Regionalization in the mammalian telencephalon

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Regionalization in the telencephalon results in the formation of functionally and anatomically distinct territories. Cell fate analysis and gene expression studies suggest these subdivisions arise relatively late in development compared with the spinal cord or hindbrain. The mechanisms underlying the commitment of telencephalic cells to specific regional identities have been examined through recent transplantation experiments.

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Abbreviations

A/P	anteroposterior
BF	brain factor
Dlx	*Distal-less*
D/V	dorsoventral
E	embryonic day
Emx	*Empty spiracles*
HES	Hairy and Enhancer-of-split homolog
HNF	hepatocyte nuclear factor
Shh	Sonic Hedgehog

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Introduction

In terms of regional patterning, the telencephalon is both the most prominent and the least studied division of the CNS. Originating from the anterior neural plate, the paired telencephalic vesicles eventually give rise to much of the forebrain. From an evolutionary perspective, the telencephalon shows a particularly wide diversity among vertebrates and is more variable phylogenetically than either the hindbrain or spinal cord [1,2]. Although the regions of the mature mammalian telencephalon are distinct in terms of cellular organization, axonal projections and neurochemical composition, the cells that comprise them share a common developmental history.

How the telencephalon becomes regionally patterned has received surprisingly little attention from developmental biologists. Although later phases of telencephalic development (i.e. when the laminar organization [3,4•] and functional areas of the cortex are established [5–7,8•,9]) are presently the focus of intense research, the issue of how the distinct regions of the telencephalon (such as the cortex, striatum and pallidum) arise remains largely unexamined. Recent studies have begun to address this problem both by providing an accurate fate map of the telencephalon in various species and by identifying a number of genes that become expressed during overt regional differentiation [10–12]. This work, combined with the analysis of mutations that disrupt forebrain organization [13•,14••,15•–21•], has given the first indication of the molecular pathways underlying telencephalic patterning. In this review, experimental approaches that have yielded hints as to the underlying mechanisms that subdivide the telencephalon will be considered.

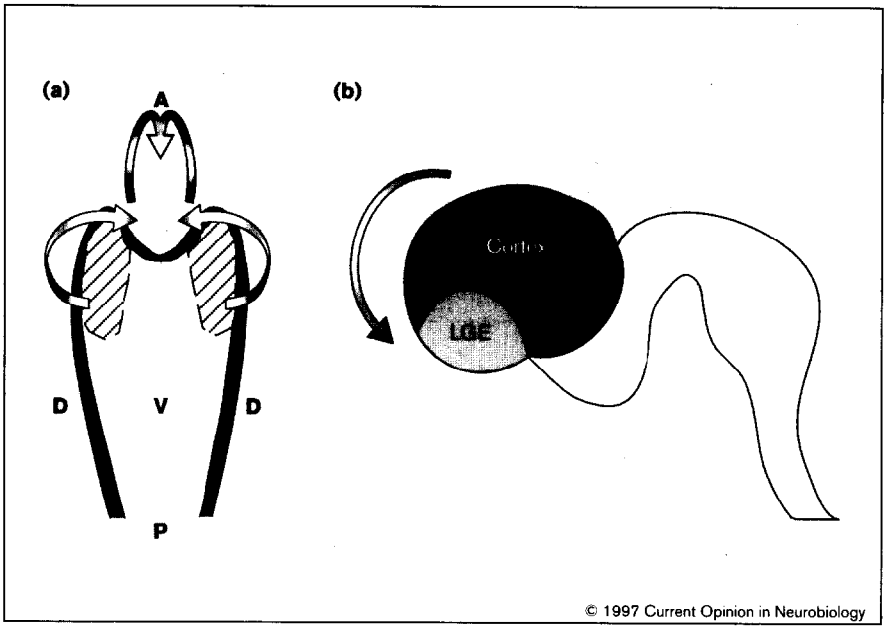
When does regionalization occur?

Fate maps of the telencephalon in a number of species (e.g. chicken [22], frog [23], zebrafish [24••]) indicate that the telencephalon is derived from the anterior lateral (i.e. alar) neural plate. Even though in frog and zebrafish a small amount of the anterior midline also appears to contribute to the telencephalon, this area is probably alar plate that has become positioned at the midline early in development [25••]. Although a fate map of the mouse telencephalon is not yet available, the fate maps in these other species suggest that the telencephalon is also derived from the alar plate and, hence, is a dorsally derived structure. Thus, what is referred to as the ventral telencephalon within this review (i.e. striatum and pallidum) is probably dorsal neural plate tissue that has moved ventrally during the morphogenetic movements associated with anterior neuropore closure.

Recent work suggests that the telencephalon is first specified as a whole and then later subdivided into specific regional territories. Thus, the transcription factor brain factor 1 (BF1) [26], the earliest expressed telencephalic marker identified to date, is expressed throughout the prospective telencephalon from embryonic day 8 (E8) in mice [25••], whereas region-specific gene expression occurs only later [27,28]. Regionalization of the telencephalon is initiated when it undergoes dramatic morphogenetic changes as a result of anterior neuropore closure. This is achieved through the characteristic migration of telencephalic cells first anteriorly and then ventrally (see Figure 1).

Subsequent to anterior neuropore closure, discrete proliferative zones appear within the dorsal (pallial) versus the ventral (striatal) telencephalon, each expressing distinct sets of regional markers (reviewed in [25••,28]). For example, the ventral telencephalon expresses members of the *Distal-less* (*Dlx*) family of genes, whereas the dorsal telencephalon expresses *Empty spiracles* genes *Emx-1* and *Emx-2*, as well as *Pax-6*. Mutations in members of both the *Dlx* and *Emx* family of genes, produced through reverse genetics and naturally occurring mutations, such as *Small eye* (which results from a mutation in the *Pax-6*

Figure 1



The folding of the anterior neural plate results in the formation of the telencephalon. **(a)** Dorsal view of the neural plate just before anterior neuropore closure. **(b)** Side view of the forebrain, at a stage immediately after the telencephalic vesicles have formed. The arrows indicate the morphogenetic movement of cells resulting in the formation of the telencephalic vesicles. Two discrete types of cellular movements occur simultaneously. First, folding movements take place in which the edges of the neural plate fold upward and toward the midline: lower arrows in (a). Second, a forward and ventrally directed movement occurs, which shifts the resulting telencephalic vesicles into the anteriormost position of the neuraxis: top arrow in (a) and arrow in (b). A, anterior; D, dorsal; LGE, lateral ganglionic eminence; P, posterior; V, ventral. Adapted from Couly and Le Douarin [22].

gene), have recently been examined [17•–21•]. This work has demonstrated that these genes are involved in both patterning the regional territories that comprise the telencephalon (in the case of *Dlx-2* and *Emx-1*, *Emx-2*) and maintaining the compartmental segregation between the dorsal and ventral telencephalon (in the case of *Pax-6*).

Is the telencephalon segmentally organized?

From the time overt regional pattern within the telencephalon becomes evident, the development of the dorsal and ventral telencephalon rapidly diverges in terms of gene expression patterns, cellular differentiation and overall organization. This raises two fundamental questions concerning telencephalic development. What initiates regional differentiation within the telencephalon? Does this result from a cell autonomous restriction of the potential of dorsal versus ventral telencephalic cells or the influence of extrinsic environmental cues? At present, the answer to the first of these questions is unclear. However, recent experiments discussed below have begun to address the latter question.

Regionalization within the telencephalon could potentially arise by two mechanisms: an intrinsic mechanism [29,30], by which telencephalic cells and their progeny are committed to specific compartments, or an extrinsic mechanism, by which positional cues induce regional identity [31–33,34•]. In the context of this review, I will argue for the latter: that is, that regional gene expression within the telencephalon acts to produce positional cues that, in turn, subdivide the telencephalon into allocation territories. Operationally, an allocation territory is an area in which the cells are fated, but not committed, to a particular identity as a result of physical constraints preventing their movement to adjacent regions. Implicit in this concept, therefore, is the notion that cells within an allocation territory do not use lineage restriction as a means of establishing regional identity, but that they are specified by inductive cues.

One current opinion favors lineage restriction as a mechanism for establishing regional territories within the telencephalon. This view suggests that such patterning results from the establishment of transient, segment-like divisions (called prosomeres) within the forebrain [28]. These divisions are proposed to be analogous to the rhombomeric divisions seen in the hindbrain ([35,36]; see also [37] for a more detailed discussion of segmentation within the CNS). On the basis of both gene expression and morphology, the existence of prosomeric divisions within the diencephalon are clear [28,38]. In contrast, the exact location of prosomeric boundaries within the telencephalon is less apparent by either criterion. As a result, their precise position (and even their existence) is presently a matter of debate. Nonetheless, distinct proliferative zones with identifiable patterns of gene expression and morphology do appear within the telencephalon, but only after the prosomeric divisions are no longer evident. These zones will eventually give rise to the five major structures comprising the telencephalon: the cortex, the striatum, the pallidum, the septum and the limbic system. Unfortunately, as the prosomeric divisions only appear transiently (E8–E11 in mice), it is uncertain how they relate to the regional territories that form at later times (i.e. E12–E17). It is unlikely that the distinct morphological regions that appear later are derived solely from individual prosomeres: as the prosomeres are subdivisions of the longitudinal (anteroposterior [A/P]) axis and the regional zones within the telencephalon are not.

Figure 2 (legend) A comparison of the proposed prosomeric model to the major structures comprising the mature telencephalon. **(a)** Regional territories of differentiation within the E10.5 (on the left) and E15.5 (on the right) mouse brain. Two orientations are shown at each age. The top set shows a sagittal view, whereas the bottom set shows a dorsal perspective. The septum, the pallidum, the limbic system and striatum are not discernible as separate structures at E10.5. Rather, they are represented according to my guess of their approximate fate map locations. **(b)** Patterns of regional gene expression at E10.5 (on the left) and E15.5 (on the right). In this case, only a sagittal view of the E10.5 brain is shown and only a dorsal view of the E15.5 brain. The sagittal views of E10.5 brain are depicted as one proposed variation of the six prosomeric divisions (P1–P6) that has been hypothesized to divide the forebrain into longitudinally arranged segments [28]. Given the number and orientation of specific regions of differentiation within the telencephalon, it is evident that individual prosomeres do not give rise solely to specific regional territories of differentiation. The arrows show the anterior (A), posterior (P), dorsal (D) and ventral (V) directions of the neuraxis, projected from the neural plate stage. CRTX, cortex; LIM, limbic system; PALL, pallidum; SEPT, septum; STR, striatum.

The most prominent of the boundaries separating discrete proliferative zones lies between the dorsal (cortical or pallial) telencephalon (dark gray region in Figure 1b and orange regions in Figure 2a) and the ventral (striatal) telencephalon (generally referred to as the LGE or lateral ganglionic eminence; light gray region in Figure 1b and yellow regions in Figure 2a). Despite its lack of repetition, the irregular shape of the domains divided by it, and the fact that it runs along the longitudinal rather than transverse axis, this boundary possesses two of the hallmarks of a compartmental border: it both restricts cell movement and separates territories with differential patterns of gene expression [39,40]. In addition, lineage mapping in mouse suggests that cells within the striatum and cortex respect this boundary [41,42]. Interestingly, lineage mapping in the chick telencephalon similarly demonstrates that, whereas cell clones can extend throughout the A/P extent of the telencephalon, they are restricted to longitudinally oriented domains [43••]. Further support for the notion that the allocation of telencephalic cells to specific territories is an early step in regional specification comes from experiments comparing the calcium-dependent adhesion systems in dorsal (cortical) versus ventral (striatal) regions [44,45••]. These experiments indicate that during early neurogenesis, cells comprising these territories can sort out from one another *in vitro*.

All of these results are consistent with dorsal (cortical) and ventral (striatal) telencephalic cells being restricted in their 'compartmental' identity. However, the way to test whether progenitors within the telencephalon are committed to a specific regional phenotype is to transplant them across the cortical/striatal boundary.

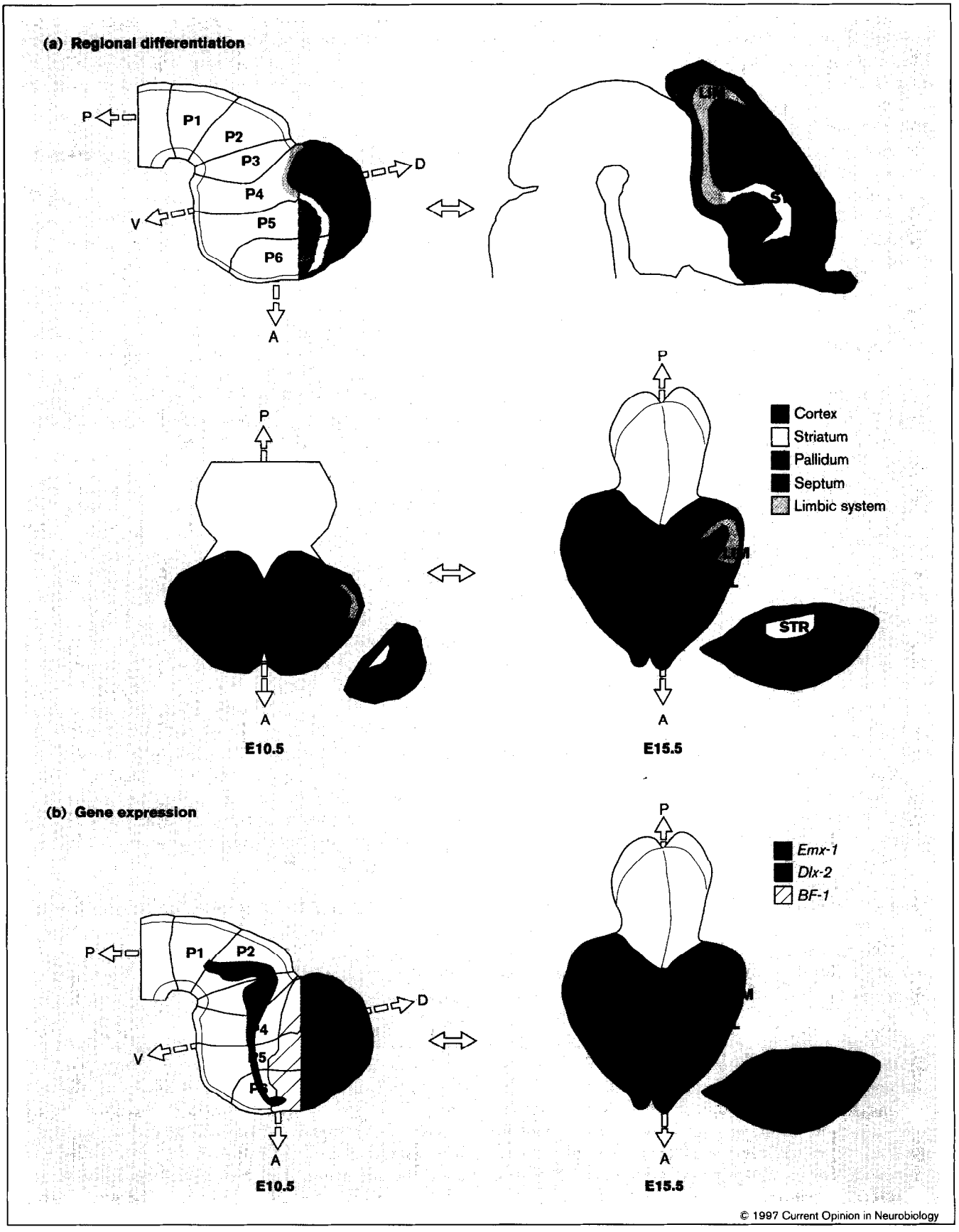
Telencephalic grafts demonstrate that regional identity is not irreversibly specified

To address the issue of whether progenitors of the dorsal versus ventral telencephalon are restricted in their potential, I and others have grafted striatal precursors at various developmental stages [4•,46••–48••]. Rather than using more traditional intraparenchymal methods (i.e. transplantation directly into brain tissue), we made these grafts by introducing cells into the cerebral ventricles and allowing them to reintegrate of their own accord. Despite introducing these grafts at various times from early to late neurogenesis, ventral telencephalic cells were consistently able to integrate widely and differentiate appropriately within a number of different telencephalic host regions. This was judged by their morphology, their expression of host-region-specific markers and their ability to make host-specific axonal projections.

However, the extent to which ventral telencephalic cells are positionally specified, as evidenced by their preferential re-incorporation into the ventral telencephalon, was found to be different. Even though I [46••] and Brüstle *et al.* [48••] found that cells showed no preference for incorporating within the telencephalon, Campbell *et al.* [47••] found that in over 90% of grafts, a percentage of the grafted progenitors integrated into the striatum. One reason that may account for these differences is that the former experiments were performed using cells at relatively late phases of neurogenesis (E16–E18 in rats, which is approximately equivalent to E15–E17 in mice) [46••,48••], whereas Campbell *et al.* [47•] performed their experiments at earlier stages (E13–E15 in rats).

In addition to age, a critical difference between these sets of experiments was that I [46••] and Brüstle *et al.* ([48••]; O Brüstle *et al.*, personal communication) removed cell surface molecules (by calcium-free protease treatment) from the donor cells before transplantation, whereas Campbell *et al.* [47••] did not. Rather, this group mechanically dissociated their donor cells or, in a subset of experiments, treated them with protease in the presence of calcium (the significance of this is that calcium-dependent adhesion systems have been shown to be protected from proteases when calcium is present [49]). This is relevant because calcium-specific adhesion systems distinguish between the dorsal versus the ventral telencephalon early but not later in development [45••]. Together, these findings suggest that calcium-dependent differences in cellular adhesion are intimately associated with the establishment of regional identities. It is important to note, however, that even though these regional identities are specified, they are not determined: a grafted cell that integrates heterotopically will differentiate according to its new environment and not to its region of origin [46••].

Figure 2


(a) Regional differentiation
P
P1
P2
P3
P4
P5
P6
D
V
A
S
P
A
E10.5
E15.5
Cortex
Striatum
Pallidum
Septum
Limbic system
STR
(b) Gene expression
Emx-1
Dlx-2
BF-1
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Interestingly, investigators who have examined the potential of grafted dorsal progenitors have also obtained different results [4•,48••]. Brüstle *et al.* [48••] saw widespread integration of dorsal (cortical) telencephalic cells (E14 mouse, early to mid neurogenesis). By contrast, Frantz and McConnell [4•] saw almost exclusive homotopic integration after grafting dorsal (cortical) telencephalic cells (E32 ferret, early to mid neurogenesis, layer 5 and E40 ferret, mid to late neurogenesis, layer 2–3). Perhaps these differences stem from the fact that these experiments were performed in different species: phylogenetic differences between mouse and ferret cortex are marked. In mice, the birthdate of cells that will occupy different cortical laminae overlaps, whereas in ferret, cells that occupy different layers of cortex are born sequentially, at distinct times, over a much more protracted period of development. The possibility that regional determination occurs at different times in different mammals warrants further investigation.

Another issue that arises from these experiments is whether all cells or only a subpopulation of more pluripotent ones are able to change their regional phenotype. Retrospective analysis of the distribution of donor cells in host animals cannot address this issue as to do so would require both knowledge of the degree of regional specification of a cell before transplantation and the ability to follow its progeny after grafting. The resolution of this issue awaits methods that are able to identity and sort subpopulations of progenitor cells before transplantation. Encouragingly, candidate markers have recently been identified by their homology with genes in *Drosophila*. Mouse genes homologous to proneural genes such as the *achaete–scute* [50] and the atonal [51,52] family of genes, as well neurogenic genes, including *notch*, *delta*, *HES-1*, *HES-3* and *jagged*, probably control neural differentiation events in vertebrates (reviewed in [53]). Similarly, the recent identification of a mammalian homolog to the *Drosophila numb* gene [54] (which is thought to be involved in asymmetric cell division) provides another excellent candidate (see Huttner and Brand, in this issue, pp 29–39). With these genes as a starting point, methods such as panning [55] and the use of green fluorescent protein (GFP; [56]) in fluorescent-activated cell sorting (FACS) open up the exciting possibility of directly addressing whether all progenitors or only a subset of pluripotent ones remain responsive to positional cues throughout the course of development.

In summary, experimental evidence from rodents suggests that cells that give rise to the regional divisions of the telencephalon are fated to populate a particular region but are not committed to doing so. Hence, at least a subpopulation of progenitors retain the ability to respond to positional cues outside of their immediate environment. These results imply that regional divisions of the telencephalon are behaving as allocation territories rather than as compartments [57]. Moreover, the adoption of specific regional phenotypes appears to result from local inductive cues rather than lineage restriction, analogous to the development of the dorsoventral (D/V) axis within the spinal cord and hindbrain [32,58,59•]. The restricted movement of progenitor cells between different telencephalic regions (by borders or selective adhesion), therefore, may facilitate commitment of cells to a particular regional fate.

How might positional cues act to regionalize the telencephalon?

Two different non-neural tissues have been implicated in imposing D/V pattern within the spinal cord. Ventral identity appears to be conferred by the action of axial mesoderm (i.e. notochord). Recently, the protein Sonic Hedgehog (Shh) has been demonstrated to activate ventral spinal cord genes, such as *Islet-1* and *HNF3*β [60–63]. Similarly, surface ectoderm has been implicated in inducing the expression of dorsal spinal cord markers, such as *dorsalin* and *slug* [64,65]. Here, the bone morphogenetic proteins BMP4 and BMP7, both of which are strongly expressed by this tissue, appear to be able to mimic the dorsal-inducing activity of ectoderm.

Are similar D/V inductive events implicated in the telencephalon? A number of lines of evidence from recent experiments suggest so. The examination of *Shh* homozygous null mutants reveals a failure to develop ventral structures along the entire extent of the neuraxis, including the telencephalon [66••]. Like the notochord, the axial mesoderm underlying diencephalon (the prechordal plate) expresses *Shh* [67••]. Removal of this structure in amphibians results in loss of midline forebrain structures [68,69]. Direct evidence for the involvement of *Shh* in ventral forebrain patterning has been demonstrated in two separate studies. Barth and Wilson [70] have demonstrated in zebrafish that Shh RNA injections can induce ectopic *nk2.2* expression (a ventral marker) in dorsal diencephalon. Similarly, a study in chick suggests that Shh can induce the expression of *nk2.1* (a gene related to *nk2.2*, with a similar expression pattern) in the diencephalon and the telencephalon [67••].

Clearly, some of the molecules that act to pattern the D/V axis in the spinal cord play a similar role in the forebrain. At present it is uncertain, however, how many of the molecular mechanisms used in these areas are conserved. That differences exist in the genetic pathways utilized would not be surprising, as analysis in *Drosophila* has revealed that the terminal regions of flies use a set of genetic determinants distinct from those used in establishing patterning within thoracic and abdominal regions [71]. In this regard, certain vertebrate genes have already been shown to be vital for forebrain development, but dispensable in the spinal cord [13•]. In addition, a number of genes that have their expression patterns largely restricted to the forebrain are necessary for the proper development of that region [14••,15•,16•]. Indeed, a novel gene, *Cerberus*, has recently been identified and

appears to be involved in directing head organization [72••]. Together, a picture is beginning to emerge that suggests that while some of the molecular mechanisms underlying forebrain regionalization may be distinct from those acting to pattern more posterior regions of the nervous system, both are determined by positional cues. This suggests that, as has been done so successfully in spinal cord and hindbrain, regional patterning in the forebrain may be achieved through mechanistic dissection by experimental means.

Conclusions

Understanding the mechanisms that establish regional pattern within the telencephalon is still in its nascent phase. Even though the prosomeric model of the telencephalon provides a framework on which to map transient morphology and early gene expression, it reveals little of how these exquisite regional patterns are established. The findings reviewed here suggest that inductive influences rather than lineage restrictions are likely to control telencephalic regionalization. Understanding the molecular and cellular nature of these positional cues will require multiple approaches, including targeted gene ablation studies in mice and large-scale mutagenesis in zebrafish. In addition, both *in vitro* and *in vivo* experimental manipulations should transform our understanding of telencephalic development from being descriptive to being mechanistic.

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