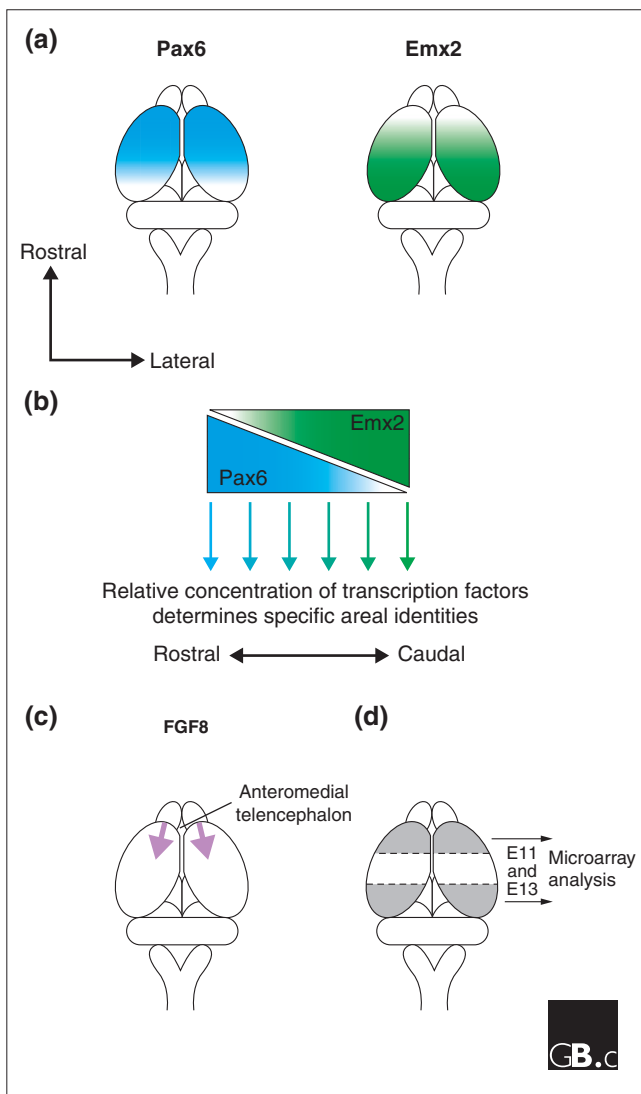
Figure 1
Factors that pattern the mammalian cortex. **(a)** Dorsal view of the mouse cerebral cortex at day E13.5 with the rostro-caudal gradients of the transcription factors Pax6 and Emx2 superimposed (see [9]). **(b)** The early cortex areal identity along the rostro-caudal axis can be imparted by the interpretation of different relative concentrations of transcription factors such as Pax6 and Emx2 at different positions along the axis. **(c)** Signals that pattern the cortex. Fibroblast growth factors (FGFs; purple) are expressed first in the anterior neural ridge and then in the anteromedial telencephalon, and may participate in early cortical patterning by signaling in the direction of the arrows (reviewed in [12]). **(d)** Sansom *et al.* [5] interrogated the genetic identity of the neocortex by performing a microarray study on discrete rostral and caudal portions of the neocortex at E11 and E13 developmental stages.

the cortex normally expressing each gene and a corresponding expansion of the remaining territory. Recent studies suggest that this is due to cross-repression of the two genes [6-10], which in turn results in the establishment of cell-autonomous regional identity within cortical cells through a process by which the actions of particular transcription

factors are determined by their expression levels relative to other transcription factors [10].

Although the sequential influences of intrinsic prepatterning and extrinsic thalamic afferent input refinement articulate a general strategy for cortical patterning, the precise genes contributing to these events and the manner in which these two processes are integrated remains poorly understood. As a means of understanding these events better, it would be particularly useful to assemble the complete complement of transcription factors with graded expression that contribute to these processes. By getting the requisite players on the table we can begin the hard work of trying to understand how they are interpreted and translated into region-specific neuronal identity and how the resulting cortical map is appropriately innervated to establish functional circuitry.

Using the latest approaches in genomic profiling, the Livesey lab [5] has sought to investigate the molecular composition - in terms of graded versus discrete domains of expression - of the mouse neocortex during initial patterning at 11 days *post coitum* (E11). This is before the onset of thalamo-cortical innervation and at a time when the neocortex consists mainly of progenitor cells. The application of oligonucleotide-based arrays consisting of 22,000 genes and expressed sequence tags (ESTs) from the mouse genome represents an unbiased approach to determining the molecular complexity of the source tissue and presents a good opportunity to assess whether the neocortex is composed exclusively of expression gradients or whether those are also discrete domains of gene expression (that prefigure areas). Such approaches are well suited to developmental neurobiology, where tissue can be harvested in both a spatially and temporally accurate manner and it is likely that a significant proportion of neuronal specification is transcriptionally based (for review see [11]).

Support for a transcriptionally defined protomap

By sampling the transcriptome of the rostral and caudal thirds of E11 mouse embryo neocortices (see Figure 1d) in an elegant dual-strain (inbred and outbred) stage-matched replicate study, Sansom *et al.* [5] have generated a high-quality dataset that reflects known transcriptional events. As such, the microarray expression profile of the major known patterning genes, including those for the transcription factors Pax6, Emx2, Lhx2 and COUP-TF1, was shown to match their relative expression levels in the neocortex. Before a dataset can be described as being truly representative, however, it must be shown to be predictive and verifiable. To this end, the authors selected 38 genes whose representation on the gene arrays was determined to be indicative of a significant change (across a spectrum of bioinformatics criteria) in gene expression along the rostro-caudal axis of the neocortex. Where an *in situ* hybridization

expression pattern could be determined (23 of 38), all of the profiles matched the rostro-caudal distribution suggested by the array data. Thus, the dataset represents an accurate molecular description of domain-restricted neocortical gene expression at stage E11.

Given the above, the most striking finding from this screen [5] is that all of the neocortical gene-expression patterns demonstrated were in clear rostro-caudal gradients across the field of progenitor cells (see Figure 1), consistent with the presence of a graded transcriptional code as required by the cooperative concentration hypothesis [10]. This hypothesis postulates that gene-expression patterns like the ones observed by Sansom *et al.* [5] are a function of the concentration-dependent differences in the binding efficacy of transcription factors such as Emx2 to their cognate promoters or repressors. No distinct compartments of gene expression were observed. Sansom *et al.* [5] acknowledge, however, that the differences in recorded expression levels can also be influenced by the developmental gradient (progenitor cells versus newly born neurons versus differential neurons) and the related difference in the ratio of neurons to progenitors at different stages. Thus, as with all microarray-based screens, it is imperative to put the data in the context of the biological system and all the variables therein to establish what can be meaningfully derived from the data. To address this issue, the E11 dataset was supplemented with a separate microarray experiment to assess the transcriptional profiles of the rostral, middle and caudal thirds of the neocortex on day E13. In this way, transcriptional events associated not only with the rostro-caudal gradients but also with area-specific maturation events were determined.

Taken together, the E11 and E13 datasets allow a high-definition appreciation of the spatial and temporal changes in gene expression across the neocortex. Using these approaches in conjunction with an additional *in situ* hybridization screen, Sansom *et al.* [5] concluded that there is little evidence for discrete compartments of neocortical progenitor cells at these time points. Although it is possible that some level of resolution may have been lost by the pooling strategy, the data strongly support the gradient-based protomap model. At stages E11 and E13, the question remains of how these transcriptional gradients are read and interpreted by the incoming axons.

What are the targets of FGF signaling in the neocortex?

A common theme in developmental biology is the role of signaling centers that secrete diffusible molecules and influence the fate of the recipient tissues. The developing neocortex is subject to the patterning effects of many diffusible signal proteins such as bone morphogenetic proteins (BMPs), Wnt family proteins, FGFs and Hedgehog-related proteins (see Figure 1c) (for reviews see [4,12]). Genetic gain- and loss-of-function

studies implicate FGF8 as the primary secreted factor that imparts positional information on the rostro-caudal axis of the developing neocortex [4,13,14]. To assess the contribution of FGF signaling, Sansom *et al.* [5] took advantage of a mouse with a *foxg1*-driven forebrain-specific mutation in the FGF receptor 1 (*Fgfr1*); the phenotype of this mutant mouse is consistent with an abrogation of FGF8-mediated patterning in the neocortical area [15,16]. Careful comparison (nine replicates at E12.5) of the whole neocortex between the *Fgfr1*-mutant line and the *foxg1*-driver lines (containing only the driver construct) identified a large number of both positively and negatively regulated genes in the mutant, including many of the expected targets altered in a manner consistent with disrupted FGF signaling. These included downregulation of Ets-domain transcription factors that are known targets of FGF signaling.

The results of these experiments were complemented by *in vitro* experiments confirming that many of the predicted targets respond appropriately to FGF8 in a cortex explant culture system. The greatest value of such a dataset is that it provides unbiased insights into the consequences of FGF signal interruption. This conclusion must, of course, be qualified by both the fact that residual signaling activity through other receptors may persist and that the expression of *foxg1* itself may be altered in these mutants (for example, see [17]). Moreover, the gene-expression changes noted may be a primary effect because of the direct loss of signal, or a secondary effect because of a downstream effect or transformation of tissue identity because of the lack of signal.

Despite these caveats, Sansom *et al.* [5] make a compelling case that their analysis has revealed many of the salient consequences of FGF signaling for gene expression. Specifically, in the *Fgfr1*-mutant cortex they report an expression level of neurogenesis-associated genes that is significantly higher than normal, suggesting that one of the functions of FGF8 is the negative regulation of neurogenesis and the maintenance of neocortical progenitor cells. The authors also used the available data to identify a previously undescribed candidate cortical patterning gene, *Mest*. Sansom *et al.* [5] show that *Mest* is directly responsive to FGF8 signaling, and also show through microarray analysis of a *Mest* mutant mouse that Mest is likely to be an antagonist of the FGF-mediated rostral patterning of the neocortex.

It now seems likely that the early neocortex is patterned by a multifaceted gradient of transcription factors, signaling molecules and other molecular determinants. But the challenge remains to understand exactly how this gradient is established and how it is subsequently interpreted by the neocortical progenitors and ultimately translated into neurons with specific areal identities. With recent advances in microarray technology allowing the reproducible generation of microarray data from restricted cell numbers (for example, see [18]), coupled with sophisticated anatomical genetic labeling

strategies, it may soon be possible to follow and transcriptionally profile individual progenitors as they go through the transition from neocortical progenitor to neuron.

In summary, the technically elegant survey by Sansom *et al.* [5] of neocortical transcriptional patterning has provided a dataset that reinforces the prevailing dogma of the protomap hypothesis and provides a foundation from which molecular insights can be drawn. Further surveys of this kind will help weave together how rostro-caudal patterns of transcriptional expression lead ultimately to the regionalization of cortical areas. It now remains for the scientific community to recognize the intrinsic value of these vast data resources and experimentally integrate these findings into existing paradigms as a means to gain novel biological insights.

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