Proto-mapping the areas of cerebral cortex: transcription factors make the grade

Edwin S. Monuki and Christopher A. Walsh

Two new studies demonstrate how the homeodomain genes ***Emx2*** and ***Pax6*** may work together to provide positional information to the developing neocortex.

How the cerebral cortex becomes parceled into discrete functional subdivisions is one of the oldest and most fundamental questions in neuroscience. For some time, we have known that the cortex is divided into discrete areas with distinct functions, such as vision and motor control. Each area shows unique patterns of cellular anatomy and specific patterns of connectivity with the thalamus: for example, the visual cortex is interconnected with the lateral geniculate nucleus of the thalamus, which in turn receives visual input directly from the retina. How do these distinct cortical areas attain their identity during development? Does the cortex represent a conglomerate of discrete 'cognitive organs' that develop independently from one another, arising from progenitor cells that 'know' to become visual cells or motor cells, before the arrival of afferent inputs (the 'protomap' model)[1]? Or, is the cortex like a brand-new hard drive, capable of performing a standard computational analysis on whatever input it receives, with the distinctive identities of cortical regions being determined by the inputs (the 'protocortex' model)[2]? In support of the latter model, altering thalamic input early in development can cause dramatic changes in cortical organization. For example, when retinal input is rerouted into the auditory centers, the auditory cortex acquires features that normally characterize visual cortex[2,3]. However, a growing body of evidence suggests that the incoming thalamic connections interact with a developing cortex that already contains intrinsic positional information[4-6]. For example, mice that lack thalamic input altogether can still develop a surprisingly normal cortical arealization[7,8]. What provides the positional information in the cortex, and how do factors intrinsic to cortical progenitor cells interact with the inputs that cortex receives?

When it comes to providing positional information, developmental biologists think first about homeodomain transcription factors, because this large family of proteins provides positional information in a host of developmental settings. To take one classic example, the front end of the Drosophila embryo is specified by a gradient of the homeodomain protein bicoid. Transcription factors have the important feature that they activate other genes, and furthermore, bicoid activates some genes at high concentration and other genes at lower concentration. Therefore, a gradient of bicoid protein can trigger a cascade of downstream genes (gap genes, segmentation genes and others) that further subdivide the embryo into distinct structures. The remarkable work by Mallamaci *et al.*[9] in this issue of *Nature Neuroscience*, together with a similar recent study by Bishop *et al.*[10], suggests that cortical areas are similarly established in large part by gradients of homeodomain proteins expressed in the cells that generate cerebral cortex.

Both groups studied Emx2, a homeodomain protein essential to mouse[11] and human[12] cortical development. The protein is expressed by cortical progenitor cells in a graded fashion along one axis (high posteromedial, low anterolateral, **Fig. 1**) and throughout the entire two-dimensional extent of developing cortex. Using an *Emx2* knockout mouse, both groups looked at area-specific or area-restricted patterns of gene expression within the cortex; they also studied connections between the cortex and thalamus. The two groups reached essentially the same conclusion: namely, that loss of Emx2 results in a marked reduction of cortical areas that normally express high Emx2 levels (posteromedial cortex, including visual cortex) and a positional shift and/or expansion of cortical areas that express lower levels of Emx2 (intermediate and anterior cortex, including somatosensory and motor cortex, **Fig. 1**). Because Emx2 is expressed in a small area of thalamus[13], both groups investigated whether a thalamic defect contributed to the observed cortical changes. Importantly, they found that it did not; the changes in cortical organization thus reflect changes in the positional information of the cortex itself.

The evidence for reduction of posterior cortex is convincing, because there is a reduction in both absolute and relative size compared to other cortical regions. Posterior cortex is not entirely lost, however, as indicated by the preservation of some connections between the lateral geniculate nucleus and presumptive visual cortex[9]. Mallamaci *et al.* show that an additional posteromedial cortical region, the hippocampus, is also present but reduced in *Emx2*$^{-/-}$ mice, confirming recent work by others on this mouse line[14]. In contrast to this posterior reduction, the somatosensory (and auditory) area, which normally expresses intermediate levels of Emx2, does not show a large change in size but is instead shifted posteromedially.

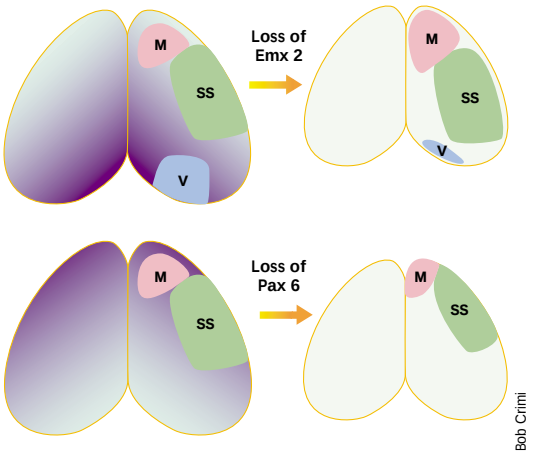


Fig. 1. Emx2 and Pax6 are expressed in opposing gradients across cortical progenitor cells, and their loss leads to distinctive shifts in the positions and sizes of cortical areas. Visual cortex is not included in the lower panel, as changes to visual cortex were not specifically addressed in the Pax6 mutant mice. M, motor cortex; SS, somatosensory cortex; V, visual cortex.

Both authors are in the Department of Neurology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, 02115, USA. Edwin S. Monuki is also in the Department of Pathology, Division of Neuropathology, Children's Hospital, Boston, Massachusetts, 02115, USA.
email: emonuki@caregroup.harvard.edu or cwalsh@caregroup.harvard.edu

Most intriguingly, both groups demonstrate a relative expansion in *Emx2*$^{-/-}$ mice of anterior cortical regions that normally express the lowest concentration of Emx2; Bishop *et al.* also demonstrate an absolute twofold expansion of anterior cortex (assessed using expression of an anterior cortical marker, Cad8) despite an overall one-third reduction of cortical surface area in these mice. Thus, cortical areas are differentially affected in *Emx2*$^{-/-}$ mice, with posteromedial regions markedly reduced, intermediate cortex shifted, and anterior cortex expanded. The loss of Emx2 does not result in complete loss of any particular cortical area, but instead results in a change of scale of cortical areas relative to each other. Most importantly, these changes correspond to the normal Emx2 expression gradient (**Fig. 1**). Because changes in areal size in the *Emx2*$^{-/-}$ mice correlate with areal position along the Emx2 gradient, it is likely that the Emx2 gradient directly regulates areal size. A further implication of these findings is that cortical areas are seemingly able to compete for space, because the areas that are least dependent on Emx2 (anterior cortex) are able to expand at the expense of those that are most dependent (posterior cortex).

This model, suggesting that parcellation of cortical areas is regulated by homeodomain protein gradients, is supported by work on a second mouse mutant, which lacks the homeodomain protein Pax6. Cortical Pax6 expression is also graded, but in a direction that opposes Emx2 (high anterolateral, low posteromedial, **Fig. 1**). In a fashion similar to the Emx2 mutants, markers of anterior and lateral cortical regions that normally express high Pax6 levels (including motor and somatosensory cortex) are reduced but not completely lost in *Pax6*-deficient mice[9] (**Fig. 1**). Although study of the Pax6 mutant line was limited (in part because thalamic axons do not reach the cortex in these mice), these findings support a model in which the complementary Emx2 and Pax6 gradients provide positional information to the developing neocortex in much the same way that gradients of other homeodomain proteins, such as bicoid, impart positional information in other developmental contexts.

The next set of questions involves how these homeodomain proteins regulate the formation of specific cortical areas. Do they influence proliferation or some other aspect of area-specific programming? Mallamaci *et al.* have begun to address this issue by studying the tissue morphology and cell cycle kinetics of anterior, intermediate and posterior cortical progenitors in *Emx2* knockout mice. Interestingly, they find that the proliferative layers are significantly thicker in these mice, but that the cell cycling times are indistinguishable from wild-type mice. This suggests that Emx2 does not regulate arealization by altering proliferation in a simple way, but may instead modulate other area-specific programs; additional experimental work will be needed to answer this question.

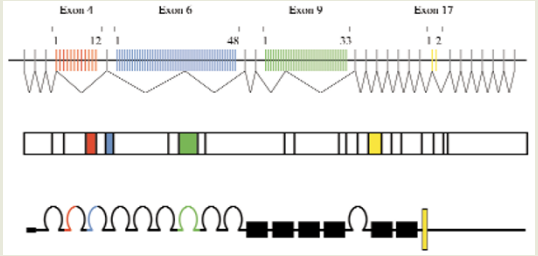
Several other outstanding questions remain unanswered. For instance, do Emx2 and Pax6 act independently or in a combinatorial manner to impart positional information? An *Emx2*/*Pax6* double knockout might be useful to answer this question. Also, how is the positional information encoded by these homeodomain protein gradients converted into the sharp areal boundaries of mature cortex? Mechanisms used to translate other homeodomain gradients (such as the bicoid gradient) into discrete subdivisions may provide insights into this problem. Another question concerns the upstream factors that establish the Emx2 and Pax6 gradients. Secreted molecules such as the Bmps, Wnts and Fgfs have been shown to act in gradients to initiate the production of transcription factors, and they may therefore be involved. Although the downstream transcriptional targets of Emx2 and Pax6 remain unknown, one potential target may be the ephrins, which show graded expression within somatosensory cortex and affect its topographic organization[15]. Finally, although the Emx2/Pax6 countergradient axis is clearly an important one, gradients along other axes must also be acting to subdivide cortex, because functional areas are not aligned solely along one axis. The identification of factors that act along other axes, and their interactions with the Emx2/Pax6 countergradient, will also be of great future interest.

1. Rakic, P. *Science* **241**, 170–176 (1988).
2. O'Leary, D. D. *Trends Neurosci.* **12**, 400–406 (1989).
3. Sharma, J., Angelucci, A. & Sur, M. *Nature* **404**, 841–847 (2000).
4. Barbe, M. F. & Levitt, P. *J. Neurosci.* **11**, 519–533 (1991).
5. Gitton, Y., Cohen-Tannoudji, M. & Wassef, M. *J. Neurosci.* **19**, 4889–4898 (1999).
6. Dehay, C., Giroud, P., Berland, M., Smart, I. & Kennedy, H. *Nature* **366**, 464–466 (1993).
7. Miyashita-Lin, E. M., Hevner, R., Wassarman, K. M., Martinez, S. & Rubenstein, J. L. *Science* **285**, 906–909 (1999).
8. Nakagawa, Y., Johnson, J. E. & O'Leary, D. D. *J. Neurosci.* **19**, 10877–10885 (1999).
9. Mallamaci, A., Muzio, L., Chan, C.-H., Parnvelas, J. & Boncinelli, E. *Nat. Neurosci.* **3**, 679–686 (2000).
10. Bishop, K. M., Goudreau, G. & O'Leary, D. D. *Science* **288**, 344–349 (2000).
11. Pellegrini, M., Mansouri, A., Simeone, A., Boncinelli, E. & Gruss, P. *Development* **122**, 3893–3898 (1996).
12. Brunelli, S. *et al. Nat. Genet.* **12**, 94–96 (1996).
13. Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A. & Boncinelli, E. *Nature* **358**, 687–690 (1992).
14. Tole, S., Goudreau, G., Assimacopoulos, S. & Grove, E. A. *J. Neurosci.* **20**, 2618–2625 (2000).
15. Vanderhaeghen, P. *et al. Nat. Neurosci.* **3**, 358–365 (2000).

A new high for alternative splicing

Alternative splicing of RNA transcripts has long been recognized as one way of generating molecular diversity. But a recent paper (D. Schmucker *et al.*, *Cell* **101**, 1–20, 2000) sets what is surely a new record, with the identification of a *Drosophila* axon guidance receptor, termed Dscam, that has no fewer than 38,000 different isoforms. The extracellular domain is assembled in modular fashion, with multiple alternatives for each module (see diagram). Although it is possible that not all combinations are expressed *in vivo*, many clearly are: among 50 randomly selected Dscam cDNAs, 49 were unique.

The authors confirm that Dscam is involved in axon guidance, but whether the isoforms have different functions remains an open question. It is tempting to think that this molecular diversity is somehow related to the complexity of the neural wiring, but how a neuron could make use of so many different possibilities—given the difficulties of specifying the splicing pattern with any precision—is still anyone's guess.



Charles Jennings