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THE SPECIFICATION OF DORSAL CELL FATES IN THE VERTEBRATE CENTRAL NERVOUS SYSTEM

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ABSTRACT

The generation of distinct classes of neurons at defined positions within the developing vertebrate nervous system depends on inductive signals provided by local cell groups that act as organizing centers. Genetic and embryological studies have begun to elucidate the processes that control the pattern and identity of neuronal cell types. Here we discuss the cellular interactions and molecular mechanisms that direct neuronal cell fates in the dorsal half of the vertebrate central nervous system. The specification of dorsal neuronal cell fates appears to depend on a cascade of inductive signals initiated by cells of the epidermal ectoderm that flank the neural plate and propagated by roof plate cells within the neural tube. Members of the transforming growth factor$^{-\beta}$ (TGFβ) family of secreted proteins have a prominent role in mediating these dorsalizing signals. Additional signals involving members of the Wnt and fibroblast growth factor (FGF) families may also contribute to the proliferation and differentiation of dorsal neuronal cell types.

INTRODUCTION

The assembly of neural circuits in the vertebrate nervous system begins with the generation of functionally distinct neuronal cell types, each at a stereotyped position within the neural tube. In recent years, considerable progress has been made in defining the mechanisms that control specification and patterning of neural cells during vertebrate development (McConnell 1995, Anderson &

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Jan 1997). These studies have revealed that neural cell fate depends critically on local environmental signals that progressively restrict the developmental potential of neural progenitor cells. Such signals are thought to direct cell fates by inducing the expression of intrinsic proteins, notably transcription factors (Bang & Goulding 1996). These proteins act in turn to regulate the expression of surface receptors and components of signal transduction pathways that provide axons and growth cones with the ability to select specific pathways and to form precise target connections (Tessier-Lavigne & Goodman 1997).

The cells of the vertebrate central nervous system (CNS) derive from the neural plate, an epithelial sheet that arises from the dorsal ectoderm of the gastrula-stage embryo. The formation of the neural plate depends on signals from the prospective axial mesoderm of the organizer. Fate mapping in different vertebrate species has shown that the most anterior region of the neural plate forms forebrain structures and progressively more posterior regions give rise to the midbrain, hindbrain, and spinal cord (Schoenwolf et al 1989, Eagleson & Harris 1990). A variety of grafting and neural plate or neural tube rotation studies (Roach 1945, Jacobson 1964, Simon et al 1995, Ensini et al 1998) have provided evidence that the identity of early neural cells is controlled by independent signaling systems that operate along the anteroposterior and dorsoventral axes of the neural tube. These studies have led to suggestions that the position a cell initially occupies along each of these two axes will define the inductive signals to which it is exposed and will thus be an important predictor of its eventual fate. This view of early neural patterning has received strong support from studies of the signaling systems that control cell fate along the rostrocaudal and dorsoventral axes of the neural tube (reviewed in Lumsden & Krumlauf 1996, Tanabe & Jessell 1996).

In this article we focus on the dorsoventral patterning of the neural tube and in particular on the mechanisms that control the identity of dorsal neural cells. We discuss emerging evidence that the specification of dorsal neural fates is initiated by inductive signals provided by epidermal ectoderm cells at the lateral margins of the neural plate. This dorsal inductive signaling pathway appears to be triggered by secreted proteins of the transforming growth factor-ß (TGFß) family. Since the process of neural induction also appears to involve regulated TGFß signaling, it is likely that the induction of neural tissue and its patterning along the dorsoventral axis are mechanistically related processes. We therefore review current ideas about the molecular basis of neural induction before turning to the patterning signals and cellular responses that control the fate of cells in the dorsal half of the developing neural tube. We discuss the mechanisms of dorsal cell fate specification first at caudal levels of the neural tube that give rise to the spinal cord and then at more rostral levels that generate the hindbrain, midbrain, and forebrain.

NEURAL INDUCTION AND BMP SIGNALING

Studies in Xenopus embryos have provided evidence that neural induction results from the inactivation of a constitutive signaling pathway that functions to repress neural differentiation in ectodermal cells. The first evidence for this initially counter-intuitive mechanism came with the finding that the dissociation of blastula-stage Xenopus ectoderm into single cells, thereby preventing cell-cell signaling, was sufficient to elicit the formation of neural tissue (Godsave & Slack 1989, Grunz & Tacke 1989, Sato & Sargent 1989). Subsequently, overexpression of a dominant negative activin type II receptor was found to induce neural differentiation of ectodermal cells (Hemmati-Brivanlou & Melton 1994). These results suggested that the antineurogenic signal transmitted between ectodermal cells might be mediated by secreted proteins of the TGFß/activin/BMP family.

The TGFß-like factor bone morphogenetic protein-4 (BMP4) has emerged as one likely candidate for an endogenous signal that functions to repress neural differentiation in Xenopus ectodermal cells. BMP4 is widely expressed in the early ectoderm, and its expression is extinguished from neural plate cells during neural induction (Fainsod et al 1994, Hemmati-Brivanlou & Thomsen 1995, Schmidt et al 1995). In addition, BMP4 inhibits the expression of neural markers and promotes epidermal differentiation in dissociated ectodermal cells (Wilson & Hemmati-Brivanlou 1995). These findings have led to the proposal that organizer-derived signals induce neural tissue through the secretion of endogenous proteins that block BMP signaling (Harland 1996).

Direct support for this idea has come from the identification of three proteins that are expressed by organizer tissue and inhibit BMP signaling. The activin- and BMP7-binding-protein follistatin is expressed by organizer cells in Xenopus embryos and can elicit neural differentiation (Hemmati-Brivanlou et al 1994). Noggin is also expressed in organizer cells (Lamb et al 1993) and binds to BMP4 with high affinity, blocking its activity (Zimmerman et al 1996). Chordin, a third protein expressed in the organizer (Sasai et al 1994), also has neural inducing activity (Sasai et al 1996) and binds BMP4, albeit at somewhat lower affinity than noggin (Piccolo et al 1996). In addition, gremlin, cerberus, and DAN have been identified as members of a fourth class of secreted proteins that can antagonize BMP signaling (Hsu et al 1998). One of these proteins, cerberus, is expressed in the anterior endoderm of the organizer and has been implicated in the induction of head structures, including anterior neural tissue (Bouwmeester et al 1996). These four classes of proteins each have neural inducing activity in Xenopus ectoderm and are likely to share a common mechanism of action, but they appear to be unrelated structurally.

The analysis of mutations in genes encoding BMPs and their endogenous antagonists has begun to test the involvement of BMP signaling and its inhibition

in neural induction. Mutations in the mouse *BMP4* gene do not show an obvious expansion in neural tissue at the expense of epidermal ectoderm (Winnier et al 1995), as might have been anticipated from studies of BMP4 function in Xenopus. This might reflect the presence of other BMPs that function in a manner similar to that proposed for BMP4 or the existence of parallel pathways of neural induction unrelated to BMP inhibition. Mice that lack *follistatin* (Matzuk et al 1995) or *noggin* function (McMahon et al 1998) do not exhibit obvious abnormalities in neural induction, although *noggin* mutants have defects in the differentiation of ventral neural cell types. The absence of neural induction defects in these mutants could again be accounted for by the compensatory actions of other organizer-derived molecules that inhibit BMP signaling. In zebrafish, however, *BMP2* (*swirl*) mutant embryos (Kishimoto et al 1997) do exhibit an expanded neuroectoderm (Mullins et al 1996) and *chordin* (*chordino*) mutants (Schulte-Merker et al 1997) have a reduced neuroectoderm (Hammerschmidt et al 1996). These findings are consistent with the idea that BMP antagonists promote neural differentiation. However, the dorsoventral axis of the entire embryo is affected in *swirl* and *chordino* mutants, and thus changes in the extent of the neuroectoderm in these mutants may be a secondary consequence of alterations in the size of organizer mesoderm. Moreover, recent studies in the chick embryo suggest that inhibition of BMP signaling by chordin is not sufficient to induce neural plate tissue (Streit et al 1998). Nonetheless, these studies, like the analysis of zebrafish *swirl* and *chordino* mutants, suggest that modulation of BMP activity by antagonists such as chordin has a role in regulating the size of the neural plate. A similar function to subdivide the ectoderm into neural and nonneural domains has been proposed for the *Drosophila* homologues Dpp and Sog (Biehs et al 1996). Thus, the precise role of endogenous BMP antagonists in neural induction remains unclear and may in fact differ between organisms.

An additional unresolved issue is whether the induction of generic neural properties and the assignment of regional fate to neural cells are inseparable or independent processes. The neural tissue induced in Xenopus ectoderm by follistatin, noggin, and chordin expresses molecular markers that are normally confined to the forebrain (Lamb et al 1993, Hemmati-Brivanlou et al 1994, Sasai et al 1994, Lamb & Harland 1995). However, an analysis of ectopic neural induction in the chick embryo indicates that it is possible to generate neural tissue that lacks any sign of an early anteroposterior pattern (Streit et al 1997). The formation of neural tissue of caudal character may require subsequent signaling events, perhaps involving secreted proteins of the fibroblast growth factor (FGF) family (Isaacs et al 1992, Cox & Hemmati-Brivanlou 1995, Kengaku & Okamoto 1995, Lamb & Harland 1995, Storey et al 1998).

Moreover, it also remains unclear whether neural plate formation and the early specification of the dorsoventral identity of neural cells occur

independently. As will be discussed below, BMP signaling has been implicated in the control of dorsal cell fate as well as in neural induction. Thus, the generation of the neural plate through the regulation of BMP signaling could establish intrinsic regional characteristics in early neural plate cells that modulate their response to signals that specify neuronal identity. In support of this idea, Xenopus ectodermal explants that have been neuralized by noggin treatment show an apparent dorsoventral organization (Knecht et al 1995) and different doses of noggin promote the generation of neural cells with distinct dorsoventral identities (Knecht & Harland 1997).

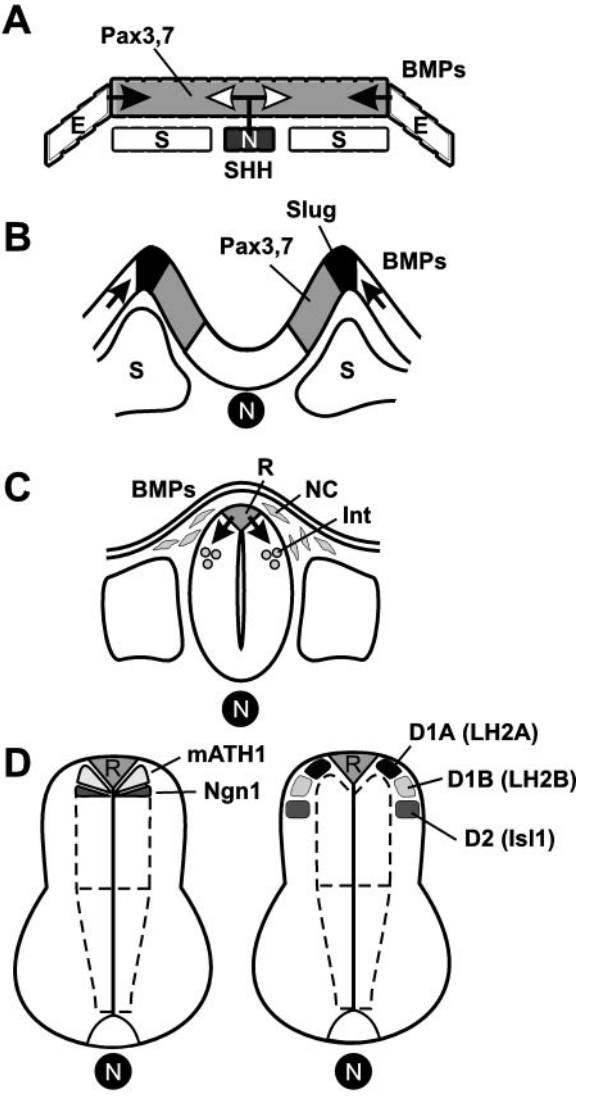
CELL PATTERNING IN THE DORSAL SPINAL CORD

To begin to examine the mechanisms of dorsal cell patterning, we focus first on the spinal cord because the early development of its component cell types has been analyzed in greater detail than in most rostral regions of the neuraxis. The neurons of the mature spinal cord serve two major functions. They relay cutaneous sensory input to higher centers in the brain, and they coordinate motor output. The neuronal circuits that mediate these functions are to a large extent segregated anatomically; neurons involved in the processing of cutaneous sensory input are located in the dorsal half of the spinal cord, whereas neurons involved in motor output reside in the ventral half of the spinal cord.

The major cell types of the spinal cord are generated at different positions along the dorsoventral axis of the early neural tube (Figure 1) (Tanabe & Jessell 1996). Ventrally, a specialized group of glial cells, the floor plate, forms at the midline of the neural tube. Motor neurons are generated at locations lateral to the floor plate, and several classes of interneurons are formed at positions dorsal to motor neurons. The dorsal half of the neural tube initially gives rise to neural crest cells, which subsequently migrate from the dorsal neuroepithelium and populate the peripheral nervous system. Roof plate cells are formed at the dorsal midline of the neural tube, and at a later stage, cells lateral to the roof plate differentiate into several classes of dorsal sensory interneurons.

Dorsal and Ventral Neural Cells Are Specified Independently

The inductive signals that control the identity and pattern of neural cell types in the spinal cord derive from two distinct groups of nonneural cells. Experimental evidence for the independence of dorsal and ventral patterning signals emerged initially from surgical manipulations in chick and amphibian embryos and from the analysis of mouse and zebrafish mutants. These studies established a requirement for inductive signals provided by the notochord and floor plate for the generation of ventral cell types in the brain and spinal cord. This



ventralizing signal appears to be mediated by the secreted glycoprotein Sonic Hedgehog (Shh). A wide range of ventral neural cell types is eliminated after surgical removal of the notochord at early stages (van Straaten & Hekking 1991, Yamada et al 1991, Ericson et al 1992), in mutant mice and zebrafish embryos that lack notochord and floor plate (Bovolenta & Dodd 1991, Beattie et al 1997), in *Shh* null mutant mice (Chiang et al 1996), or after blockade of Shh signaling with specific antibodies (Marti et al 1995, Ericson et al 1996, 1997).

The differentiation of dorsal neural cell types persists in the absence of ventralizing signals (Yamada et al 1991, Ericson et al 1992, Liem et al 1997), suggesting two possible mechanisms for the control of dorsal neural identities. First, the generation of dorsal cell types could represent a "default" program of neural differentiation. In this view, inductive signaling from the notochord could simply repress dorsal cell fates at ventral positions. Alternatively, the acquisition of dorsal neural fates could depend on inductive signaling from adjacent tissues, much as ventral fates depend on notochord signaling. If the default model were correct, the elimination of ventralizing signals should lead to an increase in the number of cells adopting dorsal fates and an expansion of these dorsal neural cells into more ventral regions of the neural tube. In fact, the elimination of notochord signaling results in the ventral expansion of the expression of certain dorsally restricted genes such as *Pax3* and *Pax7* (Yamada et al 1991, Goulding et al 1993, Chiang et al 1996, Ericson et al 1996). However, after notochord removal, the differentiation of definitive dorsal cell types such as neural crest cells, roof plate cells, and dorsal interneurons is still restricted to the dorsal neural tube (Liem et al 1997). These observations suggest that the specification of dorsal neural cell fates requires the action of "dorsalizing" inductive signals.

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Figure 1 Inductive signaling in the generation of dorsal neural cell types during spinal cord development. (*A*) The spinal cord is derived from the neural plate, an epithelial sheet overlying the notochord (N) and somitic mesoderm (S) and flanked by epidermal ectoderm (E). Neural cell patterning is controlled by ventralizing signals [Sonic Hedgehog (SHH)] (*open arrows*) from notochord cells and dorsalizing signals (BMPs) (*solid arrows*) from epidermal ectoderm. Pax3 and Pax7 are initially expressed at all mediolateral positions in the neural plate, but are repressed medially (ventrally) by SHH signaling. (*B*) At the neural fold stage, BMP signaling promotes maintenance of Pax3/Pax7 expression and induces expression of Slug in premigratory neural crest cells. (*C*) At the time of neural tube closure, neural crest cells (NC) emigrate from the dorsal neural tube and roof plate cells (R) are generated at the dorsal midline. Roof plate cells are a source of BMP signaling that controls the differentiation of dorsal interneurons (Int). (*D*) Distinct domains of dividing neuronal progenitors express the bHLH transcription factors mATH1 and Ngn1. Several classes of neurons (D1A, D1B, and D2) that derive from these progenitors are distinguished by expression of the LIM homeodomain proteins LH2A, LH2B, and Isl1.

Inductive Signals and the Generation of Neural Crest Cells

Initial studies aimed at defining the cellular source of dorsalizing signals focused on signals that induce neural crest differentiation. Neural crest cells constitute a migratory cell population that arises from the lateral margin of the neural plate. As the neural plate folds and closes at the future dorsal midline of the neural tube, neural crest cells delaminate from the neuroepithelium and migrate extensively to form the neurons and glial cells of the peripheral nervous system, melanocytes, and a variety of other nonneural cell types. Cell tracing studies have demonstrated that the lineages of premigratory neural crest and other dorsal neural cell types diverge just before neural crest emigration (Bronner-Fraser & Fraser 1988, Selleck & Bronner-Fraser 1995). Thus, signals acting around the time of neural tube closure appear to induce neural plate cells to adopt a neural crest fate. Two tissues located adjacent to the lateral neural plate at the time of neural crest generation, the nonneural (epidermal) ectoderm and the paraxial mesoderm, have been proposed to play a role in the specification of neural crest cell fates (reviewed in Baker & Bronner-Fraser 1997).

One source of a neural crest-inducing signal appears to be the nonneural or epidermal ectoderm that flanks the lateral neural plate. Grafting studies in amphibian (Moury & Jacobson 1989, 1990) and chick embryos (Dickinson et al 1995, Selleck & Bronner-Fraser 1995) have shown that the apposition of epidermal ectoderm and neural plate induces the generation of neural crest cells. In vitro studies using explants of chick neural plate from the presumptive spinal cord region have also shown that epidermal ectoderm can promote neural crest differentiation (Dickinson et al 1995, Liem et al 1995). These neural plate explants are cultured in the absence of mesoderm, thus excluding a late requirement for mesodermal signals in the induction of neural crest. Inductive signals from epidermal ectoderm therefore appear to be sufficient to induce neural crest cells in neural plate tissue at caudal levels of the neuraxis.

Inductive signals involved in neural crest generation could also be provided by the paraxial mesoderm and lateral plate mesoderm that lie beneath the lateral neural plate. Grafting studies in ovo and explant culture experiments in amphibian embryos (Raven & Kloos 1945, Mayor et al 1995, Bonstein et al 1998) have suggested that mesodermal signals have the capacity to induce neural crest cells. However, these experiments did not exclude the possibility that neural cells are also exposed to signals from epidermal ectoderm and thus do not resolve whether mesodermal signals are sufficient to induce neural crest cells. Isolated explants of chick neural plate cultured in the presence of paraxial mesoderm have, however, been reported to give rise to melanocytes (Selleck & Bronner-Fraser 1995), suggesting that mesodermal signals may be sufficient to induce some neural crest cell types.

The generation of neural crest cells is confined to levels of the neural tube caudal to the mid-diencephalon (Couly & Le Douarin 1987), suggesting that the apposition of epidermal ectoderm and neural plate at more rostral forebrain levels is not sufficient to induce neural crest cells. Recent experiments indicate that caudal paraxial mesoderm can provide a signal that "caudalizes" rostral neural plate cells, permitting them to respond to epidermal ectoderm signals with the generation of neural crest cells (Muhr et al 1997). A possible involvement of mesodermal signals in the induction of neural crest has also been suggested by studies in Xenopus in which the removal of presumptive paraxial mesoderm results in reduced neural crest generation (Bonstein et al 1998).

Taken together, these results suggest that neural crest induction is a multistep program. The process is likely to begin with the induction of the neural plate by presumptive axial mesoderm. A caudalizing signal from paraxial mesoderm may act to specify the region of neural plate that is competent to generate neural crest, and a subsequent dorsalizing signal from ectoderm may initiate neural crest generation. The involvement of later signaling events that direct neural crest migration and differentiation at target sites in the periphery has been well established (Stemple & Anderson 1993, Le Douarin et al 1994). Thus, the development of the neural crest appears to involve a series of local interactions that progressively restrict the fate of neural cells.

Inductive Signals and the Generation of Roof Plate Cells

A second cell population that is generated from lateral neural plate cells around the time of neural tube closure is the roof plate, a group of specialized dorsal midline glial cells (Altman & Bayer 1984). Lineage-tracing experiments in chicks (Bronner-Fraser & Fraser 1988) and in mice (Echelard et al 1994) have indicated that roof plate cells and neural crest cells share a common cellular precursor. In addition, at early stages of neural tube formation, markers that later define distinct roof plate and neural crest cell populations are coexpressed by dorsal midline cells. Thus, the mechanisms of induction of roof plate and neural crest cells may be related. This idea is supported by the observation that roof plate cells, like neural crest cells, are induced in neural plate explants by exposure to signals from adjacent epidermal ectoderm (Liem et al 1997).

THE MOLECULAR IDENTITY OF DORSALIZING SIGNALS

Three classes of secreted factors have been advanced as candidate signals that specify the fate of dorsal neural cells, notably neural crest cells. These are secreted proteins of the FGF, Wnt, and TGFß families. Members of each of these families of inductive factors are expressed in or adjacent to the lateral neural plate or dorsal neural tube, consistent with a role for these proteins

in the control of dorsal neural cell specification or proliferation. Much of the initial evidence for the involvement of these factors in dorsal neural patterning has come from gain-of-function studies, either from overexpression studies in Xenopus or from the analysis of neural tissue cultured in the presence of recombinant proteins. Loss-of-function studies designed to test the role of these candidate inductive factors in vivo have been hampered by the fact that these proteins are members of large multigene families with overlapping expression patterns. Nevertheless, recent genetic studies, discussed below, have begun to provide evidence that certain of these secreted inductive factors are required for dorsal neural patterning.

Fibroblast Growth Factors (FGFs)

Studies in Xenopus have indicated that FGFs can induce neural crest differentiation in ectodermal cells that have been neuralized by dissociation (Kengaku & Okamoto 1995) or by treatment with noggin (Mayor et al 1995) or chordin (LaBonne & Bronner-Fraser 1998). However, FGF is not sufficient to promote neural crest generation in isolated neural plate explants (Liem et al 1995, Mayor et al 1997, Muhr et al 1997). Neuralized (dissociated or noggin-treated) ectoderm explants are likely to contain a mixture of epidermal and neural cells, and interactions between these populations may have a role in the formation of neural crest cells in these cultures in response to FGF. Overexpression of a truncated dominant negative FGF receptor in neural plate cells has been reported to block the generation of neural crest cells in response to epidermal ectoderm (Mayor et al 1997). Thus, functional FGF receptor activity may be required for neural plate cells to respond to neural crest-inducing signals.

Because FGFs have also been implicated in the control of rostrocaudal identity in the early neural plate (Isaacs et al 1992, Cox & Hemmati-Brivanlou 1995, Kengaku & Okamoto 1995, Lamb & Harland 1995, Storey et al 1998), the apparent role of FGF signaling in neural crest generation could reflect a requirement for an FGF-mediated caudalizing signal. Moreover, the ability of FGFs to enhance the generation of neural crest cells appears to be indirect and may be mediated by Wnt family members (LaBonne & Bronner-Fraser 1998). Thus, the role of FGFs in neural crest induction remains unclear. FGFs appear not to be sufficient for neural crest specification, but may act in concert with other signaling factors in the patterning process that leads to neural crest differentiation.

Wnts

The Wnt proteins are a group of secreted cysteine-rich glycoproteins involved in a variety of embryonic cell-cell signaling events (Cadigan & Nusse 1997). Two of these factors, Wnt-1 and Wnt-3a, are expressed at the time of neural

crest generation in the dorsal neural tube from forebrain to spinal cord levels (Parr et al 1993, Hollyday et al 1995). Genetic studies in the mouse have established that Wnt-1 is required for midbrain development (McMahon & Bradley 1990, Thomas & Capecchi 1990) and Wnt-3a for paraxial mesoderm formation (Takada et al 1994). However, defects in the dorsoventral patterning of the neural tube have not been reported in *Wnt-1* and *Wnt-3a* null mutants, possibly as a result of the overlap in expression patterns and functional redundancy between individual Wnts. Evidence for a role for Wnts in dorsal neural tube development has, however, emerged from two experimental approaches. First, overexpression studies in the mouse and in Xenopus have suggested that Wnts are involved in the control of dorsal neural cell proliferation and patterning. Second, an analysis of *Wnt-1–Wnt-3a* compound mutant mouse embryos has revealed a requirement for Wnt signaling in the control of differentiation in the dorsal neural tube.

Misexpression of *Wnt-1* throughout the embryonic spinal cord in the mouse results in an enlargement of the dorsal neural tube (Dickinson et al 1994). This abnormality appears to result from an increase in cell proliferation rather than an alteration in cell identity along the dorsoventral axis of the neural tube, suggesting that Wnt-1 influences neural tube patterning by regulating the expansion of precursor cell populations.

However, three studies in Xenopus have provided evidence that Wnt proteins may have a more instructive role in the regulation of neural crest cell fate. Overexpression of *Wnt-1* or *Wnt-3a* in Xenopus embryos or in neuralized ectodermal explants leads to an increase in several neural crest cell markers (Saint-Jeannet et al 1997). In this study, an increase in the neural crest cell population was detected even when cell proliferation was blocked, perhaps reflecting a change in cell fate within the neural plate. The Xenopus *Wnt-7B* gene is expressed in the dorsal neural tube and epidermis after neural tube closure (Chang & Hemmati-Brivanlou 1998). *Wnt-7B* induces expression of the neural crest markers *Slug* and *Twist* in ectodermal explants neuralized by noggin or by dissociation (Chang & Hemmati-Brivanlou 1998). Similarly, *Wnt-8* promotes expression of *Slug* in ectodermal tissue that has been neuralized by chordin (LaBonne & Bronner-Fraser 1998). Like FGFs, Wnts have been implicated in the regulation of neural patterning along the rostrocaudal axis (McGrew et al 1995). Because the neural tissue induced by chordin or noggin appears rostral in character (Lamb et al 1993, Sasai et al 1994, Lamb & Harland 1995), a caudalizing signal may be required to make this neural tissue competent to generate neural crest in response to epidermal ectoderm signals. Thus, the ability of Wnt proteins to increase neural crest cell number may reflect their caudalizing activity. However, the caudal neural marker *HoxB9* (*Xlhbox6*) is not induced by *Wnt-7B* overexpression (Chang & Hemmati-Brivanlou 1998), and no change

occurs in the position of the hindbrain marker *Krox-20* in *Wnt-3a*-injected embryos (Saint-Jeannet et al 1997). Thus, these studies suggest a more direct activity for Wnts in dorsoventral patterning of the neural plate.

The best evidence to date for a requirement for Wnt signaling in the control of dorsal neural development has been provided by an analysis of mice that lack both *Wnt-1* and *Wnt-3a* activity (Ikeya et al 1997). *Wnt-1–Wnt-3a* double mutants show a significant reduction in the number of melanocytes and cranial and spinal sensory neurons and exhibit deficits in skeletal structures derived from the cranial neural crest. However, in these mutant embryos, a range of distinct cell types is still found at appropriate positions along the dorsoventral axis of the neural tube. Thus, this study suggests that Wnt signaling is not required for primary patterning of neural cell identity in the mouse, but rather for the expansion of dorsal neural progenitors. The many Wnt proteins that are expressed in the dorsal neural tube are therefore candidates for regionally restricted mitogens or survival factors that regulate proliferation of neural crest and CNS progenitors.

Transforming Growth Factor-ß (TGFß)-Related Proteins

The TGFß superfamily, including bone morphogenetic proteins (BMPs) and activins, constitutes a large group of highly conserved secreted signaling proteins (reviewed in Kingsley 1994, Hogan 1996). Initial screens for TGFß superfamily members expressed in the dorsal neural tube identified dorsalin-1 (Dsl-1), a secreted BMP-like factor (Basler et al 1993). Dsl-1, like epidermal ectoderm, can induce the generation of neural crest cells in chick neural plate explants in vitro. The expression of Dsl-1 is, however, initiated in the dorsal neural tube after the onset of neural crest generation, indicating that Dsl-1 does not mediate the dorsalizing activity of epidermal ectoderm in vivo, but may mimic the activity of endogenous inducing factors.

The ability of Dsl-1 to induce neural crest generation nevertheless suggested that dorsalizing signals from epidermal ectoderm are likely to involve members of the BMP family. Subsequent studies revealed that several BMPs are expressed in epidermal ectoderm at a stage more consistent with their function as endogenous dorsalizing signals. In the chick embryo, BMP4 and BMP7 are expressed in this manner and, like Dsl-1, they mimic the ability of epidermal ectoderm to induce neural crest formation in chick neural plate tissue (Liem et al 1995). In addition, these BMPs have the capacity to induce roof plate cells in neural plate explants (Liem et al 1997).

Evidence of a requirement for BMP activity in the induction of neural crest and roof plate by epidermal ectoderm has emerged from studies with secreted antagonists of BMP signaling. As described above, the study of neural induction in Xenopus has uncovered at least three putative neural inducing factors that

are structurally unrelated, but appear to share a common mechanism of action. These proteins, noggin, chordin, and follistatin, bind BMPs and activins and block their activity, apparently by preventing the activation of specific BMP and activin receptors. In vitro studies reveal that different inhibitors have specific abilities to antagonize distinct members of the TGFß family (Liem et al 1997). The neural crest-inducing activity of BMP4 is blocked by noggin, but not by follistatin, whereas the inductive activity of BMP7 in these assays is blocked by follistatin, but not by noggin. Treatment with follistatin and noggin significantly inhibits the ability of epidermal ectoderm to induce neural crest and roof plate cells in vitro (Liem et al 1997). Thus, the activity of epidermal ectoderm to promote generation of these dorsal neural cell types appears to involve BMP or TGFβ signaling (Figure 1).

These in vitro studies have provided strong support for a role for BMPs in the control of dorsal neural cell identity and pattern. However, since noggin and follistatin antagonize a number of different BMPs as well as activins, the identity of the relevant TGFß-related signaling factor(s) is not clear. Genetic studies have thus far failed to demonstrate a direct requirement for specific BMPs in the induction of neural crest cells. By virtue of their pattern and timing of expression, BMP2, BMP4, and BMP7 are the most likely mediators of the dorsalizing signal from epidermal ectoderm in mice. Mice lacking *BMP2* or *BMP4* activity die early in embryogenesis, and this early lethality precludes an easy analysis of their requirement for dorsal neural patterning. Mice that lack *BMP7* functions have defects in growth and morphogenesis of the eye, but abnormalities in neural crest and dorsal spinal cord development have not been detected. The failure to demonstrate essential functions for specific BMPs in early dorsoventral patterning of the neural plate may reflect functional redundancy between BMP family members (Dudley & Robertson 1997). A more detailed analysis of neuronal patterning in zebrafish *BMP2* (*swirl*) (Kishimoto et al 1997) and *chordin* (*chordino*) mutants (Schulte-Merker et al 1997) may shed additional light on the requirement for regulated BMP signaling in the control of early dorsal neural cell fate.

THE RESPONSE OF NEURAL PLATE CELLS TO DORSALIZING SIGNALS

How do cells in the lateral neural plate and dorsal neural tube respond to BMP signals from the adjacent epidermal ectoderm? The transcription factors *Pax3*, *Pax7*, *Msx1*, and *Msx2* are initially expressed throughout the neural plate but are repressed ventrally by Shh signaling (Figure 1). Expression of these four genes in neural plate cells is elevated by exposure to epidermal ectoderm or to BMPs (Liem et al 1995, Monsoro-Burq et al 1996). Thus, the initial phase

of neural expression of these *Pax* and *Msx* genes appears not to depend on signals from the epidermal ectoderm but may, nevertheless, be a consequence of their induction by BMPs expressed by ectodermal cells before neural plate formation.

The role of these *Pax* and *Msx* genes in the response of cells to dorsalizing signals has not been resolved. Neural plate cells that express these genes but have not yet been exposed to epidermal ectoderm signaling do not generate neural crest cells when grown in vitro (Liem et al 1995). Thus, the expression of these *Pax* and *Msx* genes by neural plate cells appears insufficient to trigger the differentiation of definitive dorsal cell types. The expression of *Msx* genes appears to be upregulated by BMPs in a variety of tissues and may therefore represent a more general component of the cellular response to BMP signaling (reviewed in Davidson 1995). Defects in dorsal neural patterning have not been reported in mice lacking *Pax3* or *Pax7* function. However, the differentiation of certain neural crest derivatives is disrupted in *Pax3* and *Pax7* mutants (Stuart et al 1994, Mansouri et al 1996), suggesting that these genes have roles at later stages of neural crest development.

Several zinc finger class transcription factors have been identified in dorsal neural tube cells, and there is emerging evidence that these proteins are involved in the initial specification of neural crest cells by BMP-mediated signals from the epidermal ectoderm. One such gene, *SLUG*, is induced in vitro and in vivo by epidermal ectoderm and by BMPs (Figure 1) (Dickinson et al 1995, Liem et al 1995). Moreover, a reduction in neural *SLUG* expression by antisense oligonucleotide treatment has been reported to impair the emigration of neural crest cells (Nieto et al 1994). However, gene-targeting studies have shown that *Slug* function is not required for neural crest generation, migration, or differentiation in mice (Jiang et al 1998). In addition, the overexpression of *Slug* in isolated *Xenopus* ectodermal tissue appears to be insufficient for the generation of neural crest cells (LaBonne & Bronner-Fraser 1998), indicating that additional factors are required to promote neural crest differentiation. The *Zic* genes represent a second class of zinc finger transcription factors with potential roles in neural crest differentiation. Vertebrate embryos express at least three *Zic* genes in dorsal regions of the neural tube (Nagai et al 1997). Studies in Xenopus embryos have shown that overexpression of *Zic3* (Nakata et al 1997) or *Zic2* (Brewster et al 1998) induces the expression of neural crest markers in ectodermal tissue. However, mice lacking *Zic1* function do not exhibit defects in neural crest cell differentiation (Aruga et al 1998), possibly reflecting the overlap in expression and function of other *Zic* genes. Defects in the differentiation of neural crest cells have been reported in mice with mutations in the gene encoding the transcription factor AP2 (Zhang et al 1996), although the stage at which AP2 acts in neural crest cell development has not been defined.

Premigratory neural crest cells that are generated in the dorsal neural tube subsequently delaminate from the dorsal neural epithelium and acquire mesenchymal properties before their long-range migration. Several of the genes that are induced in dorsal neural tube cells by BMPs may contribute to this delamination process. The ras superfamily GTP-binding protein rhoB is expressed by premigratory neural crest cells and transiently in neural crest cells after their delamination (J-P Liu & TM Jessell, submitted). Expression of rhoB is induced in neural plate tissue exposed to BMPs, although the time course of *rhoB* induction is slower than that of *Slug*, suggesting that rhoB functions at a step downstream of the initial specification of premigratory neural crest cells. Consistent with this idea, the delamination, but not the specification, of neural crest cells can be inhibited in vitro by inactivation of rho proteins with the bacterial exotoxin C3 (J-P Liu & TM Jessell, submitted). The rho class of GTP-binding proteins has been implicated in the control of the actin cytoskeleton and in cell substrate adhesion (Hall 1998). Therefore, rhoB may be involved in the reorganization of cell shape and adhesive contacts associated with the epithelial-to-mesenchymal transition of neural crest cells that occurs during delamination from the dorsal neural tube.

Expression of the cell adhesion molecule cadherin 6B is also upregulated in premigratory neural crest cells (Nakagawa & Takeichi 1995) and can be induced in neural plate tissue by BMPs (J-P Liu & TM Jessell, submitted). Around the time of neural crest delamination, expression of *cadherin 6B* is extinguished and a closely related gene, *cadherin 7* is expressed (Nakagawa & Takeichi 1995). The roles of these two cadherins in neural crest development remain unclear, but by analogy with other known activities of cadherins in selective cell adhesion (Takeichi 1995), it is possible that the delamination of neural crest cells is regulated by this switch in cadherin expression. Taken together, these findings suggest that BMPs provided by the epidermal ectoderm are able to induce the expression of genes involved both in the specification of premigratory neural crest cells and in their subsequent emigration from the neural tube.

BMP-mediated signals from the epidermal ectoderm are also involved in the induction of roof plate cells at the dorsal midline of the neural tube (Liem et al 1997). At late stages of neural tube development, roof plate cells are defined by expression of the bZIP transcription factor MAFB. Soon after neural tube closure, however, cells at the dorsal midline of the neural tube coexpress Slug and MAFB. As described earlier, lineage-tracing studies have provided evidence that individual cells at the dorsal midline of the neural tube can give rise both to neural crest and roof plate cells. How the distinct identities of roof plate and neural crest cells are established therefore remains unclear. Temporal changes in gene expression by dorsal midline cells might contribute to this choice of alternate fates. The downregulation of Slug expression in dorsal midline cells

could lead to the loss of potential of cells to generate neural crest, with the consequence that those MAFB-expressing cells that remain at the dorsal midline of the neural tube acquire roof plate identity.

INDUCTIVE SIGNALING IN THE GENERATION OF DORSAL HORN INTERNEURONS

Interneurons of the dorsal horn of the spinal cord play a role in the central processing of sensory stimuli. The majority of these dorsal interneurons are generated at later stages, after the closure of the neural tube. During the period of dorsal interneuron generation, the epidermal ectoderm is no longer in contact with the dorsal neural tube, and the ectodermal expression of BMP4 and BMP7 is downregulated (Liem et al 1995, Watanabe & Le Douarin 1996). Thus, it is unlikely that the epidermal ectoderm is directly responsible for the induction of dorsal interneurons.

Recent studies suggest that the roof plate is a source of later inductive signals that control the generation of dorsal horn neurons (Figure 1*C*). Experiments with cultured chick neural plate tissue have demonstrated that signals from the roof plate are sufficient to promote dorsal interneuron differentiation in vitro (Liem et al 1997). In these explant cultures, several classes of dorsal interneurons are induced. Strikingly, the positions at which these interneuron classes are generated relative to the roof plate in vitro recapitulates their normal relative positions in vivo. Interneurons that express the transcription factors LH2A and LH2B (D1A and D1B neurons, collectively D1 neurons) are generated close to the roof plate, whereas interneurons that express Isl1 (D2 neurons) are generated further from the roof plate (Figure 1*D*).

What is the molecular nature of the roof plate–derived inductive signal(s) for dorsal interneurons? Around the time of dorsal interneuron generation, cells in and adjacent to the roof plate in the chick neural tube express at least six members of the TGFß family (BMP4, BMP5, BMP7, DSL1, GDF6/7, and activinB) in nested domains (Liem et al 1997; Lee et al 1998). Treatment of chick neural plate tissue with BMP4, BMP5, BMP7, GDF6/7, or DSL1 induces D1 interneurons (Liem et al 1997; Lee et al 1998), whereas treatment with activin induces D2 interneurons (Liem et al 1997). Since activinB appears to be expressed in a broader domain than that of BMPs, neural progenitors close to the roof plate may be exposed to a high level of BMP activity and preferentially generate D1 neurons, whereas progenitors further from the roof plate may be exposed to low BMP and high activin doses and respond with the generation of D2 interneurons. Thus, qualitative differences in the signaling activity of TGFß-related proteins expressed in and around the roof plate may influence the position and identity of dorsal neurons. A requirement for TGFß signaling in the generation

of dorsal interneurons is also supported by studies in which the induction of D1 and D2 neurons by the roof plate is markedly inhibited by treatment with the TGFß antagonists noggin and follistatin (Liem et al 1997; Lee et al 1998).

One additional mechanism for the diversification of dorsal neuronal fates appears to be a temporal change in the response of neural cells to TGFß signaling. The choice between neural crest and dorsal interneuron fates appears to be regulated by a temporal switch in neural cell responsiveness (Liem et al 1997). At early stages of development, neural progenitors respond to BMPs with the generation of neural crest cells, but not dorsal interneurons. At later times, the same BMP signal promotes generation of dorsal interneurons, but not neural crest cells. The consequence of this changing responsiveness is that ectodermal BMPs are likely to direct neural crest and roof plate fates, whereas later roof plate–derived BMPs are likely to direct dorsal interneuron fates.

A Requirement for the BMP-Related Factor GDF7 in Dorsal Interneuron Generation

The in vitro studies described above have suggested that the induction of dorsal interneurons by the roof plate involves signaling by TGFß-related proteins, but they do not address the requirement for individual signaling molecules in this patterning process. Since several BMPs and related factors are expressed by roof plate cells, two key questions remain unresolved (Figure 2). Are specific BMPs required in vivo for the induction of dorsal interneurons? And do BMPs have distinct or redundant functions in the control of neural cell fate in the dorsal spinal cord?

Genetic analyses in mice have begun to address the requirement for specific BMPs in the control of dorsal interneuron differentiation. These studies have initially focused on GDF7, a BMP family member expressed selectively by roof plate cells after neural tube closure (Lee et al 1998). *GDF7* null mutant embryos exhibit a selective defect in neurogenesis in the dorsal spinal cord. In these mutants, a single class of dorsal interneurons, D1A neurons, is eliminated, whereas the differentiation of other identified dorsal cell types, including the roof plate itself, is unaffected. Thus, GDF7 has an essential role in the development of a specific subset of dorsal interneurons, providing genetic support for the involvement of BMP signaling in the control of neuronal identity and pattern in the dorsal spinal cord.

The bHLH transcription factor mATH1 is expressed by dividing neural progenitors adjacent to the roof plate (Figure 1*D*) (Akazawa et al 1995, Ben-Arie et al 1996). Transgenic studies in mice, using the mATH1 promoter to drive reporter gene expression, suggest that mATH1 progenitors give rise to the D1

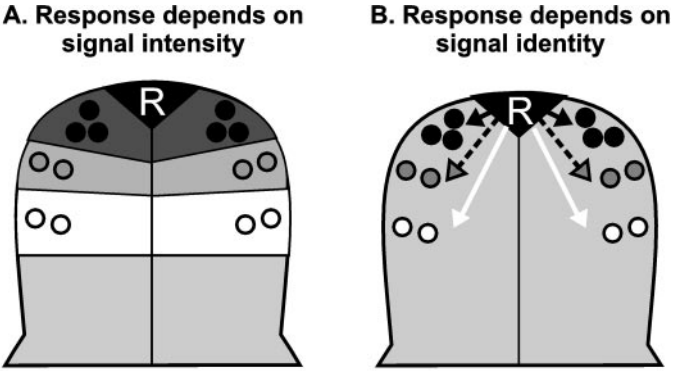

Figure 2 Two models for the generation of neuronal diversity in the dorsal spinal cord. (*A*) In this model, the BMP-related proteins expressed in and around the roof plate could have overlapping functions in patterning the dorsal neural tube. The nested expression of these proteins may establish a dorsal domain of graded BMP activity such that different cell types are specified by distinct levels of BMP signaling. (*B*) In model B, different BMP-related proteins could have qualitatively distinct roles in dorsal cell fate specification. In this view, different cell types may each depend on the activity of individual signaling factors.

classes of dorsal interneurons (Helms & Johnson 1998). In *GDF7* mutants, the initial expression of mATH1 and the generation of D1B neurons from these mATH1 progenitors are unaffected, whereas the later expression of mATH1 and the production of D1A neurons are lost (Figure 3) (Lee et al 1998). Thus, these studies suggest that GDF7 functions to regulate the formation of a population of dorsal neural progenitors that are fated to generate D1A interneurons.

GDF7 is one of three BMPs expressed by mouse roof plate cells, and the expression of BMP6 and BMP7 by roof plate cells is not affected by loss of GDF7 function. Thus, the elimination of D1A interneurons in GDF7 mutants could reflect a specific requirement for GDF7 signaling in their induction. An alternate explanation is that the generation of D1A neurons is simply dependent on high levels of net BMP activity (Figure 2*A*). In this view, the loss of GDF7 function might simply reduce BMP activity to a level below the threshold required for the generation of D1A neurons. However, the loss of D1A interneurons is not observed in *BMP7* mutants (KJ Lee et al, unpublished data). Thus, the elimination of D1A interneurons in *GDF7* mutants is most easily explained by a selective dependence on GDF7 signaling rather than a requirement for a high net level of "generic" BMP activity.

These results indicate that GDF7 has an essential signaling function that is not compensated for by other BMP family members. However, this requirement

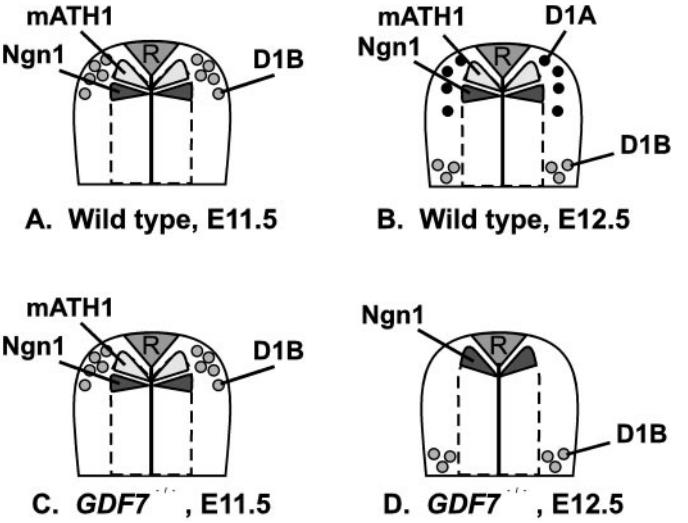

Figure 3 Elimination of GDF7, a BMP-related factor expressed by roof plate cells, results in the selective loss of a single class of dorsal interneurons. In *GDF7* homozygous null mutants, the initial appearance of mATH1$^+$ neural progenitors adjacent to the roof plate and the generation of the D1B class of interneurons from these progenitors are unaffected [embryonic day 11.5 (E11.5) shown in *panels A, C*]. However, in these mutant embryos, the maintenance of the mATH1$^+$ population and the generation of D1A interneurons from this progenitor pool is eliminated (E12.5, shown in *panels B, D*). In *GDF7* null embryos, Ngn1$^+$ progenitors, normally located just ventral to the mATH1 domain (*panel B*), occupy a position immediately adjacent to the roof plate (*panel D*). Thus, GDF7 signaling appears to direct the formation of a group of progenitor cells that give rise to a specific dorsal neuronal cell class. (From Lee et al 1998.)

for GDF7 activity does not appear to result from a unique activity of GDF7 to induce D1A neurons. Treatment of *GDF7* mutant explants with either GDF7 or BMP7 restores late expression of mATH1 (Lee et al 1998). Thus, the inability of BMP6 and BMP7 to compensate for the loss of GDF7 function may indicate that BMP6 and BMP7 are not active in vivo or are not presented to neural precursor cells at a time when GDF7 signals are required for D1A neuron generation. Taken together, these data suggest that the multiple BMPs expressed by roof plate cells have distinct and nonredundant roles in neuronal patterning in the dorsal spinal cord (Figure 2*B*).

An Essential Role for Roof Plate Signaling in Dorsal Neural Patterning

In vitro induction assays, together with an analysis of *GDF7* mutant mice, suggest that the generation of dorsal cell types depends on a cascade of

BMP-mediated signaling that originates from epidermal ectoderm and is propagated by roof plate cells (Figure 1). The patterning of neural cell types in the ventral spinal cord appears to involve a similar cascade of Shh-mediated signaling initiated in an adjacent nonneural tissue, the notochord, and propagated by a ventral midline glial population, the floor plate.

Can all neural patterning be accomplished by primary, nonneural sources of inductive signals, the notochord and epidermal ectoderm? Or do secondary, neural organizing centers, the floor plate and roof plate, have distinct roles in the patterning of neural cell types in the spinal cord? In the ventral spinal cord, early Shh signaling by the notochord and late Shh signaling by the floor plate have been proposed to serve different functions in the patterning of ventral cell types (Placzek et al 1993, Ericson et al 1996). However, an analysis of zebrafish mutant embryos has suggested that inductive signals from either notochord or floor plate are sufficient to induce the differentiation of secondary motor neurons (Beattie et al 1997). Thus, the role of the floor plate as an essential secondary neural organizing center remains unclear. In contrast, the analysis of dorsal neural patterning in *GDF7* mutants shows directly a requirement for roof plate–derived inductive signals in the differentiation of a class of dorsal interneurons. Thus, inductive signals from the roof plate appear to serve a distinct function in dorsal neural patterning that is not provided by earlier signals from the epidermal ectoderm.

MUTATIONS THAT AFFECT DORSAL NEURAL DEVELOPMENT

In addition to the many known genes that appear to regulate dorsal patterning, a number of spontaneously generated mouse mutations affect the development of the dorsal neural tube. In particular, dorsal neural tube defects have been described in mice with two mutations, *open brain (opb)* and *dreher (dr)*. The analysis of these mouse mutants may provide valuable models to study additional aspects of the mechanisms involved in dorsal neural fate specification.

Homozygous *opb* mutants exhibit a severe exencephalic malformation, apparently caused by a failure of neural tube closure at the midbrain-forebrain boundary, and have pronounced abnormalities in the morphology of the spinal cord and dorsal root ganglia (Günther et al 1994). The extent of these defects varies along the rostrocaudal axis, and, in the most severely affected regions, the spinal cord appears nearly circular in the transverse plane. In these regions, the expression of roof plate markers such as *Wnt-1* and *Wnt-3a* is lost (Günther et al 1994, Nagai et al 1997). The apparent loss of roof plate cells in *opb* mutants is also accompanied by changes in the expression patterns of *Pax3*, *Pax6*, *Msx2*, *Zic1*, and *Zic2*. In addition, expression of *Shh* and *HNF-3ß* in the ventral neural tube is expanded in *opb* mutants. These observations suggest that the *opb*

mutation leads to defects in the specification of cell fates along the dorsoventral axis of the neural tube.

Homozygous *dr* mutants have reduced viability, and adult mice are ataxic and show a variety of other neurological abnormalities, including circling behavior and hyperactivity (Green 1981). An analysis of *dr* mutant embryos has revealed that the roof plate in the caudal neural tube and at the midbrain-hindbrain junction is significantly reduced in size (JH Millonig et al, in preparation). In addition, the D1 classes of dorsal interneurons are markedly reduced in number in *dr* mutants. Homozygous *dr* mutant embryos also exhibit severe defects in early cerebellar development.

Thus, the depletion of dorsal neural cell types in *dr* and *opb* mutants is consistent with perturbations in dorsal inductive signaling. However, further analysis is needed to determine whether these defects are attributable to a lack of inductive signals from epidermal ectoderm and roof plate cells or to an abnormal response of neural progenitors to these inductive signals.

CONTROL OF DORSAL CELL FATE AT ROSTRAL LEVELS OF THE NEURAL TUBE

As discussed above, the patterning of neural cell types appears to be controlled by the position that cells occupy along the rostrocaudal as well as the dorsoventral axis. How are these two axes of positional information integrated to determine cellular identity? Two possible models may be envisioned. The signaling system that dictates dorsoventral identity may be similar at all rostrocaudal levels, and intrinsic differences in the rostrocaudal character of neural progenitors could be responsible for regionally distinct cell fates. Alternatively, dorsoventral signaling systems may vary along the rostrocaudal axis, such that neural progenitors are exposed to regionally distinct environmental signals. Experimental evidence has provided support for aspects of both models. It is likely therefore that both intrinsic differences in neural progenitors and positional differences in extrinsic signals contribute to patterning of neural identity along the rostrocaudal and dorsoventral axes of the neural tube. We next discuss recent studies that address the mechanisms of dorsal cell patterning in the hindbrain, midbrain, and forebrain, suggesting that some aspects of dorsal cell fate specification are similar at all levels of the neural tube. We also review evidence indicating that variations in this dorsalizing system at different rostrocaudal positions may contribute to regional differences in dorsal neural cell fate.

Specification of Dorsal Cell Fate in the Hindbrain

The domain of the neural plate that gives rise to the hindbrain is located rostral to the region of neural plate that generates spinal cord, but these two regions share many similarities. The neural plate at both levels is flanked by epidermal

ectoderm and paraxial mesoderm. Moreover, dorsal (initially lateral) neural plate precursors at hindbrain levels, as at spinal cord levels, give rise to neural crest cells and roof plate cells. Finally, the expression of many genes is conserved between hindbrain and spinal cord levels of the neural plate and in the respective adjacent tissues.

Recent evidence suggests that dorsal cell patterning in the hindbrain, as in the spinal cord, is controlled by inductive signals mediated by BMPs (Figure 4). In the chick embryo, epidermal ectoderm flanking the hindbrain neural plate expresses BMP4 and BMP7 (Pourquie et al 1996, Muhr et al 1997, Schultheiss

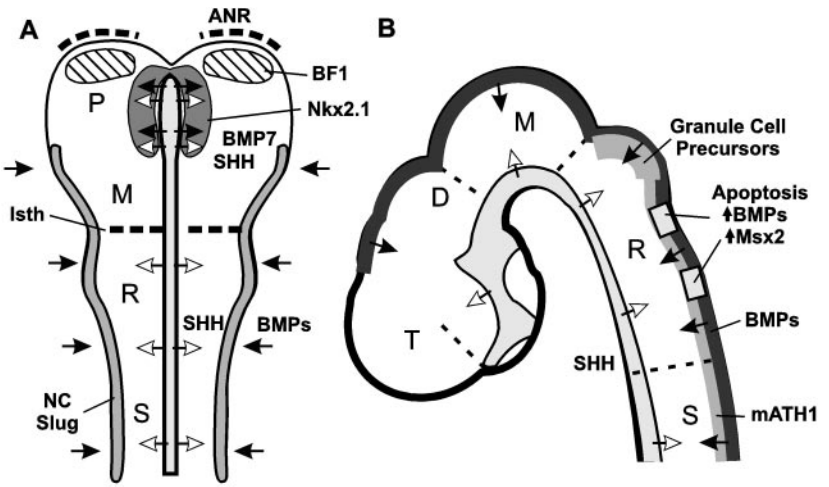

Figure 4 Inductive signaling in the specification of dorsal neural cell types at distinct rostrocaudal positions. (*A*) At neural plate stages, ventralizing signals (SHH) (*open arrows*) are provided by notochord cells at the midline. Neural crest cells are generated in response to inductive signals (BMPs) (*solid arrows*) from epidermal ectoderm cells lateral to the neural plate at spinal cord (S), hindbrain [rhombencephalon (R)], and midbrain [mesencephalon (M)] levels. The combined expression of BMP7 and SHH by prechordal plate cells is proposed to induce Nkx2.1 expression in midline cells of the prospective forebrain [prosencephalon (P)]. FGF signaling by the anterior neural ridge (ANR) appears to direct expression of BF1 in the future telencephalon, much as FGF signaling by the isthmus (Isth) is thought to direct local growth and pattern of the midbrain-hindbrain region. (*B*) At neural tube stages, BMPs provided by roof plate cells appear to impose dorsal cell fates along the neural axis from the spinal cord (S) to the diencephalon (D). mATH1 expression is induced in neural progenitors adjacent to the roof plate at spinal cord and rhombencephalic (R) levels. Inductive signals from even numbered rhombomeres induce high-level BMP and Msx2 expression and promote apoptotic elimination of neural crest cells in r3 and r5. Granule cell precursors, which also express mATH1, are induced dorsally in the rostral hindbrain. In the medial telencephalon (T), BMPs appear to reduce cell proliferation and BF1 expression and promote apoptosis and Msx1 expression.

et al 1997). Hindbrain epidermal ectoderm promotes generation of neural crest cells and increases expression of Msx genes in chick hindbrain neural plate explants, and these activities are mimicked by BMP4 (Muhr et al 1997).

Several *BMP* genes, including *BMP2*, *BMP4* and *BMP7*, are also expressed in hindbrain region epidermal ectoderm in the mouse (Lyons et al 1995, Arkell & Beddington 1997, Dudley & Robertson 1997), and their function in patterning the hindbrain neural plate has been suggested by studies of cultured mouse embryos (Arkell & Beddington 1997). In these studies, the implantation of BMP7-expressing cell pellets adjacent to the ventral hindbrain resulted in a marked hypertrophy of the neural tube and a local increase in mitotic activity. In addition, expression of the dorsal genes *Msx1* and *AP2* and ectopic neural crest cell generation was detected in the ventral hindbrain near grafts of BMP7-expressing cells. Thus, BMP7 appears to regulate growth and promote dorsal cell fates in the hindbrain. However, ventral BMP7 misexpression did not affect the dorsal restriction of *Pax3* expression, indicating that some dorsal properties may be unaffected by BMP signaling over this period of hindbrain development. Similar studies in the chick spinal cord found that ectopic BMP4 can expand the domain of *Pax3* expression in the ventral neural tube (Monsoro-Burq et al 1996). These contrasting results may reflect differences in the timing of ectopic BMP treatment or, perhaps less likely, differences in the regulation of *Pax* gene expression in hindbrain and spinal cord levels of the neuraxis. Changes in dorsoventral patterning in the hindbrain have not been reported in *BMP7* mutant embryos, which, as discussed above, may be explained by functional redundancy between BMP family members.

Signaling between Rhombomeres Influences BMP Expression and Neural Crest Cell Fate in the Hindbrain

In addition to a general role in promoting dorsal cell differentiation in the hindbrain, BMPs may also have more specialized functions in directing the fates of certain dorsal cell populations. One example of a more specialized role for BMPs occurs in the hindbrain, where the neural tube is subdivided by a series of transient constrictions into domains termed rhombomeres. The rhombomeric pattern of cells in the hindbrain appears to reflect a segmental organization and significant advances have been made in understanding the control of segmental fate in this region of the CNS (Lumsden & Krumlauf 1996). One feature that has emerged from these studies is that inductive signals from newly formed rhombomeres regulate BMP expression and, in turn, influence the survival, fate, and differentiation of cells in adjacent rhombomeres (Figure 4). Cranial neural crest cells in the hindbrain originate at three locations and migrate in discrete streams to contribute to certain cranial ganglia and to the three branchial arches. Interposed between these domains of crest generation are regions, in rhombomere (r)

3 and r5, that do not appear to contribute migrating neural crest cells. Initially, however, neural crest cell generation appears continuous along the length of the hindbrain neural tube, but neural crest cells are eliminated from r3 and r5 before emigration (Lumsden et al 1991). These domains of neural crest depletion are coincident with *Msx2* expression and with regions of increased apoptosis. In ovo and in vitro studies suggest that the apoptotic elimination of crest cells from r3 and r5 depends on repressive signaling from even numbered rhombomeres (Graham et al 1993). The elimination of neural crest cells in response to signals from even numbered rhombomeres appears to involve induction of BMP4 expression in the dorsal neural tube in r3 and r5 (Graham et al 1994). In support of this, BMP4 promotes *Msx2* expression, stimulates apoptosis, and depletes neural crest cells in isolated explants from r3 and r5 (Figure 4). However, BMP4 does not deplete neural crest from r4 explants. Thus, intrinsic differences in the response properties of hindbrain cells as well as restrictions in the expression of BMP4 may control selective neural crest elimination.

Control of Cerebellar Development by Dorsal Inductive Signaling

The varied neuronal cell types of the cerebellum appear to originate from progenitor cells in the dorsolateral portion of the neural tube at the midbrain/hindbrain border (Hallonet & Le Douarin 1993). Cerebellar development depends critically on inductive signaling involving Wnt and FGF proteins secreted by cells of the isthmic region at this midbrain-hindbrain junction (Joyner 1996). Recent studies indicate that patterning signals that specify dorsoventral identity also appear to have a role in the assignment of cerebellar cell fates.

The granule cells of the cerebellum derive from the rhombic lip at the dorsal margin of the metencephalic (anterior hindbrain) neuroepithelium (Hatten & Heintz 1995). During embryogenesis, rhombic lip cells delaminate from the metencephalic neuroepithelium and migrate rostrally to the incipient cerebellar plate to form the external germinal layer (EGL). EGL cells proliferate during early postnatal life, then exit the cell cycle and migrate into the underlying cerebellar cortex to settle in the internal granule layer (IGL).

The specification of granule cell precursors appears to take place at early stages, when these cells are located within the rhombic lip. Rhombic lip granule cell precursors express the transcription factors mATH1 (Akazawa et al 1995, Ben-Arie et al 1996) and RU49 (Yang et al 1996), and *mATH1* function is required for cerebellar granule cell generation and for formation of the EGL (Ben-Arie et al 1997). Isolated rhombic lip cells differentiate into granule cell neurons after transplantation into the postnatal EGL (Alder et al 1996), suggesting that rhombic lip cells are competent to generate granule cells when provided with differentiation signals that are present in the EGL.

The inductive signals that specify cerebellar granule cell precursors within the rhombic lip may be similar to those that pattern dorsal cell fate in the caudal neural tube (J Alder, KJ Lee, TM Jessell & ME Hatten, in preparation). Granule cell precursors expressing mATH1 arise close to the metencephalic roof plate, a source of several BMPs, including BMP6, BMP7, and GDF7. Expression of *mATH1* and three other dorsal markers, *Zic1*, *Zic2*, and *Wnt3a*, is induced in explants of ventral metencephalic neural plates treated with BMP6, BMP7, or GDF7. Moreover, exposure to BMPs appears to confer on isolated ventral mentencephalic cells the competence to generate granule cells after transplantation into postnatal EGL. The induction of rhombic lip granule cell precursors, much like the induction of dorsal interneurons in the spinal cord, may therefore depend on BMP signals that originate in the hindbrain roof plate (Figure 4). Further studies are needed to determine whether the generation of other cerebellar cell types, such as deep nuclei and Purkinje cells, also depends on BMP-mediated dorsalizing signals.

Regulation of Dorsal Cell Fate in the Midbrain and Forebrain

The neural plate at midbrain (mesencephalic) and caudal diencephalic levels is flanked by epidermal ectoderm, and several BMPs are expressed in this epidermal ectoderm as well as in dorsal midline structures (roof plate) of the mesencephalon and diencephalon (Dudley & Robertson 1997, Furuta et al 1997). Neural crest cells are generated from the dorsal neural tube at all levels caudal to the mid- diencephalon. Moreover, this rostral limit of neural crest generation corresponds to the rostral boundary of BMP expression at early neural plate stages in the chick embryo (Figure 4) (Muhr et al 1997). The spatial control of neural crest generation along the rostrocaudal axis of the neural plate may therefore result in part from the early pattern of BMP expression in flanking epidermal ectoderm. In addition, telencephalic neural plate cells do not generate neural crest when exposed to BMPs in vitro, suggesting that intrinsic differences in rostral and caudal neural progenitors also delimit neural crest generation.

Until recently, the mechanisms responsible for cellular patterning in the forebrain have remained obscure. The difficulties in understanding forebrain development are attributable in part to the complexity of the rostral CNS, to the dramatic morphological rearrangements that occur during its development, and to the lack of overt segmentation in the early prospective forebrain. Recent analyses of gene expression patterns in the forebrain, combined with genetic studies, have nevertheless begun to elucidate the organization of the forebrain (Shimamura et al 1995).

Some general principles of dorsoventral organization appear to be conserved along the neuraxis from spinal cord to forebrain. A number of genes, such as

Pax3 (Goulding et al 1991) and *Zic1* and *Zic2* (Nagai et al 1997), are expressed along the length of the dorsal neural tube in the mouse. Molecular markers that define the roof plate (Shimamura et al 1995), including *BMP* gene expression (Lyons et al 1995, Dudley & Robertson 1997, Furuta et al 1997; Lee et al 1998), extend from the caudal spinal cord to the lamina terminalis at the base of the telencephalon. These observations suggest that some aspects of dorsal neural identity in the forebrain, as in the caudal neural tube, may be controlled by BMP signaling. In support of this idea, both rostral and caudal nonneural ectodermal cells share the ability to induce *Msx* expression in prosencephalic neural plate explants (Shimamura & Rubenstein 1997), and BMP7, which is expressed in ectoderm adjacent to most or all regions of the neural plate at later stages, can mimic the ability of ectoderm to induce *Msx* expression.

Although BMPs appear to control dorsal neural identity throughout the neuraxis, *BMP7* is also expressed by prechordal mesoderm that underlies the prospective ventral midline of the forebrain (Dale et al 1997). Recent studies suggest that BMP7 function is required coordinately with Shh to induce the specialized ventral midline cells of the rostral diencephalon (Figure 4). BMP7 appears to act directly on neural plate cells so that rostral diencephalon ventral midline cells rather than floor plate cells are generated in response to Shh. Thus, the fate of cells in the ventral forebrain appears to be controlled by specialized signaling properties of anterior axial mesoderm, and this inductive signaling may be mediated by an interaction between Shh and BMPs.

BMPs may also play a later role in the control of local cell growth and regionalization in the dorsal forebrain (Figure 4). *BMP2*, *BMP4*, *BMP5*, *BMP6*, and *BMP7* are coexpressed in a region of dorsomedial telencephalon in older mouse embryos (Furuta et al 1997). The expression of *BMPs* in this region correlates with high-level *Msx* expression, an exclusion of *BF1* expression, reduced cell proliferation, and increased apoptosis. In vitro assays indicate that BMPs induce *Msx1* expression, stimulate cell death, and inhibit BF1 expression and cell proliferation in telencephalic neuroectoderm explants. *BMP* expression in the dorsomedial forebrain also coincides with choroid plexus differentiation, as marked by expression of the winged helix gene *Hfh4*. However, *Hfh4* expression is not induced in telencephalic explants exposed to BMPs, which may indicate that other signals are required in concert with BMPs to promote choroid plexus formation.

Forebrain development also appears to depend on other local signaling mechanisms that reflect the distinct properties of the forebrain and its unique position at the rostral limit of the neural plate. One specialized characteristic of the prospective forebrain is expression of the winged helix transcription factor BF1 in the anterolateral neural plate that gives rise to the telencephalon (Tao & Lai 1992, Shimamura et al 1995). BF1 function is required for telencephalic

development (Xuan et al 1995). In vitro studies using explants of mouse rostral (prosencephalic) neural plate suggest that signals from the adjacent ectoderm, termed the anterior neural ridge, are necessary and sufficient to induce *BF1* expression (Figure 4) (Shimamura & Rubenstein 1997). The ability of the anterior neural ridge to induce *BF1* expression is not mimicked by epidermal ectoderm flanking the caudal neural plate or by BMP7 treatment. FGF8 is restricted in its expression to the anterior neural ridge and can induce *BF1* expression in prosencephalic neural plate explants. Moreover, the activity of the anterior neural ridge to induce *BF1* expression and promote generation of dopaminergic neurons in forebrain explants is inhibited by treatment with a soluble, high-affinity blocking receptor for FGF8 (Ye et al 1998). The anterior neural ridge may thus represent a specialized local organizing center, analogous to the isthmus region that patterns cell identity in the mid- and hindbrain (Crossley et al 1996, Joyner 1996).

Several other signaling centers may act at various stages of neural development to direct forebrain differentiation and identity. Anterior endoderm appears to provide critical inductive signals for anterior neural plate development in mouse (Thomas & Beddington 1996, Varlet et al 1997) and Xenopus (Bouwmeester et al 1996) embryos. A specialized population of cells at the anterior neural plate boundary is necessary and sufficient for forebrain patterning in the zebrafish embryo (Houart et al 1998). The early regional fate of the forebrain may therefore depend on a series of interactions between anterior cell populations.

CONCLUSIONS AND FUTURE DIRECTIONS

Studies over the past few years have provided only a preliminary insight into the mechanisms of cell patterning in the dorsal neural tube, and several important issues remain to be resolved. First, the emerging evidence that BMP signaling has a central role in dorsal neural patterning does not exclude that other factors, notably the Wnt proteins, may have accessory or independent functions in expanding the dorsal progenitor cell population or in specifying distinct dorsal fates. Since Wnt proteins are induced by BMPs (Dickinson et al 1995), the complete program of dorsal cell differentiation may necessarily involve the coordinated action of these two classes of secreted factors. Second, it remains unclear whether all cell populations generated in the dorsal neural tube depend on BMP signals from ectodermal or roof plate cells or both. Several neuronal cell types in the developing spinal cord, for example the local interneurons that populate dorsal horn laminae II and III, are generated from progenitor cells located in the intermediate region of the spinal cord (Nornes & Das 1974). These neuronal progenitors are quite distant from the dorsal

domains of BMP expression. Thus, it will be important to determine whether the generation of laminae II and III neurons and other intermediate-region cell types is directed by a distinct program, independent of dorsally-derived BMP signals.

The mechanisms by which BMP signaling controls dorsal cell fates have not yet been addressed. Many details of the pathway by which BMP signals are transduced have emerged in recent years from the study of nonneural cells (reviewed in Massagué 1996), and it will be important to apply these advances to the study of BMP signaling in neural systems. In particular, we need to know which BMP receptors are activated during dorsal patterning and how the intracellular mediators of BMP signals, the Smad proteins, define the response of neural cells to dorsalizing signals. One attractive possibility is that changes in BMP receptor or Smad expression, or in both, underlie the marked temporal change in competence of neural cells to BMP signals that has been revealed from in vitro studies of dorsal cell differentiation.

An additional striking finding has been the identification of a large and structurally diverse group of secreted proteins that bind to BMPs. In many cases, these BMP-binding proteins have been shown to block the signaling activity of BMPs (Piccolo et al 1996, Zimmerman et al 1996). However, there have also been suggestions that such proteins may instead facilitate the diffusion of BMPs from local sources of BMP synthesis and thus potentiate the activity or range of action of BMPs (Holley et al 1996). One of these proteins, noggin, is expressed at high levels by roof plate cells, suggestive of a role in the modulation of dorsal patterning (Shimamura et al 1995). *Noggin* mutant mice have defects in the development and patterning of the ventral neural tube (McMahon et al 1998), but the role of noggin or other BMP-binding proteins in dorsal neural patterning remains largely unexplored. Three other BMP-binding proteins, chordin, follistatin, and flik, are expressed by the notochord (Sasai et al 1994, Patel et al 1996, Streit et al 1998), raising the possibility that the secretion of these factors by notochord cells may also contribute to the establishment of early dorsoventral patterns in the neural tube.

The contribution of TGFß-like proteins other than the BMPs to dorsal patterning also needs further study. ActivinB is expressed at high levels by cells in the dorsal neural tube, and in vitro studies have suggested that activins have inductive activities that are distinct from those of the BMPs (Liem et al 1997). A detailed analysis of mice lacking activin function may help to define the role of this class of TGFß proteins in dorsal patterning. A more divergent member of the TGFß family, glial-derived neurotrophic factor (GDNF), has also been shown to be expressed by dorsal neural tube cells (Hellmich et al 1996), but its function here is unknown. In other neural tissues, GDNF has been shown to exert a potent neurotrophic activity (Lin et al 1993, Henderson et al 1994,

Buj-Bello et al 1995), raising the possibility that some of the BMPs implicated in early dorsal patterning may have later roles in the promotion of neural cell survival. The notion that BMPs have later functions in the control of neural differentiation is supported by observations that BMPs have dramatic effects on the elaboration of dendrites on cultured CNS neurons (Lein et al 1995). Determining the relevance of these observations to the development of neurons in vivo may require the analysis of conditional mutations in *BMP* genes, to circumvent earlier patterning defects.

The differentiation of cells in the dorsal half of the vertebrate CNS could therefore involve BMP signaling at many successive developmental stages: in the initial induction and patterning of neural cells, in the subsequent maintenance of neural cell survival, and finally in the elaboration of neuronal polarity. The availability of sophisticated molecular genetic methods and refined in vitro assays should permit each of these unresolved but intriguing issues to be addressed experimentally.

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