Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord

Kevin J. Lee, Monica Mendelsohn, and Thomas M. Jessell

Howard Hughes Medical Institute (HHMI), Department of Biochemistry and Molecular Biophysics, Center for Neurobiology and Behavior, Columbia University, New York, New York 10032 USA

Inductive factors are known to direct the regional differentiation of the vertebrate central nervous system (CNS) but their role in the specification of individual neuronal cell types is less clear. We have examined the function of GDF7, a BMP family member expressed selectively by roof plate cells, in the generation of neuronal cell types in the dorsal spinal cord. We find that GDF7 can promote the differentiation in vitro of two dorsal sensory interneuron classes, D1A and D1B neurons. In Gdf7-null mutant embryos, the generation of D1A neurons is eliminated but D1B neurons and other identified dorsal interneurons are unaffected. These findings show that GDF7 is an inductive signal from the roof plate required for the specification of neuronal identity in the dorsal spinal cord and that GDF7 and other BMP family members expressed by the roof plate have non-redundant functions in vivo. More generally, these results suggest that BMP signaling may have a prominent role in the assignment of neuronal identity within the mammalian CNS.

[Key Words: BMPs; neural patterning; spinal cord; commissural neurons]

Received July 22, 1998; accepted in revised form September 1, 1998.
BMPS (Jones et al. 1991; Liem et al. 1995, 1997; Lyons et al. 1995; Arkell and Beddington 1997; Dudley and Robertson 1997) and in vitro assays have shown that BMPS expressed by roof plate cells can promote the differentiation of dorsal spinal cord interneurons (Liem et al. 1997). These findings have led to the idea that neuronal patterning in the dorsal spinal cord depends on a cascade of TGFβ-related inductive signaling that is initiated by the epidermal ectoderm and propagated by the roof plate.

Gene targeting studies in mice have demonstrated a number of essential functions for BMPS in mammalian development, notably in the differentiation of mesodermal tissues (King et al. 1994; Storm et al. 1994; Dudley et al. 1995; Luo et al. 1995; Winnier et al. 1995; Zhang and Bradley 1996). These studies, however, have not been informative in defining the contribution of BMPS to neural tube patterning. Two major issues concerning the role of BMPS as dorsalizing signals in the CNS remain unresolved. Are the BMPS expressed by the roof plate required in vivo for the generation of dorsal neuronal cell types? And, if so, do roof plate-derived BMPS have distinct or overlapping functions in the generation of dorsal neuronal cell types?

We have taken a genetic approach to define the requirement for individual BMPS in the control of neuronal identity in the dorsal spinal cord. This study focuses on GDF7, a BMP family member that we have found to be expressed selectively by roof plate cells in the embryonic CNS. GDF7 can mimic the ability of the roof plate to induce the differentiation in vitro of two classes of dorsal commissural interneurons, D1A and D1B neurons. To define the requirement for GDF7 signaling we generated a targeted mutation in the mouse Gdf7 gene. Gdf7 mutant embryos lack D1A neurons, but D1B neurons and other types of dorsal interneurons differentiate normally. Our results establish that GDF7 activity is required for the specification of neuronal identity in the spinal cord. They also show that roof plate signaling has an essential role in dorsal neural patterning, distinct from that provided by epidermal ectoderm signals.

**Results**

Selective expression of Gdf7 by roof plate cells

To identify inductive factors that control the differentiation of dorsal interneurons in the mouse spinal cord, we analyzed the neural expression of BM P family members between E8.5 and E12.5, the period of generation of most dorsal interneurons (Nornes and Das 1974; Altman and Bayer 1984). Of nine BMP genes analyzed, only one, Gdf7 (Storm et al. 1994), was found to be expressed selectively by roof plate cells at the dorsal midline of the neural tube. Gdf7 expression in roof plate cells was first evident at E9 in hindbrain and rostral spinal cord levels (Fig. 1A) and was subsequently detected at all axial levels (Fig. 1B; data not shown) from the diencephalon to the sacral spinal cord.

**Figure 1.** Expression of Gdf7 in the embryonic CNS. (A–C) Distribution of Gdf7 in the embryonic mouse CNS; (D) distribution of the related chick GDF6/7 gene in the developing chick spinal cord (SC). (A, B) Transverse sections through the mouse caudal neural tube showing Gdf7 expression in the roof plate (RP) at E9.5 (A) and at E11.5 (B). (C) Parasagittal section through the E15.5 mouse hindbrain (HB) showing Gdf7 expression in the choroid plexus (CP) of the fourth ventricle. (D) Transverse section through stage 20 chick spinal cord showing selective expression of GDF6/7 in the roof plate. Although chick GDF6/7 is slightly more similar in sequence to mouse Gdf6 than mouse Gdf7 (see F), its expression pattern resembles that of Gdf7. (E) Sequence alignment of the predicted amino acid sequences of the mature carboxy-terminal domains of mouse (m) GDF7 (Storm et al. 1994), human (h) GDF7, chick (c) GDF6/7 and zebrafish (z) RADAR (Rissi et al. 1995). (F) Sequence relationship between mouse GDF7, human GDF7, chick GDF6/7, zebrafish RADAR, and other mouse BMP and GDF family members (Hogan 1996). Sequence similarity was determined by comparison of predicted amino acid sequences following the first cysteine residue of the mature carboxy-terminal domains. This dendrogram was generated by use of the Genetics Computer Group program PileUp.
ral axis until E15.5, the latest stage examined (data not shown). The related genes Gdf5 and Gdf6 (Storm et al. 1994) are not expressed in the embryonic spinal cord (data not shown).

Two other BMP genes, Bmp6 and Bmp7, are also expressed in roof plate cells (Fig. 4C,D, below; Jones et al. 1991; Lyons et al. 1995; Arkell and Beddington 1997; Dudley and Robertson 1997). Within the roof plate, the expression of Gdf7, Bmp6, and Bmp7 overlapped temporally and spatially, but Bmp6 and Bmp7 were both expressed more widely than Gdf7 in other regions of the CNS (data not shown). In addition, expression of Bmp7 was detected in epidermal ectoderm cells flanking the neural folds (data not shown; Dudley et al. 1995; Lyons et al. 1995; Arkell and Beddington 1997). Beginning at E13, expression of Gdf7, as well as Bmp6 and Bmp7 (Furuta et al. 1997), was detected in choroid plexus cells. Gdf7 was expressed solely in the choroid plexus of the fourth ventricle (Fig. 1C), whereas expression of Bmp6 and Bmp7 was also detected in lateral ventricle choroid plexus (data not shown; Furuta et al. 1997).

The patterns of neural expression of many members of the BMP family vary among different vertebrate species (Jones et al. 1991; Basler et al. 1993; Dudley and Robertson 1997; Liem et al. 1997). To determine whether the pattern of Gdf7 expression is conserved, we isolated a chick gene that encodes a BMP family member closely related to mouse GDF6 and GDF7 (Fig. 1E,F). This gene is also expressed selectively by roof plate cells (Fig. 1D). A closely related zebrafish gene, Radar, is expressed in the dorsal midline of the hindbrain and spinal cord (Rissi et al. 1995). Thus, the roof-plate-specific expression of a member of the Gdf7/Gdf6/Radar subfamily of Bmp genes appears conserved in vertebrate evolution.

Expression of mATH1 and LH2 transcription factors defines two classes of dorsal commissural interneurons

The time of onset and selectivity of expression of Gdf7 by roof plate cells suggested a role for this gene in the differentiation of interneurons generated close to the dorsal midline of the spinal cord. To begin to address this issue, we used the expression of transcription factors to define successive steps in the differentiation of distinct classes of dorsal interneurons in the spinal cord. At this stage, neurons that emerge from the mATH1+ progenitor domain express both LH2A and LH2B (Fig. 2J–L; data not shown). Thus, both D1A and D1B neurons appear to arise from mATH1+/cATH1+ dorsal progenitors but acquire distinct profiles of transcription factor expression and occupy different positions in the dorsal spinal cord. A third class of dorsal interneurons (termed D2 neurons) expresses the LIM homeobox gene Isl1 and appears to arise from a more ventral position (Liem et al. 1997).

GDF7 induces the differentiation of dorsal commissural neurons in vitro

To examine whether GDF7 can promote the generation of D1A and D1B interneurons, stage 10 chick intermediate neural plate ([i]) explants (Yamada et al. 1993) were cultured alone or in the presence of GDF7 for 36–48 hr. Explants grown alone did not give rise to cATH1+ or LH2A+/LH2B+ cells (Fig. 2Q,S). Treatment with GDF7 induced cATH1+ cells (Fig. 2R) as well as neurons that expressed LH2A and/or LH2B (Fig. 2T). Because the available anti-LH2 antibody detects both LH2A and LH2B proteins, we used an RT-PCR assay to distinguish the induction of LH2A and LH2B gene expression. Treatment of [i] explants with GDF7 induced both LH2A and LH2B (Fig. 2U). Thus, GDF7 induces the expression of transcription factors that define the D1A and D1B classes of interneurons.

Gdf7 mutant mice develop hydrocephalus

To address the requirement for GDF7 function in cell
patterning in the dorsal spinal cord, we generated a null mutation in the mouse Gdf7 gene (Fig. 3A–C). Mice heterozygous for the targeted Gdf7 allele (termed Gdf7m1) were overtly normal and fertile. These mice were interbred to generate homozygous Gdf7m1/Gdf7m1 mutant offspring. In two different genetic backgrounds, Gdf7m1 homozygous mutant mice were born in normal numbers and survived the immediate postnatal period (Table 1).

From 14 to 21 days after birth, however, a significant fraction of the homozygous mutant animals developed severe hydrocephalus (Fig. 3F–I). The penetrance of this phenotype varied between 20% and 37%, depending on the genetic background (Table 1). Similar hydrocephalic abnormalities were not detected in heterozygous (Gdf7m1/+ or homozygous wild-type siblings in either genetic background. Further details of the hydrocephalic phenotype are provided in the legend to Figure 3.

A selective defect in neurogenesis in the dorsal spinal cord of Gdf7 mutant mice

To analyze the role of GDF7-mediated signaling in embryonic neuronal patterning, we examined neural differentiation in the dorsal spinal cord of Gdf7m1 mutant embryos. We first determined whether the development of the roof plate itself is perturbed in Gdf7m1 mutants. The roof plate expression of the homeobox genes Msx1 and Msx2 and the secreted growth factor gene Wnt1 was similar in wild-type and Gdf7m1 homozygous mutant embryos analyzed from E11.5 to E13.5 (Fig. 4A,B; data...
Lee et al.

Figure 3. Generation of a Gdf7-null allele and hydrocephalic abnormalities in Gdf7 mutant mice. (A) Strategy for targeted disruption of the mouse Gdf7 locus by homologous recombination. The diagram indicates the position of the Gdf7 exon encoding half of the pro-domain and the entire mature carboxy-terminal region (coding region, solid rectangle; 3’ UTR, open rectangle). Restriction sites: (H) HindIII; (R) EcoRV; (S) SphI; (X) XbaI. (B) Southern blot analysis of DNA from ES clones digested with XbaI and EcoRV and probed with the fragment indicated in A. The presence of the wild-type allele is indicated by an 8.5-kb band and the disrupted allele by a 6.9-kb band. (C) PCR analysis of DNA from progeny of heterozygote matings with primers indicated in A. The wild-type allele is indicated by a 130-bp band and the mutant allele by a 260-bp band. (D,E) In situ hybridization of wild-type (D) and Gdf7m1 homozgous mutant (E) embryos with a Gdf7 coding region probe. Hybridization was not detected in the roof plate in Gdf7m1 homozygous mutant embryos. (F-I) Parasagittal sections of brains from wild-type (F,H) and Gdf7m1 homozygous mutant (G,I) mice stained with hematoxylin/eosin. Twenty-five percent of Gdf7 mutant brains (n = 8) analyzed at postnatal (P) day 15 showed a marked dilation of the lateral ventricles (LV). No obvious defects in morphology of the cerebellum and cerebral cortex were detected at this age. By P40, a severe hydrocephalus that is associated with dilation of the fourth (4V) and lateral ventricles (cf. H and I) was observed in 25%–37% of Gdf7 mutants (see Table 1). Hydrocephalic mutant animals showed considerable variation in the extent to which the different ventricles were enlarged. In some mutants (I) the fourth ventricle was dilated, and the organization and foliation of the cerebellum was disrupted. In all affected brains examined, however, the cerebral cortex was thinned and the hippocampus was displaced dorsally. Despite these abnormalities, histological analysis showed that the cellular architecture of the cerebellum and cerebral cortex was grossly normal in Gdf7-null mutants (data not shown). The late onset of brain defects and the relatively normal cellular organization in Gdf7 mutants suggest that the disruption of brain morphology is a secondary consequence of hydrocephalus. The expression of Gdf7 in the fourth ventricle choroid plexus (Fig. 1C) raises the possibility that defects in choroid plexus development may underlie the hydrocephalus observed in Gdf7m1 mutant mice. Histological analysis indicated that the fourth ventricle choroid plexus epithelium was still present in affected Gdf7m1 mutant mice and expression of Msx1, Bmp6, and Bmp7 in the choroid plexus appeared normal (data not shown). Thus, the cellular basis of the hydrocephalus is unclear, but it is likely to reflect a requirement for Gdf7 function in choroid plexus cells.

Table 1. Analysis of progeny from heterozygote matings (Gdf7m1/+ × Gdf7m1/+)

|       | +/- | +/- | +/- (% of total +/-) |
|-------|-----|-----|----------------------|
| N. of progeny: mixed background (129Sv × C57BL/6) | 127 (50%) | 60 (24%) | 22 (37%) |
| 66 (26%) | | |

|       | +/- | +/- | +/- (% of total +/-) |
|-------|-----|-----|----------------------|
| N. of progeny: inbred background (129Sv) | 32 (52%) | 15 (24%) | 3 (20%) |
| 15 (24%) | | |

Animals were genotyped 10 days after birth. No differences were noted in the results of crosses of animals that derive from two independent ES cell targeting events, and both sets of results are combined here. Hydrocephalus was not observed in any wild-type or heterozygous animals in these crosses. Data not shown). Thus, the differentiation of the roof plate and its expression of other signaling molecules appear not to be affected by the loss of Gdf7 function.

Next, we examined the development of the mATH1 progenitor population in Gdf7m1 mutants and in wild-type and heterozygous siblings. The early phase (E10–E10.5) of generation of mATH1+ cells in the dorsal spinal cord appeared normal in Gdf7m1 mutants (Fig. 4E,H). However, by E11.5, the number of mATH1+ progenitors in Gdf7m1 mutant embryos was reduced to ~40% (Fig. 4F,H) and by E12.5, to ~10% (Fig. 4G,H) of the number found in wild-type embryos. The loss of the later phase of mATH1 expression was a fully penetrant phenotype.

This analysis left unresolved whether the elimination of late mATH1 expression in Gdf7m1 mutants reflects the deletion of this progenitor population or simply the loss of an individual molecular marker. To examine this issue, we made use of the observation that mATH1+ cells are normally interposed between Msx1+ roof plate cells and a more ventral group of progenitor cells defined by...
expression of the bHLH gene Neurogenin1 (Ngn1) (Fig. 4M). If the cells that normally express mATH1 are still present in Gdf7m1 mutant embryos, we would expect that the Msx1+ roof plate cells and the Ngn1+ progenitors are separated by a domain of cells that express neither gene. Instead we observed that the loss of mATH1 expression (Fig. 4I) was accompanied by a dorsal shift in the Msx1+ population of mATH1+ neural cells and dorsal neural progenitors in the cervical (forelimb level) spinal cord in wild-type (top panels) and in Gdf7m1 homozygous mutant embryos (bottom panels). (A–D) In situ hybridization showing that roof plate expression of Msx1 (A), Wnt1 (B), Bmp6 (C), and Bmp7 (D) is normal in Gdf7 mutant embryos examined at E12.5 (A,B) and at E11.5 (C,D). (E–H) Generation of mATH1+ progenitors adjacent to the roof plate. At E10.5 (E,H), mATH1+ cell number is normal in Gdf7 mutants. By E11.5 (F,H), the number of mATH1+ cells in Gdf7 mutant embryos is significantly reduced (to ~40%), and at E12.5 (G,H), Gdf7 mutants have only 10% as many mATH1+ cells as wild-type siblings. The depletion of mATH1+ cells in the cervical spinal cord was detected in all E12.5 Gdf7 homozygous mutants examined (n=8–20 sections from 18 embryos). (H) Quantitative analysis of the mATH1+ progenitor population in wild-type and homozygous mutant embryos (mean ± s.d., n=10–25 sections from two to six embryos of each genotype). In addition, we detected no difference in the number of mATH1+ progenitors in Gdf7m1+/+ heterozygotes as compared with wild-type siblings. (I–K) In situ hybridization showing the location of mATH1+ and Ngn1+ cells in wild-type and Gdf7m1 mutant embryos at E12.5. In wild-type embryos, mATH1+ progenitors (I, top) are interposed between Ngn1+ progenitors (J) and Msx1+ roof plate cells (summarized in M). Thus, the mATH1+ progenitor domain appears as a region of unlabeled cells (between broken lines) in the wild-type spinal cord hybridized with Ngn1 and Msx1 probes (K, top). In Gdf7m1 mutant embryos, the loss of mATH1+ cells (I, bottom) is accompanied by a dorsal shift in the position of Ngn1+ progenitors (J), and the unlabeled region between Ngn1+ neural cells and Msx1+ roof plate cells is eliminated (K, top, summarized in N). The distance between the domains of Ngn1 progenitors (indicated by broken lines in J) is 89 ± 14 μm in wild-type and 43 ± 5 μm in Gdf7m1 mutants.

To address whether the absence of late mATH1+ progenitors in Gdf7m1 mutants is a consequence of apoptotic cell death, we compared patterns of cell death in wild-type and mutant embryos. Very few apoptotic cells were detected in the dorsal spinal cord of E12.5 wild-type and mutant embryos. (Mean ± S.D., n=10–25 sections from two to six embryos of each genotype). There is no difference in the mean number of apoptotic cells in or near the mATH1+ progenitor domain in wild-type embryos or in mutant littermates that lack late mATH1+ cells. In contrast, abundant cell death was detected in other tissues, for example, in the tail bud and in the distal limb, in both wild-type and mutant embryos (data not shown). (M,N) Summary showing positions of neural progenitors in the dorsal spinal cord in wild-type and Gdf7m1 mutant embryos at E12.5.

Gdf7m1 homozygote embryos (mean ± s.d.; n =10 sections from four embryos of each genotype). There is no difference in the mean width of the Msx1+ roof plate in wild-type and mutant embryos (see A). (L) Detection of apoptotic cell death by end labeling of fragmented DNA (TUNEL assay) in the dorsal spinal cord in E12.5 wild-type and Gdf7m1 mutant embryos. Very few dying cells are detected in or near the mATH1+ progenitor domain in wild-type embryos or in mutant littermates that lack late mATH1+ cells. In contrast, abundant cell death was detected in other tissues, for example, in the tail bud and in the distal limb, in both wild-type and mutant embryos (data not shown). (M,N) Summary showing positions of neural progenitors in the dorsal spinal cord in wild-type and Gdf7m1 mutant embryos at E12.5.
layed lumbar level, where the loss of mATH1+ cells was first observed at E13.5. Thus the loss of Gdf7 function does not appear to result in increased apoptosis in the region of D1 neuron generation in the dorsal spinal cord.

To address whether the loss of late mATH1 progenitor cells in Gdf7m1 mutants is accompanied by defects in the generation of dorsal interneurons, we examined the differentiation of D1A and D1B neurons, the two cell populations defined by the differential expression of the LH2 transcription factors. In E11.5 wild-type embryos, LH2A and LH2B are co-expressed by neurons lateral to the roof plate whereas more ventrally, D1B neurons express LH2B but not LH2A (Fig. 5A,B). In Gdf7m1 homozygous mutant embryos examined at E11.5, we found no change in expression of LH2A or LH2B (Fig. 5A,B), in the number of LH2A/LH2B neurons close to the roof plate (Fig. 5C,D) or in the number of ventrally located D1B (LH2B/LH2A) neurons (data not shown). Older Gdf7m1 mutant embryos, however, exhibit a striking defect in dorsal interneuron generation. In E12.5 wild-type embryos, LH2A and LH2B expression has segregated into two distinct populations of neurons. Most D1A (LH2A/LH2B) neurons are located lateral to the roof plate, whereas D1B neurons are found in a more ventral position deep in the dorsal horn (Fig. 5F,J). The number of dorsal D1A neurons in E12.5 Gdf7m1 homozygotes was reduced to ~8% of that in wild-type littermates (Fig. 5F–I). In contrast, the number of LH2A/LH2B neurons in the deep dorsal horn, which at E12.5 consist primarily of D1B neurons, was not significantly altered in Gdf7m1 mutants (Fig. 5I–L). A similar depletion of D1A neurons was seen in Gdf7m1 mutant embryos examined at E13.5 and at E14.5, again with no apparent change in the number of D1B neurons (data not shown). Several other classes of dorsal cells were also unaffected in Gdf7m1 mutants, including Isl1+ (D2) interneurons (wild type, 123 ± 17 D2 neurons/section; Gdf7m1 homozygotes, 106 ± 13 D2 neurons/section; mean ± S.D. for n = 12 sections of four embryos) and dorsal spinal cord interneurons expressing the LIM homeobox genes Lmx1b or Lim1/2 (data not shown).

Taken together, these data show that Gdf7 function is required selectively in the pathway of D1A interneuron differentiation.

Other TGFβ-related signals are required for the induction of D1B interneurons

Our analysis of neurogenesis in the dorsal spinal cord of Gdf7m1 mutant embryos indicates that the generation of D1B neurons does not require GDF7 signaling. Previous studies have demonstrated that the ability of chick roof
plate to induce LH2A/LH2B neurons (D1 neurons) is blocked only partially by treatment with the BMP binding proteins Noggin and Follistatin (Liem et al. 1997). This partial block could reflect the expression by the chick roof plate of TGFβ family members such as Dsl-1 that are insensitive to Noggin and Follistatin (Liem et al. 1997) or that some D1 neurons can be induced by non-TGFβ signals from the roof plate.

To begin to address whether the generation of D1B neurons depends on inductive signals mediated by other TGFβ family members, we examined whether the inductive activities of the three known BMPs expressed by mouse roof plate cells can be inhibited by BMP-binding proteins. Noggin binds and inactivates BMP4 (Zimmerman et al. 1996) and Follistatin blocks signaling by activin and BMP7 (Nakamura et al. 1990; Yamashita et al. 1995). We found that the GDF7-mediated induction of LH2A/LH2B neurons in chick [i] explants was blocked by Noggin but not by Follistatin (Fig. 6A). Exposure of [i] explants to either BMP6 (Fig. 6B) or BMP7 (Liem et al. 1997) also induced LH2A/LH2B neurons. The activity of BMP6 and BMP7 in these assays, in contrast to that of GDF7, was blocked by Follistatin but not by Noggin (Fig. 6B; Liem et al. 1997). Thus, the three known BMP family members expressed by the mouse roof plate are sensitive to the inhibitory actions of either Noggin or Follistatin.

Next, we examined whether inductive signals from mouse roof plate that promote LH2A/LH2B neuron generation are inhibited by Noggin and Follistatin. Many LH2A/LH2B neurons were induced in [i] explants grown in contact with mouse roof plate (Fig. 6D,F). The induction of LH2A/LH2B neurons by the roof plate was markedly suppressed by treatment with either Noggin or Follistatin and was inhibited by ~97% with combined application of both antagonists (Fig. 6E,F). Thus, the generation of both the D1A and D1B classes of neurons in response to roof plate signals appears to depend on inductive signaling mediated by TGFβ family members. The persistence of D1B neuronal differentiation in Gdf7m1 mutant embryos is therefore likely to result from residual TGFβ-related signaling by the roof plate.

The generation of D1A neurons in Gdf7 mutants is restored by exogenous GDF7 or BMP7

Our results indicate that GDF7 has an essential signaling function in the generation of D1A neurons. However, both Bmp6 and Bmp7 are expressed normally by roof plate cells in Gdf7m1 mutants (Fig. 4C,D), raising the issue of why BMP6 and BMP7 fail to compensate for the loss of GDF7 activity in vivo. One possible explanation is that in mouse neural tissue, GDF7 alone is capable of promoting D1A neuron formation. To test this idea, we compared the ability of GDF7 and BMP7 to induce D1A neurons in wild-type neural tissue and to rescue D1A neuron generation in neural tissue isolated from Gdf7m1 mutant embryos.

First, we asked whether exogenous GDF7 can restore late mATH1 and LH2A expression, the two markers of D1A neuron differentiation, in Gdf7m1 mutant neural tissue. Explants of mouse dorsal neural tube containing roof plate cells ([d+rp] explants) were isolated from Gdf7m1 homozygous mutants and wild-type siblings at E11.5, a time when a reduction in mATH1 expression is first detected in mutant embryos. These explants were cultured for 60 hr, either alone or in the presence of GDF7. When cultured alone, Gdf7m1 mutant [d+rp] explants generated only ~10% as many mATH1+ cells as wild-type [d+rp] explants (Fig. 7A,B,G), consistent with the loss of late mATH1+ cells in Gdf7m1 mutants in vivo. Moreover, there was a threefold reduction in LH2A expression in [d+rp] explants from Gdf7m1 mutants, relative to the level detected in wild-type explants (Fig. 7H). Addition of GDF7 to wild-type [d+rp] explants resulted in a marked increase in the number of mATH1+ cells.
Gdf7m1 type (A,C,E) and Gdf7m1 homozygous mutant (B,D,F) embryos. Mx1+ roof plate cells are green. Gdf7 mutant [d+rp] explants isolated at E11.5 (44 somites) (B) and cultured in COS (control) supernatant for 60 hr generate <10% as many mATH1+ cells as explants from age-matched wild-type siblings (A,G). Wild-type explants respond to added GDF7 with the generation of ~5-fold more mATH1+ cells (C,G). Culture with GDF7 increases the number of mATH1+ cells in mutant explants to a level similar to that in treated wild-type explants (D,G). Treatment with BMP7 similarly increases mATH1 cell number in wild-type and mutant explants (E,F,G). In these experiments, explants were treated with equivalent doses of GDF7 and BMP7, as judged by their activity in inducing LH2A+/LH2B+ neurons in chick neural plate assays (data not shown). In untreated wild-type and mutant [d+rp] explants, mATH1+ cells are restricted to the region adjacent to the roof plate (A,B). After treatment with GDF7 or BMP7, mATH1+ cells are found at all dorsoventral positions within the explants, independent of genotype (C–F). Thus, at E11.5, cells more distant from the roof plate respond to GDF7 or BMP7 with the expression of mATH1. GDF7 treatment of [d+rp] explants isolated from E12.5 wild-type or mutant embryos did not increase mATH1+ cell number (data not shown), suggesting that GDF7 signaling normally acts prior to E12.5 to induce or maintain mATH1+ progenitors. (G) Histograms show mean ± S.D. of results from four to six explants of each genotype. (H) RT-PCR analysis of LH2A and ribosomal protein S12 gene (control) expression in E11.5 wild-type and Gdf7 mutant [d+rp] explants cultured for 60 hr with COS cell (control) supernatant or supernatant containing GDF7 or BMP7. Treatment of mutant explants with GDF7 or BMP7 restores LH2A expression to wild-type levels.

Discussion

BM Ps have been implicated in the induction and patterning of neuronal cell types in the dorsal spinal cord but their precise functions in this process have not been established. We have examined whether BM Ps are required in vivo to promote the differentiation of dorsal interneurons and whether different BM Ps have redundant or distinct functions in the generation of specific dorsal cell types. Our results show that Gdf7-null mutant mice lack a single identified class of dorsal commissural neurons, D1A neurons, and thus establish that BMP-mediated signals from the roof plate are required for the differentiation of specific neuronal subtypes in the dorsal spinal cord. Furthermore, our studies provide evidence that the multiple BM Ps expressed by the roof plate do indeed have nonredundant functions in controlling the generation of selective subsets of spinal neurons. The diversity of TGFβ-related molecules expressed in discrete regions within the embryonic CNS thus raises the possibility that this class of signals is employed more widely in the specification of neuronal subtype identity.
GDF7 is required for the generation of D1A interneurons

Genetic studies on the function of other vertebrate BMP family members have established certain essential roles for this class of secreted proteins, but have not resolved their contribution, if any, to the control of neuronal identity. Targeted mutations in mouse Bmp2 and Bmp4 result in early embryonic lethality, which has so far precluded an analysis of their later function in neuronal development (Winnier et al. 1995; Zhang and Bradley 1996). Eye growth and development is perturbed in Bmp7 mutants (Dudley et al. 1995; Luo et al. 1995) but neuronal patterning defects in the brain and spinal cord have not been reported. Mutations of other BMP family members appear not to be associated with neural abnormalities (King et al. 1994; Storm et al. 1994). One interpretation of these results is that there is functional redundancy between co-expressed BMP family members (Dudley and Robertson 1997). Alternatively, the BMPs that have been analyzed to date may not play essential roles in the particular neural structures that have been studied. Our results establish that GDF7 has a critical function in the development of a specific subset of dorsal spinal cord interneurons and thus provide genetic evidence for the involvement of BMP signaling in the control of neuronal identity and pattern.

The expression of Bmp6 and Bmp7 by roof plate cells is not changed in Gdf7 mutants. Therefore, the loss of D1A neurons in Gdf7 mutants could reflect a specific requirement for GDF7 signaling. Alternatively, the inductive activity of GDF7 and other roof-plate-derived BMPs could be equivalent, with the diffusion of multiple BMPs from the roof plate establishing a BMP activity gradient in the dorsal neural tube. If the specification of D1A neurons were to require the highest level of BMP activity, the loss of GDF7 function might simply reduce net BMP activity to a level below the threshold sufficient to generate D1A neurons. An examination of dorsal neural tube patterning in Bmp6 and Bmp7 mutants has, however, revealed no apparent defect in the differentiation of D1A or D1B neurons (K.J. Lee, T.M. Jessell, A. Dudley, M. Solloway, and E. Robertson, unpubl.). Thus, the loss of D1A interneurons in Gdf7 mutants is most easily explained by a selective dependence on GDF7 signaling, in turn indicating that roof-plate-derived BMPs have non-equivalent functions in vivo.

It remains unclear why Bmp6 and Bmp7 fail to substitute for Gdf7 in vivo. The specific requirement for GDF7 could result either from selectivity in the ability of these BMPs to promote D1A neuron generation or from a difference in the presentation of these signaling molecules in vivo. We find that D1A neuron generation can be restored in Gdf7m1 mutant explants by treatment with either GDF7 or BMP7. Similarly, GDF7, BMP6, and BMP7 have indistinguishable inductive activities on chick neural tissue. Thus, the exposure of neural tissue to recombinant BMPs in vitro appears to obscure the distinction in the functions of GDF7 and other roof-plate-derived BMPs in vivo.

GDF7, BMP6, and BMP7 have apparently similar activities on cultured neural tissue, yet serve different functions in vivo. One possible explanation for this observation is that although Bmp6 and Bmp7 are expressed by roof-plate cells, the level of BM P6 and BM P7 produced is below the threshold for D1A neuron generation. A second possibility is that the activities of BMPs in vivo require accessory factors that are available in the vicinity of the roof plate only for GDF7. One potential class of accessory factor may be a BMP heterodimer partner that enhances inductive activity (Aono et al. 1995; Suzuki et al. 1997), and the roof plate might express such a factor that dimerizes with GDF7, but not with other BMPs. A second class of accessory factor might be one required to facilitate the secretion, diffusion, or presentation of BMPs. The Drosophila BMP-binding protein Sog has been suggested to function in vivo not simply as a BMP antagonist, but rather as part of a transport mechanism for the BMP family member Dpp (Holley et al. 1996). Indeed, Noggin, a protein that we have found to bind GDF7 but not BMP6 and BMP7, is expressed by roof-plate cells (Shimamura et al. 1995; McMahon et al. 1998). It is possible, therefore, that Noggin facilitates the diffusion or presentation of GDF7, and the failure of BMP6 and BMP7 to substitute for GDF7 reflects the absence of corresponding accessory factors for these BMPs. Mice lacking Noggin function have defects in ventral neural tube patterning (McMahon et al. 1998), but the differentiation of dorsal interneurons has not been analyzed in these mutants.

The generation of other classes of dorsal interneurons depends on TGFβ-related signals independent of GDF7

The generation of D1B neurons is unaffected in Gdf7m1 mutants, indicating that other signals are sufficient for D1B neuron generation. One likely source of signals that direct D1B neuron generation is the roof plate. In addition, because D1B neurons are generated prior to D1A neurons, earlier BMP signals produced by epidermal ectoderm cells (Liem et al. 1995; Lyons et al. 1995; Dudley and Robertson 1997) may also contribute to the generation of D1B neurons. The induction of LH2A/LH2B+ neurons by roof-plate cells (Fig. 6) and by epidermal ectoderm (Liem et al. 1997) is blocked by treatment with follistatin and Noggin. Thus, inductive signals for D1B neuron differentiation, whether provided by the roof plate or by the epidermal ectoderm, appear to be mediated by TGFβ family members.

Other classes of dorsal interneurons, notably D2 interneurons, also differentiate normally in the absence of GDF7 function. A different TGFβ-related protein, activinB, has been implicated in the induction of D2 interneurons (Liem et al. 1997). Members of different subclasses of the TGFβ superfamily can also act as qualitatively distinct signals in the promotion of diverse neural crest derivatives (Shah et al. 1996). Collectively, these observations support the idea that the activities of distinct TGFβ family members contribute to the diversifi-
cation of cell types in both the central and peripheral nervous systems.

GDF7 signaling and the pathway of D1 interneuron specification

Our results indicate that the loss of mATH1 expression reflects the deletion of the neuronal progenitors normally marked by mATH1. It follows that the D1A neurons that normally derive from these progenitors are also lacking. The elimination of the late mATH1+ cells does not appear to result from apoptosis of these dorsal neural progenitors as no increase in cell death was observed in the dorsal spinal cord in Gdf7m1 mutants. In addition, we found no increase in the Ngn1+ population or in the number of D1B or D2 interneurons in Gdf7 mutants. Thus, the loss of mATH1+ cells and D1A neurons is likely to result from a failure in the generation of this cell population rather than from cell death or from a switch in cell fate.

There are two possible steps at which GDF7 may influence the differentiation of interneurons. Neither the initial generation of mATH1+ progenitors in the dorsal neural tube nor the differentiation of D1B interneurons from these progenitors is affected by loss of GDF7 function. In contrast, the maintenance of the mATH1+ progenitor population and the differentiation of D1A neurons from this late population of mATH1+ progenitors depends on GDF7 signaling. Thus, GDF7 activity could be required to induce late mATH1+ progenitors from the pool of dorsal Pax3+, Pax7+ precursors (Fig. 8, Model 1). Alternatively, GDF7 could be required for the conversion of early D1B neuron-producing mATH1+ cells to a second mATH1+ progenitor cell type that gives rise instead to D1A neurons (Fig. 8, Model 2).

An essential role for the roof plate as a secondary signaling center

The patterning of cell types in both the dorsal and ventral halves of the spinal cord appears to involve a cascade of inductive signaling initiated in adjacent non-neural tissue and propagated by midline glial cells (Tanabe and Jessel 1996). Ventrally, Shh secreted by the notochord induces floor plate cells, thereby establishing a secondary, neural source of Shh. Shh function is required for ventral cell patterning in the spinal cord (Marti et al. 1995; Chiang et al. 1996; Ericson et al. 1996). However, it remains unclear whether early Shh signaling by the notochord and late Shh signaling by the floor plate have separate roles in the patterning of the ventral spinal cord. In the dorsal spinal cord, BMPs produced by cells of the epidermal ectoderm induce roof-plate cells, which in turn express additional BMPs (Liem et al. 1997). Inductive signaling in the dorsal neural tube, however, appears to differ from that in the ventral neural tube, in that certain BMPs such as Gdf7 are expressed solely by roof-plate cells and not by the epidermal ectoderm. Our genetic analysis of GDF7 function has established a requirement for roof-plate-derived signals in the differentiation of one subclass of dorsal interneurons. Thus, primary and secondary dorsal signaling centers, the epidermal ectoderm and roof plate, have distinct roles in the regulation of neuronal identity and pattern in the mammalian CNS.

Materials and methods

Cloning of chick and human Gdf7-related genes

A 75-nucleotide fragment of chick GDF6/7 was cloned by degenerate PCR with the primers: 5'-CCGCTGGATTAGG-GCATAC-3' and 5'-GGTTGGGGCTCCAGGTGGG-3'. This fragment was used to screen a chicken genomic DNA library (Stratagene), and two overlapping clones were isolated and mapped. A fragment of human GDF7 (gift of Se-Jin Lee) was used to isolate overlapping clones from a human genomic DNA library. Human GDF7 was mapped by FISH (BIOS Labs) to 2p23-2p24, a position that is syntenic to that reported previously for mouse Gdf7 on the proximal end of chromosome 12 (Storm et al. 1994).

Generation of a targeted mutation in Gdf7

A mouse 129/Sv genomic library (Stratagene) was screened with a 400-nucleotide Gdf7 genomic fragment (Storm et al. 1994), and overlapping phage clones were isolated and mapped (Fig. 3). The Gdf7 targeting vector was constructed by flanking pgk-neo with a 3.5-kb HindIII fragment and a 5.5-kb Sphl-NotI fragment (where the NotI site is provided by the phage λ polylinker). This targeting vector is designed to delete the Gdf7 exon that encodes half of the propeptide and the mature carboxy-terminal domain. Linearized plasmid (30 µg) was used to electroporate 3×10^6 E14 ES cells (Hooper et al. 1987) at 800 V and 3 µF with a BioRad Gene Pulser. One hundred and sixty G418-resistant

Figure 8. Potential roles for GDF7 in the regulation of interneuron fate in the dorsal spinal cord. The generation of late mATH1+ progenitors and D1A neurons depends on GDF7 activity. In contrast, other BMP signals from the epidermal ectoderm and/or roof plate appear to be sufficient for the induction of early mATH1+ progenitors and the differentiation of D1B neurons. GDF7 could function in the generation of D1A neurons in one of two ways: GDF7 may act on dorsal Pax3+, Pax7+ neural plate cells to induce the late population of mATH1+ progenitors that differentiate into D1A neurons (Model 1). Alternatively, GDF7 may be required to convert the early D1B-fated mATH1+ progenitors into the late class of mATH1+ progenitors that generates D1A neurons (Model 2).
colonies were isolated and expanded. Genomic DNA was prepared and digested with XbaI and EcoRV and probed with a 200-nucleotide Smal fragment. Six recombinant clones were identified and confirmed by further Southern blot analysis. Two of these clones were injected into C57BL/6J blastocysts to produce a total of three chimeric founders that transmitted the mutated allele.

Indistinguishable phenotypes were observed in animals derived from the independently targeted clones, both in a mixed (129Sv x C57Bl/6) and an inbred (129Sv) background. Genotyping of embryos and offspring was performed by PCR with the following primers: Neo220, 5'-AAGCTCTCTGACTAGGGAG-GAG-3'; GDF-C3, 5'-ATTCCTCTACATGGATGCCC-3'; KO-B1, 5'-ATGACACCTGCTTGTGCTAGGG-3'. Thirty-three cycles of PCR were performed with annealing at 65°C. The mutant allele generated a 260-nucleotide product (between Neo220 and KO-B1) and the wild-type allele a 130-nucleotide product (between GDF-C3 and KO-B1).

In situ hybridization

In situ hybridization was performed essentially as described (Schraen-Wiemers and Gerfin-Moser 1993). For detection of mouse Gdf7, a 400-nucleotide genomic fragment (Storm et al. 1994) or a 1.2-kb HindIII fragment was used as a probe. A 5-kb XbaI genomic fragment was used to detect chick GDF6/7. Other probes for in situ hybridization were Bmp2 and Bmp6 (Lyons et al. 1989), Bmp4 (Jones et al. 1991), Bmp5 (King et al. 1994), Bmp7 (Lyons et al. 1995), Gdf5 and Gdf6 (Storm et al. 1994), mouse Ds1 (Basler et al. 1993), mATH1 (Heim and Johnson 1998), rat LH2A (Xu et al. 1993), mouse LH2B [gift of L. Carlsson (Umeå University, Sweden) and H. Okamoto (Keio University School of Medicine, Japan)], chick LH2A [gift of J.C. Izpisua-Belmonte, Salk Institute, La Jolla, CA], chick LH2B (Liem et al. 1997), Wnt1 (Parr et al. 1993), Msx2 (Mackenzie et al. 1991), Ngn1 (Ma et al. 1996), and Lmx1b (Chen et al. 1998).

Immunofluorescence and cell death (TUNEL) assays

Immunocytochemistry was performed as described (Yamada et al. 1993; Pfaff et al. 1996). Antibodies used were: L1, rabbit anti-LH2A/LH2B (Liem et al. 1997); mouse anti-AH2A/LH2B (G. Tremml and T.M. Jessell, unpubl.); guinea pig anti-LH2A (K.J. Lee and T.M. Jessell, unpubl.); anti-mATH1/cATH1 (G. Tremml and T.M. Jessell, unpubl.), guinea pig anti-LH2B (K.J. Lee and T.M. Jessell, unpubl.); anti-LH2A/LH2B (Liem et al. 1997); anti-LH2B/LH2A (Liem et al. 1997); anti-LH2A/LH2B (Liem et al. 1997); anti-LH2B (Liem et al. 1997), Wnt1 (Parr et al. 1993), Msx2 (Mackenzie et al. 1991), Ngn1 (Ma et al. 1996), and Lmx1b (Chen et al. 1998).

Neural explant culture and induction assays

Explants of chick neural plate ([i] explants) were isolated and cultured as described previously (Yamada et al. 1993; Liem et al. 1995). Explants of mouse neural tube (d+rp) explants) from the cervical (forelimb) region were dissected in disase, and surrounding tissues were removed. The neural tube was isolated at the ventral midline, and the roof plate and dorsal one-quarter of the neural tube was isolated. Mouse explants were cultured in collagen matrices in Iscove's Modified Dulbecco's Medium (GIBCO) supplemented with Mito serum extender (Collaborative Research).

Mouse GDF7, human BM7, and mouse BM6 were obtained by transfection of COS-7 cells (Basler et al. 1993). To prepare a Myc-tagged GDF7 expression construct, the GDF7 carboxy-terminal mature peptide coding region was first amplified by PCR with the primers: 5'-CGGAATTCCTGATCCGAGGA-GAGCTAGGCCTGCTAGCGAAGTC-3' and 5'-AGCATACTCATCTACGAGCC-3'. This fragment was digested with EcoRV and SpeI underlined sites) and ligated to an Asp718-EcoRV fragment encoding the amino-terminal propeptide region of DSL1 (Basler et al. 1993) to recreate the myc epitope at the junction between DSL1 and GDF7. A similar construct that expresses a fusion of the GDF7 mature peptide with the propeptide region of BM4 was also prepared. Both fusions were cloned into the expression vector pMT21 and used to transfect COS-7 cells. The resulting supernatants containing DSL1-GDF7 or BM4-GDF7 had qualitatively identical activities in all assays. Noggin was provided by Richard Harland (University of California, Berkeley) and Follistatin by the National Hormone and Pituitary Program.

Acknowledgments

We thank Sejin Lee for providing mouse and human GDF7 sequences in advance of publication and for helpful discussions, A. Dudley, M. Solloway, and E. Robertson for Bmp mutants and cDNAs, J. Johnson for mATH1 reagents and information, G. Tremml for LH2 antisera, P. Mombaerts for ES cells and advice, D. Kingsley for communication of unpublished data, L. Carlsson and H. Okamoto for mouse LH2B cDNA, J.C. Izpisua-Belmonte for chick LH2A cDNA, Q. Ma and D. Anderson for Ngn-1 cDNA, K. Lyons for Bmp cDNAs, and R. Harland and J. De Jesus-Escobar for Noggin. We are grateful to B. Han for help in cell culture, S. Brenner-Morton for advice on immunocytochemistry, R. Alcaraz for histology, and S. Kaplan, C. Lee, and M. Schift for technical assistance. We thank B. Axel, J. Briscoe, J. Dodd, A. Hemmati-Brivanlou, K. Liem, N. Shah, C. Stern, A. Streit, and L. Vosshall for discussion and comments on this manuscript. K.J.L. was an HHMI Fellow of the Life Sciences Research Institute and T.M.J. is an Investigator of HHMI. The sequence of chick GDF6/7 has been deposited in GenBank under accession no. AF089086.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

Akazawa, C., M. Ishibashi, C. Shimizu, S. Nakanishi, and R. Kageyama. 1995. A mammalian helix-loop-helix factor structurally related to the product of the Drosophila proneural gene aonal is a positive transcriptional regulator expressed in the developing nervous system. J. Biol. Chem. 270: 8730-8738.
Lee et al.

Altman, J. and S.A. Bayer. 1984. The development of the rat spinal cord. Adv. Anat. Embryol. Cell Biol. 85: 1–164.

Aono, A., M. Hazama, K. Notoya, S. Taketomi, H. Yamasaki, R. Tsukuda, S. Sasaki, and Y. Fujisawa. 1995. Potent ectopic bone-inducing activity of bone morphogenetic protein-4/7 heterodimer. Biochem. Biophys. Res. Commun. 210: 670–677.

Arkell, R. and R.S.P. Beddington. 1997. BMP7 influences pattern and growth of the developing hindbrain of mouse embryos. Development 124: 1–12.

Basler, K., T. Edlund, T.M. Jessell, and T. Yamada. 1993. Control of cell pattern in the neural tube: Regulation of cell differentiation by dorsalin-1, a novel TGF beta family member. Cell 73: 687–702.

Ben-Arie, N., A.E. McCall, S. Berkman, G. Eichele, H.J. Bellen, Ericson, J., P. Rashbass, A. Schedl, S. Brenner-Morton, A. Pepicelli, L. Gan, B. Lee, and R.L. Johnson. 1998. Limb and kidney defects in Lmx1b mutant mice suggest an involvement of LMX1B in human nail patella syndrome. Nature Genet. 19: 51–55.

Chiang, C., Y. Litingtung, E. Lee, K.E. Young, J.L. Corden, H. Westphal, and P.A. Beachy. 1996. Cycloplasia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383: 407–413.

Crossley, P.H., S. Martinez, and G.R. Martin. 1996. Midbrain development induced by FGFR8 in the chick embryo. Nature 380: 66–68.

Dickinson, M.E., M.A. Selfak, A.P. McMahon, and F.M. Bronner. 1995. Dorsalization of the neural tube by the non-neural ectoderm. Development 121: 2099–2106.

Dudley, A.T. and E.J. Robertson. 1997. Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. Dev. Dyn. 208: 349–362.

Dudley, A.T., K.M. Lyons, and E.J. Robertson. 1995. Requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes & Dev. 9: 2795–2807.

Ericson, J., S. Morton, A. Kawakami, H. Roelink, and T.M. Jessell. 1996. Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. Cell 87: 661–673.

Ericson, J., P. Rashbass, A. Schell, S. Brenner-Morton, A. Kawakami, V. van Heyningen, T.M. Jessell, and J. Briscoe. 1997. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell 90: 169–180.

Furuta, Y., D.W. Piston, and B.L.M. Hogan. 1997. Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. Development 124: 2203–2212.

Helfs, A.W. and J.E. Johnson. 1998. Progenitors of dorsal commissural interneurons are defined by mATH1 expression. Development 125: 919–928.

Hogan, B.L. 1996. Bone morphogenetic proteins in development. Curr. Opin. Gen. Dev. 6: 432–438.

Holley, S.A., J.L. Neul, L. Attisano, J.L. Wran, Y. Sasai, M.B. O’Connor, E.M. De Robertis, and E.L. Ferguson. 1996. The Xenopus dorsalizing factor noggin ventralizes Drosophila embryos by preventing DPP from activating its receptor. Cell 86: 607–617.

Hooper, M., K. Hardy, A. Handyside, S. Hunter, and M. Monk. 1987. HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. Nature 326: 292–295.

Ikeya, M., S.M.K. Lee, J.E. Johnson, A.P. McMahon, and S. Takada. 1997. Wnt signaling required for expansion of neural crest and CNS progenitors. Nature 389: 966–970.

Jones, C.M., K.M. Lyons, and B.L. Hogan. 1991. Involvement of bone morphogenetic protein-4 (BMP-4) and Vgr-1 in morphogenesis and neurogenesis in the mouse. Development 111: 531–542.

King, J.A., P.C. Marker, K.J. Seung, and D.M. Kingsley. 1994. BMP5 and the molecular, skeletal and soft-tissue alterations in short ear mice. Dev. Biol. 166: 112–122.

Kingsley, D. 1994. The TGFβ superfamily: New members, new receptors, and new genetic tests of function in different organisms. Genes & Dev. 8: 133–146.

Leber, S.M. and J.R. Sanes. 1995. Migratory paths of neurons and glia in the embryonic chick spinal cord. J. Neurosci. 15: 1236–1248.

Lee, S.M.K., P.S. Danielian, B. Fritsch, and A.P. McMahon. 1997. Evidence that FGFR8 signaling from the midbrain-hindbrain junction regulates growth and polarity in the developing midbrain. Development 124: 959–969.

Liem, K.F., G. Tremml, H. Roelink, and T.M. Jessell. 1995. dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82: 969–979.

Liem, K.F., G. Tremml, and T.M. Jessell. 1997. A role for the roof plate and its resident TGFβ-related proteins in neuronal patterning in the dorsal spinal cord. Cell 91: 127–138.

Lumsden, A. and R. Krumlauf. 1996. Patterning the vertebrate neuraxis. Science 274: 1109–1115.

Luo, G., C. Hofmann, A.L.J. Bronckers, M. Sohocki, A. Bradley, and G. Karsenty. 1995. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes & Dev. 9: 2808–2820.

Lyons, K.M., R.W.Pelton, and B.L.M. Hogan. 1989. Patterns of expression of murine Vgr-1 and BMP-2a RNA suggest that transforming growth factor-β-like genes coordinately regulate aspects of embryonic development. Genes & Dev. 3: 1657–1668.

Lyons, K.M., B.L. Hogan, and E.J. Robertson. 1995. Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. Mech. Dev. 50: 71–83.

Ma, Q., C. Kintner, and D.J. Anderson. 1996. Identification of neurogenin, a vertebrate neuronal determination gene. Cell 87: 43–52.

MacKenzie, A., M.W.J. Ferguson, and P.T. Sharpe. 1991. Hox-7 expression during murine craniofacial development. Development 113: 601–611.

Martí, E., D.A. Bumcrot, R. Takada, and A.P. McMahon. 1995. Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types. Nature 375: 322–325.

McMahon, A.P. and A. Bradley. 1990. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. Cell 62: 1073–1085.

McMahon, J.A., S. Takada, L.B. Zimmerman, C.M. Fan, R.M. Harland, and A.P. McMahon. 1998. Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. Genes & Dev. 12: 1438–1452.

Meyers, E.N., M. Lewandoski, and G.R. Martin. 1998. An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. Nature Genet. 18: 136–141.

Monaghan, A.P., D.R. Davidson, C. Sime, E. Graham, R. Baldock, S.S. Bhattacharya, and R.E. Hill. 1993. The Msh-like homeobox genes define domains in the developing vertebrate eye. Development 112: 1053–1061.

3046 GENES & DEVELOPMENT
Moury, J.D. and A.G. Jacobson. 1989. Neural fold formation at newly created boundaries between neural plate and epidermis in the axolotl. Dev. Biol. 133: 44–57.

Nakamura, T., K. Takio, Y. Eto, H. Shibai, K. Títani, and H. Sugino. 1990. Activin-binding protein from rat ovary is follistatin. Science 247: 836–838.

Nornes, H.O. and G.D. Das. 1974. Temporal pattern of neurogenesis in spinal cord of rat. I. An autoradiographic study—time and sites of origin and migration and settling patterns of neuroblasts. Brain Res. 73: 121–138.

Parr, B.A., M.J. Shea, and A.P. McMahon. 1993. Mouse Wnt genes exhibit discrete domains of expression in its early embryonic CNS and limb buds. Development 119: 247–261.

Pfaff, S.L., M. Mendelsohn, C.L. Stewart, T. Edlund, and T.M. Jessell. 1996. Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. Cell 84: 1–20.

Riddle, R.D., M. Ensini, C. Nelson, T. Tsuchida, T.M. Jessell, and C. Tabin. 1995. Induction of the LIM homeobox gene Lmx1 by Wnt17a establishes dorsoventral pattern in the vertebrate limb. Cell 83: 631–640.

Rissi, M., J. Wittbrodt, E. Delot, M. Naegeli, and F.M. Rosa. 1995. Zebrafish Radar: A new member of the TGF-beta superfamily defines dorsal regions of the neural plate and the embryonic retina. Mech. Dev. 49: 223–234.

Roelink, H., J.A. Porter, C. Chiang, Y. Tanabe, D.T. Chang, P.A. Beachy, and T.M. Jessell. 1995. Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. Cell 81: 445–455.

Schaeren-Wiemers, N. and A. Gerfin-Moser. 1993. A single protocol to detect transcripts of various types and expression levels in neural tissue and cultured cells: In situ hybridization using digoxigenin-labelled cRNA probes. Histochemistry 100: 431–440.

Shah, N.M., A.K. Groves, and D.J. Anderson. 1996. Alternative neural crest cell fates are instructively promoted by TGFB superfamily members. Cell 85: 331–343.

Shimamura, K., D.J. Hartigan, S. Martinez, L. Puelles, and J.L. Rubenstein. 1995. Longitudinal organization of the anterior neural plate and neural tube. Development 121: 3923–3933.

Storm, E.E., T.V. Huynh, N.G. Copeland, N.A. Jenkins, D.M. Kingsley, and S.-J. Lee. 1994. Limb alterations in mice due to mutations in a new member of the TGF beta superfamily. Nature 368: 639–643.

Suzuki, A., E. Kaneko, J. Maeda, and N. Ueno. 1997. Mesoderm induction by BMP-4 and -7 heterodimers. Biochem. Biophys. Res. Commun. 232: 153–156.

Tanabe, Y. and T.M. Jessell. 1996. Diversity and pattern in the developing spinal cord. Science 274: 1115–1123.

Tanabe, Y., H. Roelink, and T. Jessell. 1995. Induction of motor neurons by Sonic hedgehog is independent of floor plate differentiation. Curr. Biol. 5: 651–658.

Thomas, K.R. and M.R. Capecci. 1990. Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. Nature 346: 847–850.

Tsuzuki, T., M. Ensini, S.B. Morton, M. Baldassare, T. Edlund, T.M. Jessell, and S.L. Pfaff. 1994. Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. Cell 79: 957–970.

Winnier, G., M. Blessing, P.A. Labosky, and B.L. Hogan. 1995. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes & Dev. 9: 2105–2116.

Xu, Y., M. Baldassare, P. Fisher, G. Rathbun, E.M. Oltz, G.D. Tanabe, Y., H. Roelink, and T. Jessell. 1995. Induction of motor cell pattern in the neural tube. Motor neuron induction by diffusible factors from notochord and floor plate. Cell 73: 673–686.

Yamashita, H., P. ten Dijke, D. Huylebroeck, T.K. Sampath, M. Andries, J.C. Smith, C.H. Heldin, and K. Miyazono. 1995. Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. J. Cell Biol. 130: 217–226.

Ye, W., K. Shimamura, J.L.R. Rubenstein, M.A. Hynes, and A. Rosenthal. 1998. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93: 755–766.

Zhang, H., and A. Bradley. 1996. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development 122: 2977–2986.

Zimmerman, L.B., J.M. De Jesus-Escobar, and R.M. Harland. 1996. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell 86: 599–606.
Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord

Kevin J. Lee, Monica Mendelsohn and Thomas M. Jessell

Genes Dev. 1998, 12:
Access the most recent version at doi:10.1101/gad.12.21.3394

References
This article cites 62 articles, 24 of which can be accessed free at: http://genesdev.cshlp.org/content/12/21/3394.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.