Late-emigrating neural crest cells in the roof plate are restricted to a sensory fate by GDF7

Liching Lo*†, Emma L. Dormand*, and David J. Anderson*†‡

*Division of Biology 216-76 and †Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125

Communicated by Fred H. Gage, The Salk Institute for Biological Studies, San Diego, CA, April 5, 2005 (received for review January 6, 2005)

Lineage-tracing experiments have shown that some premigratory neural crest cells generate both sensory (S) and autonomic (A) derivatives, whereas others generate only S derivatives. Whether this lineage heterogeneity reflects random variation in a homogeneous population or an early sensory specification of some premigratory crest cells has not been clear. Using Cre recombinase-based fate mapping, we show that GDF7, which is exclusively expressed in the roof plate, marks neural crest cells with a 10-fold higher bias to the sensory lineage than those marked (at the same stage of development) by an inducible Wnt1-Cre, which is expressed more broadly in the dorsal neural tube. *In vitro*, GDF7 has potent sensory neuron-inducing activity. These data suggest that some premigratory crest cells are deterministically restricted to the S lineage and implicate GDF7 itself in this restriction process.

sensory neurons | neural stem cells | BMPs | spinal cord

Neural crest cells emigrate from the dorsal neural tube to generate neurons and glia in the sensory (S) and autonomic (A) branches of the peripheral nervous system (1). A major question in neural crest biology has been to understand when and how these two neural sublineages are specified (2). Single-cell lineage tracing experiments demonstrated that some premigratory, and even early migrating, neural crest cells can generate both S and A derivatives (3–5), suggesting that these lineages segregate primarily after emigration from the neural tube. However, some premigratory neural crest cells generated only S, and not A, derivatives (3, 4). Whether such cell fate heterogeneity reflected stochastic variation in an otherwise homogenous cell population or rather the existence of distinct subpopulations of neural crest cells with predictable fate restrictions was not clear.

In the mouse embryo, recombinase-based fate-mapping by using a Wnt1-Cre driver has suggested that neural crest cells originate from both the roof plate, a midline structure, and more lateral regions of the dorsal neural tube (6, 7). The roof plate is a signaling center that produces a number of diffusible factors, such as Wnts and BMP-family members including GDF7 (reviewed in ref. 8). Genetic ablation studies have shown that the roof plate is required for the generation of several classes of spinal cord dorsal interneurons, and fate mapping experiments have indicated that some of these interneurons derive from the roof plate itself (9). This requirement for the roof plate, in turn, reflects a requirement for GDF7 (10). Thus, the roof plate is a source of both signals and CNS progenitor cells.

Here we have used *Gdf7-Cre* mice to fate map neural crest cells derived from the roof plate. Our data suggest that a strong S lineage bias is imposed on these premigratory neural crest cells and implicate GDF7 itself in this lineage restriction.

Materials and Methods

Mouse Manipulations. *GDF7-Cre* knock-in mice have been described in ref. 9, as have *Wnt1-Cre-ER^{TM}* transgenic mice (11). Both lines were crossed to *Rosa26-loxp* reporter mice (12) to generate embryos for analysis. Dissociated neural tube (dNT) cultures and antibody staining were carried out as described in refs. 13–15. Approximately 15,000 cells were plated per 0.126-cm^2 ring. Where indicated, the culture medium was supplemented with BMP2, BMP6 (Genetics Institute), BMP7 (R & D Systems), or GDF7 (R & D Systems). Neural tubes from embryonic day (E)9.0 mouse embryos were prepared as described in ref. 13, except that 1 mg/ml dispase was used, and explanted onto fibronectin-coated dishes in neural crest medium (14) containing 50 ng/ml BMP2. Cultures were fixed in 4% paraformaldehyde. The principal immune reagents used were antibodies to Brn3a (mouse IgG_1, Chemicon) and Phox2B (rabbit, C. Goridis and J.-F. Brunet, Centre National de la Recherche Scientifique, Paris).

Supporting Information. Details of other antibody sources and dilutions used are available in *Supporting Materials*, which is published as supporting information on the PNAS web site.

Results

***Gdf7-Cre*-Derived Neural Crest Cells Are Restricted to S Fates.** We fate mapped premigratory neural crest cells in the roof plate by using mice in which Cre recombinase is knocked into the 3′ UTR of the *Gdf7* locus (9). This knock-in is exclusively expressed in the roof plate, faithfully recapitulating the expression of the endogenous gene (10). Crossing of these mice to R26R reporter mice, containing a Cre-dependent *lacZ* permanent lineage tracer (12), revealed that β-gal$^+$ cells could be detected first in the dorsal neural tube at E9.0 and, at this stage, were restricted to rostral regions of the neuraxis (Fig. 1 *A* and *B*). Examination of the distribution of *lacZ*$^+$ cells in the periphery at E12.5 (a stage by which neural crest migration has ceased) revealed a strong bias (≈50-fold) of *Gdf7-Cre*-derived neural crest cells to a S vs. sympathetic (A) fate (Fig. 1 *G*, *I*, and *K* and Table 1).

Neural crest cells are known to generate primarily S derivatives at late stages of migration (16). The S restriction of cells marked by *Gdf7-Cre* could thus reflect the late expression of this gene, relative to the timing of crest migration. As a control, therefore, we compared the fate of crest cells marked by using an inducible *Wnt1-CreER^{TM}* transgene (6, 11), activated by injection of 4-OH tamoxifen at E9–9.5, the stage at which *Gdf7* first becomes expressed (17). In contrast to *Gdf7*, *Wnt1-Cre* is expressed not only in the roof plate but also in adjacent regions of the dorsal neural tube (6, 11). This comparison indicated that *Gdf7-Cre*-derived neural crest cells were almost 10 times more likely than *Wnt1-CreER*-derived crest cells to colonize S than sympathetic ganglia (Fig. 1 *H*, *J*, and *L* and Table 1). This S fate bias was seen not only rostrally, but also at caudal axial levels where *Gdf7-Cre* is expressed (Fig. 1*M*, arrow, and *Inset*) and where both S and A neurons are being generated at this stage (16) (Table 1 and data not shown).

These data suggest that *Gdf7*$^+$ premigratory neural crest cells are strongly biased to S fates. To determine whether they generate only S neurons or also glia, double-labeling was performed by using antibodies to β-gal and Brn3a, a marker of S

Freely available online through the PNAS open access option.

Abbreviations: A, autonomic; dNT, dissociated neural tube; E*n*, embryonic day *n*; S, sensory.

‡To whom correspondence should be addressed. E-mail: wuwei@caltech.edu.

www.pnas.org/cgi/doi/10.1073/pnas.0502581102

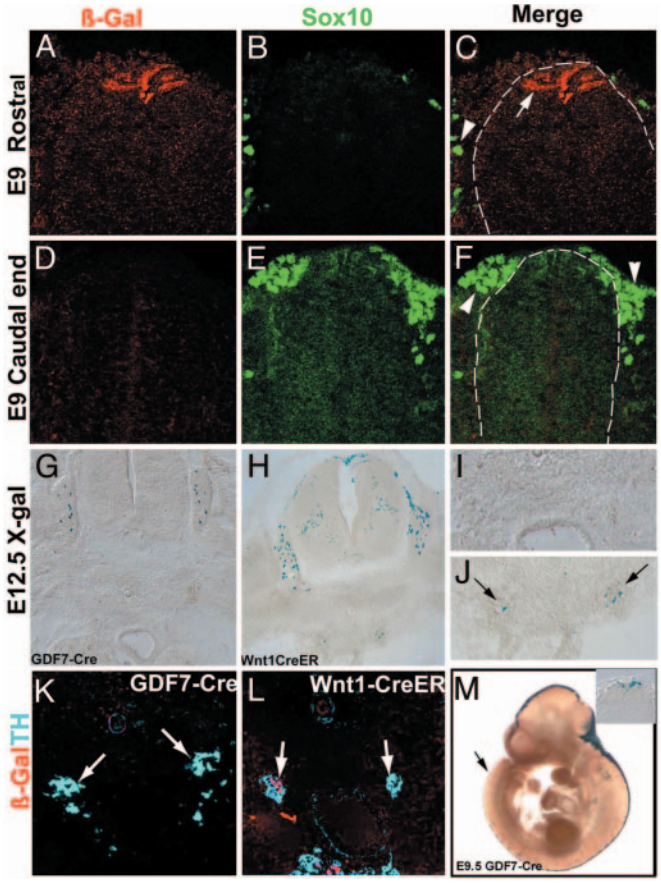

Fig. 1. GDF7 marks a premigratory neural crest subpopulation that is strongly biased to a S fate. (*A–F*) Double-label immunofluorescence of cross sections through an E9 *GDF7Cre; Rosa26STOPlacZ* embryo stained with antibodies to β-galactosidase (*A* and *D*) or Sox10 (*B* and *E*). (*A–C*) Note that β-gal$^+$ cells are restricted to the roof-plate (*A*). (*G* and *I*) Section of E12.5 *GDF7-Cre; Rosa26STOPlacZ* embryo stained with X-gal shows β-gal$^+$ cells in the dorsal root ganglia (*G*) but not in the sympathetic ganglia (*I*). (*H* and *J*) Section through a control E12.5 *Wnt1-CreER; Rosa26STOPlacZ* embryo injected with 4-OH Tamoxifen at E9.5 reveals β-gal$^+$ cells in both the dorsal root ganglia (*H*) and sympathetic ganglia (*J*, arrows). (*K* and *L*) Double-labeling for β-gal and TH, a marker of sympathetic neurons (arrows). See Table 1 for quantification. (*M*) Whole-mount staining of a *Gdf7-Cre; Rosa26STOPlacZ* embryo at E9.5 reveals that *Gdf7*-expressing cells extend along the dorsal neural tube to caudal-most regions of the embryo (arrow and *Inset*), where sympathetic as well as S neurons are being generated at this stage.

neurons (18, 19). This analysis revealed Brn3a$^+$, β-gal$^+$ cells in the dorsal root ganglia of *Gdf7-Cre*; *R26R* as well as *Wnt1-CreER*; *R26R* embryos (Fig. 2 *A–A″* and *B–B″*, arrows and yellow cells). Costaining for β-gal and Sox10, a marker of neural crest-derived glia (20, 21), also revealed double-positive cells (Fig. 2 *C–C″*, arrows and yellow cells). β-Gal$^+$ cells were also observed that coexpressed either BFABP or S100β, two other glial markers (Fig. 7, which is published as supporting information on the PNAS web site). These data suggest that neural crest cells derived from the roof plate can generate both S neurons and glia. However, because the Cre-recombinase fate-mapping method is not clonal, these data do not establish whether individual *Gdf7*$^+$ cells generate both cell types.

Many *Gdf7*-Expressing Cells Are Restricted from an A Fate *in Vitro*. The observation that roof-plate neural crest cells are specified for an S fate *in vivo* leaves open the question of whether these cells are intrinsically restricted to this fate or still retain A capacity. To address this question, we exposed *Gdf7-Cre*-derived neural crest cells *in vitro* to high concentrations of BMP2, an A-inducing signal (14, 15, 22–24). Double-labeling of such cultures for β-gal and Phox2b, an A marker (25, 26), indicated that, although a relatively large proportion of all crest cells expressed Phox2b (28 ± 10%), only a tiny proportion (1.6%) of these cells were derived from *Gdf7-Cre*-expressing progenitors (Table 2). Conversely, the vast majority (≈84%) of *Gdf7-Cre*-derived cells did not express Phox2b. These data suggest that the lack of A differentiation by roof plate-derived neural crest cells *in vivo* is unlikely to reflect simply a lack of access to A-inducing signals.

Table 1. Relative contributions of GDF7 and Wnt1-derived neural crest cells to S and sympathetic ganglia

	GDF7-Cre	Wnt1-CreER	DRG ratio
Mean of cells in DRG, % (*n*)	98 ± 1 (4)	84 ± 3 (3)	—
Mean of cells in SG, %	2 ± 1	16 ± 3	—
Rel.DRG colonization*	49	5.3	9.2†

The percentage of cells in the DRG or SG was first calculated for each embryo as (no. cells in DRG)/(no. cells in DRG + no. cells in SG) × 100% or (no. cells in SG/no. cells in DRG + no. cells in SG) × 100%, respectively. The mean of this percentage was then calculated for all embryos of a given genotype. The mean percentage of cells in the DRG, or in the SG, in the GDF7-Cre embryos ($n = 4$) was statistically significantly different from that in Wnt1-CreER embryos ($n = 3$) ($P < 0.01$).

*The relative colonization of the DRG compared with the SG was then calculated as (mean % of cells in DRG/mean % of cells in the SG).

†The relative preference of GDF7-derived cells to colonize the DRG compared with Wnt1 derived cells (at E9.5) is calculated as the ratio of the numbers in the first two columns.

GDF7 Selectively Promotes a S Fate *in Vitro*. The foregoing experiments suggested that roof plate-derived neural crest cells,

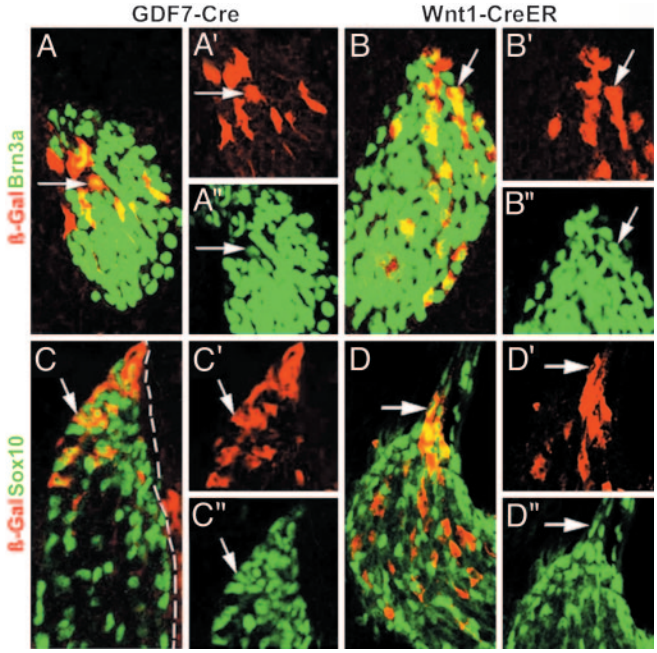

Fig. 2. GDF7-Cre-derived neural crest cells generate both S neurons and glia. Analysis of *GDF7-Cre;Rosa26STOPlacZ* embryos (*A–A″* and *C–C″*) or *Wnt1CreER;Rosa26STOPlacZ* embryos (*B–B″* and *D–D″*) at E12.5. Double-labeling with antibodies to Brn3a (*A*, *A″*, *B*, and *B″*, green) and β-gal (*A*, *A′*, *B*, and *B′*, red) reveals S neurons (*A* and *B*, yellow). Double-labeling with antibodies to Sox10 (*C″* and *D″*, green) and β-gal (*C′* and *D′*, red) and shows that GDF7-Cre- and Wnt1-CreER-derived neural crest cells also generate glia (*C* and *D*, yellow).

Table 2. GDF7-derived neural crest cells are resistant to induction of A neurogenesis by BMP2

Cells	Crest cell expression, %
Phox2b$^+$ neurons	28 ± 10
X-gal$^+$ cells	10 ± 2
Predicted Phox2b$^+$ X-gal$^+$ neurons*	2.9
Actual Phox2b$^+$ X-gal$^+$ neurons	1.6 ± 0.5†

The percentage of Phox2b$^+$, X-gal$^+$, or Phox2b$^+$ X-gal$^+$ double-positive cells was first measured in each neural tube explant as (no. marker cells/total DAPI cells) × 100%. The mean of this percentage was then calculated for all explants (n = 5). Five to seven fields (×40) in the Phox2b rich area of the explants were randomly selected per explant. BMP2 was used at 50 ng/ml, and cells were cultured for 5 days.

*The predicted percentage of Phox2b$^+$ X-gal$^+$ neurons is calculated as the product of the numbers in the first two rows.

†The mean percentage of the actual contribution to A neurons is significantly different from the predicted contribution (assuming there is no bias, $P = 0.03$ from five explants).

marked by expression of *Gdf7*, are strongly biased toward a S fate. Because GDF7 itself is a signaling molecule, it might act as an S-inducing signal. Alternatively, it could bias the migration of crest cells to a location containing S-inducing signals. These alternatives cannot be distinguished by examining the fate of neural crest cells in *Gdf7* knockout mice (10). We therefore turned to an *in vitro* system to directly test whether GDF7 protein may promote a S fate.

In previous work, we demonstrated that E10 rat dNT cultures contain a population of Sox10$^+$ neural crest cells with both S and A differentiation capacity. BMP2 can promote S or A neurogenesis, at low (10 ng/ml) or high (50–100 ng/ml) concentrations, respectively (ref. 15 and Figs. 3 *A*, *A′*, *C*, *C′* and 4 *A* and *B*). Similar results were obtained with BMP6 and BMP7 (Fig. 4 *A* and *B*). In contrast, purified recombinant GDF7 protein promoted S neurogenesis at all concentrations tested (10–100 ng/ml; Figs. 3 *B′* and *D′* and 4*A*) and did not promote A neurogenesis (Figs. 3 *B* and *D* and 4*B*). GDF7 thus has a unique activity among all BMP family members thus far tested to specifically promote differentiation of Brn3a$^+$ neurons.

GDF7 is known to promote the differentiation of dorsal commissural (CNS) neurons expressing Lhx2 and Lhx9 (10), and *in vivo* some of those neurons, like S neurons (19), coexpress Brn3a (Fig. 3*F*). In GDF7-treated dNT cultures, however, expression of Lhx2/9 was not detected in the Brn3a$^+$ cells that developed (Fig. 3 *E* and *E′*). Moreover, many of the Brn3a$^+$ cells coexpressed TrkA, a marker of peripheral S neurons (27) not expressed by Brn3a$^+$ spinal cord dorsal interneurons (Fig. 3 *G*–*G′′*). The Brn3a$^+$ neurons that develop in GDF7-treated E10 dNT cultures are, therefore, most likely peripheral S neurons and not CNS dorsal commissural neurons.

Transient Exposure to GDF7 Commits Some Multipotent Neural Crest Cells to a S Fate. We next asked whether a transient exposure to GDF7 might be sufficient to induce a stable restriction to an S fate, such that crest cells preexposed to this signal would no longer respond to A-inducing signals. To this end, dNT cultures were incubated in 100 ng/ml GDF7 for only 48 h, by which time most cells do not yet express Brn3a (Fig. 5*D*, day 2 vs. day 3). After this preincubation, the GDF7 was washed out and the cells were further incubated for 24 h in medium with or without 100

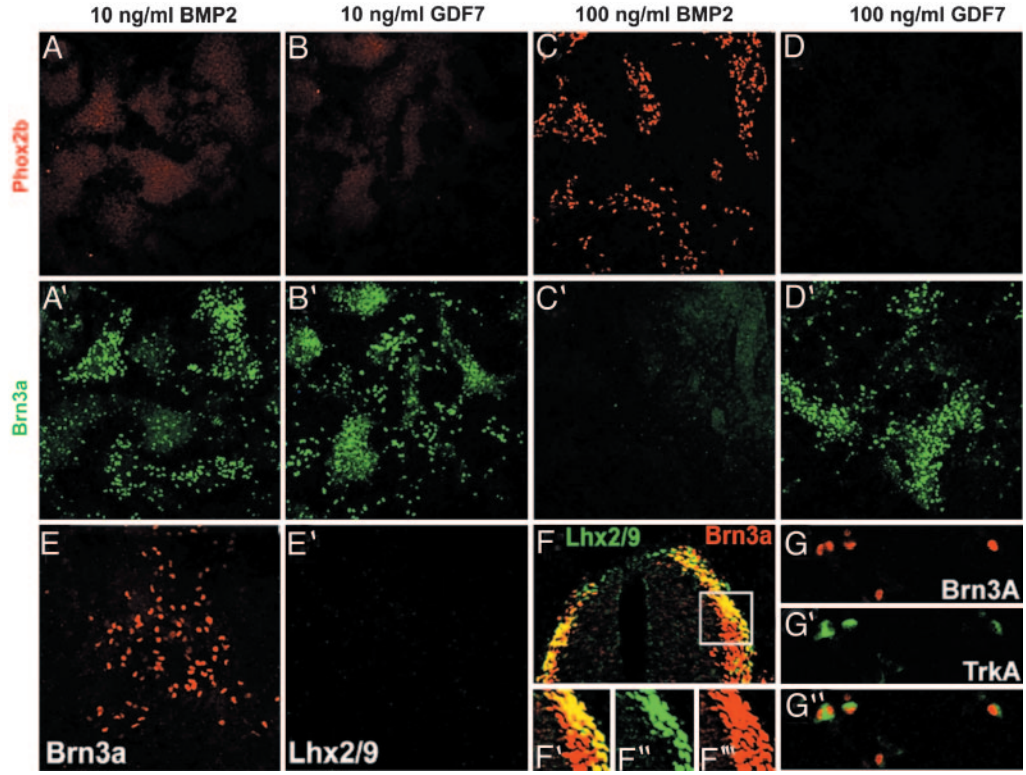

Fig. 3. GDF7 selectively promotes S differentiation in dNT cultures. E10 rat dNT cultures were grown for 3 days in the indicated concentrations of growth factors, fixed, and double-labeled with anti-Phox2b (*A*–*D*) and anti-Brn3a (*A′*–*D′*). See Fig. 4 for quantification. (*E*–*E′*) GDF7 induces Brn3a$^+$ peripheral S neurons and not Brn3a$^+$ dorsal interneurons (Lhx2/9$^+$). (*F*) Control staining of E12.5 rat spinal cord double-labeled for Lhx2/9 (*F′′*, green) and Brn3a (*F′′′*, red) shows Lhx2/9-positive dorsal interneurons. (*G*–*G′′*) dNT cells cultured for 3 days in 100 ng/ml GDF7. Most Brn3a$^+$ cells (*G*) are TrkA$^+$ (*G′*), indicating that they are peripheral S neurons. (Magnification: *A*–*E′*, ×10; *G*–*G′′*, ×20).

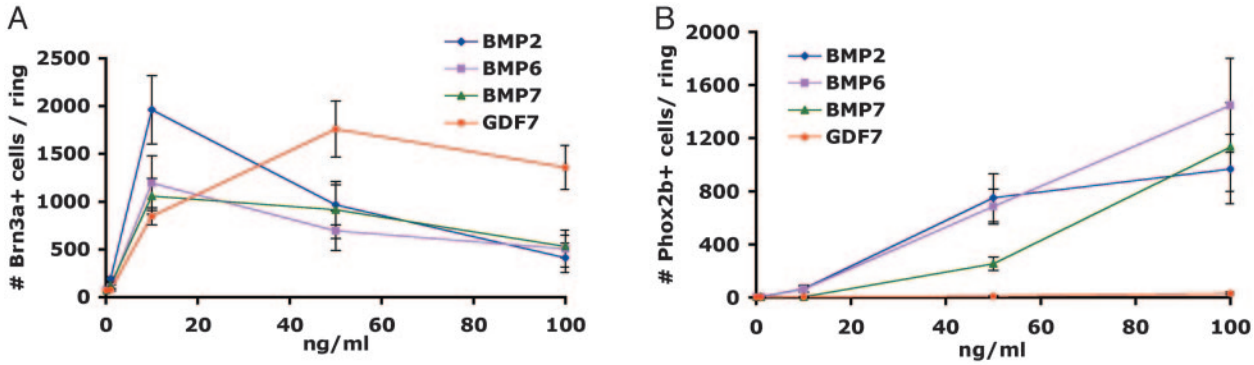

Fig. 4. GDF7 and BMPs exhibit distinct neuronal subtype-inducing profiles in dNT cultures. Dissociated E10.5 rat neural tube cultures were grown in the presence of the indicated growth factor for 3 days, then fixed and double-labeled with antibodies to Brn3a (*A*) or Phox2b (*B*). The total number of Phox2b or Brn3a expressing cells per culture is indicated. The data were pooled from six experiments with three to six cultures per experiment per concentration, although not all concentrations were tested in every experiment. (*A*) In BMP2, BMP6, and BMP7, the induction of Brn3a peaks at 10 ng/ml and declines with increasing concentrations, whereas that in GDF7 increases over this same concentration range ($P < 0.02$). (*B*) Phox2b expression increases with progressively higher concentrations of BMP2, BMP6, and BMP7, whereas little or no expression is seen in GDF7. $P < 0.03$. Error bars indicate SEM.

ng/ml BMP2 (Fig. 5, left flow diagram). After this washout, numerous Brn3a$^+$ cells still differentiated in control medium (Fig. 5 *B′* and *C*, compare 0→0 vs. G→0), and there was no significant reduction in the number of such cells that developed in comparison to cultures reexposed to GDF7 (Fig. 5*C*, G→0 vs. G→G). Importantly, in cultures transferred to BMP2, the number of Brn3a$^+$ cells that developed was also not reduced in comparison with controls (Fig. 5 *B′* and *C*, G→BMP vs. G→G).

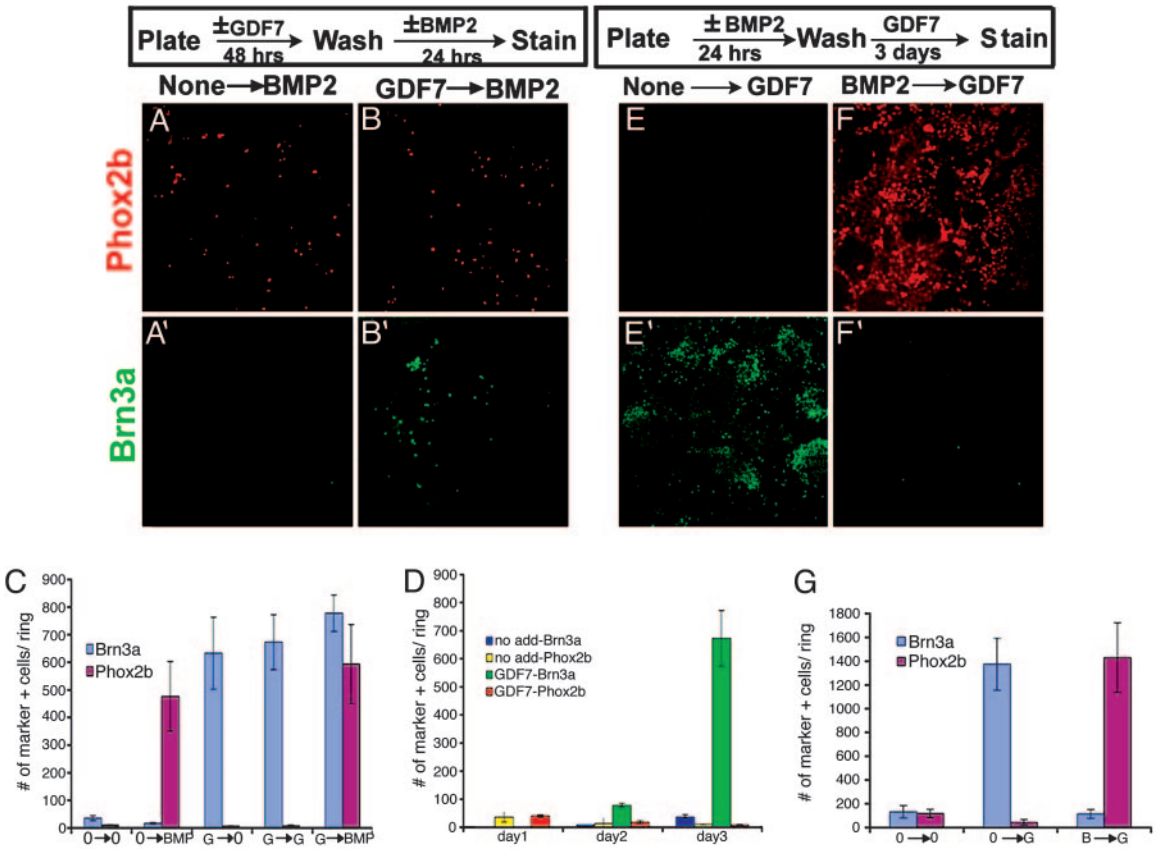

Fig. 5. Transient exposure to GDF7 commits multipotent S/A progenitors to a S fate. (*A–D*) Cultures were preincubated with or without GDF7 (100 ng/ml) for 48 h, then washed and switched to medium with or without BMP2 (100 ng/ml) for an additional 24 h (left flow diagram) before double-labeling for Brn3a (green) and Phox2b (red). (*C*) BMP2 did not reduce the number of Brn3a$^+$ neurons that develop after GDF7 washout (G→0 vs. G→BMP). Data represent two different experiments each containing five replicates. (*D*) Time course of Brn3a induction by GDF7 compared with the control (''no add''). Brn3a is only weakly expressed at day 2, the time of GDF7 washout (flow diagram). (*E–G*) dNT cultures were preincubated with or without 100 ng/ml BMP2 for 24 h, washed, and then incubated in 100 ng/ml GDF7 for 3 additional days (right flow diagram). (*E* and *E′*) S neurons develop in cultures preincubated in control medium, and no A neurons develop. (*F* and *F′*) S neuron differentiation is strongly inhibited if the cultures are preincubated in BMP2, and primarily A neurons develop. (*G*) Quantification. Note the reciprocity in the number of S vs. A neurons under the two conditions.

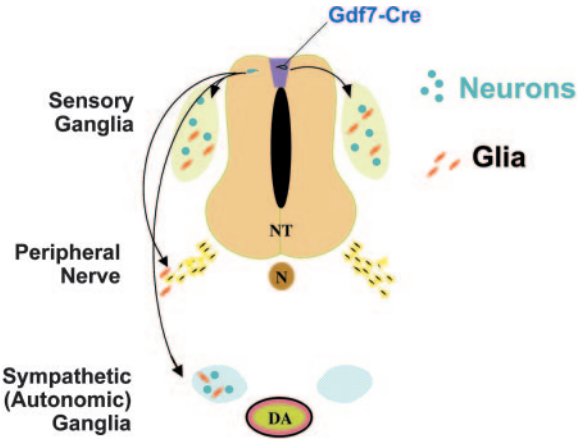

Fig. 6. Pleuripotent and restricted S progenitors coexist in the premigratory neural crest. Premigratory neural crest cells in the roof plate (purple) marked by expression of *Gdf7* are restricted to S fates (both neuronal and glial; right). The data do not exclude the possibility that some S fate-restricted progenitors are also present outside the roof plate. GDF7 itself may play a role in S fate restriction. NT, neural tube; DA, dorsal aorta.

The number of Phox2b$^+$ cells that developed after the switch to BMP2 was not diminished by GDF7 preincubation (Fig. 5 *A–C*, purple bars), indicating that some dNT cells had escaped S fate restriction by GDF7. This finding is not surprising, because only a subpopulation of cells expressed Brn3a after GDF7 pretreatment for 48 h (data not shown). Taken together, these results suggest that transient exposure to GDF7 is sufficient to restrict at least some crest cells to a S lineage, such that they cannot be diverted to an A fate by BMP2.

The foregoing experiments did not distinguish whether GDF7 simply acts as a permissive factor, which promotes overt differentiation of a latent subpopulation of precommitted S progenitors or, rather, can commit multipotent cells, which also have A capacity, to an S fate. To distinguish among these alternatives, we asked whether preincubation of dNT cultures in conditions that induce A neurogenesis (high BMP2) could prevent S differentiation upon subsequent exposure to GDF7. Such a preincubation in BMP2 virtually abolished GDF7-induced S neurogenesis (Fig. 5 *E′*, *F′*, and *G*, O→G vs. B→G). Control experiments indicated that this loss of S differentiation capacity by BMP2 preexposure could not be explained by increased cell death or decreased proliferation of neural crest cells (Fig. 8, which is published as supporting information on the PNAS web site). These data are consistent with the idea that GDF7 can promote S lineage commitment in a multipotent crest population.

Discussion

The data presented here suggest that late-emigrating neural crest cells derived from the roof plate are strongly biased, if not restricted, to an S fate and implicate locally synthesized GDF7 in this fate restriction. Thus, the S vs. A lineage heterogeneity observed in prior *in vivo* clonal analyses of neural crest fate (28) may reflect, at least in part, deterministic differences in the fate specification of premigratory neural crest cells (Fig. 6). More generally, our data are consistent with the idea that neural crest cells originating from the dorsal midline have different fates from those delaminating from more lateral aspects of the dorsal neural tube. A similar conclusion was previously reached from lineage-tracing studies in the zebrafish (29, 30). However, in that system, the midline-derived neural crest cells were biased to a melanocyte, rather than a neuronal, fate (29). Therefore, the present data provide evidence that some neural crest cells are predictably specified, if not restricted, to the S lineage before they emigrate from the neural tube. Interestingly, this lineage restriction does not appear to commit cells to a neuronal fate, because glia, as well as S neurons, are generated from this population. Although these results are not based on a clonal analysis, they are consistent with the emerging idea that, counterintuitively, neuronal subtype specification occurs before commitment to neuronal or glial fates (31).

Our observations also implicate GDF7 itself in late sensory lineage specification. In dNT cultures, unlike other BMP family members that can promote either S or A fates, depending on concentration, GDF7 promotes only an S fate at all concentrations tested. Furthermore, GDF7 is the only growth factor we have tested that exclusively promotes S neurogenesis in our dNT culture system. GDF7 has also been shown to promote the differentiation of D1A dorsal commissural neurons in cultures of E12.5 neural tubes (10), and some of those neurons also express Brn3a *in vivo*. Therefore, it is possible that GDF7 promotes only one aspect of S identity, represented by Brn3a expression, and that other factors determine whether these Brn3a$^+$ cells will become peripheral S neurons or CNS dorsal commissural neurons. Nevertheless, we did not observe any Brn3a$^+$, Lhx2/Lhx9$^+$ neurons in our GDF7-treated cultures. Thus, at some stages of development, GDF7 may be sufficient to promote peripheral S neuron differentiation from dorsal neural tube progenitors. The diffusible nature of GDF7 further suggests that it may act in an autocrine or paracrine mode. Although our *in vitro* data do not prove that GDF7 acts as an S-inducing signal *in vivo*, they provide corroborative evidence that the S fate bias of *Gdf7-Cre*-expressing progenitors *in vivo* is not simply coincidental with the expression of *Gdf7* at relatively late stages of crest migration (16).

The prespecification of *Gdf7-Cre*-expressing neural crest cells cannot explain the origin of all S neurons, because many such neurons are generated before *Gdf7* is first expressed. Indeed, a relatively small proportion of late-generated S neurons is derived from *Gdf7*-expressing crest cells. Other signals must, therefore, specify S differentiation at earlier stages of development. The fact that *Bmp6* and *Bmp7* are also expressed in the dorsal neural tube (8, 10), and that these factors can also promote S differentiation *in vitro* (at low concentrations), makes them interesting candidates for additional S fate-promoting signals. Wnt1 has also been implicated in S neuron fate specification (32, 33). BMPs and Wnts have been shown to cooperate in the initial specification of the neural crest (34, 35). Further studies should clarify the relative roles of Wnt family and BMP family members in S fate determination.

We thank A. McMahon and J. McMahon (Harvard University) for providing control *Wnt1-CreER; Rosa26* mice, K. Lee and T. M. Jessell for providing *Gdf7-Cre* mice and a *Gdf7* expression plasmid, Y. Moukouyama for immunostaining the sections shown in Fig. 7 *D–F*, and M. Bronner-Fraser for helpful comments on the manuscript. D.J.A. is an Investigator of the Howard Hughes Medical Institute.

1. Le Douarin, N. M. & Kalcheim, C. (1999) *The Neural Crest* (Cambridge Univ. Press, Cambridge, U.K.).
2. Le Douarin, N. M. (1986) *Science* **231,** 1515–1522.
3. Bronner-Fraser, M. & Fraser, S. (1988) *Nature* **335,** 161–164.
4. Bronner-Fraser, M. & Fraser, S. (1989) *Neuron* **3,** 755–766.
5. Fraser, S. E. & Bronner-Fraser, M. E. (1991) *Development (Cambridge, U.K.)* **112,** 913–920.
6. Jiang, X., Rowitch, D. H., Soriano, P., McMahon, A. P. & Sucov, H. M. (2000) *Development (Cambridge, U.K.)* **127,** 1607–1616.
7. Chai, Y., Jiang, X., Ito, Y., Bringas, P., Jr., Han, J., Rowitch, D. H., Soriano, P., McMahon, A. P. & Sucov, H. M. (2000) *Development (Cambridge, U.K.)* **127,** 1671–1679.
8. Lee, K. J. & Jessell, T. M. (1999) *Annu. Rev. Neurosci.* **22,** 261–294.
9. Lee, K. J., Dietrich, P. & Jessell, T. M. (2000) *Nature* **403,** 734–740.

10. Lee, K. J., Mendelsohn, M. & Jessell, T. M. (1998) *Genes Dev.* **12,** 3394–3407.
11. Danielian, P. S., Muccino, D., Rowitch, D. H., Michael, S. K. & McMahon, A. P. (1998) *Curr. Biol.* **8,** 1323–1326.
12. Soriano, P. (1999) *Nat. Genet.* **21,** 70–71.
13. Lo, L., Tiveron, M.-C. & Anderson, D. J. (1998) *Development (Cambridge, U.K.)* **125,** 609–620.
14. Shah, N. M., Groves, A. & Anderson, D. J. (1996) *Cell* **85,** 331–343.
15. Lo, L., Dormand, E., Greenwood, A. & Anderson, D. J. (2002) *Development (Cambridge, U.K.)* **129,** 1553–1567.
16. Serbedzija, G. N., Fraser, S. E. & Bronner-Fraser, M. (1990) *Development (Cambridge, U.K.)* **108,** 605–612.
17. Zirlinger, M., Lo, L., McMahon, J., McMahon, A. P. & Anderson, D. J. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 8084–8089.
18. Gerrero, M. R., McEvilly, R. J., Turner, E., Lin, C. R., O'Connell, S., Jenne, K. J., Hobbs, M. V. & Rosenfeld, M. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10841–10845.
19. Fedtsova, N. G. & Turner, E. E. (1995) *Mech. Dev.* **53,** 291–304.
20. Kuhlbrodt, K., Herbarth, B., Sock, E., Hermans-Borgmeyer, I. & Wegner, M. (1998) *J. Neurosci.* **18,** 237–250.
21. Kim, J., Lo, L., Dormand, E. & Anderson, D. J. (2003) *Neuron* **38,** 17–31.
22. Reissman, E., Ernsberger, U., Francis-West, P. H., Rueger, D., Brickell, P. D. & Rohrer, H. (1996) *Development (Cambridge, U.K.)* **122,** 2079–2088.
23. Schneider, C., Wicht, H., Enderich, J., Wegner, M. & Rohrer, H. (1999) *Neuron* **24,** 861–870.
24. Varley, J. E. & Maxwell, G. D. (1996) *Exp. Neurol.* **140,** 84–94.
25. Pattyn, A., Morin, X., Cremer, H., Goridis, C. & Brunet, J.-F. (1997) *Development (Cambridge, U.K.)* **124,** 4065–4075.
26. Pattyn, A., Morin, X., Cremer, H., Goridis, C. & Brunet, J.-F. (1999) *Nature* **399,** 366–370.
27. Martin-Zanca, D., Barbacid, M. & Parada, L. F. (1990) *Genes Dev.* **4,** 683–694.
28. Bronner-Fraser, M. & Fraser, S. E. (1991) *Development* **2,** S17–S22.
29. Dorsky, R. I., Moon, R. T. & Raible, D. W. (1998) *Nature* **396,** 370–373.
30. Dorsky, R. I., Moon, R. T. & Raible, D. W. (2000) *Bioessays* **22,** 708–716.
31. Anderson, D. J., Lo, L.-C., Zirlinger, M., Choi, G. & Zhou, Q. (2004) in *Stem Cells in the Nervous System: Functional and Clinical Implications*, ed. Gage, F. (Springer, Berlin), pp. 25–41.
32. Hari, L., Brault, V., Kleber, M., Lee, H. Y., Ille, F., Leimeroth, R., Paratore, C., Suter, U., Kemler, R. & Sommer, L. (2002) *J. Cell Biol.* **159,** 867–880.
33. Lee, H. Y., Kleber, M., Hari, L., Brault, V., Suter, U., Taketo, M. M., Kemler, R. & Sommer, L. (2004) *Science* **303,** 1020–1023.
34. Garcia-Castro, M. I., Marcelle, C. & Bronner-Fraser, M. (2002) *Science* **297,** 848–851.
35. LaBonne, C. & Bronner-Fraser, M. (1998) *Development (Cambridge, U.K.)* **125,** 2403–2414.