

Early Steps in the Development of the Forebrain

Review

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The tremendous complexity of the adult forebrain makes it a challenging task to elucidate how this structure forms during embryonic development. Nevertheless, we are beginning to understand how a simple epithelial sheet of ectoderm gives rise to the labyrinthine network of cells that constitutes the functional forebrain. Here, we discuss early events in forebrain development—those that lead to the establishment of the anterior neural plate and the regional subdivision of this territory into the different domains of the prospective forebrain.

The Structure, Origins, and Morphogenesis of the Forebrain

Our conscious thoughts, our emotions, and many of our memories reside within the forebrain, and indeed it is this region of our CNS that confers many uniquely human attributes. Despite this, the general organization of the forebrain is conserved in all vertebrates. What makes the brain of each species unique is not the initial presence or absence of different subdomains of the CNS; rather, it is the extent to which these domains are elaborated as they form the various structures that comprise the mature brain. Early steps in CNS patterning are largely conserved, and studies primarily undertaken in chick, fish, frog, and mouse are beginning to unravel the mechanisms by which the forebrain is induced and patterned.

The forebrain arises from anterior neuroectoderm during gastrulation, and by the end of somitogenesis it comprises the dorsally positioned telencephalon and eyes, the ventrally positioned hypothalamus, and the more caudally located diencephalon (Figures 1D–1G). The diencephalon contains, from rostral to caudal, the prethalamus (or ventral thalamus), the thalamus (or dorsal thalamus), and the pretectum. Sitting between the prethalamus and thalamus is a prominent boundary region termed the zona limitans intrathalamica (zli), a structure that may constitute a separate subdivision of the diencephalon (Figure 1E; Zeltser et al., 2001). In recent years, the prosomeric model of forebrain organization has provided a framework for understanding many studies of forebrain development, and a thorough

discussion of the latest incarnation of this model can be found elsewhere (Puelles and Rubenstein, 2003).

Despite its complexity, the forebrain derives from a simple sheet of neuroepithelial cells. However, morphogenesis is more complex than in other regions of the CNS. In consequence, from looking at the mature forebrain, the derivation and topological relationships of its component parts are not immediately obvious. In this regard, fate-mapping studies, although incomplete in all model species, have helped to reveal the neural plate origins of the cells that comprise the different regions of the forebrain. An unexpected finding of these studies is that the dorsal and ventral forebrain have different origins within the gastrula ectoderm. In fish, cells destined to contribute to the hypothalamus are located caudal to prospective dorsal forebrain tissue (telencephalon and eye field) and are close to the organizer where they are intermingled with prospective floorplate cells (Figure 2A; Mathieu et al., 2002; Woo and Fraser, 1995). From this location, prospective hypothalamic cells move rostrally within the neural plate, displacing more dorsal forebrain tissue laterally (Figures 1A and 1B; Varga et al., 1999). In chick, the anlage of the entire forebrain moves rostrally (Figures 2D–2G; Foley and Stern, 2001), but, as in fish, movements may occur to a greater extent in prospective ventral than in more dorsal neural tissue (e.g., Patten et al., 2003).

One consequence of the rostral movement of ventral forebrain cells is the lateral displacement of eye field cells from an initially unitary field spanning the midline toward the prospective left and right optic vesicles (Figures 1A and 1B; Varga et al., 1999). Subsequent to this, the optic vesicles evaginate from the lateral wall of the forebrain and the most distal cells invaginate to form the neural retinal layer of the optic cup. The cells that line the back of the optic cup differentiate as pigmented epithelium and the cells that connect the optic cup to the rest of the forebrain form the optic stalk and differentiate as glial cells of the optic nerve (Figure 1G).

Telencephalic precursors are located rostral and lateral to the eye field, adjacent to the anterior margin of the neural plate (Figures 1A–1C; Cobos et al., 2001; Eagleson et al., 1995; Fernandez-Garre et al., 2002; Inoue et al., 2000; Rubenstein et al., 1998; Varga et al., 1999; Whitlock and Westerfield, 2000). Prospective subpallial (ventral) telencephalic cells are located rostrally, directly in front of the eye field, whereas prospective pallial precursors are positioned more caudally along the neural plate margin, in continuity with the dorsal diencephalon (Cobos et al., 2001; Whitlock and Westerfield, 2000). Diencephalic precursor cells are located caudal to the eye field (Figures 1A and 1B), but it is not known if the prethalamic, thalamic, and pretectal subdivisions are fully elaborated at the neural plate stage.

The Anterior Neural Plate Is “Protected” from the Influence of Caudalizing Factors

There are at least three major steps in the formation of the prospective forebrain. Ectodermal cells must acquire neural identity, rostrally positioned neural tissue

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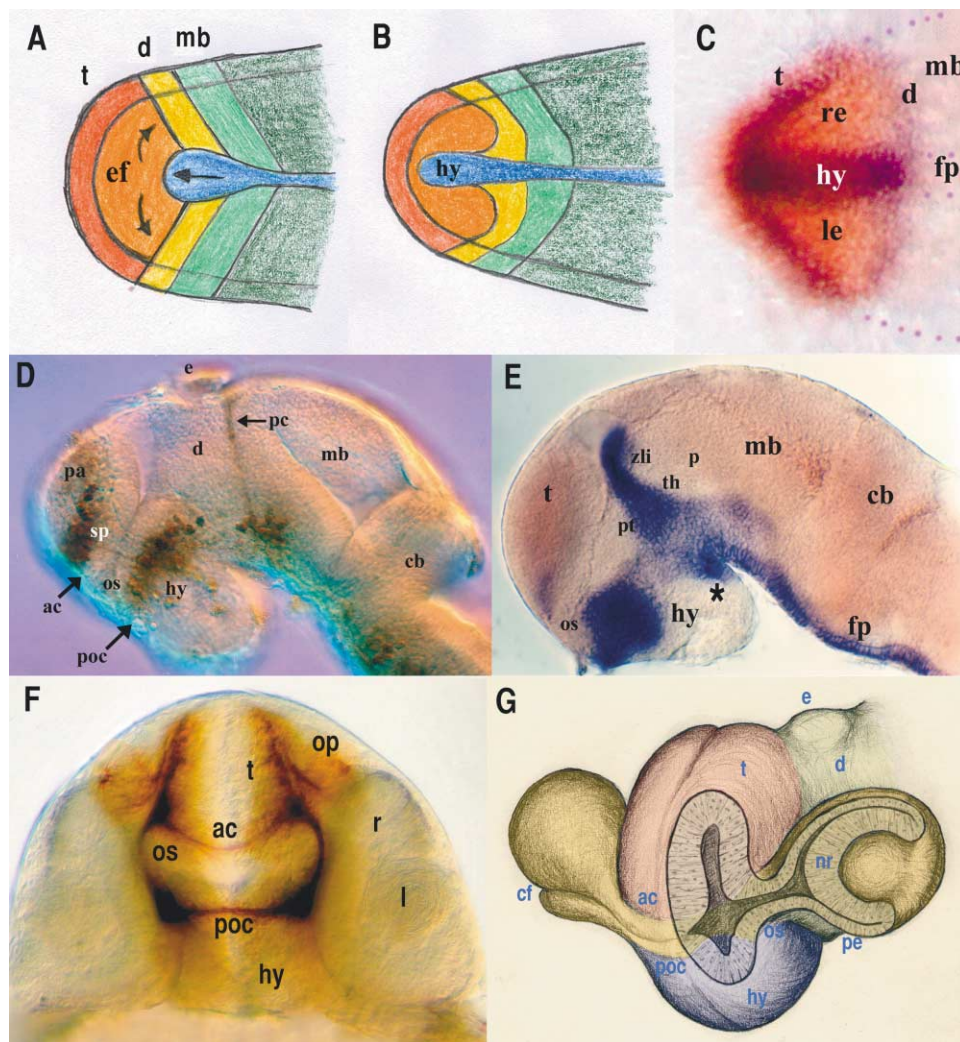


Figure 1. Organization of the Rostral Neural Plate and Forebrain in Embryonic Zebrafish

(A and B) Cartoons of the rostral neural plate of a zebrafish embryo with anterior to the left. The approximate locations of cells destined to give rise to various territories are shown in different colors. The topological relationships of prospective forebrain domains are conserved between vertebrate species, although the relative sizes of the neural plate domains vary. In (A), axial midline neural tissue (blue) is shown moving rostrally within the neural plate deflecting cells within the eye field (orange) laterally (arrows) from where they will contribute to the left and right optic vesicles. The radial organization of the most rostral neural plate makes terminology of axes problematic. Telencephalon and eye field are both considered to be dorsal (alar) structures in the mature forebrain (G). On the neural plate, prospective telencephalon can be considered to be both anterior and/or lateral (dorsal) to the eye field. Prospective telencephalon can therefore be considered to be both a prospective dorsal, and the most rostral, neural plate domain.

(C) Expression of two transcripts that mark the various territories in the rostral neural plate of a zebrafish embryo at same stage and in same orientation as shown in (B).

(D–F) Lateral with rostral to the left (D and E) and frontal (F) views of embryonic zebrafish brains. (D) Early differentiating neurons visualized with an antibody to the neurotransmitter GABA. (E) Expression of *shh* (blue). In caudal regions, expression is restricted to the floorplate whereas in the midbrain expression includes other basal plate cells. In the diencephalon, *shh* expression extends dorsally along the zli, dividing the rostral prethalamic region from the more caudal thalamic and pretectal regions. Within the hypothalamus, only the anterior and dorsal regions express *shh* at this stage. In the text, we define the rostral limit of the floorplate as being located approximately at the caudal border of the hypothalamus (asterisk)—the rostral limit of *shh* expression in midline cells. Hypothalamus may well have specialized midline cells that run in continuity with more caudal floorplate. At gastrula and neural plate stages, it is not known if the different regions of the prospective hypothalamus are already specified. (F) Optic stalk tissue that connects the eyes to the brain is shown in continuity with a domain of cells that spans the midline. Cells in this medial location most likely originate from the medial parts of the eye field directly rostral to the prospective hypothalamus (see [B]), and differentiate as preoptic regions at the interface between telencephalon and diencephalon.

(G) Schematic view of the front of the forebrain showing the relationship between the eye and the brain.

Abbreviations: ac, anterior commissure; cb, cerebellum; cf, choroid fissure; d, diencephalon; e, epiphysis; ef, eye field; fp, floor plate; hy, hypothalamus; l, lens; le, prospective left eye; m, midbrain; nr, neural retina; os, optic stalk; p, pretectum; pa, pallium; pc, posterior commissure; pe, pigment epithelium; poc, postoptic commissure; pt, prethalamus; re, prospective right eye; sp, subpallium; t, telencephalon; th, thalamus; zli, zona limitans intrathalamica.

Figure panels are adapted from Barth and Wilson (1995) and Macdonald et al. (1994) and unpublished data from N. Staudt and C.H.

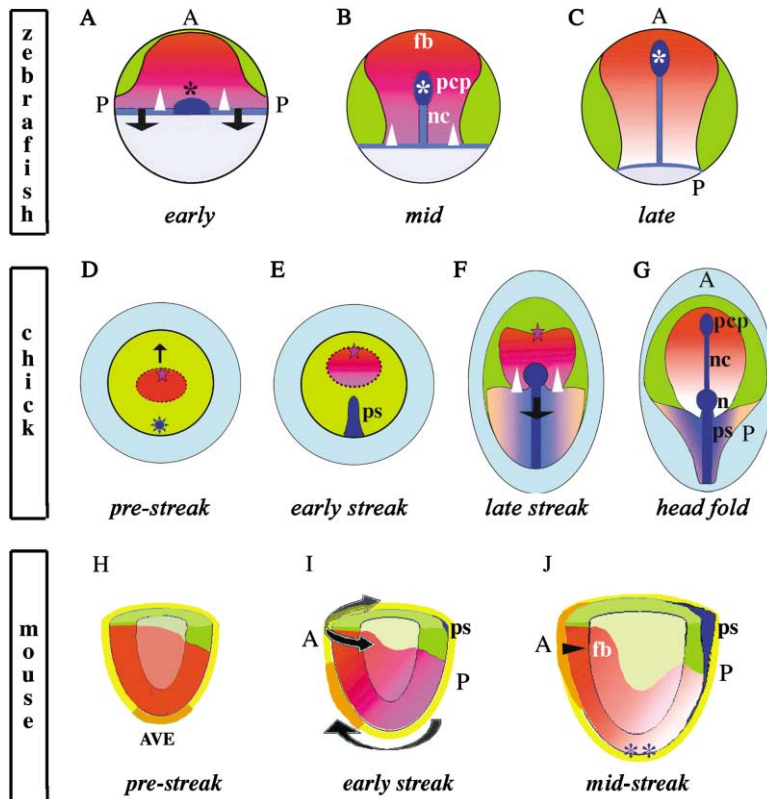


Figure 2. Cell Movements Separate the Prospective Forebrain from Sources of Caudalizing Factors

(A–C) Dorsal views of fish embryos during gastrulation. In this and other panels, epiblast/nonneural ectoderm is shown in green whereas neural ectoderm is shown in red/pink. The germ ring (blue) is a source of caudalizing signals (white arrowheads) and so as epiboly moves the germ ring vegetally (black arrows); the prospective forebrain becomes positioned further distant from the caudalizing signals. The prechordal plate mesoderm migrates rostrally beneath the neural plate and is a source of antagonists of caudalizing signals. Prospective hypothalamic cells (*) also move rostrally within the neural plate. Although not illustrated on the figure, they lag behind the advancing prechordal plate (Varga et al., 1999).

(D–G) Dorsal views of chick embryos prior to and during gastrulation. The pink star indicates the approximate rostral limit of the prospective forebrain within the epiblast. At very early stages (D and E), the prospective forebrain moves rostrally in response to signals from underlying hypoblast tissues. This distances the prospective forebrain from precursors of the primitive streak and organizer (blue star) and from caudalizing signals. By mid-late streak stage (F), the organization of tissues is similar to that shown for fish in (A). The node sits caudal to the rostral neural plate, and, as it regresses (black arrow), the prospective forebrain is distanced from caudalizing signals (white arrowheads).

(H–J) Lateral views of sagittally bisected mouse embryos prior to and during gastrulation. The arrows indicate movements in the extraembryonic layer (yellow) that shift the AVE (orange) rostrally. This tissue is a source of signals (black arrowhead) that protect the adjacent prospective forebrain from the influence of caudalizing signals. The position of the future organizer/node is shown by the double asterisk.

(D)–(J) are adapted from Foley et al. (2000), Stern (2001), and Lu et al. (2001) with modifications based on the fate map of Fernandez-Garre et al. (2002) and interpretations of Rubenstein et al. (1998).

Abbreviations: AVE, anterior visceral endoderm; fb, prospective forebrain; n, node; nc, prospective notochord; pcp, prechordal plate mesoderm; ps, primitive streak; A, anterior; P, posterior.

must adopt anterior character, and regional patterning must take place within the rostral neural plate. There remains much controversy and discussion regarding the extent to which these events, particularly the first two, are independent or intrinsically linked to each other, and a comprehensive discussion of this issue can be found in other reviews (De Robertis et al., 2000; Foley and Stern, 2001; Stern, 2002). Below, we summarize some salient issues and concepts regarding the specification of the anterior neural plate, and in subsequent sections we elaborate a more detailed discussion of regional patterning within the anterior neural plate once it has formed.

Bmp Antagonists and Fgfs Promote Neural Development

Despite being a topic of intensive study, there is still no consensus in the field with respect to the mechanisms and signals involved in neural induction (Munoz-Sanjuan and Brivanlou, 2002; Stern, 2002; Wilson and Edlund, 2001). The Bmp and Fgf signaling pathways take center stage in these arguments, with some investigators suggesting additional roles for other signals in the early specification of neural tissue (Bally-Cuif and Hammerschmidt, 2003). In frogs and fish, high levels of Bmp

activity suppress anterior neural development, and, conversely, abrogation of Bmp activity can promote neural specification. These and other observations led to the proposition of the neural default model, which suggests that ectodermal cells become neural unless exposed to Bmps (Munoz-Sanjuan and Brivanlou, 2002). In the embryo, it is proposed that antagonists of Bmp signaling within prospective neural ectoderm ensure that Bmp activity is maintained at a level sufficiently low to allow neural development. However, evidence from studies in chick (Streit et al., 2000) and other species (Akai and Storey, 2003; Wilson and Edlund, 2001; Ying et al., 2003) suggests that suppression of Bmp activity is not sufficient to induce neural identity and that earlier signals, most likely Fgfs, promote a “prospective” or “pre” neural state prior to gastrulation (Stern, 2002). Subsequent to this, Bmp antagonists (and maybe other signals) are proposed to cement neural identity.

Neural Tissue Develops Anterior Character Unless Exposed to Caudalizing Signals

In many assays, whenever neural tissue is induced, it expresses transcripts that are later restricted to forebrain and midbrain territories. Expression of these “anterior markers” raises the possibility of an obligate link

between induction of neural identity and acquisition of anterior character (discussed in Foley and Stern, 2001). In such a scenario, neural inducing signals are proposed to impart both neural and anterior identity to the ectoderm and it is later events that posteriorize the anterior neural tissue to generate the full range of CNS structures. However, in many experiments linking neural induction to acquisition of anterior character, the induction occurs in tissue isolated (physically or in time) from the signals that are believed to impart caudal identity to the forming CNS. In some situations, induced neural tissue apparently never passes through a phase of expression of anterior markers. For instance, frog ectodermal explants exposed contemporaneously both to neural inducing and caudalizing signals express posterior, but not anterior, neural markers (e.g., Papalopulu and Kintner, 1996). Furthermore, the same anterior-posterior (AP) patterning signals appear to be active both in non-neural as well as neural tissues (Read et al., 1998; Koshida et al., 1998), suggesting that neural induction may simply “reveal” the AP character already acquired by all ectodermal cells. Our favored interpretation of these and other data is that neural induction in the presence of caudalizing factors will lead to formation of neural tissue with caudal character, whereas neural induction in assays or in regions of the embryo lacking caudalizing factors will lead to induction of neural tissue with anterior identity or lacking regional character.

Anterior Neural Tissue Avoids Exposure to Caudalizing Factors

Rostral neural tissue must be “protected” from the influence of caudalizing factors if it is to acquire and retain anterior character. This is done in three ways: through localized expression of the caudalizing factors, through localized expression of antagonists of the caudalizing factors, and by morphogenetic movements that keep the anterior neural plate out of the range of the factors (Figure 2). At gastrula stages, there is considerable variation in the morphology and tissue organization of different vertebrate embryos. As a consequence, the tissue movements and the sources of both the caudalizing signals and their antagonists vary between animals (Beddington and Robertson, 1998; Foley and Stern, 2001). The organizer (Spemann’s organizer in frog, the shield in fish, and node in chick and mouse) and its early derivatives (such as the prechordal mesoderm) may initially be a source of antagonists of caudalizing signals. However, at late stages, when the organizer is contributing to more posterior tissue, it loses its ability to promote rostral fates and likely becomes a source of caudalizing signals. In addition to the early derivatives of the organizer, other tissues help protect the anterior neural plate from the influence of caudalizing factors (Chapman et al., 2003; Martinez-Barbera and Beddington, 2001; Stern, 2001; Tam and Steiner, 1999). For instance, in the chick, signals from the extraembryonic hypoblast initiate movements in the overlying epiblast that direct the prospective forebrain away from the source of caudalizing signals (Figures 2D–2G; Foley et al., 2000).

In mice, the anterior visceral endoderm (AVE) is an important source of anticaudalizing signals (Figures 2H–2J; Kimura et al., 2000; Perea-Gomez et al., 2001). The AVE is a population of extraembryonic cells that migrate rostrally from the distal tip of the blastula to underlie the anterior neural plate (Thomas et al., 1998).

Most mouse mutants in which AVE signaling and movement are disrupted lack anterior CNS structures (Stern, 2001), potentially due to exposure of anterior cells to caudalizing signals. One revealing exception is the *cripto* mutant in which prospective AVE cells remain at the distal tip of the embryo and fail to migrate rostrally. Despite this, anterior neural tissue develops, albeit further caudally than in wild-type embryos (Ding et al., 1998; Liguori et al., 2003). A related phenotype occurs in fish embryos lacking the activity of *Oep*, an ortholog of *Cripto*, or lacking activity of Nodal ligands. In these embryos, the forebrain again develops further caudally within the embryo, closer to where one would expect caudalizing factors to be present (Feldman et al., 2000; Gritsman et al., 1999). *Oep* and *Cripto* are proteins essential for Nodal signaling (Schier, 2003), and the likely explanation of these phenotypes lies in the complexity of roles for Nodal signals during early development. Nodal signaling is required for the cell movements that extend tissues rostrally but, crucially, it is also required for development of mesendodermal tissue that is a source of factors that caudalize the CNS. Thus, in Nodal-signaling mutants, tissues fail to move rostrally (away from the usual source of caudalizing signals), but the depletion of caudalizing signals still allows forebrain development to occur, albeit in a different position to wild-type.

Nodal mutants highlight the importance of the balance between caudalizing factors and their antagonists in early specification of the anterior neural plate. Although fish mutants that lack Nodal activity possess large forebrains (Gritsman et al., 1999), mutants with partially reduced Nodal activity can have smaller forebrains (Shimizu et al., 2000; Sirotkin et al., 2000). In this case, the likely explanation lies in Nodal signals being important for regulating the expression of both the caudalizing factors and their antagonists (Wilson and Rubenstein, 2000). Partial reduction of Nodal activity primarily affects specification of tissues such as the prechordal mesoderm (e.g., Gritsman et al., 2000) that are required for antagonizing posteriorizing factors and so leads to enhanced caudalization and reduced forebrain development. However, with more severe abrogation of Nodal activity, the expression of the caudalizing signals themselves is lost (e.g., Erter et al., 2001), making the absence of antagonists of these signals redundant.

Wnts, Fgfs, Bmps, and Retinoic Acid Are Caudalizing Factors

The identity of the factors that caudalize the neural plate has been surprisingly difficult to resolve. This is in part because the various signaling pathways that modulate early AP pattern are often involved in many events (as illustrated by the preceding discussion on Nodals), and so their effects on AP patterning could be either direct or indirect. Fgfs, Wnts, Retinoic Acid (RA), Nodals, and Bmps are among the signaling molecules that have been proposed as caudalizing factors (Figure 3A; Agathon et al., 2003; Munoz-Sanjuan and Brivanlou, 2001), and it is very likely that the combinatorial activity of several pathways is required to establish early AP pattern (e.g., Haremaki et al., 2003; Kudoh et al., 2002). At early stages, the activity of these pathways is likely to impart only crude AP pattern to the forming neural plate, and, as we discuss below, later signals acting locally within the neural plate refine this early pattern to establish discrete CNS subdivisions.

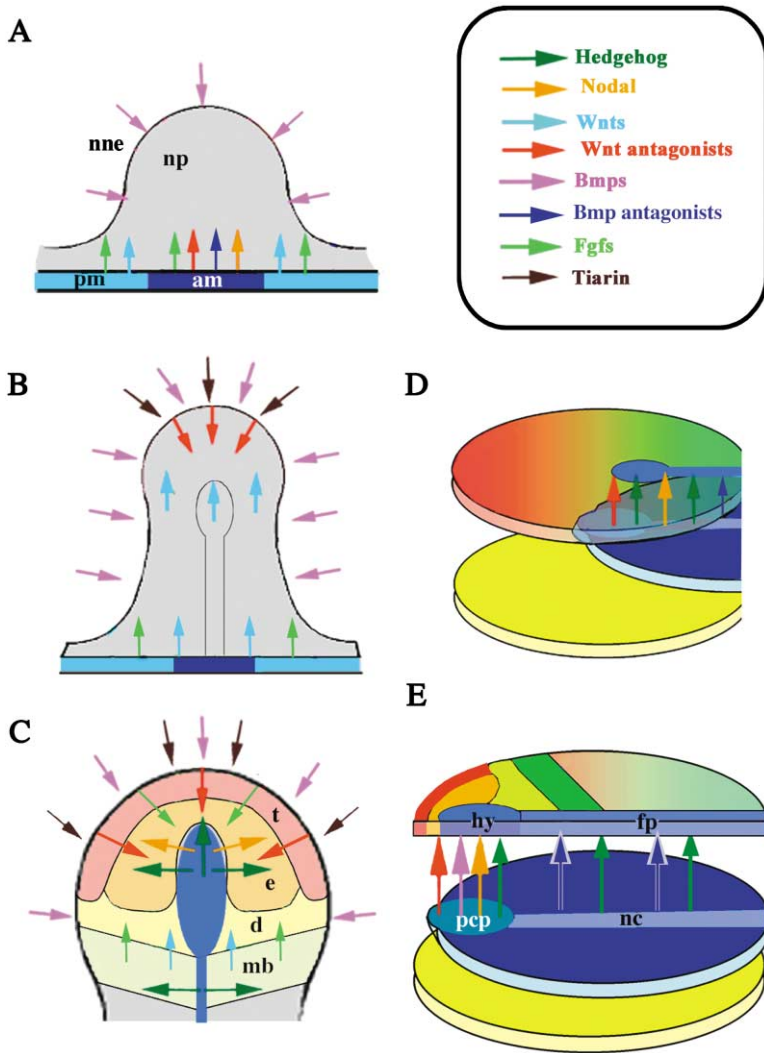


Figure 3. Signals that Regulate Regionalization of the Rostral Neural Plate

(A–C) Schematics of the neural plate (gray), nascent mesendoderm in the germ ring (pale blue), organizer (dark blue), and midline neural tissue (blue in [C], [D], and [E]) at successive stages of gastrulation. Anterior (including prospective forebrain) is to the top. The panels are based upon neural development in fish but similar events occur in other species. Arrows indicate various secreted signals, and although we show directionality, there is no evidence that these signals only act in the directions indicated.

(A) During early gastrulation, caudalizing signals (including Wnts, RA, and Fgfs) arise from the germ ring. Antagonists to at least some of these signals are produced in the organizer and its early derivatives (prechordal plate mesendoderm). Bmp signals from nonneural ectoderm encroach into the neural plate at this and later stages.

(B) By mid- to late gastrulation, Wnt antagonists and the secreted protein Tiarin are secreted from cells at and/or near the anterior margin of the rostral neural plate. Expression of Fgfs (not shown) and Wnts is initiated in neural plate domains caudal to the prospective forebrain. At this stage, midline neural tissue is extending rostrally in the neural plate (outlined) and may already be a source of Hh and Nodal signals (not shown).

(C) By neural plate stage, Fgfs are expressed in the ANB and Hh and Nodal ligands are expressed in prospective hypothalamic tissue (blue) that has extended close to the rostral limit of the neural plate.

(D and E) Schematics showing some of the signals arising from tissues beneath the neural plate (top layer) that potentially influence forebrain development. Mesoderm and axial mesendoderm (prechordal plate and notochord) are shown in blue, and endoderm and extraembryonic tissues are shown in yellow.

(D) During gastrulation, axial mesendoderm

produces both Nodal and Hh proteins that signal to the overlying neural plate. The effects of Nodal activity upon midline neural tissue are moderated by the activity of Nodal antagonists (not shown, see Feldman et al., 2002). Endodermal and extraembryonic tissues are probably also a source of signals (not shown) that influence neural plate development in all species. (E) By neural plate stage, axial mesendoderm has reached the rostral limit of the anterior neural plate. In addition to Nodals and Hhs, prechordal plate mesendoderm also expresses Bmps whereas the more caudal axial tissues express Bmp antagonists.

Abbreviations: am, prospective axial mesendoderm; d, prospective diencephalon; e, eye field; mb, prospective midbrain; nne, nonneural ectoderm; np, neural plate; pm, paraxial mesoderm; t, telencephalon.

Caudalizing signals could potentially influence the initial AP patterning of the neural plate by affecting cell fate and/or cell behavior. For instance, Wnts and Wnt antagonists may regulate the initial establishment of AP positional values (Yamaguchi, 2001) through the establishment of a global gradient of secreted Wnt activity that defines positional fates in the forming neural plate (Kiecker and Niehrs, 2001a; Nordstrom et al., 2002). Conversely, the mechanisms by which Bmp signals promote caudal development may involve the regulation of cell behaviors. For instance, within nascent mesoderm of fish embryos, the level of Bmp activity determines the extent to which cells converge dorsally and extend along the AP axis (Marlow et al., 2004; Myers et al., 2002). When exposed to high levels of Bmp activity, cells fail to converge and consequently end up in more caudal regions of the embryo. If similar regulation of cell movements occurs in ectodermal cells, this may provide a

mechanism by which Bmp signals promote development of caudal neural tissue. We return to Wnts, Bmps, and other signals when we discuss their roles in local patterning of anterior neural plate derivatives.

In summary, prior to and during gastrulation, AP pattern starts to emerge within the embryo, and within the context of this emerging AP pattern, neural induction occurs. In regions of the embryo protected from the influence of caudalizing signals, this neural tissue forms the prospective forebrain.

Local Signaling Events Lead to Regional AP Patterning within the Anterior Neural Plate

One consequence of the initial regionalization of the neural plate is the establishment of cell populations, such as the floorplate and isthmus organizer, that are local sources of signals within the neuroectoderm.

These local “organizers” modulate and refine initial regional patterning such that, by the end of gastrulation, the expression domains of many newly induced genes begin to subdivide the neural plate into discrete territories that prefigure the various structures of the mature CNS. In recent years, there has been considerable progress in elucidating the signaling events that lead to regional subdivision of the anterior neural plate (Figure 3). One concept emerging from these studies is that cells at the anterior border of the neural plate—the ANB (also referred to as the anterior neural ridge or ANR) are a source of signals that promote telencephalic gene expression (Echevarria et al., 2003; Houart et al., 1998; Shimamura and Rubenstein, 1997; Tian et al., 2002). Several genes encoding secreted proteins are expressed in the ANB and/or adjacent tissues, and below we discuss the roles played by signaling pathways implicated in anterior neural plate patterning. We focus upon the signals that regulate regional fate, do not discuss growth and morphogenesis, and only briefly describe some of the transcriptional responses downstream of the signaling pathways.

Wnt Antagonists Promote Telencephalic Development

In zebrafish, one of the secreted proteins responsible for the activity of the ANB is *Tlc* (Houart et al., 2002), a member of the secreted Frizzled Related Protein (sFRP) family. *Tlc*-expressing cells are able to restore telencephalic identity to embryos lacking endogenous ANB cells and can induce telencephalic gene expression in neural plate territories normally fated to become diencephalon or midbrain. Furthermore, abrogation of *Tlc* activity leads to delayed and reduced expression of telencephalic marker genes. Although it is unclear which sFRP family genes are orthologous to *tlc* in other species, several are expressed in the anterior neural plate and/or in underlying extraembryonic and embryonic endodermal tissues. This suggests both a conserved role for this family of proteins and a possible functional equivalence or cooperation between the ANB and endodermal tissues in anterior neural plate patterning.

sFRPs are generally considered to antagonize Wnt activity by sequestering secreted Wnts (Uren et al., 2000), and indeed the ability of *Tlc* to promote telencephalic gene expression is mimicked by other putative secreted Wnt antagonists (Houart et al., 2002). This suggests that the establishment of telencephalic identity requires local suppression of Wnt activity. Several other lines of evidence support a role for local antagonism of Wnt signaling in the specification of the telencephalon. Zebrafish *masterblind* (*mb1^{-/-}*) embryos that carry a mutation in the intracellular Wnt pathway scaffolding protein Axin1 lack a telencephalon (Heisenberg et al., 2001; van de Water et al., 2001), most likely due to a local requirement for Axin1 to suppress Wnt signaling in the anterior neural plate (Heisenberg et al., 2001). Zebrafish mutants lacking activity of *Tcf3*, a transcriptional repressor of Wnt target genes, also lack telencephalon (Kim et al., 2000), and local activation of canonical Wnt signaling suppresses telencephalic gene expression (Houart et al., 2002). Studies in frog and chick also suggest that the telencephalon is established in a domain of low Wnt activity (Kiecker and Niehrs, 2001a; Lupo et al., 2002; Nordstrom et al., 2002), and, in mouse, absence of *Six3*

function in the prospective forebrain leads to locally enhanced Wnt activity, causing a loss of telencephalic tissue (Lagutin et al., 2003).

The requirement for Wnt antagonists during establishment of telencephalic identity implies the presence of a source of Wnt signals that need to be antagonized. One such source may be neural plate tissue caudal to the telencephalon and eye field that expresses several Wnt genes, including *wnt8b*. Abrogation of *Wnt8b* activity in fish leads to a slight increase in telencephalic size, but, more dramatically, it restores telencephalic fates in *mb1^{-/-}* embryos (Houart et al., 2002). This rescue is somewhat surprising given that the increased Wnt signaling in *mb1^{-/-}* embryos would be predicted to be due to activation of the pathway downstream of Axin1. However, Wnt activity regulates expression of genes that modulate Wnt signaling (Dorsky et al., 2003; Houart et al., 2002; Kim et al., 2002; Nordstrom et al., 2002). Thus, in *mb1^{-/-}* mutants, *wnt8b* is ectopically expressed throughout the anterior neural plate, and this excess of ligand contributes to the overactivation of the Wnt pathway and suppression of telencephalic development. ***Graded Responses to Levels and Timing of Wnt Activity May Contribute to the Regional Subdivision of the Anterior Neural Plate into Telencephalon, Eyes, and Diencephalon***

In *mb1^{-/-}* fish embryos, the loss of telencephalon is accompanied by loss of eyes and expansion of diencephalic fates to the front of the neural plate (Masai et al., 1997), indicating that enhanced Wnt activity can lead to a fate transformation of telencephalon and eye field to diencephalon. Supporting this conclusion, local activation of Wnt signaling suppresses eye formation in fish (Houart et al., 2002). The ability of high levels of Wnt signaling to promote diencephalic development in fish is supported by studies in chick and frog, where Wnt signaling promotes posterior and suppresses anterior forebrain markers (Braun et al., 2003; Kiecker and Niehrs, 2001a; Nordstrom et al., 2002), and in mice, where absence of *Six3* function leads to enhanced Wnt signaling and promotion of midbrain and posterior diencephalic fates (Lagutin et al., 2003). Overall, these studies support the idea that within the prospective forebrain, telencephalon and eyes are specified in regions of low or no Wnt activity, whereas more posterior diencephalic fates are promoted by Wnt signaling (Figure 3). Although the Wnt pathway regulates proliferation of neural cells (Chenn and Walsh, 2003; Megason and McMahon, 2002), the early changes in neural plate patterning upon manipulation of Wnt activity are primarily due to altered fate specification rather than changes in proliferation or apoptosis.

An appealing possibility is that discrete levels of Wnt signaling subdivide the rostral neural plate into telencephalon, eye field, and diencephalon. Indeed, increasing Wnt antagonist activity within the ANB can drive expression of telencephalic markers into the eye field (Houart et al., 2002), consistent with the possibility that severe abrogation of Wnt activity promotes telencephalic gene expression at the expense of eye field markers. As enhanced Wnt activity can also suppress eye formation, specification of the eye field may require a level of Wnt activity intermediate between that encountered by prospective telencephalic and diencephalic

cells. Furthermore, within the telencephalon itself, Wnt signaling may have a graded role in promoting dorsal at the expense of ventral identity (Gunhaga et al., 2003; Theil et al., 2002), consistent with fate mapping data showing that prospective dorsal telencephalon is located more caudally than prospective ventral telencephalon (Cobos et al., 2001), placing it closer to sources of Wnts.

How does the Wnt pathway regulate CNS patterning? The canonical Wnt pathway is used reiteratively during the development of the forebrain. Wnt signaling contributes to initial regionalization of the forming neural plate into crude AP subdivisions, then locally promotes caudalization of the forebrain anlage and later still induces proliferation and modulates patterning within individual forebrain domains. Although these roles are now reasonably well documented, the mechanisms by which Wnt signals regulate regional fate determination within the forming CNS are still poorly understood.

Perhaps the simplest model of Wnt pathway activity is that the initial AP regionalization of the neural plate is established by a gradient of secreted Wnts (and other caudalizing signals) acting throughout the nascent neural plate (Dorsky et al., 2003; Kiecker and Niehrs, 2001a; Nordstrom et al., 2002). Subsequent to this, spatially localized expression of agonists and antagonists of Wnt signaling could locally establish or refine gradients of Wnt activity and consequently further refine regional patterning (e.g., Houart et al., 2002). However, although the canonical Wnt pathway is certainly used reiteratively in the progressive refinement of CNS patterning, as yet it is uncertain if Wnt proteins act in a graded way, promoting different fates at different concentrations.

In favor of Wnts establishing a gradient of activity are the observations that chick neural plate explants express different regional markers in response to different concentrations of Wnt-conditioned medium (Nordstrom et al., 2002) and that nuclear localization of β -catenin, a transcriptional activator of the canonical Wnt signaling pathway, is graded in the neural plate, high caudally and low rostrally (Kiecker and Niehrs, 2001a). However, it is not certain that β -catenin-dependent transcriptional activation is required for anterior neural plate patterning. For instance, a β -catenin-activated transgene shows no enhanced expression in fish embryos lacking Tcf3 function, despite the severe caudalization of the anterior neural plate in these embryos (Dorsky et al., 2002). Tcfs are required for transcriptional repression of caudally expressed neural genes in anterior regions; however, Wnt-dependent nuclear localization of β -catenin may not be required for activation of these genes in absence of Tcf-mediated repression (Dorsky et al., 2003). Therefore a key consequence of activation of the Wnt pathway may be to relieve Tcf-mediated repression of caudally expressed genes, whereas other signals mediate transcriptional activation of these genes.

Six3 Homeodomain Proteins Are Activated When Wnt Signaling Is Suppressed and Suppress Wnt Activity in the Anterior Neural Plate

There are undoubtedly many transcription factors that mediate patterning of forebrain structures downstream of the activity of Wnts and Wnt antagonists. Here, we focus on just two families of proteins, Sine-oculis (Six) and Iroquois (Irx), that promote anterior and posterior

fates within the prospective forebrain- and midbrain-forming regions of the neural plate, respectively.

Six3 homeodomain-containing transcription factors (and related Six6/Optx2 proteins) are expressed in the telencephalic and eye field regions of the prospective forebrain and are crucial for the formation of these structures in both fish and mice (Carl et al., 2002; Lagutin et al., 2003). Complementarily, exogenous Six3 is able to expand forebrain structures and ectopically induce eyes and forebrain markers in more caudal CNS tissue (Bernier et al., 2000; Kobayashi et al., 1998; Loosli et al., 1999).

six3 expression in the anterior neural plate is suppressed by enhanced Wnt activity (Braun et al., 2003; Kim et al., 2000) and by removal of ANB cells (C.H., unpublished data), and, reciprocally, Six3 activity directly represses transcription of *wnt* genes (Braun et al., 2003; Lagutin et al., 2003). Six3 proteins therefore function in a positive feedback pathway in which they are activated in regions of low Wnt activity, and subsequently one of their functions is to suppress Wnt signaling, thereby promoting rostral forebrain fates. The ability of exogenous Six3 to restore telencephalon and eyes to fish embryos lacking the activity of the Tcf3 transcriptional repressor may therefore be due both to repression of ectopic *wnt* gene expression (Lagutin et al., 2003) and potentially through the ability of Six3 to function downstream of the Wnt pathway to specify rostral forebrain fates.

Mutually Repressive Interactions between Six and Irx Proteins Contribute to the Establishment of Anterior and Posterior Forebrain Identity, Respectively

Wnt signaling promotes caudal diencephalic identity, and the *Irx* family of homeodomain proteins are among the potential effectors of the Wnt pathway in this role. Complementary to *six3* expression in rostral regions of the neural plate, prospective caudal diencephalon and midbrain express various *irx* genes. This expression is expanded rostrally both in fish embryos lacking Tcf3 function (Itoh et al., 2002) and in chick explants exposed to Wnt protein (Braun et al., 2003). Conversely, suppression of Wnt signaling leads to loss of *irx3* expression in chick neural plate explants (Braun et al., 2003). Therefore *six3* and *irx* genes are expressed in mutually exclusive domains of the neural plate and behave in opposite ways in response to Wnt activity.

Misexpression studies have revealed that exogenous Six3 can suppress *irx3* expression and that, conversely, *Irx3* can suppress *six3* transcription (Kobayashi et al., 2002). This mutual repression provides a mechanism for sharpening the intracellular response to extracellular signals. For instance, cells positioned at the interface between *six3* and *irx* expression domains may receive signals that initially activate both genes; however, competitive crossrepressive transcriptional interactions would help ensure that expression of only one gene would be maintained. Such transcriptional mechanisms provide an effective way to translate the activity of graded extracellular signals into sharply defined responses within discrete neuroepithelial domains (Lee and Pfaff, 2001). It is, however, worth noting that the posterior border of *six3* expression is very dynamic and appears to regress rostrally over time (Kobayashi et al.,

2002; Zuber et al., 2003; N. Staudt and C.H., unpublished data), leaving a diencephalic domain free of both *irx* and *six3* transcripts.

The transcriptional interactions that subdivide the *six3*-expressing anterior forebrain into telencephalon and eyes are not well understood, but rapid progress is being made in clarifying the roles and regulatory interactions of transcription factors involved in specification of the eye field (Chow and Lang, 2001; DelBene et al., 2004; Zhang et al., 2002; Zuber et al., 2003) and development of the telencephalon (Zaki et al., 2003). Similarly, the subdivision of the *irx* expression domain into posterior diencephalon and midbrain probably involves mutually repressive interactions between Pax6 within the forebrain and Engrailed proteins within the prospective midbrain (Scholpp et al., 2003; and references within). These observations suggest a model whereby Wnt pathway activity (and other signals) promotes subdivision of the prospective forebrain into various domains by inducing or suppressing expression of various transcription factors with mutually repressive activities.

The Interface between the six3 and irx Expression Domains May Prefigure the Position of the zli

Fate mapping studies have yet to reveal the destiny of cells located between the *six* and *irx* expression domains; however, gene expression analysis suggests that this interface approximates the position at which the *zli* later forms (Braun et al., 2003; Kobayashi et al., 2002). The *zli* is a prominent boundary cell population (Zeltser et al., 2001) dividing the rostral forebrain (telencephalon, eyes, hypothalamus, and prethalamus) from caudal diencephalon (thalamus and pretectum). It is likely that the *zli* locally regulates development of dorsal forebrain tissue through expression of Shh (Figure 1E) and possibly other signaling proteins. For instance, mice compromised in Hh activity lack expression of *fgf* genes adjacent to the dorsal *zli* and exhibit subsequent defects in the growth and differentiation of the dorsal diencephalon (Hashimoto-Torii et al., 2003; Ishibashi and McMahon, 2002). It is currently unclear if the boundary between prospective prethalamus and prospective thalamus has any roles in forebrain patterning prior to formation of the *zli*. However, ablation of cells in the prospective diencephalon as early as late gastrulation leads to disrupted forebrain development in fish (Houart et al., 1998).

Fgf Signaling Regulates Regionalization of the Telencephalon

In addition to Wnt antagonists, the ANB expresses both Fgf3 and Fgf8, two potent signaling proteins with roles in a wide variety of developmental events. The ability of Wnt antagonists to locally induce *fgf8* expression in the anterior neural plate (Houart et al., 2002) suggests that establishment of the ANB as a source of Fgf signals is a downstream consequence of local repression of Wnt activity. Fgfs are able to rescue telencephalic gene expression in mouse explants lacking an ANB (Shimamura and Rubenstein, 1997). However, ANB-derived Fgf signaling may not be required for induction of the telencephalon, since telencephalic tissue is present in all described fish and mouse embryos with compromised Fgf signaling in the neural plate.

In all species examined, Fgf signaling does have profound roles in the regional patterning and polarization

of the telencephalic neuroepithelium. Telencephalic phenotypes attributable to reduced Fgf activity include proliferation and apoptosis defects (Storm et al., 2003), midline defects (Shanmugalingam et al., 2000; Walshe and Mason, 2003), and defects in morphogenesis of the olfactory bulb (Hebert et al., 2003). Most studies concur that high levels of Fgf activity promote rostroventral at the expense of dorsocaudal telencephalic fates (e.g., Kuschel et al., 2003). However, at least some Fgf activity does appear to be required both for ventral (e.g., Garel et al., 2003; Lupo et al., 2002; Shinya et al., 2001; Storm et al., 2003) and dorsal (Galli et al., 2003; Gunhaga et al., 2003; Walshe and Mason, 2003) region-specific gene expression.

One of the most intriguing roles proposed for Fgf signals is to polarize the neocortex, with high levels of Fgf activity promoting anterior cortical fates (Grove and Fukuchi-Shimogori, 2003). This is similar to the role proposed for Fgf8 in the polarization of the midbrain tectum where Fgf activity regulates expression of Ephrins and probably other proteins that influence retinotectal map formation (e.g., Picker et al., 1999). In the telencephalon, Fgf activity may contribute to the polarization of cortical territories through repression of Emx2 (Crossley et al., 2001; Garel et al., 2003; Storm et al., 2003), a transcription factor that reciprocally modulates Fgf activity (Fukuchi-Shimogori and Grove, 2003). Although cortical organization is a relatively late manifestation of dorsal telencephalic pattern, it is feasible that polarization of the entire telencephalic neuroepithelium is initiated at neural plate stage by ANB-derived Fgf signals acting upon both prospective pallial and subpallial domains.

Expression of *fgfs* in neural plate tissue and the competence of adjacent cells to respond to Fgf signals may both be regulated by Six3 and Irx proteins. *fgf8* expression in the ANB is dependent upon Six3 activity in mice (Lagutin et al., 2003) and probably fish (Carl et al., 2002), whereas *fgf8* expression at the isthmus is dependent upon Irx activity (Glavic et al., 2002; Itoh et al., 2002). Furthermore, exogenous Fgf triggers expression of posterior neural markers in *irx*-expressing cells, whereas it induces anterior markers in *six3*-expressing cells (Kobayashi et al., 2002). In a similar way to Six3, Otx2 may confer the ability of the anterior neural plate to respond to ANB signals. Indeed, the ANB of hypomorphic *otx2* mutant mice is functionally intact, but the neural plate of such embryos lacks the ability to respond to ANB signals or exogenous Fgfs (Tian et al., 2002).

Bmp Signaling May Be Required to Establish the ANB

Suppression of high levels of Bmp activity is an important step in the establishment of the prospective forebrain (e.g., Bachiller et al., 2000). However, within the forming neural plate, lower levels of graded Bmp activity contribute to the initial establishment of lateral (marginal) to medial pattern. Consequently, at least in fish and frog embryos, specific thresholds of Bmp activity appear to be required for establishment of fates that derive from the margins of the neural plate, such as telencephalon, dorsal eye, and epithalamus rostrally (Barth et al., 1999; Hammerschmidt et al., 2003) and dorsal sensory neurons and neural crest cells caudally (Aybar and Mayor, 2002; Nguyen et al., 2000). These observations suggest that a window of Bmp activity

during gastrulation enables development of marginal neural plate fates—if Bmp signaling is too high, nonneural fates are promoted, whereas if it is too low, then more medial neural plate fates are promoted. Thus establishment of the ANB itself may depend upon rostral marginal neural plate cells being exposed to appropriate levels of Bmp activity (Anderson et al., 2002; Houart et al., 2002).

Tiarin Promotes Marginal, Prospective Dorsal, Neural Plate Fates

tiarin encodes a putative secreted protein that is expressed in cells adjacent to the rostral margin of the neural plate in frog embryos (Figures 3B and 3C; Tsuda et al., 2002). In gain-of-function assays, *tiarin* expands the fates of CNS structures located at or near the margin of the neural plate and suppresses more medial fates. Although loss-of-function studies have yet to be performed, these results suggest that Tiarin functions similarly to Bmps within the neural plate. However, in other assays, Tiarin does not have the same activity as Bmps, suggesting that these proteins have similar functional consequences but may act in different pathways. Tiarin can also antagonize the ventralizing activity of Hh proteins, but this does not appear to be due to direct interference in the Hh signal transduction cascade. Potentially, therefore, Tiarin is a component of a novel signaling pathway that promotes marginal fates (such as telencephalon) within the anterior neural plate.

Axial Tissue Is a Source of Signals that Influence DV Pattern in the Prospective Forebrain

Mesendoderm underlying the developing forebrain influences the induction, movements, patterning, and maintenance of rostral CNS structures. For instance, mesodermal tissues such as the AVE in mouse express various secreted Wnt antagonists that potentially influence levels of Wnt activity in the overlying neural plate. Mesendoderm also mediates induction of ventral brain structures (e.g., Kiecker and Niehrs, 2001b; Muenke and Beachy, 2000) and is required for patterning and perhaps maintenance of the remaining dorsal forebrain tissue (Hallonet et al., 2002; Martinez-Barbera and Beddington, 2001; Ohkubo et al., 2002; Rohr et al., 2001). However, AP pattern in the dorsal forebrain can be established independent of underlying axial mesendoderm (Feldman et al., 2000; Gritsman et al., 1999; Masai et al., 2000; Liguori et al., 2003).

Hh and Nodal Signals Induce and Pattern the Hypothalamus

Hh genes are expressed in the prechordal mesodermal tissues that induce the hypothalamus, all hypothalamic tissue is absent in mice lacking Shh activity (Chiang et al., 1996; Ohkubo et al., 2002) and Hh protein can induce hypothalamic gene expression in both in vitro and in vivo assays (e.g., Barth and Wilson, 1995; Dale et al., 1997; Ericson et al., 1995). Although Hh signaling is essential for hypothalamus formation in mammals, it is not known if Hh signals directly mediate all aspects of hypothalamus induction. For instance, if Hh signaling is required for differentiation of the anterior mesodermal tissues that induce the hypothalamus, then abrogation of signals other than Hhs may contribute to the ventral forebrain phenotypes in Hh pathway mutants.

In addition to a role in hypothalamus induction, Hh signaling subsequently regulates DV patterning of the hypothalamus and other forebrain structures. The severity of hypothalamic deficits in mice with compromised Hh activity has precluded investigation of regional patterning of the basal forebrain in these mutants. However, mutant analyses in fish have shown that markers of dorsal and anterior hypothalamus are most sensitive to reductions in the level of Hh activity (Figures 4F and 4G; Karlstrom et al., 2003; Mathieu et al., 2002; Rohr et al., 2001). Indeed, abrogation of Hh activity leads to expansion of markers of posterior ventral hypothalamus (Mathieu et al., 2002; Varga et al., 2001). These results imply that high levels of Hh activity suppress posterior ventral markers and suggest that, subsequent to hypothalamic induction, the highest levels of maintained Hh activity are not in ventral midline cells but instead are likely to occur in more dorsal and anterior regions (Figure 1E).

Fish lacking all Nodal activity still possess a forebrain but hypothalamic tissue is absent (Figures 4A–4C; Rohr et al., 2001). Therefore Nodal signaling is required for development of the entire hypothalamus in fish, and probably also in other species (Hayhurst and McConnell, 2003). However, the depletion of axial mesendoderm in Nodal mutants (Gritsman et al., 2000; Lowe et al., 2001) means that all signals derived from this tissue are compromised, and because of this Nodal mutants lack both Nodal and Hh signaling in rostral axial tissue (Rohr et al., 2001). Chimera analysis has shown that it is only posterior/ventral hypothalamic cells which need to cell-autonomously receive Nodal signals for their specification (Figures 4D and 4E). Thus dorsal hypothalamic tissue can be restored when prechordal plate tissue is rescued beneath CNS tissue unable to receive Nodal signals (Mathieu et al., 2002). Therefore it appears that for the posterior/ventral region of the hypothalamus, the Nodal and Hh pathways have somewhat opposite roles, with Nodal signaling promoting, and high levels of Hh activity suppressing, development. Current experiments have not ruled out a direct role for Nodal signaling in the development of more dorsal and anterior regions of the hypothalamus, and, indeed, it is possible that Nodal and Hh signals may cooperate to specify and maintain some ventral brain structures (Mathieu et al., 2002; Paten et al., 2003).

The Identity of Signals that Impart Hypothalamic versus Floorplate Identity to Axial CNS Tissue Is Uncertain

The most obvious AP subdivision of the ventral CNS occurs between hypothalamus rostrally and floorplate (and adjacent basal plate cells; see Figure 1E) caudally, and, surprisingly, we know very little about how or when this distinction is made. There are currently two principal hypotheses to explain the acquisition of hypothalamic versus floorplate identity by axial neural tissue. The first is that signals from underlying mesendoderm differ along the AP axis with midline neuroectoderm differentiating as either floorplate or hypothalamus depending upon whether it receives signals from prechordal mesendoderm or from chordamesoderm (e.g., Dale et al., 1997, 1999). The second proposes that the inducing signals are the same at all axial levels and that it is

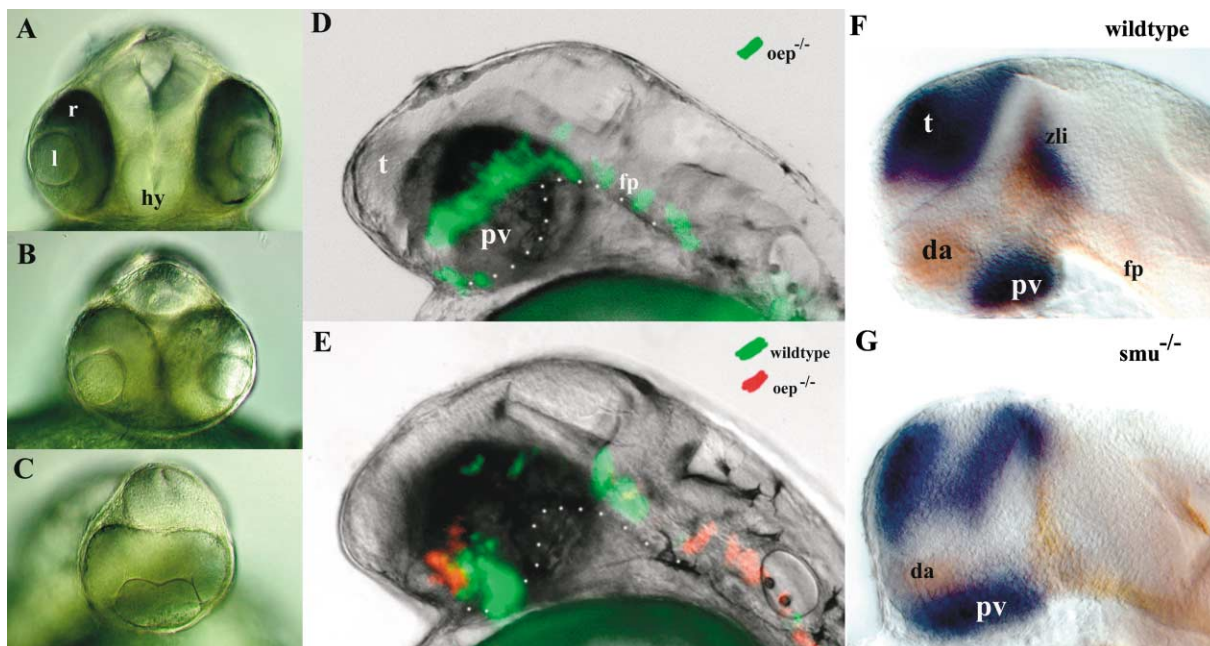


Figure 4. Nodal and Hh Signals Affect Hypothalamic Induction and Patterning

(A–C) Frontal views of a wild-type zebrafish embryo (A) and two embryos (B and C) homozygous for mutations affecting Nodal signaling. In the mutants, the hypothalamic tissue that is normally located between the eyes is absent and the retinæ are partially (B) or completely (C) fused. (D and E) Lateral views of living wild-type zebrafish embryos in which wild-type cells or cells unable to receive Nodal signals (*oep*^{-/-}) were transplanted into the prospective hypothalamus during gastrulation. Both wild-type and mutant cells have extended rostrally along the ventral neural tube contributing to both floorplate and hypothalamus. However, the cells unable to receive Nodal signals (green in [D] and red in [E]) are excluded from the posterior and ventral regions of the hypothalamus. The ventral midline of the CNS is indicated by dots.

(F and G) Lateral views of brains of a wild-type zebrafish embryo (F) and an embryo (G) homozygous for a mutation in the *smoothened* gene (*smu*^{-/-}) in which Hh signaling is severely reduced. Expression of *shh* (red) in dorsal and anterior hypothalamic tissue is reduced in the mutant, whereas *emx2* expression (blue) in posterior and ventral hypothalamus is expanded.

Abbreviations: da, anterior and dorsal hypothalamus; fp, floorplate; l, lens; pv, posterior and ventral hypothalamus; r, retina; t, telencephalon; zli, zona limitans intrathalamica.

Figure panels adapted from Masai et al. (2000) and Mathieu et al. (2002).

differential competence of anterior versus posterior neural tissue that determines whether midline cells differentiate with hypothalamic or floorplate identity (Kobayashi et al., 2002; Shimamura and Rubenstein, 1997; Tian et al., 2002). This second hypothesis still requires a mechanism of AP patterning by which anterior and posterior axial neuroectodermal cells acquire differential competence.

The Bmp and Wnt pathways are candidates for imparting hypothalamic versus floorplate identity to axial neural tissue. Bmps are expressed in prechordal plate tissue underlying the hypothalamus, but expression is absent or activity is inhibited in more caudal axial mesoderm (Anderson et al., 2002; Dale et al., 1999; Vesque et al., 2000). Furthermore, addition of Bmp7 along with Shh promotes hypothalamic at the expense of floorplate marker genes in tissue explants (Dale et al., 1997, 1999). However, hypothalamic gene expression is initially induced in fish embryos with abrogated Bmp activity (Barth et al., 1999), raising the possibility that Bmp signaling may play a more restricted role in hypothalamic patterning subsequent to its induction.

To date, most data on the role of Wnt signaling in regional patterning of the rostral brain have focused upon dorsal, alar plate, regions (such as telencephalon

and eye), and it is not yet known if this pathway influences regional subdivision of ventral, basal plate, forebrain structures. Indeed, at stages when Wnts and Wnt antagonists are already influencing anterior neural plate patterning in fish, prospective hypothalamic cells are still located caudal to the eye field (Mathieu et al., 2002; Varga et al., 1999; Woo and Fraser, 1995), a long way from their final destination in the rostral brain. This disjunction between the AP location of dorsal and ventral forebrain precursors during gastrulation challenges the notion that the same signals could contemporaneously establish AP pattern in both territories. Nevertheless, *six3*, a gene negatively regulated by Wnt activity, promotes expression of hypothalamic rather than floorplate markers in midline neural cells in response to Hh activity in chick (Kobayashi et al., 2002). Similarly, in mouse, *Otx2* is required for neural plate cells to induce hypothalamic gene expression in response to signals from prechordal plate tissue (Tian et al., 2002).

The Rostral Neural Plate Is Uniquely Positioned to Be Influenced Both by Axial and ANB Signals

Through expression of Hh, Nodal, and perhaps other signals, axial tissues influence DV patterning, fate specification, and proliferation throughout the forebrain. DV

patterning of forebrain derivatives is perhaps best understood within the telencephalon, and as this topic is extensively reviewed elsewhere (Campbell, 2003; Marin and Rubenstein, 2002; Rallu et al., 2002a; Schuurmans and Guillemot, 2002) we do not cover it here. We finish by discussing the role of axial tissue and signals in the partitioning of the eye field and the interactions between signals originating from the ANB and from axial cell populations.

Axial Tissue Regulates Bilateral Partitioning of the Eye Field and Induction of Proximal/Ventral Optic Vesicle Fates

The movements of, and signals produced by axial tissues (prospective hypothalamus and underlying mesendoderm) affect the subsequent development of other regions of the forebrain. This is most evident in the development of the optic vesicles from the eye field. In the absence of axial tissues, the single eye field never separates into two bilateral eyes and the optic stalks fail to form (e.g., Adelmann, 1936; Figures 4B and 4C). This failure in morphogenesis and fate specification is likely to be a consequence of two roles for axial tissue. First, as prospective hypothalamic cells move rostrally within the neural plate, medially positioned eye field cells are displaced laterally from where they subsequently contribute to left and right optic vesicles (Figures 1A and 1B; Varga et al., 1999). The failure of these movements in zebrafish mutants affecting either gastrulation movements (Heisenberg et al., 2000), or Nodal signaling (Varga et al., 1999), has the consequence that eye field cells fail to be displaced from the midline. Although hypothalamic cell movements have not been studied in great detail in other species, they probably do occur (e.g., Dale et al., 1999; Patten et al., 2003) and their disruption potentially contributes to the cyclopia/holoprosencephaly phenotypes seen in many species when axial tissues/signals are disturbed (Hayhurst and McConnell, 2003; Roessler and Muenke, 2001). An alternative proposition is that splitting of the eye field is simply a consequence of signals from underlying prechordal plate mesendoderm inducing medial neural plate cells to adopt nonretinal fates (Li et al., 1997; Pera and Kessel, 1997), and it remains possible that both movements and signaling contribute to eye field separation.

A second role for axial tissues is to produce signals that divert medially positioned eye field cells to form optic stalk tissue rather than retina (reviewed in Chow and Lang, 2001). In embryos with defects in formation of, or signaling by, axial tissues, the medial region of the eye field is specified as retina instead of ventral optic stalk (Chiang et al., 1996; Macdonald et al., 1995), leading to formation of a single neural retina fused across the midline (Figure 4C). One of the axial signals that patterns the developing eye field and optic vesicles is Shh.

Loss of Hh signaling leads to a failure in induction of optic stalk marker genes (Chiang et al., 1996; Varga et al., 2001), while conversely overexpression of Shh leads to expansion of ventral optic stalk tissue at the expense of retina (Ekker et al., 1995; Macdonald et al., 1995). As in other regions of the CNS, it appears that Hh signaling acts in a graded way in the forming optic vesicle. Ventral optic stalk fates are induced in regions with the highest

levels of Hh activity, whereas ventral and dorsal neural retina are established progressively further distant from axial tissue and presumably are exposed to progressively lower Hh activity (Sasagawa et al., 2002; Zhang and Yang, 2001; reviewed in Chow and Lang, 2001). Several other signaling pathways may cooperate with Hhs to promote ventral optic stalk and ventral retinal fates. First, while the optic vesicle defects of Nodal pathway mutants are no doubt in part due to stalled movements of prospective hypothalamic cells and the reduction/loss of Hh activity, it remains possible that Nodal signals have direct activity on eye field cells. Second, both RA and Fgfs modulate ventral optic vesicle development (e.g., Sasagawa et al., 2002, and see below). The epistatic and regulatory relationships between these various signaling pathways remain to be resolved.

ANB Signals May Cooperate with Axial-Tissue-Derived Signals to Specify Optic Stalk and Ventral Telencephalic Fates

Cells in medial regions of the anterior neural plate are uniquely positioned at the intersection between the rostral limit of axial tissue and the ANB (Figure 3C), both sources of a variety of signaling molecules. Indeed, recent studies have raised the possibility that there is cooperation between axial- and ANB-derived signals in the specification of the ventral optic stalks, the commissural plate, preoptic area, and ventral telencephalon.

In fish, various Nodal and Hh pathway mutants lack expression of optic stalk markers, consistent with a role for these signals in the induction of ventral optic stalk fate. Surprisingly, however, complete abrogation of Nodal activity leads to restoration of optic stalk specific gene expression at the interface between telencephalon and the fused retina, even though there appears to be no restoration of Hh signaling in such embryos (Feldman et al., 2000; Masai et al., 2000; Take-uchi et al., 2003). The most likely explanation of this phenotype is that signals from the ANB, which may be enlarged in the absence of Nodal activity (Gritsman et al., 1999; Houart et al., 2002), compensate for the loss of axial signals and locally induce optic stalk specific gene expression. Among the candidate signals produced by the ANB are Fgfs, which are known to promote optic stalk gene expression (Shanmugalingam et al., 2000; Walshe and Mason, 2003).

Cooperation between Fgf and Hh signals is also likely to occur during fate specification in the prospective ventral (subpallial) telencephalon (Ohkubo et al., 2002). Ventral telencephalic gene expression is reduced or lost in mice and fish lacking either Hh activity or Fgf3/Fgf8 activity (Rallu et al., 2002a). The source of Hh and Fgf signals that mediate ventral telencephalic development is still uncertain and may vary between species (see Gunhaga et al., 2000; Rallu et al., 2002a), but axial tissue and the ANB are excellent candidates for providing such signals. The potential redundancy between the Fgf and Hh pathways in ventral telencephalic development has been most clearly demonstrated in compound mouse mutants. Removal of the activity of Gli3 restores ventral telencephalic fates in both Shh and Smoothed mutants, indicating that a key role for Shh is to antagonize Gli3-mediated repression of ventral telencephalic gene expression (Rallu et al., 2002b). The restoration of ventral fates in these double mutants implies another source

of ventralizing signals and, once again, Fgfs are good candidates (Aoto et al., 2002; Kuschel et al., 2003).

Concluding Remarks

Considerable progress has been made in the identification of signaling pathways that influence cell fate in the anterior neural plate, although we are still some way from understanding the exact mechanisms by which patterning occurs. For instance, the Wnt pathway influences regional patterning at various different times and places during anterior CNS development—does this mean that cells show several discrete and independent responses to Wnt pathway activity at different developmental stages? Or is Wnt signaling a continuous and dynamic process in which levels of pathway activity are modulated over time through the activity of various agonists and antagonists expressed at different times and places during forebrain development? If the latter, how are discrete read-outs of pathway activity achieved? We do not know if the Wnt pathway really plays a role in the global patterning of the entire CNS, and it will be important, for instance, to determine if the pathway has a role in ventral forebrain development comparable to that in more dorsal regions. If Wnt signaling does have graded activity throughout the CNS, then how is this achieved? Can Wnts act as morphogens during neural plate patterning, and, if so, how are gradients of Wnt activity established over potentially long distances? We can ask similar questions about the other signaling pathways we have discussed—we are at a point where we know many of the regulators of early forebrain development, but have yet to fully understand how they work. Our review ignores left-right patterning in the developing forebrain. Lateralization is a highly conserved feature of forebrain development and is fundamental to nervous system function (e.g., Toga and Thompson, 2003). Although some advances in our understanding of how forebrain lateralization develops are coming from studies in fish (e.g., Concha et al., 2003; Gamse et al., 2003; Halpern et al., 2003), this area of study is still in its infancy and needs input from other model systems. Given its complexity of form, the mechanisms that regulate morphogenesis and growth of the forebrain are of course as important as those that govern cell fate decisions. As yet, there is little to discuss on this topic, but it is surely one that will see major advances in the not-too-distant future.

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