Longitudinal organization of the anterior neural plate and neural tube

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SUMMARY

Over the last century, several morphological models of forebrain organization have been proposed that hypothesize alternative topological solutions for the relationships of the histogenic primordia. Central to all of these models are their definitions of the longitudinal axis and the longitudinal organization of the neural plate and neural tube. To understand the longitudinal organization of the anterior brain, we have sought to identify molecular properties that are continuous along the entire longitudinal axis of the embryonic CNS. In this essay, we describe studies of the expression of several genes in the mouse between 7.5 (presomite stage) and 10.5 days post coitum (dpc) that provide evidence for the trajectory of the anterior-posterior axis and the longitudinal organization of the anterior CNS.

Specifically, we report that the expression of noggin, sonic hedgehog and Nkx-2.2 define longitudinal columns of cells that are present along the entire CNS axis. Within the forebrain, the expression of these genes, as well as that of Nkx-2.1 and BF-1, are in distinct longitudinal regions in the neural plate and tube. We demonstrate that the earliest longitudinal axon pathways of the forebrain are spatially correlated with the longitudinal domain defined by Nkx-2.2. Finally, expression of the former genes, and Otx-1 and Emx-2, suggests that the cephalic neural plate is organized into molecularly distinct domains delimited by longitudinal and transverse borders; these results provide a foundation for defining the mechanisms that pattern the neural plate.

Key words: forebrain, prosencephalon, pattern formation, axis, brain development, hedgehog, homeobox genes

INTRODUCTION

There is renewed interest in understanding the organization of the embryonic forebrain. Over the last century, prominent neuroembryologists have attempted to elucidate the primary forebrain subdivisions and their morphological relationships with more caudal brain parts (reviewed in Keyser, 1972; Puelles and Rubenstein, 1993). However, discrepancies regarding several basic morphological aspects, including the organization of longitudinal domains of the forebrain, has led to divergent conclusions.

Recently, the discovery that putative regulatory genes are expressed in regionally restricted patterns in the developing forebrain has provided new tools for defining, at higher resolution, histogenetic domains and their boundaries. The gene expression patterns provide tests of the explicit or implicit predictions of the diverse morphological conceptions of forebrain organization.

In this essay, we discuss the evidence: (1) that longitudinal zones traverse the forebrain and extend through the midbrain, hindbrain and into the spinal cord; (2) that the neural plate is subdivided by topologically longitudinal and transverse zones of gene expression and (3) that the early longitudinal axonal scaffold generally correlates with the cells that express a particular longitudinal gene marker.

HISTORICAL CONCEPTS OF THE LONGITUDINAL AXIS AND ORGANIZATION OF THE FOREBRAIN

The complex and dynamic morphology of the developing forebrain has led to multiple interpretations of the longitudinal axis in the forebrain. Some authors who have focused their studies on the closed neural tube have conceived the longitudinal axis as a bent line centred in the ventricular lumen. This line has been held to end anteriorly at different positions: the optic recess, lamina terminalis, the neuroporic recess or within the telencephalic vesicle (for example, von Kupffer, 1906; Kuhlenbeck, 1973; Altman and Bayer, 1986, 1995). The abstract nature of this sort of axis limits its usefulness to didactic purposes and to serving as an operational reference for describing planes of section. Other concepts of the longitudinal axis can be based on a topological analysis of the neural plate following the ideas of His (1893) (Fig. 1). In this formulation, the neural plate is organized in symmetric halves relative to a median axial region referred to as the prospective floor plate of the closed neural tube. This region overlies the axial mesodermal organizers (prechordal plate and notochord). Within this schema, there are four principal longitudinal zones in the neural plate: the primordia of the floor, basal, alar and roof plates. Different models of the longitudinal organization of the anterior CNS can be represented by different topological arrangements of these zones (Fig. 1).
two lines correspond to schemas A and C. The schema to the left (A) shows one of the main current conceptions, in which the alar/basal boundary does not converge upon the axis of symmetry and extends to the rostral border of the neural plate. Here it would intersect the prospective telencephalic area according to available fate maps. The rostral border of the neural plate would thus theoretically contain distinct portions with basal or alar properties, before passing into the roof plate anlage. The vertically striped line identifies where the anterior-most end of the brain lies in this schema. The schema to the right (C) shows another option, in which the alar/basal limit converges upon the anterior part of the symmetry axis in front of the end of the prospective floor plate and concentric to the roof anlage. The anterior-most end of the brain is represented by the horizontally striped line. Even within this sort of conception, variations exist due to options placing the end of the floor plate ends with the prerubral tegmentum (PTG). This leaves

Theoretically, the rostral end of the limit between the alar and basal plates may be conceived in different ways (Fig. 1B), although most models propose that it is either non-convergent (Fig. 1A) or convergent (Fig. 1C) upon the median axis of the neural plate. The non-convergent option extends this boundary to the anterior border of the neural plate, and implies that basal and alar plates reach the rostral end of the neural plate (Fig. 1A). Contrarily, the convergent option implies that the roof, alar and basal plates concentrically cross the midline of the anterior-most neural plate (Fig. 1C).

Each model predicts different arrangements of the longitudinal domains as the neural plate develops into the neural tube. One can reduce the main alternatives of the longitudinal organization of the anterior CNS to the three representative options (Fig. 2). Various other elements of each schema (i.e., transverse boundaries or ventricular sulci) are left out here for clarity. The arrangement of the primary zones of the neural plate in Fig. 1A would result in the forebrain organization shown in Fig. 2A, whereas those in Fig. 1C lead to the models shown in Fig. 2B,C.

Model 2A extends the basal plate into the so-called basal telencephalon. This was chosen by Keyser (1972) and others cited by him, because of the relatively precocious differentiation in the ganglionic eminences in the telencephalon, in accordance with the proposal of His that basal plate structures differentiate first. Neither Altman and Bayer (1986, 1995) nor Swanson (1992) has given specific reasons for their similar choice. The floor plate is assumed to end at the isthmic fovea, following Kingsbury’s conclusion (Kingsbury, 1920).

Model 2B postulates, contrary to Model 2A, that the basal plate ends with the prerubral tegmentum (PTG). This leaves the forebrain as an alar plate territory, devoid of floor and basal plates.

Model 2C follows closely the criterium of His (1893) on the basal plate (Puelles et al., 1987a) and also postulates, contrary to the other models, the more rostral extension of the floor region, consisting of epichordal and prechordal parts (Puelles and Rubenstein, 1993; Rubenstein et al., 1994).

If any one of these morphological/embryological conceptions is correct, one expects to find cellular and/or molecular properties that are totally or partially isomorphic with the proposed longitudinal domains (in the simplest case, from the caudal end of the spinal cord to the rostralmost forebrain). Thus, we sought to identify molecular characteristics that are expressed in longitudinal stripes along the anterior-posterior (A-P) axis.

There are several genes that are expressed in longitudinal stripes in different dorsoventral (D-V) positions in the spinal cord that extend into the brain. These include 

- **Dbx** (Lu et al., 1992), **Msx-1** (Hill et al., 1989), **Nkx-2.2** (Price et al., 1992), **Pax-3**, **Pax-6**, **Pax-7** (Stoykova and Gruss, 1994), **sonic hedgehog** (Shh) (Echelard et al., 1993), and **Wnt-1**, **Wnt-3** and **Wnt-3a** (Parr et al., 1993). However, among these, only **Shh** and **Nkx-2.2** extend into the rostralmost areas of the prosencephalon. Thus, we chose to study their expression in the mouse in detail.

**Nkx-2.2 AND Shh ARE EXPRESSED IN PARALLEL ADJACENT STRIPES ALONG THE ENTIRE ANTERIOR-POSTERIOR AXIS OF THE CNS**

Nkx-2.2 is a homeobox gene (Price et al., 1992), whose expression is first detectable at the 1-somite stage in a median rostral region of the neural plate, just anterior to the underlying rostral tip of the notochordal plate (Fig. 3A). Shh (also known as **vhh-1**) encodes a molecule implicated in floor plate and motor neuron induction (Echelard et al., 1993; Krauss et
Longitudinal organization of the brain al., 1993; Roelink et al., 1994, 1995; Martí et al., 1995), as well as ventral forebrain markers (Ericson et al., 1995). The previous studies established that it is expressed in a continuous stripe in the neural tube, apparently close to the Nkx-2.2 stripe. We have extended these studies by comparing Nkx-2.2 expression with that of Shh.

Nkx-2.2 and Shh expression patterns define adjacent and non-overlapping longitudinal neuroepithelial zones that extend along the entire CNS and end anteriorly, where they cross the midline in the optochiasmatic region (see legend to Fig. 3 for details). The spatiotemporal changes in expression patterns of both genes are schematically summarized in Fig. 3L.

The D-V position of their expression changes at transitions between major brain regions. In the spinal cord and hindbrain, Shh is expressed in the ventral midline cells (floor plate) and Nkx-2.2 is expressed in the adjacent paramedian plate cells (Fig. 3K). At the isthmus (midbrain-hindbrain transition zone), expression of both genes gradually expands dorsally, so that the Nkx-2.2 and Shh stripes occupy larger ventral portions in the midbrain and forebrain (Fig. 3J). At the zona limitans, the future interthalamic boundary, both genes show a dorsal extension along the boundary that becomes increasingly prominent from 9.5 dpc (Fig. 3G,H). Unlike in more caudal regions of the CNS, Shh expression in the secondary prosencephalon does not extend to the ventral midline (Fig. 3G-I) (Echelard et al., 1993; Roelink et al., 1994).

While there are complexities in these expression patterns (discontinuities, dorsal deflections and a major change in D-V position), the basic finding is that the expression of Nkx-2.2 and Shh provide evidence for the trajectory of the longitudinal axis of the CNS and for the continuity of certain longitudinal properties in two large regions of the CNS (forebrain/midbrain and hindbrain/spinal cord). Similar findings have recently been reported for these genes in zebrafish (Barth and Wilson, 1995); this study also provided evidence that Shh can regulate Nkx-2.2 expression. In a later section, we address whether the...
Fig. 3. Expression of *Nkx-2.2* and *Shh* define adjacent longitudinal domains along the entire CNS. (A-C) Dorsorostral views of neural plate stage embryos; (D-F) lateral views of neurulation stage embryos; (G-H) mid-sagittal views of the brain at 10.0 and 11.5 dpc, respectively; (I-K) transverse sections at 11.5 dpc. (A) *Nkx-2.2* at 2 somites; expression begins in front of the head process (small arrow) and extends near to the anterior edge of the neural plate (arrowhead). Anterior is towards the bottom of the panel. (B) *Nkx-2.2* at 8 somites; bilateral stripes of expression cross the midline (arrowhead) and posteriorly end at the presumptive zona limitans. (C) *Shh* at 8 somites; transcripts occupy a median position of the anterior neural plate, where it forms a narrow loop in the floor of the neural groove (arrowhead). (D) *Nkx-2.2* at 11 somites; separate domains of expression are present in the forebrain, midbrain/posterior diencephalon, hindbrain and anterior spinal cord (arrows). (E) *Shh* at 12 somites; expression extends from the anterior forebrain to a posterior limit in the anterior hindbrain. (F-H) Simultaneous detection of *Nkx-2.2* (brown in F, purple in G,H) and *Shh* (purple in F, brown in G,H) at 13 somites (F), 10.0 dpc (G), and 11.5 dpc (H). Both genes are expressed in adjacent domains and cross the midline just ventral to the optic stalk (large arrows in G,H). At 13 somites (F), their expression in the prosencephalon is not in adjacent domains (asterisk), whereas at later stages they are expressed in adjacent stripes along the entire A-P extent of the CNS (G-K). They have a dorsal deflection at the zona limitans (large arrowheads in G,H) and *Nkx-2.2* has dorsal deflections at the interrhombomeric boundaries (small arrowheads in G). The rostral limit of *Shh* expression at the ventral midline is marked by an open arrow in G. *Shh* expression also begins in a separate domain in the medial ganglionic eminence by 9.5 dpc (small arrows in G,H). Transverse sections demonstrate that the *Nkx-2.2* and *Shh* domains are adjacent at the level of the postoptic forebrain (I), caudal diencephalon (J), and hindbrain and spinal cord (K). The planes of section are indicated in G, except for K, which sectioned the spinal cord transversely and the hindbrain at the level of cervical flexure.
(L) Schematic representation of spatiotemporal changes in Nkx-2.2 (red) and Shh (green) expression in the axial mesoderm and the neuroectoderm, from early somitic stages to 10.0 dpc. Abbreviations: di, diencephalon; dt, dorsal thalamus; f, forebrain; fp, floor plate; h, hindbrain; he, heart; hf, neural plate; hp, head process; is, isthmus; m, midbrain; ma, mammillary area; me, mesencephalon; mg, medial ganglionic eminence; n, notochord; oc, optic cup; os, optic stalk; pos, postotic sulcus; pt, pretectum; r2/3, rhombomere 2/3 boundary; rh, rhombencephalon; rt, Rathke’s pouch; sc, spinal cord; sp, secondary prosencephalon; t, telencephalic vesicle; tb, tailbud; tu, tuberal hypothalamus; vt, ventral thalamus; zl, zona limitans. Scale bars, 200 μm for A–H and 100 μm for I–K.

Methods. 2,026 bp of cDNA encoding murine Nkx-2.2 were isolated and sequenced. Whole-mount in situ RNA hybridization was carried out according to Shimamura et al. (1994) and Jowett and Lettice (1994). As chromogenic substrates for alkaline phosphatase, NBT/BCIP and Vector alkaline phosphatase substrate kit II (Vector Laboratories) were used. Stained samples were embedded and sectioned with a cryostat at 15 μm.

Fig. 4. Longitudinal axonal tracts compared to the Nkx-2.2-positive stripe.

(A) Lateral view of 10.5 dpc brain stained for αN-catenin protein (brown) and Nkx-2.2 mRNA (purple). Selected transverse sections of a similar preparation are shown in B–E; section planes are indicated in A, except for E which shows the cervical spinal cord. A cluster of neurons ventral to the median end of the Nkx-2.2 domain, the anterobasal nucleus (abn), produces the axons that project caudally as the tract of the postoptic commissure (tpoc). The dorsal margin of the TPOC lies near the dorsal boundary of the Nkx-2.2 stripe (B,C). Rostrally, in the region around the optic stalk, the TPOC forms a tight fascicle that is largely contained within the Nkx-2.2-expressing domain, but spreads slightly ventralwards (A–C). Caudal to the zona limitans, the TPOC mixes with other tracts, both ventral and dorsal to the

Nkx-2.2 stripe (Fig. 4A); the ventral diencephalon contains many axons originating in the presumptive mammillary area, the posterior commissure or the interstitial nucleus of Cajal (Chédotal et al., 1995). Abbreviations are the same as the former figures. abn, anterobasal nucleus; ac, anlage of anterior commissure; cf, commissural fibers; drg, dorsal root ganglion; dvdt, dorsoventral diencephalic tract; mc, motor column; mlf, medial longitudinal fasciculus; nV, exit point of the trigeminal nerve; poc, postoptic commissure; rp, roof plate; sot, supraoptico tract; tmesV, mesencephalic trigeminal tract; tpoc, tract of postoptic commissure. Scale bars, 200 μm for A, and 100 μm for the others.

Methods. Simultaneous detection of protein and mRNA was done as described in Shimamura et al. (1994).
expression of Nkx-2.2, Shh and other molecules suggest the continuity of the principal longitudinal zones along the entire A-P axis.

LONGITUDINAL AXON TRACTS AND THE STRIPE OF Nkx-2.2 EXPRESSION FOLLOW THE SAME TRAJECTORY

We noted the striking similarity in the position of some of the earliest axon tracts (Easter et al., 1993) with the stripe of Nkx-2.2 expression. Accordingly, we examined this spatial relationship at 10.5 dpc by simultaneous in situ RNA hybridization for Nkx-2.2 and immunohistochemistry for $\alpha$N-catenin. The latter is a cadherin-associated molecule predominantly expressed in developing neurons and axons (Uchida et al., 1994).

The earliest major longitudinal tract (Fig. 4A) originates from the anterobasal nucleus (Abn; Fig. 4B) just ventral to the prospective optic chiasma (Easter et al., 1993). These neurons produce axons that project caudally as the tract of the postoptic commissure (TPOC), whose trajectory is highly correlated with the Nkx-2.2-positive stripe (Fig. 4A-C) (see details in the legend to Fig. 4). Thus, one of the earliest axon pathways of the forebrain follows a course that partly overlaps the ventricular stripe of Nkx-2.2 expression. This result suggests that the mechanisms guiding longitudinal axonal growth may be regulated by longitudinally arranged neuroepithelial domains. There is considerable interest in the possibility that boundary zones separating neuroepithelial domains may provide spatial information that direct the growth of axons (Wilson et al., 1993; Chien and Harris, 1994; MacDonald et al., 1994; Chédotal et al., 1995). Nkx-2.2 expression might define such a molecularly distinct longitudinal boundary zone.

LONGITUDINAL COLUMNS OF THE FOREBRAIN

As discussed earlier, there are varying ideas regarding the trajectory of the roof, alar, basal and floor plates into the forebrain as shown in Figs 1 and 2. To address these options, we have sought to identify molecular markers for each longitudinal domain and to determine the A-P extend of their expression.

We used the expression of noggin to follow the course of the roof plate. noggin encodes a secreted polypeptide with neural-inducing properties (Lamb et al., 1993). noggin is expressed in the roof plate along the entire A-P extent; its anterior-most expression approximates the end of the prosencephalic roof plate and does not enter lamina terminalis (Fig. 5A,E). This site corresponds topographically the anterior-most border of the neural plate suggested by marking experiments in the chick embryo (Puelles et al., 1987b). These findings are consistent with the model in Fig. 2C.

There are several genes that are expressed in the alar plate of the spinal cord that show continuous expression into the brain. For instance, Pax-3 is expressed in a continuous column from the spinal cord into the caudal forebrain (Goulding et al., 1991). Recently, a zinc finger-containing gene named Zic has been shown to be expressed in the alar plate along the entire A-P axis at 9.0-10.0 dpc (Aruga et al., 1994). Zic’s anterior-most expression ends in a region that is dorsal to where the stripe of Nkx-2.2-expressing cells cross the midline. This result is consistent with the interpretation that Nkx-2.2 expression
approximates with the alar/basal boundary of the forebrain, a point that we address in greater detail below.

The patterns of gene expression also show that the basal plate exhibits both continuous and region-specific molecular properties along the axis. In the spinal cord, distinct patterns of Lim-homeobox gene expression are correlated with the functional organization of the motor columns (Tsuchida et al., 1994). The expression of several of these genes, as well as Wnt-7b (Parr et al., 1993; Shimamura and Rubenstein, data not shown), extends from the basal plate of the spinal cord into the basal plate of the hindbrain, midbrain, diencephalon and mammillary area. Other histological markers, such as acetylcholinesterase and neuronal birth date measurements show continuity of basal plate properties (i.e. early differentiation) along the entire CNS (Puelles et al., 1987a, and references therein).

The transition zone between the alar and basal plates of the spinal cord is approximated by a morphological feature named the sulcus limitans. There is evidence that cells in this zone have distinct molecular properties. A homeobox gene named Dbx is expressed in a longitudinal stripe that is just ventral to the sulcus limitans (Lu et al., 1992; Shimamura and Rubenstein, data not shown). Dbx expression in this longitudinal column ends in the isthmic region at 9.5 dpc. Nkx-2.2 expression also exhibits a discontinuity at the isthmus. Posterior to the isthmus, Nkx-2.2 is expressed in paramedian stripes that separate the floor plate from the longitudinal columns of motor neurons (Fig. 3K); anterior to the isthmus, its expression extends to the front of the brain in a more dorsal position (Fig. 3G-J). Because the Nkx-2.2 stripe is dorsal to the oculomotor nucleus and midbrain tegmentum (Fig. 4A), it may correspond to the alar/basal boundary of the midbrain and forebrain.

The floor plate, which is the most ventral longitudinal column in the spinal cord, is specified by the notochord (reviewed in Jessell and Dodd, 1992; Smith, 1993). Previous studies showed that the floor plate ends at the caudal mammillary area; this region is near to the anterior end of the notochord (Puelles et al., 1987a; Hatta et al., 1991; Placecz et al., 1993). It is not established that a floor plate-like structure exists in the anterior forebrain. The prosencephalon rests on prechordal mesodermal tissues (prechordal plate and floor plate) that express noggin, BF-1 (Tao and Lai, 1992) and Shh, respectively, as molecular markers for these longitudinal zones.

What happens to the primary longitudinal zones at the anterior extreme of the CNS? Do they extend to the front of the brain? We have used the expression of several genes as markers for each of the major longitudinal domains as they project towards the front of the brain. For instance, consider the results of Nkx-2.2 expression described in the previous sections. If the line defined by Nkx-2.2 expression is parallel to the A-P axis of the CNS, then the front of the brain would be in the region of the optic chiasm, near to where Nkx-2.2 expression begins (Fig. 5A,B). To examine this idea in greater detail, we determined the region in the prosencephalon, where the roof, alar and basal plates converge, using the expression of noggin, BF-1 (Tao and Lai, 1992) and Shh, respectively, as molecular markers for these longitudinal zones.

The most anterior limit of noggin expression is dorsal to the lamina terminalis and the preoptic recess (Fig. 5A,E). The tissue just ventral to the noggin-positive roof plate expresses BF-1. The BF-1-expressing domain crosses the midline of the anterior brain (lamina terminalis) and includes the dorsal half of the chiasmatic plate. Note that the roof plate does not express BF-1. The stripe of Nkx-2.2 expression (Fig. 5C) is parallel to the ventral border of BF-1 expression and also crosses the anterior midline. The anterior expression of the basal plate marker (Shh) crosses the midline of the anterior neural tube just ventral to the stripe of Nkx-2.2 expression.

The topological relationship of these regional markers is summarized in Fig. 5E. Our data supports Model 2C which hypothesizes that the front of the brain should be conceived not as a single point, but as a median region where the roof and floor plates end and where the alar and basal plates cross the midline of the anterior-most part of the brain. To further address this point, we have investigated the organization of the primary longitudinal zones in the neural plate.

PATTERNS OF GENE EXPRESSION IN THE NEURAL PLATE HAVE LONGITUDINAL AND TRANSVERSE BOUNDARIES

Fate maps of the axolotl, Xenopus and chicken cephalic neural plate have been reported (Jacobson, 1959; Eagleson and Harris, 1990; Couly and De Douarin, 1988). However, the resolution of these studies does not clearly establish how the different primordia relate topologically to the two axial possibilities in Fig. 1A,C (i.e. do they represent longitudinal and/or transverse subdivisions within the anterior neural plate). Gene expression studies may prove useful in detecting transverse and longitudinal subdivisions of the anterior neural plate. For instance,
Expression of transcription factors defines an early regionalization in the cephalic neural plate and the embryonic brain. (A,D,G,J) The 7-somite stage embryos viewed dorsorostrally. Lateral (B) or oblique rostral views (E,H,K) of the 12- to 13-somite (B,E) or the 10-somite embryo (H,K). (C,F,I,L) Lateral views of 10.5 dpc dissected brains. (A-C) Expression of Nkx-2.1 is first detected at about the 1-somite stage (data not shown) in a median strip of the anterior neural plate and ends caudally near the cephalic flexure. At the beginning of anterior neuropore closure, two discontinuous domains (ventral and dorsal) of Nkx-2.1 expression are apparent (C); the ventral domain derives from the initial region of expression in the anteromedial region of the neural plate; it covers the postoptic hypothalamic area; the dorsal domain of Nkx-2.1 is found in the rostroventral telencephalon and includes the preoptic area, medial ganglionic eminence and anterior entopeduncular area. (D-F) Expression of BF-1 is first detectable in the ectoderm underlying the rostral edge of the neural plate at around the 3-somite stage (data not shown). Neuroectodermal expression of BF-1 begins at about the 5-somite stage, in the rostral margin of the neural plate (D). By 10.5 dpc (F), BF-1 is expressed in the preoptic area, the adjoining half of the optic stalk and most of the telencephalon, except for a caudomedial region of the pallium (Tao and Lai, 1992). (G-I) Expression of Otx-1 is in a transverse band in the region of the presumptive forebrain and midbrain at the 2- to 3-somite stage (data not shown). By 7 somites, its expression has an inverted U-shaped appearance in the prosencephalon (G). Its rostral limit has a sharp boundary that surrounds caudolaterally the incipient optic cup and expression extends down to the presumptive midbrain-hindbrain boundary (G,H). Otx-1 continues to be expressed in the forebrain and midbrain after neuropore closure, with sharp transverse boundaries within the secondary prosencephalon and at the isthmus (I). (J-L) Expression of Emx-2 in the neural plate is detected in a restricted region of the lateroaudal forebrain primordia (J,K) beginning at around the 3-somite stage (data not shown). After neural tube closure, Emx-2 is expressed in various forebrain domains as reported previously (Simeone et al., 1992a, 1993). These regions include a large part of the telencephalic pallium, the ventral thalamus placed anterior to the zona limitans intrathalamica, an anterior mammillary region and an intermediate preptectal population (L). Arrows in Otx-1 (G-I) and Emx-2 (J-K) panels: the small arrows show the posterior expression limit; the large arrows show the anterior expression limit; the arrowhead indicates a transverse line of decreased expression in the ridge of the cephalic flexure. Staining in the medial ganglionic eminence is an artifact (L). Abbreviations are same as the former figures. he, heart. Magnification is the same for each vertical column of panels. Scale bars, 100 μm for A and B, 200 μm for C.
Xash-3 is expressed in longitudinal stripes in the Xenopus neural plate (Zimmerman et al., 1993), and Wnt-1 is expressed in a segment-like domain in the mouse midbrain primordium (Echelard et al., 1994).

To determine whether the forebrain primordium is organized into molecularly distinct territories, which may relate to mature structures, we studied the expression patterns of Nkx-2.1, Nkx-2.2, Shh, Otx-1, Emx-2, noggin and BF-1 beginning at the late primitive streak stage up to 10.5 dpc (see legend to Fig. 6 for a more complete description).

Nkx-2.1 encodes a homeodomain-containing transcription factor whose expression in the developing forebrain was first described by Lazzaro et al. (1991) and Price et al. (1992). In the neural plate, it is expressed in an anteromedial domain whose expression does not extend to the anterior edge of the neural plate. Thus, its expression in the neural plate supports the axial conception in Fig. 1C. The other models in Figs 1 and 2 do not provide a satisfactory interpretation of the Nkx-2.1 pattern and its relationship to the Nkx-2.2 stripe (see Fig. 7A).

Neuroectodermal expression of BF-1 begins at about the 5-somite stage, in the rostral margin of the neural plate (Fig. 6D). The expression domains of Nkx-2.2 and BF-1 do not overlap and both cross the midline of the anterior neural plate (Fig. 7A). The BF-1 expression pattern can be conceived of as an alar plate marker of the prosencephalon (although not the entire alar plate) and is consistent with the Fig. 1C model.

Otx-1 and Emx-2 encode homeodomain proteins that are homologues of the Drosophila orthodontic (odt) and empty spiracles (ems) genes, respectively, that are expressed in restricted regions of the developing mid- and forebrain (Simeone et al., 1992a,b; Simeone et al., 1993). We found that both genes are expressed in simple patterns in the neural plate.

Otx-1 expression at 7 somites (G) has an inverted U-shaped appearance in the prosencephalic neural plate, which spans alar, basal and floor domains of the neural plate. Its rostral boundary may reflect topological orthogonality to the longitudinal axis. Expression of Emx-2 in the neural plate is found in laterocaudal forebrain primordia beginning at around the 3-somite stage (Fig. 6I).

These results, summarized in Fig. 7, show that the anterior neural plate is subdivided into molecularly distinct domains that express specific combinations of genes. Some of these domains have borders that can be interpreted as being parallel (BF-1, Nkx-2.2, Shh and Nkx-2.1) and others perpendicular (Nkx-2.1, Otx-1 and Emx-2) to the longitudinal axis.

**MECHANISMS THAT REGULATE THE LONGITUDINAL ORGANIZATION OF THE BRAIN**

The role of the notochord in D-V patterning of the spinal cord has now been firmly established (reviewed in Jessell and Dodd, 1992; Smith, 1993). In this essay, we discussed the evidence that the primary longitudinal subdivisions of the spinal cord extend to the front of the brain, implying that axial organizers have some common properties along the entire A-P axis. The recent finding that Shh can induce ventral neural tube properties at all axial levels is consistent with this hypothesis (Ericson et al., 1995). Although common properties of the axial mesodermal tissues can set up the longitudinal organization of the entire CNS, distinct properties remain at different positions along the A-P axis.

Two mechanisms have been postulated to regulate region-specific patterning of the longitudinal zones. One mechanism
(vertical induction model) would be due to spatiotemporal differences in the patterning signals produced by the axial organizer tissues: the notochord, prechordal plate and perhaps the foregut. The other mechanism (planar induction model) would rest upon intrinsic differences in the capacity of the neuroepithelium to respond to the patterning signals. Evidence supporting intrinsic differences in the competence of distinct regions of the neuroepithelium to respond to signals from axial organizers comes from the study of Hynes et al. (1995), who showed that tyrosine hydroxylase can be induced in the midbrain primordium using notochord pieces taken from midbrain or spinal cord levels. In addition, the neuroepithelium may lack competence to respond to signals from axial organizers in specific regions, as suggested by the lack of induction of floor plate markers in the prosencephalon by the notochord (Placzek et al., 1993).

Evidence supporting regional specification along the A-P axis via vertical induction comes from the work of Ang and Rossant (1993), who showed that the anterior notochord and not the posterior notochord is capable of inducing the expression of midbrain markers (En-1 and En-2) in pre-to-early streak ectoderm. In addition, Ang et al. (1994) showed evidence that anterior and posterior axial mesoderm produce signals that induce or repress Otx-2 expression, respectively. More recent studies also support this finding (Pannese et al., 1995; Blitz and Cho, 1995). In addition, mutation of Lim-1 homeobox gene leads to complete absence of anterior CNS structures without affecting caudal CNS structures, possibly by disrupting the prechordal mesoderm (Shawlot and Behringer, 1995). These studies provide evidence that the anterior mesoderm has unique properties that may lead to the induction of those markers that only appear in the anterior CNS.

Discontinuities in the expression patterns of several genes provide clues for the position of transitions in the inductive properties of axial organizers and/or the competence of the neuroepithelium to respond to these signals. For instance, at the isthmus and the zona limitans Nkx-2.2 has a gap or a deflection in its expression (Fig. 3G,H). Other genes, such as Shh (Fig. 3G,H), also show abrupt changes in their expression at these boundary regions. Both the isthmus and zona limitans have been implicated as functionally significant transition zones. For instance, transplantation studies have demonstrated that the isthmus produces morphogenetic factor(s) capable of planar patterning of the midbrain (Martinez et al., 1991) and that the zona limitans serves as a boundary for this inductive process.

Thus, both planar and vertical inductive processes appear to have roles in region-specific patterning within longitudinal columns. Therefore, combinations of these signals can provide additional complexity to allow for the specification of the diverse tissues of the CNS.

We thank the following people for gifts of probes: Drs R. Harland (noggin), E. Lai (BF-1), A. McMahon (sonic hedgehog), A. Simeone (Ems-2 and Ots-1) and M. Takeichi (αN-catenin). We also acknowledge Drs T. Doniach and C.-M. Fan for teaching us the two-color in situ hybridization method, and S. Liu for her technical support. This work was supported by the research grants: to J. L. R. R. from March of Dimes, NARSAD, the John Merck Fund, and NIMH RO1 MH49428-01, RO1 MH51561-01A1 and K02 MH01046-01, to K. S. from the Ministry of Education, Science and Culture of Japan, to S. M. from European Biotech BIO2-CT93-0012 and NATO, and to L. P. from Spanish DGICYT PB93-1137 and European Biomed BMH-CT94-1378. K. S. is a recipient of JSPS postdoctoral fellowships for research abroad.

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(Accepted 21 August 1995)