Transient expression of the bHLH factor neurogenin-2 marks a subpopulation of neural crest cells biased for a sensory but not a neuronal fate

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Lineage-tracing experiments have indicated that some premigratory neural crest cells (NCCs) are pleuripotent, generating sensory and sympathetic neurons and their associated glia. Using an inducible Cre recombinase-based fate mapping system, we have permanently marked a subpopulation of NCCs that expresses *Ngn2*, a bHLH transcription factor required for sensory neurogenesis, and compared its fate to the bulk NCC population marked by expression of *Wnt1*. *Ngn2*$^+$ progenitors were four times more likely than *Wnt1*$^+$ NCCs to contribute to sensory rather than sympathetic ganglia. Within dorsal root ganglia, however, both *Ngn2*- and *Wnt1*-expressing cells were equally likely to generate neurons or glia. These data suggest that *Ngn2* marks an NCC subpopulation with a predictable fate bias, early in migration. Taken together with previous work, these data suggest that NCCs become restricted to sensory or autonomic sublineages before becoming committed to neuronal or glial fates.

The neural crest presents a model system for studying neural cell lineage segregation. Neural crest cells (NCCs) generate the sensory and autonomic neurons of the peripheral nervous system, and their associated glia, after migrating from the dorsolateral neural tube (1). Single-cell lineage-marking experiments *in vivo* have indicated that some premigratory trunk NCCs are pleuripotent, generating neurons and glia in both sensory and autonomic (sympathetic) ganglia (2–4). However in these experiments, other NCCs generated only a subset of these derivatives—e.g., in sensory but not autonomic ganglia. Because the marked cells were selected at random, it could not be distinguished whether NCCs are homogeneous and pleuripotent, but exhibit heterogeneity in their fates because of stochastic variation, or rather are heterogeneous and comprise subpopulations with deterministic fate restrictions that could not be prospectively identified. Lineage-marking studies performed on migrating NCCs *in vitro* have revealed evidence of rapid restriction to neuronal or glial fates (5), but the neuronal subtype(s) involved were not examined.

Evidence of molecular heterogeneity among early NCCs has been suggested by the often transient expression of various antigenic markers and genes (reviewed in ref. 1; e.g., ref. 6). However, without some way to convert the expression of such markers into a permanent lineage tracer, it remained unclear whether this heterogeneity reflected early fate-specification. Here we have achieved such a conversion by using a conditional form of Cre recombinase to permanently mark a subset of NCCs that transiently express the proneural bHLH transcription factor *Neurogenin2* (*Ngn2*) (7, 8). *Ngn2* is required for the differentiation of a subset of sensory neurons (9), but is dispensable for autonomic neurons and for Schwann (glial) cells in peripheral nerves (10). The genetic requirement for *Ngn2* in sensory neurons, however, leaves open the question of whether it is expressed by progenitors restricted to a sensory neuron fate (Fig. 1*A* 4), or rather by cells with a broader developmental potential (Fig. 1*A* 1–3).

To address this question, we have used a conditional, binary system for fate-mapping of *Ngn2*-expressing progenitor cells *in vivo* based on Cre-lox-mediated DNA recombination (11, 12). We have expressed a 4-hydroxy tamoxifen (4-OH Txf)-inducible form of Cre recombinase, CreER^{TM} (12–14), from *Ngn2* genomic regulatory elements in mice (Fig. 1*B*). Such *Ngn2-CreER^{TM}* mice are then crossed to mice carrying a ubiquitously expressed, Cre-dependent *lacZ* reporter gene (15). In embryos from this intercross, *lacZ* expression can be activated only in *Ngn2*-expressing progenitors. Because activation of the reporter gene involves a cell-heritable DNA rearrangement event, *lacZ* expression will persist in the progeny of transiently *Ngn2*-expressing cells (Fig. 1*C*). Because 4-OH Txf has a half-life of only 0.5–2 h *in vivo* (12), activation of CreER^{TM} can be further restricted to a relatively narrow developmental time window when the ligand is injected. Thus, the final expression pattern of the *lacZ* reporter will exclusively identify the progeny of cells expressing *Ngn2-CreER^{TM}* during this time window, and will not include cells that express *Ngn2* at later times (Fig. 1*C*, large lower oval).

Materials and Methods

Mouse Manipulations. Homologous recombination in embryonic stem cells was used to replace the *Ngn2* coding sequence with *CreER^{TM}* (12), by means of the same strategy used to generate *Ngn2* knockouts (10). *Ngn2-CreER^{TM}* mice bred into a C57Bl6/J background were crossed to Rosa26-loxp reporter mice (15) to generate embryos for analysis. In other experiments, mice harboring a *Wnt1-CreER^{TM}* transgene (12) were bred to the same line of reporter mice. 4-OH Txf was injected i.p. (1 mg per mouse) to activate Cre-ER^{TM}. Injections to activate CreER^{TM} at embryonic day (E)8.75–E9.0 were performed between 5 p.m. and midnight (counting the morning, the vaginal plug was identified as E0.5), and to activate it at E9.5 were performed between 8 a.m. and noon the following day. Embryos were analyzed at E12.5, or in some experiments at E10.5.

Histology. Embryos were fixed in 4% paraformaldehyde (PFA) for 1–2 h at room temperature, embedded, frozen, and sectioned at 15 μm. For double-labeling (16), sections were first X-gal-stained (17), post-fixed in 4% PFA, preblocked, and then incubated for 1.5 h at room temperature with the appropriate primary antibodies: mouse monoclonal anti-Isl-1 (IgG1, Developmental Studies Hybridoma Bank, 1:1 dilution); rabbit anti-TH (Sigma, 1:1,000 dilution); rabbit anti-brain fatty acid binding protein (BFABP; C. Birchmeier, Max Delbrück Centrum for Molecular Medicine, Berlin; 1:1,000 dilution); rabbit anti-trk-A (L. Reichardt, University of California, San Francisco; 1:2,000 dilution); rabbit anti-trk-B (L. Reichardt, 1:1,000 dilution); goat

Abbreviations: DRG, dorsal root ganglia; SG, sympathetic ganglia; NCCs, neural crest cells; 4-OH Txf, 4-hydroxy tamoxifen; BFABP, brain fatty acid binding protein; E, embryonic day.

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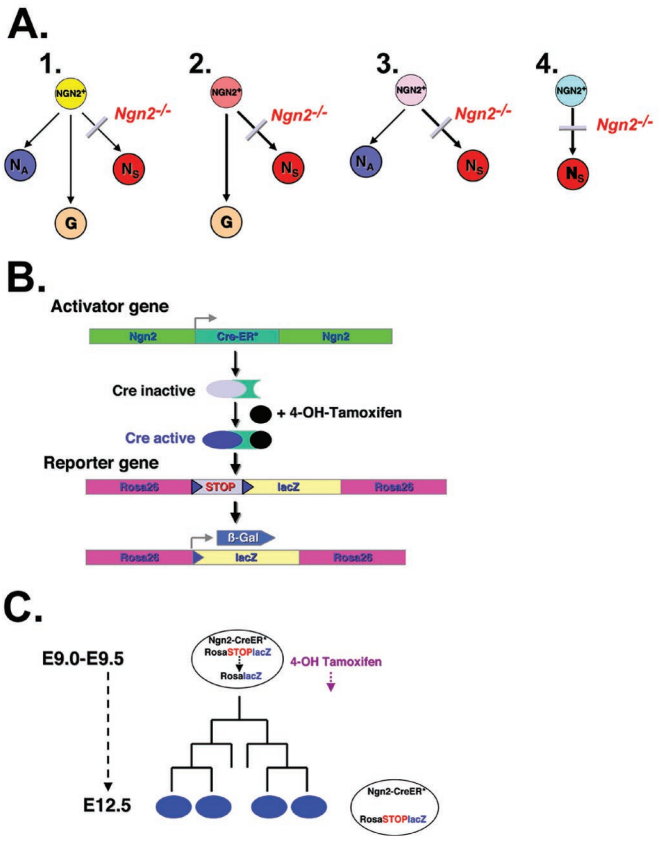

Fig. 1. Mapping the fate of *Ngn2*-expressing cells. (*A*) *Ngn2* is required for the development of a subset of sensory neurons (N_S; refs. 9 and 10), but may be transiently expressed by progenitors with a broader developmental potential (examples illustrated in 1–4). N_A, autonomic neuron; G, glia. (*B*) Strategy for inducible activation of the *Rosa26-lacZ* reporter gene by 4-OH Txf-inducible Cre expressed from the *Ngn2* locus. (*C*) Because 4-OH Txf has a half-life *in vivo* of only 0.5–2 h (12), CreER^{TM} will be active in *Ngn2*-expressing cells only at the time of injection of the steroid ligand. The progeny of these cells can be detected by expression of *lacZ* at later times (E12.5, blue ovals). Other cells expressing *Ngn2* at these later times will not express *lacZ* because of the absence of 4-OH Txf (E12.5, white oval).

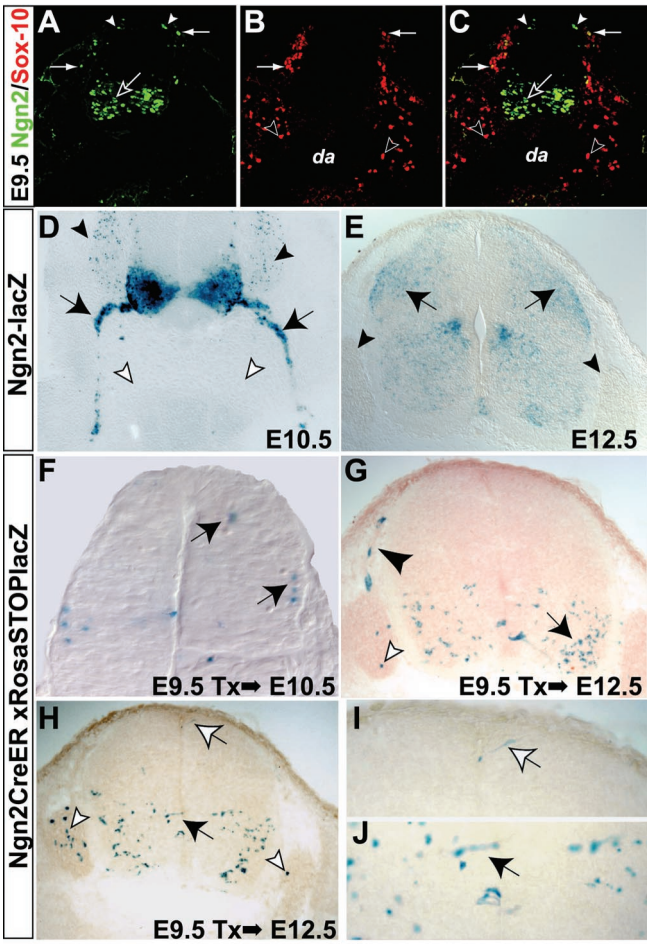

Fig. 2. Fate of transiently *Ngn2*-expressing cells in the neural tube. (*A–C*) Double-label immunofluorescence staining of a section through the caudal trunk region of an E9.5 mouse embryo with antibodies to NGN2 (*A*, green) and the pan-neural crest marker Sox10 (*B*, red). White arrowheads indicates NGN2$^+$Sox10$^-$ cells in the dorsal neural tube, open arrow indicates NGN2$^+$Sox10$^-$ motoneuron precursors in the ventral neural tube; white arrows indicate NGN2$^+$Sox10$^+$ NCCs in the dorsal migration pathway, open arrowheads indicate NGN2$^-$Sox10$^+$ cells migrating ventrally toward the dorsal aorta (da). (*D* and *E*) Analysis of conventional *Ngn2lacZ* knock-in embryos (20, 21). (*D*) At E10.5, numerous lacZ$^+$ cells are visible in the DRG (black arrowheads) and ventral nerve roots (arrows), but not in the region of the SG (white arrowheads). (*E*) By E12.5, expression in the DRG is no longer detectable (arrowheads) and there is extensive expression in the dorsolateral neural tube (arrows). (*F–J*) Analysis of *Ngn2CreER^{TM}* × *RosaSTOPlacZ* embryos injected with 4-OH Txf at E9.5 and analyzed at E10.5 (*F*) or E12 5 (*G–J*). (*F*) Some Ngn2-CreER^{TM}-expressing cells were in the dorsal neural tube at the time of Cre activation (arrows). (*G* and *H*) By E12.5, there are very few lacZ$^+$ cells in the dorsal neural tube and numerous labeled cells in DRG (white arrowhead) and dorsal roots (black arrowhead); contrast this with the pattern seen when using the conventional *Ngn2lacZ* reporter (*E*). The arrow in *G* indicates motorneurons. (*H–J*) Occasional elongated, lacZ$^+$ cells with an endfoot in the ventricular zone are seen at E12.5 in the dorsal (*H* and *I*, white arrow) and ventral (*H* and *J*, black arrow) regions of the spinal cord; *I* and *J* are higher-magnification views of the dorsal and ventral regions of the section shown in *H*. Such cells are not seen in conventional *Ngn2-lacZ* knock-in mice at the same age (compare *E* and *H*).

anti-trk-C (L. Reichardt, 1:500 dilution). Antibody staining was developed with diaminobenzidine by using the Vecstatin ABC kit (Vector Laboratories). Fluorescent secondary antibodies were Alexa red anti-rabbit (Molecular Probes) or FITC-conjugated anti-mouse IgG1 (Southern Biotechnology Associates, 1:250 dilution).

Results

In E9.5 embryos, NGN2 is expressed in both the dorsal neural tube (Fig. 2 *A* and *C*; white arrowheads), and in a subset of migrating NCCs marked by Sox10 (refs. 18 and 19; Fig. 2 *A* and *C*, white arrows). NGN2$^+$Sox10$^+$ NCCs were exclusively found in the dorsal part of the neural crest migration stream (Fig. 2*B*, arrows), and never detected near the sympathetic ganglia (SG; Fig. 2 *B* and *C*, open arrowheads). Similarly, examination of embryos from a conventional *Ngn2lacZ* knock-in line (20, 21) at E10.5 revealed numerous lacZ$^+$ cells in the dorsal root ganglia (DRG; Fig. 2*D*, black arrowheads), but none in the vicinity of the SG (Fig. 2*D*, white arrowheads). These data could indicate that *Ngn2*-expressing cells never give rise to sympathetic neurons, or that *Ngn2* is very transiently expressed in precursors of such autonomic neurons. To resolve this issue, we permanently marked the progeny of *Ngn2*-expressing cells by injecting pregnant mothers carrying embryos from an *Ngn2-CreER^{TM}* × *Rosa-loxPSTOPloxP-lacZ* intercross with 4-OH Txf, at E9.0 (n = 6 embryos) and E9.5 (n = 15 embryos). In such embryos, a very small proportion (<5%) of lacZ$^+$ neural crest-derived cells were observed in the SG, identified by counterstaining with antibody to tyrosine hydroxylase (TH; Fig. 3 *C* and *D*, arrows). Thus, some

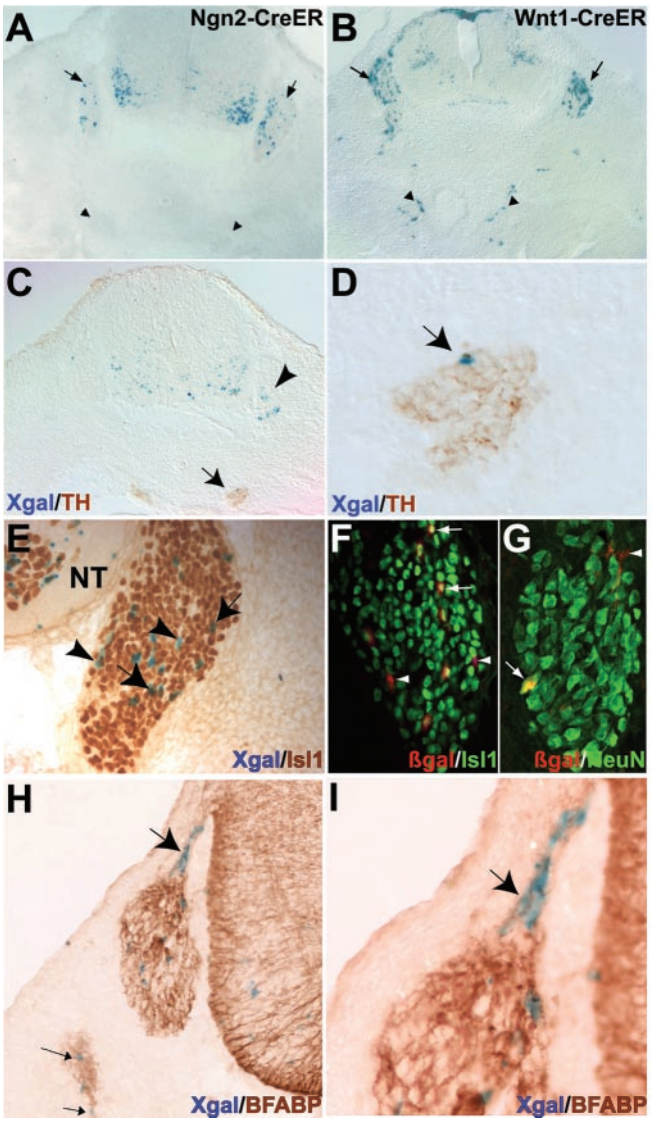

Fig. 3. Distribution of progeny derived from *Ngn2CreER^{TM}*-expressing NCCs in the PNS. All panels are from *Ngn2-CreER^{TM}* × *RosaSTOPlacZ* embryos injected at E9.5 and analyzed at E12.5, except for *B*, which is from a control *Wnt1-CreER^{TM}* × *RosaSTOPlacZ* embryo similarly injected and analyzed. Arrows in *A* and *B* indicate DRG, arrowheads SG. (*C* and *D*) Example of a rare section from *Ngn2-CreER^{TM}* embryos with a lacZ^{+} cell in the SG (arrow), visualized by counterstaining for TH (brown); *D* shows a higher-magnification view of the SG. Arrowhead indicates DRG. See Table 1 for quantification. (*E–G*) Double-labeling reveals that lacZ^{+} cells in the DRG include both neuronal (Isl1^{+} or NeuN^{+}, arrows) and non-neuronal (Isl1^{-} or NeuN^{-}, arrowheads) cells. NT indicates neural tube. The magnification in *G* is slightly higher than in *F*. (*H* and *I*) *Ngn2*-expressing NCCs generate BFABP^{+} glial cells in dorsal root (large arrow) and peripheral nerve (*H*, small arrows), as well as in the DRG. Neurons and non-neuronal cells are generated in statistically indistinguishable proportions (see Table 2).

Ngn2-expressing cells do appear to generate sympathetic neurons.

To determine whether the relative contribution of *Ngn2*-expressing cells to DRG vs. SG was quantitatively different from the NCC population as a whole, we performed a similar analysis on embryos containing a *Wnt1-CreER^{TM}* transgene (12). *Wnt1* is expressed in the dorsal neural tube and roof-plate from the onset of neural crest migration (22), and *Wnt1*-expressing NCCs populate all of the tissues derived from the neural crest (23). Injections of embryos from *Wnt1-CreER^{TM}* × *Rosa-loxPSTOPloxP-lacZ* intercrosses with 4-OH Txf were performed at similar times to those in the *Ngn2-CreER^{TM}* experiments (E8.75–E9.0 and E9.5), and the embryos were analyzed at E12.5 (Fig. 3*B*).

Table 1. Relative contributions of Ngn2- and Wnt1-derived neural crest cells to sensory and sympathetic ganglia

Injection stage	Rel. DRG col.: Ngn2-CreER*	Rel. DRG col.: Wnt1-CreER*	DRG ratio [Ngn2/Wnt1]†
E8.75-E9.0	11.2	3.7	3.0
E9.5	27.6	6.7	4.1
Combined	19.5	4.8	4.0

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 Ngn2-CreER embryos (n = 21) was statistically significantly different from that in Wnt1-CreER embryos (n = 4) at all stages ($P < 0.01$).

*The relative colonization of the DRG compared to the SG was then calculated as the [mean % of cells in DRG/mean % of cells in the SG].

†The relative preference of Ngn2-derived cells to colonize the DRG compared to Wnt1-derived cells is calculated as the ratio of the numbers in the first two columns.

To quantify the results, we counted the total number of lacZ^{+} cells in the DRG plus SG in every fourth section through the trunk region. There were many more (≈18-fold) lacZ^{+} neural crest-derived cells in *Wnt1-CreER^{TM}*-expressing embryos, presumably reflecting both the expression of *Wnt1* in more cells than *Ngn2*, and at higher levels from the multicopy transgene. Therefore, we first normalized the data by calculating the percentage of labeled ganglionic cells in either the DRG or SG for each embryo, and then taking the mean of these values across all embryos of a given genotype (n = 21 *Ngn2-CreER^{TM}* embryos; n = 4 *Wnt1-CreER^{TM}* embryos). As expected, in *Wnt1-CreER^{TM}* embryos the average percent of cells in the DRG was ≈5-fold higher than that in the SG (Table 1), reflecting the larger size of the sensory ganglia. Furthermore, this percentage was higher when 4-OH Txf injection was carried out at E9.5 (6.7-fold) than at E8.75–E9.0 (3.7-fold; Table 1), consistent with the fact that NCCs continue to populate the DRGs after they have stopped colonizing the SGs (24, 25).

Despite this overall DRG-bias of the bulk NCC population, however, in *Ngn2-CreER^{TM}* embryos the percentage of labeled cells in the DRG was ≈20-fold higher than in the SG (Table 1). Thus, cells derived from *Ngn2*-expressing progenitors contributed to sensory ganglia rather than sympathetic ganglia at a frequency 3- to 4-fold higher than that of *Wnt1*-expressing progenitors, at both early and late injection times (Table 1). To ensure that this difference was not an artifact of the smaller number of labeled cells in *Ngn2-CreER^{TM}* embryos (≈67 cells per embryo in DRG+SG vs. 1,258 cells per embryo in DRG+SG of *Wnt1-CreER^{TM}* embryos), we performed a Monte Carlo simulation to randomize the data, using the actual number of cells counted in each of the 21 embryos examined but assuming that the distribution of cells between the DRG and SG was actually the same as that measured in *Wnt1-CreER^{TM}* embryos [83% in DRG, 17% in SG, a ratio of 4.8 (Table 1)]. After 10,000 iterations of this simulation, the probability that the ratio [% cells in DRG]/[% in SG] of ≈20 that we actually measured in *Ngn2-CreER^{TM}* embryos (Table 1) was due to chance was <0.001. Consistent with this, the percentage of *Wnt1*-derived cells in SG was ≈3.5-fold higher than the percentage of *Ngn2*-derived cells in these autonomic ganglia. Thus, by several measures NCCs expressing *Ngn2* are more likely to colonize sensory ganglia than are those expressing *Wnt1*. This difference

Table 2. Relative contribution of *Ngn2*-derived neural crest cells to neuronal vs. non-neuronal derivatives in sensory ganglia

Stage of injection (no. of embryos)	Neurons	Non-neuronal cells
E9 (5)	56% ± 6%*	44% ± 6%*
E9.5 (11)	52% ± 5%	48% ± 5%
Combined	53% ± 4%	47% ± 4%

The percentage of lacZ-labeled cells that were neurons ($Isl1^+$) or non-neuronal cells ($Isl1^-$) was measured in the DRG of *Ngn2-CreER*™ embryos injected at the indicated stages; the numbers represent the mean ± SEM.
*There was no statistically significant difference in the percentage of neurons vs. non-neuronal cells at either stage. Similar results were obtained in *Wnt1-CreER*™ embryos (see text).

is likely to be an underestimate, because the *Wnt1*-expressing NCC population itself presumably contains *Ngn2*-expressing cells.

At the time of 4-OH-Txf injection, $NGN2^+$ cells are located in both the dorsal and ventral neural tube, as well as in the neural crest (Fig. 2 *A–C*). Strikingly, in embryos analyzed at E12.5 there were no labeled neurons in the dorsal neural tube; rather, they were found in neural crest derivatives and in the ventral neural tube (Fig. 2 *G* and *H*). That reporter activation did occur in some dorsal neural tube cells at E9.5 is supported by the fact that $lacZ^+$ cells could still be detected in this location in embryos analyzed at E10.5 (Fig. 2*F*), and by the persistence at E12.5 of a few labeled progenitor-like cells with an elongated morphology and endfoot in the dorsal ventricular zone (Fig. 2 *H* and *I*, white arrow). These data suggest that most $NGN2^+$ cells in the dorsal neural tube at E9.5 may be premigratory neural crest or dual crest-CNS progenitor cells (2, 3). However, we cannot formally exclude that some of these cells die, or migrate ventrally.

We next asked whether there was any bias in the differentiation of *Ngn2*-expressing cells to neuronal vs. glial fates in the sensory ganglia. To do this, we performed double-labeling for lacZ expression and the pan-neuronal markers Isl1 (Fig. 3 *E* and *F*) or NeuN (Fig. 3*G*) to identify neurons, or anti-BFABP antibody staining to identify peripheral glia (ref. 18; Fig. 3 *H* and *I*). Double-labeled $lacZ^+Isl1^+$ (or $lacZ^+NeuN^+$) neurons were clearly distinguishable from $lacZ^+Isl1^-$ non-neuronal cells (Fig. 3 *E–G*, arrows vs. arrowheads, respectively). Expression of lacZ in these latter, non-neuronal cells was colocalized with that of BFABP; this was particularly clear in the dorsal roots and peripheral nerve (Fig. 3 *H* and *I*, arrows), where there are no neuronal cell bodies. Quantification indicated that the percentage of $lacZ^+$ neurons and non-neuronal DRG cells was statistically indistinguishable in embryos injected with 4-OH Txf at either E9.0 or E9.5 (Table 2). A similar result was obtained in Wnt1-CreER™ embryos (52 ± 3% $LacZ^+Isl^+$ and 48 ± 3% $LacZ^+Isl^-$ cells; $n = 2$ embryos). Therefore, $Ngn2^+$-expressing cells give rise to both neurons and non-neuronal cells in similar proportions, as does the bulk NCC population. These data indicate that although *Ngn2*-expressing NCCs are strongly biased toward generating sensory derivatives, they are not biased toward generating neuronal vs. glial derivatives.

Finally, we examined the distribution of *Ngn2*-derived cells among different sensory neuron subtypes by double-labeling using antibodies to the NGF receptor trkA, the BDNF receptor trkB, or the NT-3 receptor trkC. These receptors are predominantly, although not exclusively, expressed by nociceptive, mechanoceptive, and proprioceptive sensory neurons, respectively (reviewed in ref. 26). We estimated that ≈68% of $lacZ^+$ neurons in the DRG are $trkA^+$ (Fig. 4 *A* and *B*, arrows; $n = 10$ embryos), a number remarkably close to the percentage of all neurons that express the receptor at this stage (≈70%; ref. 27). A much smaller proportion of $lacZ^+$ cells appeared to be $trkB^+$ or $trkC^+$ (Fig. 4 *C–F*, arrows), consistent with the fact that these receptors are expressed by a smaller proportion of all sensory neurons at this stage (27). Thus, these data suggest that *Ngn2*-expressing NCCs contribute to multiple classes of sensory neurons in DRG, without any apparent bias toward a particular subtype.

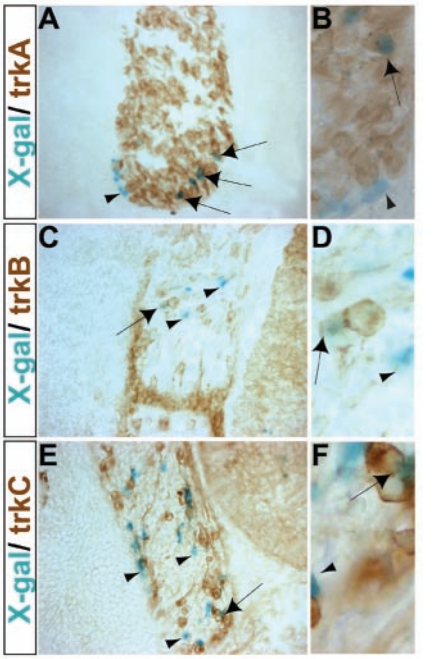

Fig. 4. *Ngn2*-expressing NCCs generate multiple sensory neuron subtypes in the DRG. Sections through DRG of E12.5 *Ngn2-CreER*^{TM} embryos injected with 4-OH Txf at E9.5. (*A* and *B*) Sections double-labeled to reveal $lacZ^+$ (blue) and $trkA^+$ (brown) cells at low (*A*) and high (*B*) magnification. Arrows indicate double-positive cells, arrowheads a $lacZ^+$, $trkA^-$ cell. (*C* and *D*) Double-labeling for $lacZ^+$ and $trkB^+$ cells. (*E* and *F*) Double-labeling for $lacZ^+$ and $trkC^+$ cells. In each case, analysis in multiple focal planes confirmed the colocalization of X-gal staining and anti-trk antibody staining. Arrows indicate double-positive cells, arrowheads a $lacZ^+trk^-$ cell.

Discussion

Classical lineage tracing studies have left open the question of whether the premigratory and early migrating neural crest is a homogeneous population of pleuripotent cells, or whether it is heterogeneous and contains both pleuripotent and intrinsically fate-restricted cells. The results presented here identify at least one subpopulation of NCCs, namely that marked by expression of *Ngn2*, which exhibits a predictable bias in its differentiated fate from an early stage in migration. *Ngn2*-expressing cells are ≈20 times more likely to generate sensory than autonomic (sympathetic) derivatives, and this bias is 3- to 4-fold greater than that of bulk NCCs marked by expression of *Wnt1*. This conclusion is supported even when taking into account the fact that there are more labeled NCCs in *Wnt1-CreER*^{TM} embryos.

The fact that even a few *Ngn2*-expressing NCCs give rise to autonomic derivatives indicates that *Ngn2* does not mark inevitable commitment to a sensory fate, but rather a strong bias to such a fate. This bias may reflect an inherently probabilistic influence of *Ngn2* on sensory fate specification, or the influence of additional unknown but deterministic factors. For example, forced expression of *Ngns* promotes sensory differentiation both *in vivo* (28) and in cultured PNS progenitor cells, but *in vitro* this effect can be overridden by environmental signals that promote autonomic neurogenesis (29). These data suggest that there are other determinants of sensory identity required in addition to *Ngns* to commit cells to

a sensory fate. Perhaps the small fraction of *Ngn2CreER*^{TM}-expressing NCCs that generated autonomic derivatives represents those cells in which such putative sensory identity codeterminants had not yet been expressed at the time of 4-OH Txf injection. Alternatively, commitment to a sensory fate may require prolonged or enhanced *Ngn2* expression, analogous to the commitment to a neural precursor fate of *Drosophila* epithelial cells expressing high levels of proneural genes (30).

The identification of *Ngn2*-expressing cells as strongly biased to a sensory fate suggests that those marked NCCs that generated only sensory derivatives in the chick lineage tracing experiments (2–4) may have been intrinsically restricted to this fate, and could be equivalent to or overlapping with the *Ngn2*-expressing population. The fact, moreover, that *Ngn2* is expressed in premigratory as well as in migrating NCCs (Fig. 2*A*; refs. 8 and 9) further suggests that such a developmental bias may be acquired by NCCs at a relatively early stage in their ontogeny, perhaps even before they exit the neural tube (Fig. 5*A*). However, we cannot exclude that such premigratory NGN2^{+} cells contribute most of the sympathetic progeny derived from the *Ngn2*-expressing population. Thus, the exact time and place at which restriction to a sensory fate is imposed remain to be determined. Whatever the case, our results suggest that from the earliest stages of migration, the neural crest is not a homogeneous population of pleuripotent cells, but rather contains deterministically distinct subpopulations with predictable fate biases.

Our results further suggest that *Ngn2*-expressing NCCs are not committed to a neuronal fate, despite their bias for a sensory identity. Consistent with this conclusion, CNS precursors isolated on the basis of *Ngn2* expression can generate glia and neurons *in vitro* (31), although it was not determined whether these cells are restricted to generating specific neuronal subtypes. Previously, we showed that postmigratory neural crest stem cells in the sciatic nerve are restricted to autonomic fates but can still generate both neurons and glia (32). Those studies, however, left open the converse question of whether all sensory progenitors also generate autonomic derivatives, or whether there exists a complementary population of sensory-restricted multipotent neuro-glial progenitors. *In vitro* studies have provided evidence of sensory-restricted progenitors, but whether these cells also generated glia could not be determined (33). The present results indicate that sensory-restricted precursors generate neurons and glia with equal probabilities, in a similar fashion as the bulk neural crest population marked by *Wnt1* expression. Taken together, these data imply that, at least in the PNS, multipotential neural progenitors become specified for certain aspects of neuronal subtype identity (Fig. 5*B*, P_S and P_A) before they have become committed to neuronal or glial fates.

This conclusion challenges current models of neural cell lineage diversification, in which progenitors are typically depicted as first committing to neuronal or glial fates (Fig. 5*C*, P_N and P_G), before acquiring particular neuronal subtype identities (reviewed in refs. 34 and 35). Evidence for such restricted neuronal and glial precursors has been provided by *in vitro* studies of embryonic spinal cord-derived progenitor cells (36–39). However, recent studies suggest that *in vivo*, spinal cord progenitors are specified for particular neuronal and glial subtype identities before choosing between neuronal and glial fates (40, 41). Thus, although some finer aspects of neuronal subtype identity may not be acquired until after precursors commit to a neuronal fate, at least certain broad subclasses (e.g., sensory vs. autonomic, motoneuron vs. interneuron) are specified while progenitors still possess both neuronal and glial capacities. Such a decision logic could reflect the dual role of bHLH proneural genes in controlling the neuron vs. glial fate decision (reviewed in ref. 42), and in neuronal identity determination (e.g., ref. 43). The need to coordinate the latter role with other transcriptional programs that specify neuronal identity (21, 44) may necessitate that such programs are established at the time proneural genes are first expressed, when neural progenitors are competent for, but not committed to, a neuronal fate (45).

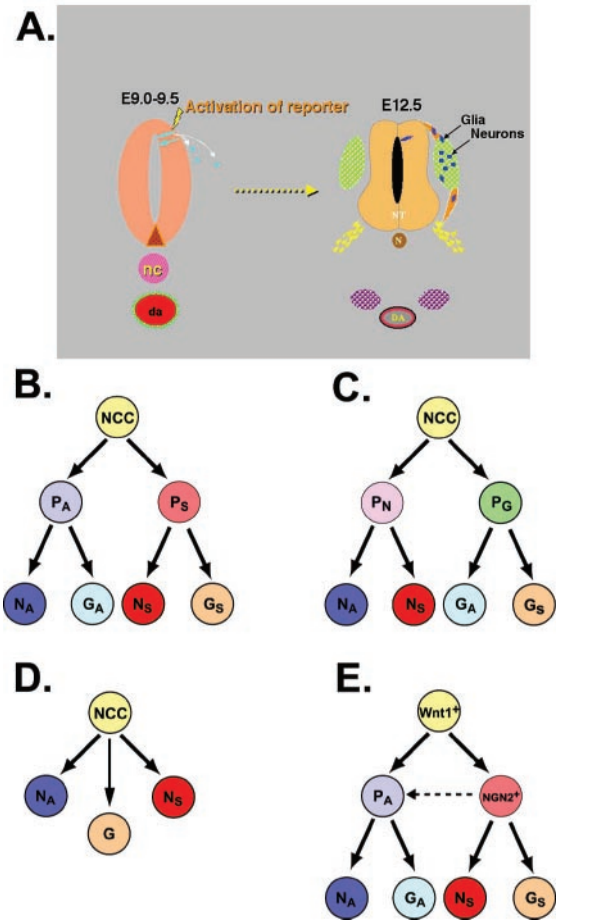

Fig. 5. Summary diagram. (*A*) Illustration of experimental results. Transient activation of Cre recombinase in *Ngn2*-expressing cells in the dorsal neural tube and/or migrating neural crest at E9.0–E9.5 leads to progeny that by E12.5 have predominantly populated the DRG and surrounding nerve roots; both neurons (blue circles) and glia (blue diamonds) are found in this location. (*B–D*) Possible modes of neural crest lineage segregation. (*B*) NCCs first generate autonomic- and sensory-restricted progenitors (P_A and P_S, respectively). These progenitors then each generate neurons (N_A, N_S) and glia (G_A and G_S). (*C*) NCCs first generate neuronal- and glial-restricted progenitors (P_N and P_G, respectively), which then differentiate into autonomic and sensory neurons or glia. (*D*) NCCs directly generate autonomic and sensory neurons and glia without restricted intermediates. (*E*) Wnt1 expression marks NCCs, whereas NGN2 is predominantly expressed by sensory-restricted progenitors, favoring the model in *B*. Dotted arrow indicates that some NGN2^{+} cells can still generate autonomic derivatives at low frequency.

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