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Disruption of Segmental Neural Crest Migration and Ephrin Expression in Delta-1 Null Mice

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Neural crest cells migrate segmentally through the rostral half of each trunk somite due to inhibitory influences of ephrins and other molecules present in the caudal-half of somites. To examine the potential role of Notch/Delta signaling in establishing the segmental distribution of ephrins, we examined neural crest migration and ephrin expression in Delta-1 mutant mice. Using Sox-10 as a marker, we noted that neural crest cells moved through both rostral and caudal halves of the somites in mutants, consistent with the finding that ephrinB2 levels are significantly reduced in the caudal-half somites. Later, mutant embryos had aberrantly fused and/or reduced dorsal root and sympathetic ganglia, with a marked diminution in peripheral glia. These results show that Delta-1 is essential for proper migration and differentiation of neural crest cells. Interestingly, absence of Delta-1 leads to diminution of both neurons and glia in peripheral ganglia, suggesting a general depletion of the ganglion precursor pool in mutant mice.

INTRODUCTION

The vertebrate neural crest is a migratory population of cells that gives rise to a wide range of cell types, including neurons, glia, adrenal, and pigment cells, as well as skeletal and connective tissue in the head. After leaving the dorsal neural tube, neural crest cells migrate along specific pathways en route to their final destinations. Although they emerge uniformly from the neural tube, the pattern of neural crest migration becomes discontinuous and segmental as they selectively invade the rostral but not caudal half of each somitic sclerotome (Bronner-Fraser, 1986; Rickmann *et al.*, 1985).

Cues intrinsic to the somites are responsible for the segmental patterning of motor and sensory axons as well as neural crest cells (Bronner-Fraser and Stern, 1991; Keynes and Stern, 1984; Teillet *et al.*, 1987). Several classes of guiding molecules have spatiotemporal patterns in the somites consistent with a role in neural crest and/or axon guidance; these include peanut agglutinin (PNA)-binding molecules (Oakley and Tosney, 1991), chondroitin sulfate proteoglycans (Oakley *et al.*, 1994; Perris *et al.*, 1991), T-cadherin (Ranscht and Bronner-Fraser, 1991), Semaphorin3A (Eickholt *et al.*, 1999), and ephrins (Krull *et al.*, 1997; Wang and Anderson, 1997).

The Eph-related family of receptor tyrosine kinases and their ligands has been suggested to play important roles in axon guidance and cell migration (Flanagan and Vanderhaeghen, 1998; Holder and Klein, 1999). Interactions between these receptors and ligands appear to mediate repulsive signals that restrict cells or growth cones to particular regions in the developing nervous system (Wilkinson, 2001). The caudal sclerotome cells express ephrins (ephrinB2 in mammals and ephrinB1 in chick), while neural crest cells express cognate Eph receptors (EphB2 in mammals and EphB3 in chick). Perturbations studies have demonstrated that Eph/ephrin interactions are required for segmental migration of neural crest cells through the somites (Krull *et al.*, 1997; Wang and Anderson, 1997). This nonuniform pattern of neural crest migration leads to the characteristic metameric pattern of peripheral ganglia.

The molecular mechanisms that establish the nonequivalent distribution of chemorepellants and/or attractants in the somites are largely unknown. One way to approach this question is to examine embryos with specific mutations that affect somite development. The recently characterized murine *Delta-1* gene (*Dll1*), a homologue of the *Drosophila* Delta gene, encodes a ligand for the Notch receptor. *Dll1* is

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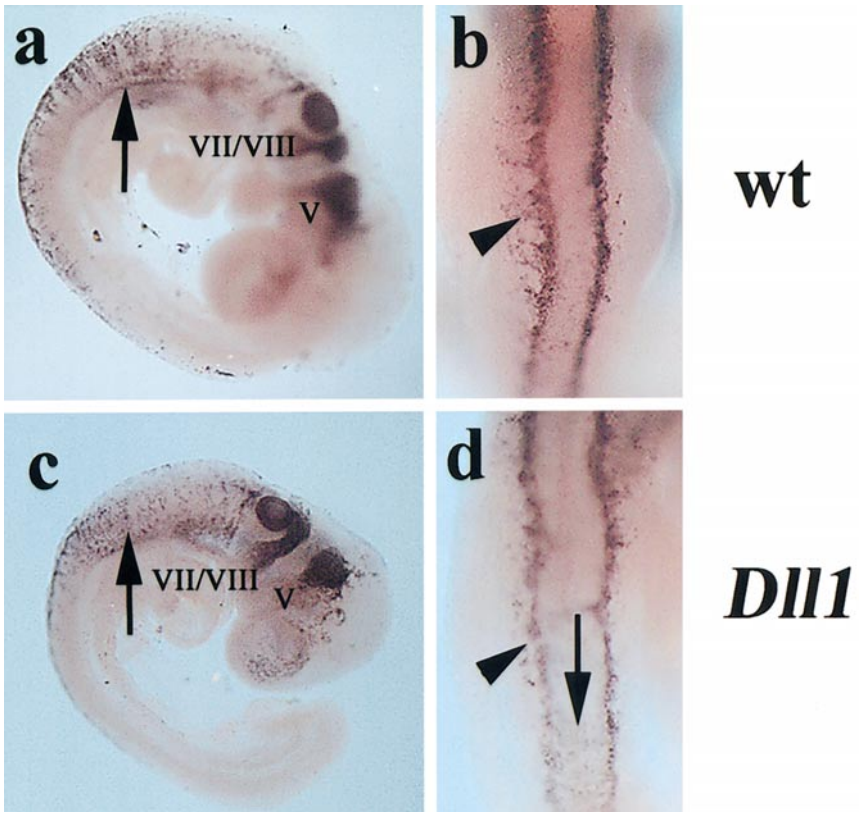


FIG. 1. Neural crest migration is delayed and nonsegmental in *Delta-1* mutant mice. Whole-mount *in situ* hybridization at E9 with Sox10 probe in wild type (wt) and *Dll1* (*Dll1*) mutant mouse. (a) Migrating neural crest in orderly streams in the anterior trunk (arrow) of a wild type mouse, while neural crest in a *Dll1* mutant mouse is random and disorderly (arrow in c). Dorsal view at higher magnification of wild type (b) and mutant *Dll1* (d) mouse shows reduced amounts of Sox10-positive neural crest cells emerging from the dorsal neural tube (arrowheads) in *Dll1* mice compared with a wild type littermate. Note also an increase in numbers of Sox10 cells crossing the dorsal midline in *Dll1* mice (arrow in d). V and VII/VIII correspond to the forming trigeminal and facial/auditory cranial ganglia, respectively.

expressed in the caudal half of the somites (Bettenhausen *et al.*, 1995). Mutations in the Notch/Delta pathway have been shown to cause severe segmentation defects and loss of rostrocaudal somite polarity (Hrabe de Angelis *et al.*, 1997).

Here, we examine the effects of disruption of the *Dll1* gene on neural crest migration patterns and the distribution of ephrin molecules. The results show that both the distribution pattern of ephrins and the subsequent migration of neural crest cells are abnormal in these mice, suggesting that Notch/Delta signaling via the Delta-1 ligand is essential for establishing the segmental pattern of neural crest migration. These findings are the first to establish a clear link between Notch signaling, somite patterning, and neural crest migration. In addition, we note a depletion in neural crest-derived ganglia, suggesting a role for Notch signaling in expansion of ganglionic precursors.

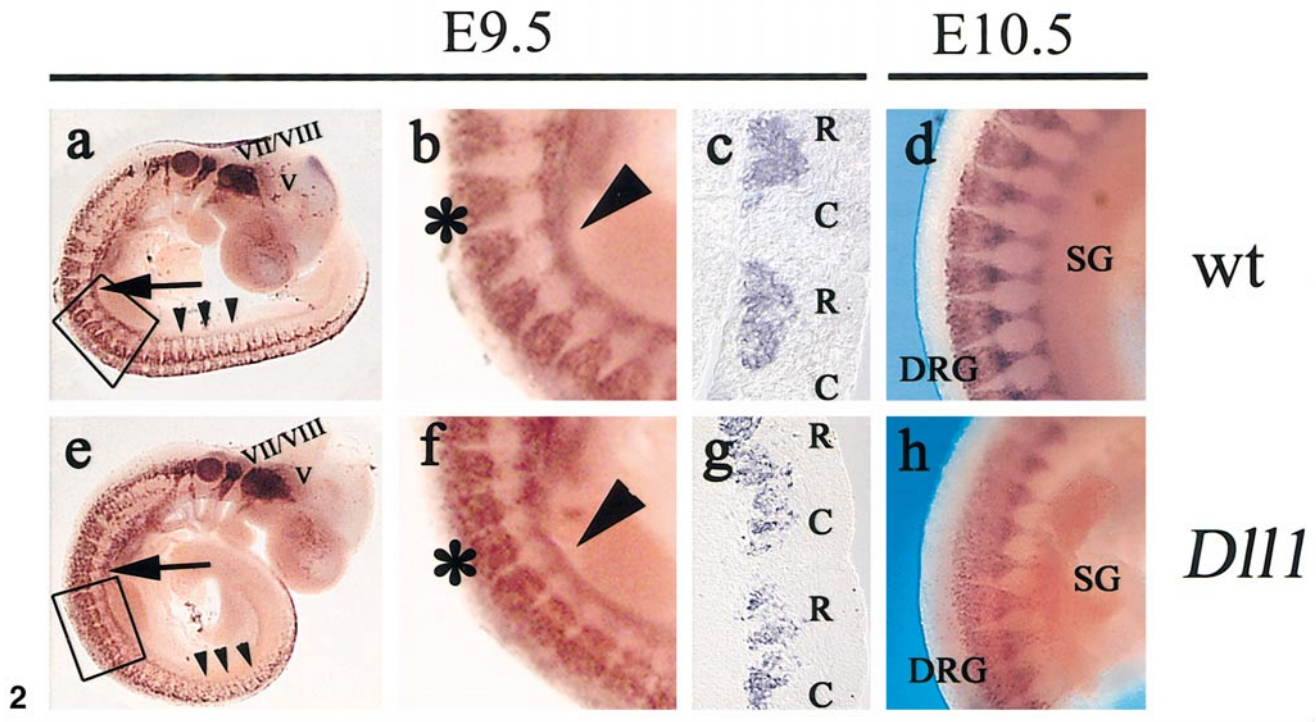
MATERIALS AND METHODS

Analysis of Mutant Embryos

Heterozygous *Dll1* mutant mice were crossed to obtain homozygous littermates. Day 0.5 of gestation was designated as the first day that the vaginal plug was observed. Since the developmental stages of mice even within the same litter can vary, embryos were staged by discrete developmental criteria, including the number of somites and the degree of limb bud formation and head development (Kaufman and Bard, 1999).

Embryos were dissected between E9 and E11 in Leibovitz-15 media and fixed overnight in fresh 4% paraformaldehyde (PF) at 4°C. Small holes were punctured in the forebrain and hindbrain region of the embryos to prevent trapping of the probe during *in situ* hybridization. At the end of the staining process, embryos or sections were incubated in a solution containing DAPI for nuclear detection.

Mice were genotyped by polymerase chain reaction (PCR) of



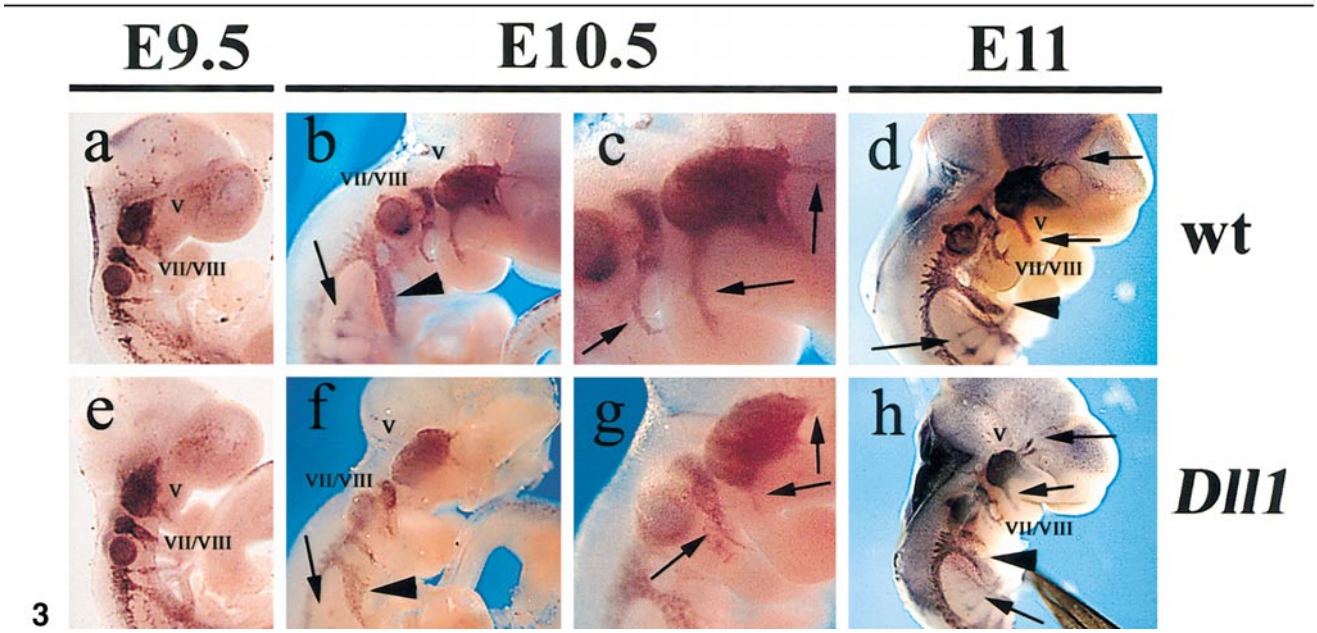


FIG. 2. *Delta-1* mutant embryos have partially fused DRG and reduced amount of peripheral glia. Whole-mount *in situ* hybridization at E9.5 and E10.5 with Sox10 probe in wild type (wt) and *Dll1* (*Dll1*) mutant mouse. (a) Neural crest cells at E9.5 migrating in orderly streams through the rostral half of the somites (arrowheads) in wild type embryos, while neural crest cells in mutants *Dll1* (e) migrate in a dispersed fashion through both halves of the somites (arrowheads). Note the discrete condensations of Sox10-positive cells in wild type (arrow in a) compared with relatively uniform Sox10 population in a *Dll1* embryo (arrow in e). (b, e) Higher magnification of the boxed area in (a) and (e), at the most rostral trunk region shows DRG beginning to condense (asterisks) and position of the future sympathetic ganglia (arrowhead in b and f). Note the neural crest streams appear fused and there is reduced Sox10 staining in the future ventral root and sympathetic regions. (c, g) Histological section through this region shows the neural crest migrating exclusively in the rostral half of the somite in a wild type embryo (R for rostral and C for caudal in c and g), while in the *Dll1* mutant, the neural crest cells are entering both the rostral and caudal half of the somites (c, g). (d, f) Later at E10.5, condensing DRG and sympathetic ganglia (DRG and SG, respectively) in wild type embryos (d); while *Dll1* mutants are missing, the SG and the DRG are mostly fused (f). V and VII/VIII correspond to trigeminal and facial/auditory cranial ganglia, respectively.

FIG. 3. *Delta-1* mutant embryos have abnormal cranial glia and reduced numbers of peripheral glia. Whole-mount *in situ* hybridization at E9.5, E10.5, and E11 with Sox10 probe in control (wt) and *Dll1* (*Dll1*) mutant mouse. Cranial ganglia in wt (a–d) and *Dll1* mutant embryos (e–h) form at their correct positions. However, as development progresses, *Dll1* embryos showed a marked reduction in Sox10 staining of cranial nerves (arrows in all figures), suggesting a reduction in peripheral glia. Note the abnormal shape of trigeminal (V) and facial/auditory (VII/VIII) ganglia and reduced numbers of neural crest cells migrating into the heart (arrowheads in all figures). (c) and (g) correspond to higher magnification of E10.5 embryos.

genomic DNA from the tail of adults and the heart of embryos; DNA was isolated by using the Gentra Puregene DNA isolation kit. Primers were designed to detect the wild-type 482-bp fragment of *Dll1*/38, which binds upstream of the *Dll1* translation initiation codon, and *Dll1*/39, which binds in exon 2. The primers used to detect the 578-bp mutant allele fragment were *Dll1*/38 and *lacZ1*.

Whole-Mount in Situ Hybridization

Embryos were removed from the mother, stripped of their membranes, and fixed in 4% paraformaldehyde overnight. Patterns of gene expression were determined by whole-mount *in situ* hybridization using DIG-labeled RNA antisense probes as described by Henrique *et al.* (1995). A 1-kb mSox10 probe was hydrolyzed before use. The *Sox10* transcripts are expressed by migrating neural crest cells during early development and by neural crest derivatives later(Cheng *et al.*, 2000; Pusch *et al.*, 1998). Eph receptor and ephrin expression patterns were observed with antisense RNA probe for the ligand ephrinB2 and the receptor EphB2.

Ephrin-Fc Whole Mount

Mouse embryos were blocked in Dulbecco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS) for 30 min before incubating them overnight at 4°C in DMEM/ephrinB1-Fc or ephrinA5-Fc (10 μg/ml). The next day, after washing with phosphate-buffered saline (PBS), the embryos were fixed for 30 min in paraformaldehyde and then incubated at 70°C for 45 min to inactivate endogenous phosphatases. The embryos were incubated with 1:1000 anti-human-Fc-alkaline phosphatase-conjugated antibody (Promega) overnight in PBS/10% FBS and washed extensively the next day with PBS then with AP buffer before 5-bromo-4-chloro-indolyl-phosphatase (BCIP) nitroblue tetrazolium (NBT) color reaction.

Neural Crest Cultures

Mouse neural tubes from E9.5 were dissociated in 1.5 mg/ml of Dispase for 30 min and washed in Leibovitz-15 media. The neural tubes were cut in small pieces (size of 2–3 somites) and pipetted in the center of wells coated with fibronectin (10 μg/ml). Neural tubes were cultured in DMEM and 10% FBS and 100 mg/ml and 100 U of penicillin and streptomycin, respectively. The next day, the cultures were washed with L-15 and incubated with 5 μg/ml of ephrinB2-Fc (R&D Systems) at 4°C for 60 min, washed twice with L15, and fixed in 4% PF for 30 min. Slides were blocked for 30 min with PBS, 1% Triton X-100, and 10% FBS. The primary antibody was rabbit anti-p75 for visualizing neural crest cells, followed by an anti-mouse IgG-Alexa 564 secondary (Molecular Probes) and neutravidin Oregon Green 488 for visualizing the biotinylated ephrinB2-Fc.

Whole-Mount Immunohistochemistry

E9.5–E10.5 mouse embryos were fixed in 4% PF for 18 h and washed three times in PBS for 60 min each. Then, embryos were blocked overnight at 4°C in PBS containing 0.1% H_2O_2, 1% Triton X-100, and 5% FBS. Embryos were washed three times for 60 min each in PBS + 1% Triton + 5% FBS. Tuj1 antibody was used at 1:500 + 10% FBS + 1% Triton for 2 days at 4°C. Embryos were washed three times for 60 min each in PBS + 1% Triton + 1% NGS, then they were incubated with 1:100 anti-mouse HRP conjugated in PBS + 1% Triton + 1% NGS overnight. Embryos were then washed three times for 60 min each in PBS + 1% Triton + 1% NGS. For DAB, embryos were incubated for 3 h at 4°C in the dark; then, to activate the DAB, H_2O_2 was added to 0.03% and embryos were observed at room temperature under a dissecting scope until cranial ganglia were visible (~5 min). Embryos were washed extensively in abundant PBS and photographed immediately.

RESULTS

Neural crest cells emerge from the neural tube in a rostrocaudal progression, such that several stages of neural crest migration can be observed concurrently in the same embryo. While migration is initiating in the caudal trunk, it is well underway in more rostral regions of the embryo. The extent of neural crest migration was assessed by using Sox10, a molecular marker that selectively identifies early migrating neural crest cells and is later observed in peripheral glia (Pusch *et al.*, 1998). Mouse embryos were analyzed from E9 to E11, with E9–E10 corresponding to the peak time of neural crest migration and E11 to the initial stages of gangliogenesis. Delta-1 homozygote embryos did not live beyond E11 (Hrabe de Angelis *et al.*, 1997).

Neural Crest Migration Is Delayed and Nonsegmental in Delta-1 Mutant Mice

The first obvious difference between control and mutant littermates was a smaller size of the mutant embryos, with some beginning to die as early as E9. At this stage, neural crest cells in wild type embryos were migrating neatly through the rostral half of the first somites. In contrast, mutant neural crest cells were only starting to penetrate the somites and appeared to be in disarray (Figs. 1a and 1c, arrows). Similarly, the emergence of the trunk neural crest was significantly delayed as seen by Sox10 labeling (arrowheads in Figs. 1b and 1d). In dorsal view, the line of Sox10-positive cells in the midline of the trunk neural tube of mutant embryos appeared thinner and some Sox10-expressing cells seemed to cross the dorsal midline (arrow in Fig. 1d). In contrast, cranial neural crest cells looked comparatively normal; those migrating into the first and second branchial arches followed their usual pathways to form the V and VII/VIII ganglia in their correct locations. However, the Sox10 cells appeared more scattered in the rostral trunk region of mutant embryos compared with those in control littermates of the same stage (arrows in Figs. 1a and 1c). The decrease in the neural crest population did not appear to be due to an increase in cell death, since DAPI staining revealed no differences in the numbers of pycnotic nuclei in mutant versus control embryos (data not show) at any stage examined in this study. In addition, other aspects of the embryo appeared normal, suggesting that there was no global developmental delay, but rather one that selectively affected particular tissue, such as the trunk neural crest.

By E9.5, neural crest cell migration is nearly complete at the rostral end of wild type embryos, and cells within the somites are aggregating at the site of the future dorsal root (DRG) and sympathetic ganglia (Fig. 2a, arrow). Concomitantly, at the more caudal regions, trunk neural crest cells are beginning to migrate through the rostral half of the somites (Fig. 2a, arrowheads). In mutant embryos, neural crest migration remained delayed; rather than clearly defined streams of migrating neural crest cells, they seemed to be continuous (arrow in Fig. 2e). Furthermore, rather than selectively migrating through the rostral half of each somite, neural crest cells appeared to be dispersed through both halves of the somites in a disorganized fashion (arrowheads in Fig. 2e). Sections through embryos confirmed that the neural crest was distributed in the caudal as well rostral halves of the somites (Figs. 2c and 2g). However, there appeared to be fewer neural crest cells at the presumptive borders between somites, maintaining a pseudosegmental quality to the migration pattern.

Delta-1 Mutant Embryos Have Partially Fused DRG and Reduced Numbers of Peripheral Glia

By E10–E10.5, neural crest migration in wild type embryos is nearly complete, with most of the peripheral ganglia (DRG and sympathetic chain) condensed, with the exception of those in the more caudal parts of the embryo (Fig. 2d). In contrast, *Dll1* mutant embryos have partially fused DRG and a dramatic reduction of the sympathetic chain (SG) (Fig. 2h). In addition, many embryos had reduced numbers of Sox10-positive cells in the spinal nerves, which at this stage are largely composed of peripheral glial precursors. In addition, the numbers of neurons in the dorsal root ganglia appeared reduced.

Although cranial ganglia initially appeared normal (Figs. 3a and 3e), we later noticed marked differences between wild type and *Dll1* littermates. First, Sox10 staining revealed significantly smaller and unusually shaped cranial ganglia (V and VII/VIII in Figs. 3b and 3f), with branches either atrophied or completely missing (arrows in Figs. 3c and 3g). Second, the numbers of vagal neural crest cells migrating into the heart and gut appeared significantly reduced in the mutant embryos (arrowheads in Figs. 3b and 3f). Third, the cervical ganglia appeared affected in the *Dll1* mutant mice (arrows in Figs. 3b and 3f). The significant reduction in Sox10 staining suggests substantial glial loss.

Neural crest migration is completed by E11, such that most of the Sox10 staining at this stage corresponds to Schwann and satellite cell precursors present in the ganglia and peripheral nerves (Britsch *et al.*, 2001; Kuhlbrodt *et al.*, 1998), which will differentiate into glia after E14 (Dong *et al.*, 1999). A few mutant embryos survived to E11. In one such embryo, the trigeminal ganglion showed a 30% reduction in size and the temporal ophthalmic branch of the trigeminal ganglion appeared to be split into two smaller nerves instead of one (arrows in Figs. 3d and 3h). Overall, the anatomical location of the cranial ganglia and most of the nerves appeared normal, indicating that they condensed at their proper sites, albeit being of a smaller size.

Loss of ephrinB2 and EphB2 in Delta-1 Mutant Mice

The most severe defect in the *Dll1* mutations is the loss of rostrocaudal polarity in the somites. Therefore, we examined the distribution of Eph receptors and ligands in the *Dll1* mice to determine whether they were altered in the mutants and, therefore, downstream of *Dll1*. Two methods were used: (1) *in situ* hybridizations with probes for EphB2 and ephrinB2, and (2) binding of ephrinB1-Fc and ephrinA5-Fc chimeric proteins to recognize the cognate receptors. *In situ* hybridization of wild-type embryos confirmed previous reports that ephrinB2 is expressed in the caudal half of the somites in rodents, whereas EphB2 is expressed by the migratory neural crest (Wang and Anderson, 1997) (Figs. 4a and 4e for ephrinB2, and 4b and 4f for EphB2). At E9, the ephrinB2 expression was much lower in mutant than in wild type embryos; however, some staining was still discernible at low levels in a region that anatomically, but not morphologically, corresponds to the caudal half of the sclerotome. Interestingly, by E9.5–E10, corresponding to the peak of neural crest migration, ephrinB2 was no longer detectable in the caudal sclerotome of mutant embryos (data not shown). These results suggest that ephrinB2 is only expressed at low levels and fails to be maintained in the somites of *Dll1* null mice.

To detect the complementary receptor(s) for ephrinB2, we performed EphB2 *in situ* as well as binding with an ephrinB-Fc cocktail (Gale *et al.*, 1996), which recognizes the EphB family of receptors that binds to transmembrane ligands. The results demonstrated a significant reduction in receptor levels on migratory neural crest of mutant mice at E9.5–E10 compared with wild type littermates (Figs. 4b and 4f for EphB2; data not shown for ephrinB-Fc cocktail). Similarly, EphB class receptor levels were reduced in the condensed DRG at E10.5 (Figs. 4c and 4g), with the most rostral DRGs being practically unstained (Fig. 4g). To determine whether neural crest cells from mutant embryos had reduced Eph receptor expression even at early stages of migration, we cultured neural tubes from wild type and mutant embryos. Neural crest cells emigrated from both normal and mutant neural tubes, suggesting that neural crest cells were produced efficiently by the *Dll1* neural tubes, as assayed by labeling with the p75 antibody, which recognizes migrating murine neural crest cells (Figs. 5a and 5b). However, the levels of EphB family receptors, assayed by ephrinB2-Fc binding, were greatly reduced or missing from the early migrating neural crest population of the mutants (Figs. 5c and 5d).

EphA subfamily receptors are present on differentiated DRGs (Gale *et al.*, 1996). We examined the distribution of this class of receptors using an ephrinA5-Fc-chimeric probe (AL-1/RAGS) (Drescher *et al.*, 1995). Similar to the results with EphB subfamily receptors, DRG in *Dll1* mutant mice

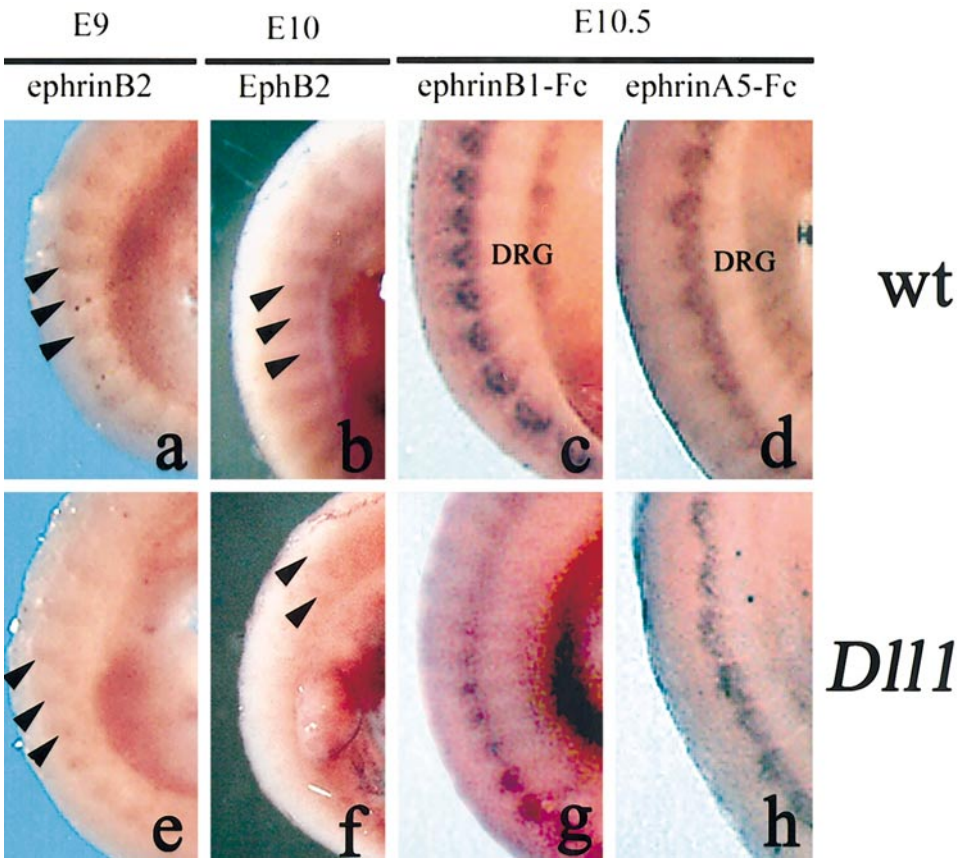


FIG. 4. EphrinB2 and EphB2 expression is lost in *Delta-1* mutant mice. Whole-mount *in situ* hybridization at E9 with ephrinB2 and E10 with EphB2 probes in control (wt) and *Dll1* (*Dll1*) mutant mouse. Trunk regions at the level of forelimb are shown; note that the ephrinB2 signal is in the caudal half of the sclerotome (arrowheads in b) in wild type embryos (a), but is greatly reduced in the *Dll1* mutants (arrowheads in e). Control embryos showed normal EphB2 levels in the rostral half of the somites, while *Dll1* levels were barely detectable (arrowheads in b and f). Binding of ephrinB1-Fc (c, g) and ephrinA5-Fc (d, h) at E10.5 in forelimb trunk region. Both probes gave robust signal, indicating the presence of EphB and EphA subclass receptors, respectively, in the forming DRG of control embryos (c, d). In contrast, *Dll1* mutant had greatly reduced levels of Eph receptors and the DRG appeared severely diminished and fused (g, h).

showed reduced levels of EphA class receptors (Figs. 4d and 4h), as reflected by smaller ganglion size and less well-defined boundaries. Moreover, staining for neurofilaments revealed a loss of neurons in both cranial and trunk peripheral ganglia (Fig. 6). By E9.5, cranial ganglia neurogenesis is well underway (Easter *et al.*, 1993) (Fig. 6a). However, *Dll1* mutant mice showed a marked reduction in the size and density of neurofilament staining in the ganglia (arrows in Figs. 6a and 6b), suggesting a decrease in the amount of neurons in the trigeminal ganglia. Later on, at E10, the delay and reduction in neurogenesis is more apparent at the level of the trunk (Figs. 6c and 6d). Note that the trunk ganglia in the *Dll1* mouse not only showed fewer positive cells, but there was also a marked absence of staining of the spinal nerves. Altogether, *Dll1* mutant mice have abnormal peripheral ganglia with substantial glial and neuronal deficits.

DISCUSSION

Delta-Notch signaling has been shown to play a key role in cell–cell signaling and cell-fate determination during many aspects of development, including formation of somites and neural/epidermal and neural/glia lineage decisions (Morrison *et al.*, 2000; Scheer *et al.*, 2001; Van De Bor and Giangrande, 2001). Active forms of Notch have been shown to inhibit neurogenesis, while repressing Notch signaling increases neuronal differentiation (Artavanis-Tsakonas *et al.*, 1999; Austin *et al.*, 1995; Chitnis *et al.*, 1995; Coffman *et al.*, 1993; Haddon *et al.*, 1998; Henrique *et al.*, 1997). Recently, it was shown that activation of Notch by a soluble form of *Delta-1* can instruct neural crest stem cells into a glial fate at the expense of neurons (Morrison *et al.*, 2000). Null mutation in *Delta-1* (*Dll1*) result in somite abnormalities and other deficits that lead to embryonic

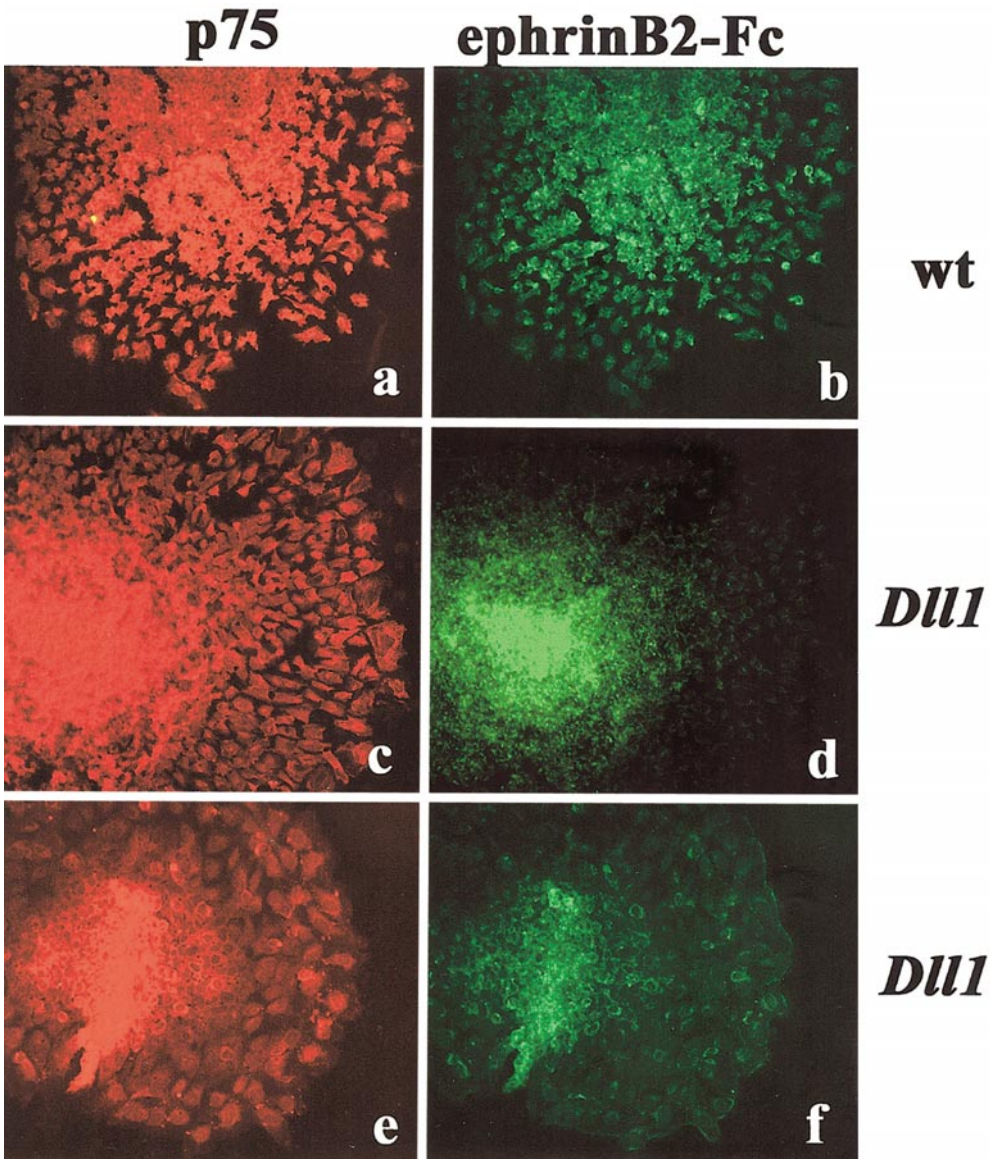


FIG. 5. Neural crest cells migrated normally *in vitro* on fibronectin substrates but have reduced ephrinB2-Fc binding in *Delta-1* mutant mice. Neural crest cultures from wild type and *Dll1* mutant mice were incubated with ephrinB2-Fc to recognize the cognate EphB class receptors and anti-p75 antibody to recognize neural crest cells. Control (a, b) and mutant (c–f) cells showed normal levels of p75 neural crest marker and similar profiles of neural crest outgrowth, while Dll1 neural crest cells (d, f) showed significant reduction of ephrinB2 binding to EphB receptor on the cell surface compared with controls.

lethality by E11 (Hrabe de Angelis *et al.*, 1997). The present study demonstrates that mutations in *Dll1* also have major defects in the neural crest. Loss of *Dll1* causes severe disruption of neural crest migration, likely by reducing ephrin ligand expression in the caudal portion of the somitic sclerotome, as well as a loss of neural crest-derived peripheral glia. Both the size of DRGs and their level of Eph receptor expression were reduced.

Neural crest migration is a complex process involving interactions between the migrating cells and cues in their local environment, especially with the caudal half of the somites, which these cells actively avoid (Bronner-Fraser, 1996). In the present study, we confirm that key components for neural crest migration in the somites of *Dll1* null mice are disrupted (Hrabe de Angelis *et al.*, 1997). We further show that the mutant mice are delayed in their development, typically being smaller with a concomitant reduction in the initial numbers of neural crest cells enter-

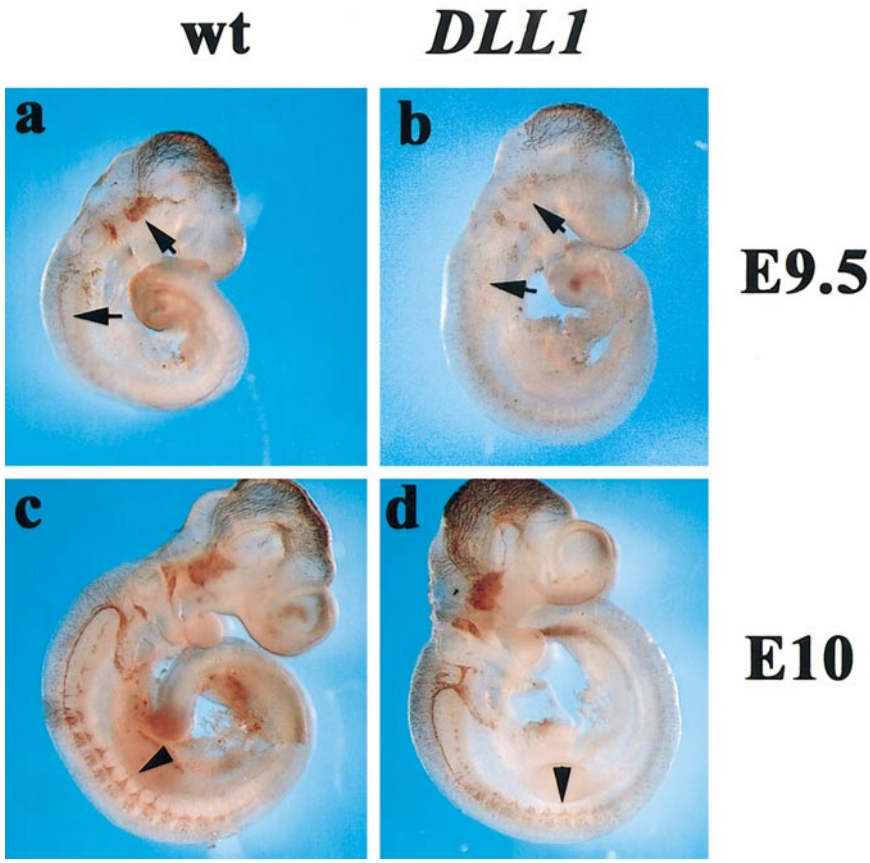


FIG. 6. *Delta-1* mutant embryos have reduced neurogenesis. Whole-mount immunohistochemistry with anti-β-tubulinIII (Tuj1) at E9.5 and E10 in control (wt) (a, c) and *Dll1* mutant mouse (b, d). At E9.5, cranial ganglia are significantly reduced in *Dll1* mutant embryos compared with a wild type control littermate (arrows in a and b, pointing to trigeminal and XII ganglia). As development progresses (E10), *Dll1* embryos continue showing a marked reduction of the neuronal marker expression in the fused trunk ganglia, indicating a reduction in peripheral neurogenesis. Note also the lack of spinal nerves at the level of the forelimb (arrowheads in c and d).

ing the somites compared with stage-matched littermates. One possibility is that the total number of neural crest generated by the neural tube is reduced due to a failure to produce neural crest cells in these embryos. If this is the case, there appears to be compensation later since the Sox10 signal in the early DRG was usually comparable to control littermates, despite the fact that they were not properly segmented. Because neural crest cells are formed in mutant embryos, *Dll1* does not seem to be absolutely required for induction of the neural crest. Alternatively, the *Dll1* mutant may only affect neural crest cells indirectly by altering the somites such that they become nonpermissive for entry of neural crest cells.

Our results show that *Dll1* is necessary for proper neural crest migration. Neural crest cells in mutant mice were randomly dispersed through the somites rather than moving selectively through only the rostral portion of each sclerotome. Although the somites formed in *Dll1* mutant mice, they were highly disorganized. The finding that neural crest cells were not able to follow their normal pathways implies that lack of rostral–caudal polarity is fundamental for their proper migration. In order to further understand this defect, we examined the expression pattern of ephrinB2, a known neural crest chemorepellant found in the caudal half of the somites which plays an important role in restricting neural crest cells from this territory (Krull *et al.*, 1997; Wang and Anderson, 1997). Although we did detect some initial expression of this molecule, in what would correspond to the caudal half of the somite, the levels were significantly reduced. Concomitantly, levels of Eph receptors were also significantly reduced on neural crest cells both during their migration and as cells were condensing into dorsal root ganglia. This implies that the *Dll1* ligand is important for maintaining normal levels of both ephrinB2 expression and EphB2 receptor expression, though is not required for its initial expression in the caudal

half of the somite. Thus, there must be other signals besides *Dll1* that tell certain molecules where they should be expressed.

An intriguing finding from our analysis of *Dll1* mutant mice is that dorsal root ganglion condensation could still take place in a pseudosegmented manner, despite somite malformations and disruption of segmental migration, concurrent with a significant reduction in the numbers of Eph-expressing neural crest descendants. This suggests that the process of ganglionic condensation may be *Dll1* independent and separate from that of neural crest migration.

After cessation of neural crest migration, changes were noted in peripheral ganglia of *Dll1* mice. First, there was a reduction in the number of glia in the peripheral nerves (as assessed by Sox10 expression) (Britsch *et al.*, 2001). This is consistent with recent findings showing that *Dll1* signaling through the Notch receptor has been found both to promote and/or impair gliogenesis in the fly depending on the context (Gaiano *et al.*, 2000; Morrison *et al.*, 2000; Scheer *et al.*, 2001; Van De Bor and Giangrande, 2001; Wakamatsu *et al.*, 2000). Our present results in *Dll1* null mice demonstrate a diminution, but not elimination, of peripheral glia. This suggests that *Dll1* is not the sole determinant of neural versus glial fate decisions, but that other molecules, perhaps other members of the Delta family, like Delta-3 and Jagged, other Notch receptor types, or yet to be characterized molecules, may also play a role in gliogenesis of neural crest-derived cells (Tanigaki *et al.*, 2001). In the mouse, for example, *Dll3* is also expressed in the presomitic mesoderm and early somites, as well as in mature DRG (Dunwoodie *et al.*, 1997). The paucity of EphA and B receptors of DRG of mutant mice also suggests that *Dll1* signaling is crucial for neuronal differentiation of the neural crest. A role for Notch signaling in formation of both neurons and glia has been suggested in *Drosophila* and zebrafish, where Notch mutants have reduction in both gliogenesis and neurogenesis (Chitnis *et al.*, 1995; Hartenstein and Posakony, 1990; Lieber *et al.*, 1993; Van De Bor and Giangrande, 2001). Thus, rather than merely influencing the neuron versus glia decision by lateral inhibition, Notch/Delta signaling may be important for maintaining a common ganglionic precursor pool for both neurons and glia. We see no evidence of increased cell death in the neural crest of mutant embryos, suggesting that the population is not depleted due to apoptosis. Furthermore, the developmental delays observed in Delta-1 null embryos are selective to certain cell populations like the trunk neural crest. Thus, our data support the idea that smaller ganglia in the Delta-1 null mice are due to a general depletion of a common neural/glial precursor pool. It should be noted that Notch signaling is involved at multiple stages and locations during development and that the interpretation of the signal may well be cell type-specific.

In summary, we have shown that neural crest migration in *Dll1* mutant mice is severely disrupted, suggesting that its proper expression is necessary for confining migrating neural crest cells to the rostral half of the somites. Because ephrinB2 is reduced in the caudal sclerotome of mutant mice, this provides one likely mechanism whereby segmental migration may be affected. However, we cannot exclude the involvement of other inhibitory and/or attractive molecules, which may also be disrupted by *Dll1* mutation (Eickholt *et al.*, 1999). We also show that peripheral neuron and glial development is diminished in *Dll1* mice, corroborating previous studies on the instructive though not selective role of Notch activation by *Dll1* in neurogenesis and gliogenesis (Anderson *et al.*, 1997; Wang and Barres, 2000).

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