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Lineage-specific requirements of β-*catenin* in neural crest development

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β-Catenin plays a pivotal role in cadherin-mediated cell adhesion. Moreover, it is a downstream signaling component of Wnt that controls multiple developmental processes such as cell proliferation, apoptosis, and fate decisions. To study the role of *β-catenin* in neural crest development, we used the *Cre/loxP* system to ablate *β-catenin* specifically in neural crest stem cells. Although several neural crest–derived structures develop normally, mutant animals lack melanocytes and dorsal root ganglia (DRG). In vivo and in vitro analyses revealed that mutant neural crest cells emigrate but fail to generate an early wave of sensory neurogenesis that is normally marked by the transcription factor neurogenin (ngn) 2. This indicates a role of *β-catenin* in premigratory or early migratory neural crest and points to heterogeneity of neural crest cells at the earliest stages of crest development. In addition, migratory neural crest cells lateral to the neural tube do not aggregate to form DRG and are unable to produce a later wave of sensory neurogenesis usually marked by the transcription factor ngn1. We propose that the requirement of *β-catenin* for the specification of melanocytes and sensory neuronal lineages reflects roles of β-catenin both in Wnt signaling and in mediating cell–cell interactions.

Introduction

Multipotent stem cells have to generate various differentiated cell types in the correct number and sequence during neural development (Sommer and Rao, 2002). The neural crest has turned out to be a valuable model system to study the mechanisms controlling this process. Neural crest cells in vertebrates give rise to neuronal and glial cells of the peripheral nervous system (PNS)* and generate nonneural cells such as pigment and smooth muscle–like cells. Many signals have been described that promote the formation of particular cell fates in migratory and postmigratory neural crest stem cells (for reviews see Anderson et al., 1997; Dorsky et al., 2000a; Sommer, 2001). Melanocyte formation is induced by Wnt signaling (Dorsky et al., 1998; Jin et al., 2001), whereas TGFβ promotes the development of smooth muscle–like cells (Shah et al., 1996). Notch signaling and NRG1 isoforms promote the generation of satellite glia and Schwann cells (Shah et al., 1994; Hagedorn et al., 2000b; Morrison et al., 2000; Leimeroth et al., 2002), whereas members of the TGFβ family promote the differentiation of autonomic neurons (Reissmann et al., 1996; Shah et al., 1996; Hagedorn et al., 2000a). Signals specifying sensory neurons from neural crest cells, however, have not yet been reported. There is increasing evidence that the fate of multipotent neural crest stem cells is not only dependent on the action of individual signals but is also influenced by the synergistic activity of multiple signals (Sommer and Rao, 2002). Hitherto unknown signals provided by cell–cell interactions alter the biological activity of instructive growth factors such as TGFβ, indicating that neural crest cells are able to integrate multiple cues (Hagedorn et al., 1999, 2000a). Moreover, cell-intrinsic changes affect fate decisions during neural crest development by changing the sensitivity of neural crest cells to specific extracellular signals (Paratore et al., 2001, 2002; White et al., 2001; Kubu et al., 2002).

β-Catenin is a good candidate to be involved in lineage decisions in neural crest development, given its dual function in cadherin-dependent cell–cell interactions and in mediating Wnt signaling, and that both cadherins and Wnt molecules have been implicated in neural development (Cadigan and Nusse, 1997; Yagi and Takeichi, 2000). By binding to the cytoplasmic domain of cadherins and to α-catenin, β-catenin

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*Abbreviations in this paper: bHLH, basic helix-loop-helix; DRG, dorsal root ganglia; E, embryonic day; mitf, microphtalmia-associated transcription factor; ngn, neurogenin; nf, neurofilament; PNS, peripheral nervous system; trk, tyrosine receptor kinase; trp2, tyrosinase-related protein 2.

Key words: β-catenin; Wnt; cadherin; melanocytes; sensory neurons

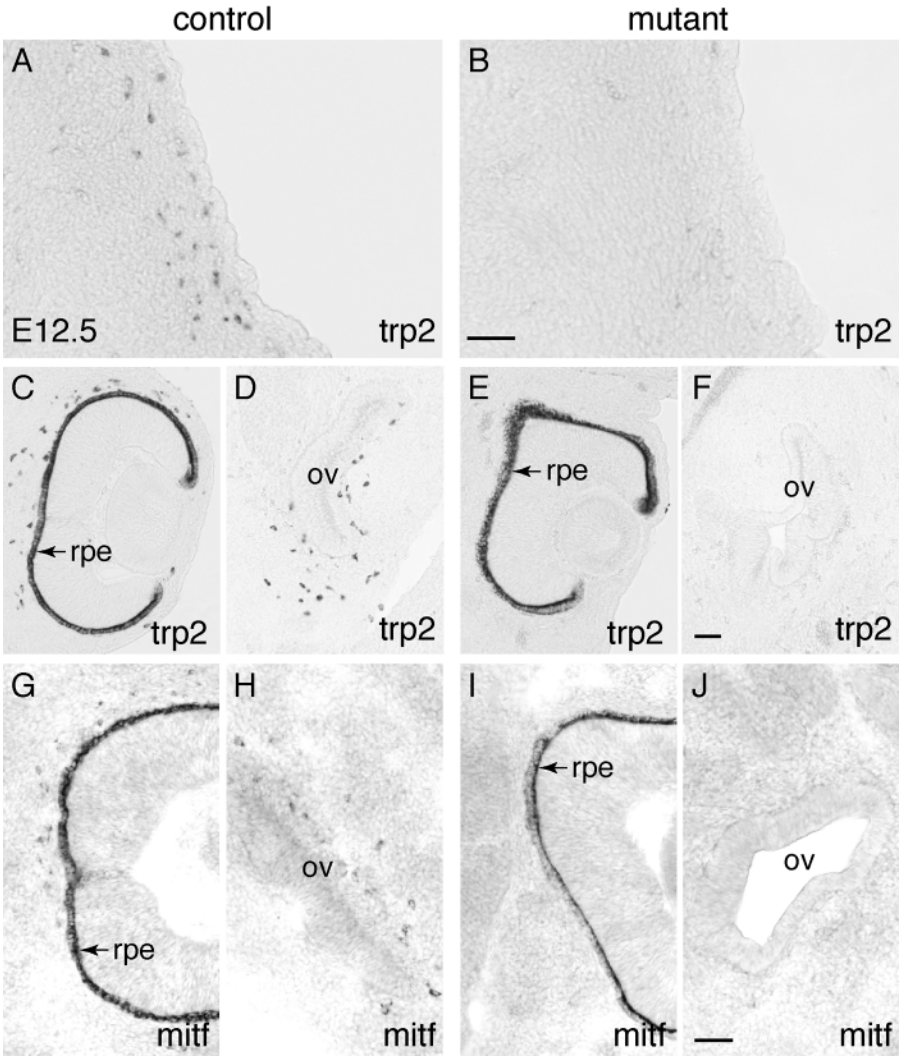


Figure 1. **Absence of melanocytes in *β-catenin* mutant embryos.** Melanocytes and their precursors were marked by in situ hybridization analysis on transverse sections of E12.5 control (A, C, D, G, and H) and mutant (B, E, F, I, and J) embryos using trp2 (A–F) and mitf (G–J) riboprobes. In mutant embryos, trp2-positive melanocytes were absent underneath the surface ectoderm (B), in areas surrounding the retinal pigment epithelium (rpe, E), and around the otic vesicle (ov, F), whereas many melanocytes were present in control embryos (A, C, and D). Many mitf-expressing melanoblasts were found around the eye (G) and the otic vesicle (H) of control but not mutant embryos (I and J). Bars, 50 μm.

links the cadherin-dependent adhesion complex to the cytoskeleton and thereby strengthens cellular interactions (Vleminckx and Kemler, 1999; Gumbiner, 2000). Wnt signaling has been implicated in the regulation of cell proliferation, apoptosis, and cell fate decisions (Cadigan and Nusse, 1997; Uusitalo et al., 1999). Activation of this pathway leads to the stabilization of β-catenin and to its translocation into the nucleus, where it associates with other nuclear effectors to form a transcriptional activator complex. In various biological systems, Wnt/β-catenin signaling has been identified as part of a signaling network that involves interactions with other signal transduction pathways such as TGFβ and Notch signaling (Hecht and Kemler, 2000; De Strooper and Annaert, 2001). A putative role of β-catenin in neural crest development is thus likely to reflect functions of cadherin-mediated adhesion and Wnt signaling. Cadherins influence neural crest specification and emigration from the neural tube (Nakagawa and Takeichi, 1998; Borchers et al., 2001). Moreover, the expression of cadherins in aggregating dorsal root ganglia (DRG) and in Schwann cells is consistent with a role at later stages of neural crest development (Pla et al., 2001). Wnt signaling is involved early in neural crest development and regulates neural crest induction and expansion (Ikeya et al., 1997; Saint-Jeannet et al., 1997; LaBonne and Bronner-Fraser, 1998), apart from the generation of melanocytes mentioned above.

To investigate the role of β-catenin in neural crest stem cells and their derivatives, we performed conditional gene ablation of *β-catenin* in premigratory neural crest. Such an approach circumvents the early embryonic lethality of mutants generated by gene deletion in the germ line (Haegel et al., 1995; Huelsken et al., 2000) and has been successfully applied before to reveal a requirement of *β-catenin* in brain and craniofacial development, skin stem cell differentiation, and fate decisions between endoderm and precardiac mesoderm (Brault et al., 2001; Huelsken et al., 2001; Lickert et al., 2002). The present study identifies β-catenin as a crucial regulator of sensory neuron specification and melanocyte formation.

Results

Inactivation of *β-catenin* in neural crest

The role of *β-catenin* in neural crest development was addressed by conditional gene inactivation in neural crest stem cells using the *Cre/loxP* recombination system (Gu et al.,

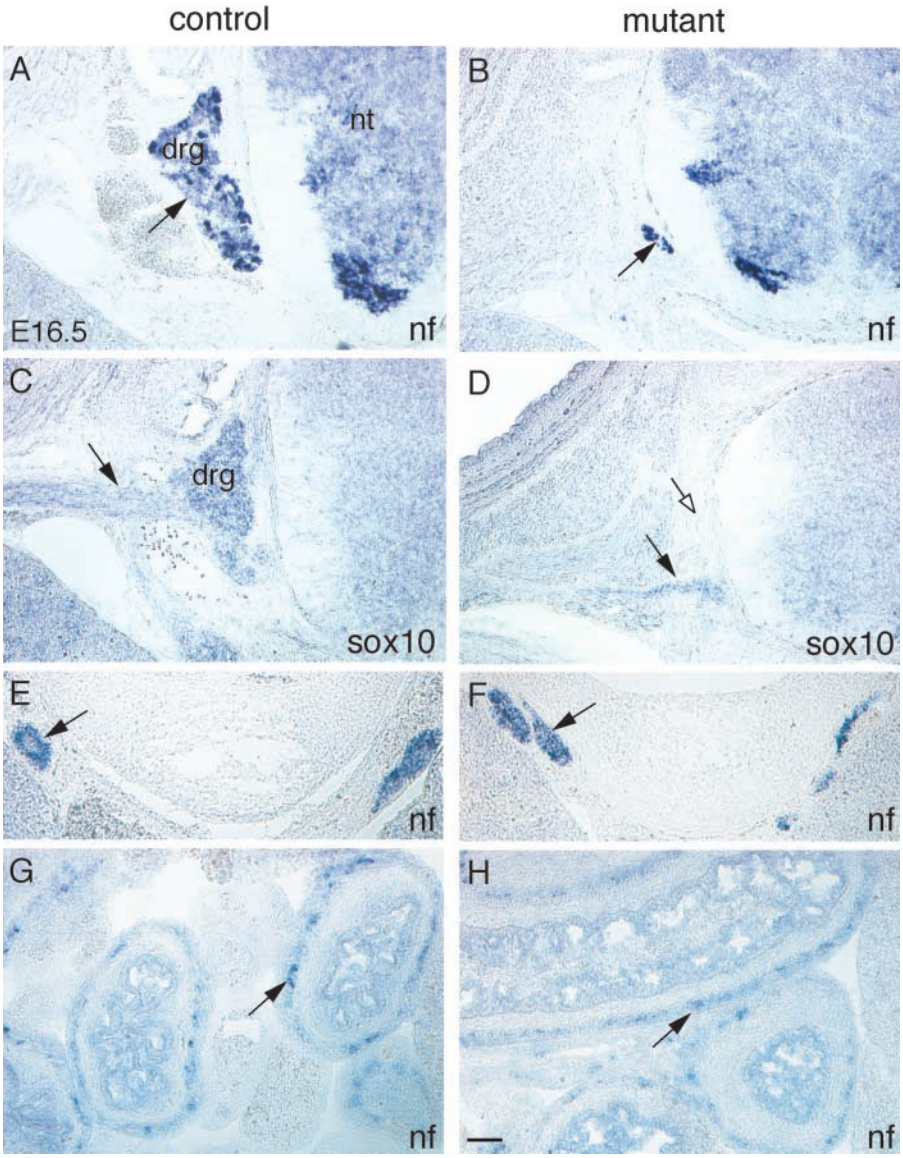


Figure 2. **Analysis of control and mutant PNS.** In situ hybridization experiments on transverse sections of E16.5 embryos with nf (A and B and E–H) and sox10 (C and D) riboprobes revealed a reduction of neuronal (A and B, arrows) and complete absence of glial (C and D, open arrow) lineages in dorsal root ganglia (drg). Peripheral nerves marked by sox10 (C and D, arrows) were reduced in diameter, whereas other crest derivatives, such as sympathetic ganglia (E and F, arrows) and the enteric nervous system (G and H, arrows), appeared to develop normally. nt, neural tube. Bar, 50 μm.

1994). Cre-mediated recombination of a floxed allele in which essential sequences of the *β-catenin* gene are flanked by *loxP* sites generates the *β-catenin* floxdel allele, from which no functional β-catenin protein is expressed (Brault et al., 2001). In *wnt1-Cre* mice, Cre recombinase is active in the entire neural crest population (Danielian et al., 1998). To generate neural crest–specific *β-catenin* mutant embryos, we crossed *wnt1-Cre* animals heterozygous for the *β-catenin* floxdel allele with animals homozygous for the *β-catenin* floxed allele. In *wnt1-Cre*/*β-catenin*$^{floxed/floxdel}$ embryos derived from such breeding, β-catenin expression is efficiently eliminated in virtually all neural crest stem cells (Brault et al., 2001). In contrast, littermates lacking the *wnt1-Cre* transgene or carrying a wild-type *β-catenin* allele express β-catenin normally and serve as control animals.

Loss of the melanocyte lineage in *β-catenin* mutant embryos

We first investigated the role of *β-catenin* in melanocyte development. Neural crest cells give rise to melanocytes of the skin, inner ear, and part of the iris (Wehrle-Haller and Weston, 1997). Wnt signaling promotes melanocyte formation from neural crest cells and absence of *wnt1* and *wnt3a* in compound mutant mice results in the loss of the melanocyte differentiation marker tyrosinase-related protein 2 (trp2) (Ikeya et al., 1997; Dorsky et al., 1998; Dunn et al., 2000; Jin et al., 2001). Similarly, we observed a complete absence of trp2 expression in *β-catenin* mutant embryos at embryonic day (E) 10.5 and E12.5 (Fig. 1; unpublished data). This phenotype was apparent at all sites of neural crest–derived melanocyte formation, such as underneath the surface ectoderm (Fig. 1, A and B), around the retinal pigment epithelium (Fig. 1, C and E), and around the otic vesicle (Fig. 1, D and F). In contrast, trp2-positive cells in the retinal pigment epithelium, which are not generated from neural crest, were not affected in the mutant.

To address whether the lack of trp2 expression reflects a requirement for *β-catenin* in early or late melanocyte differentiation, the expression of microphtalmia-associated transcription factor (mitf) was analyzed. Mitf activates pig-

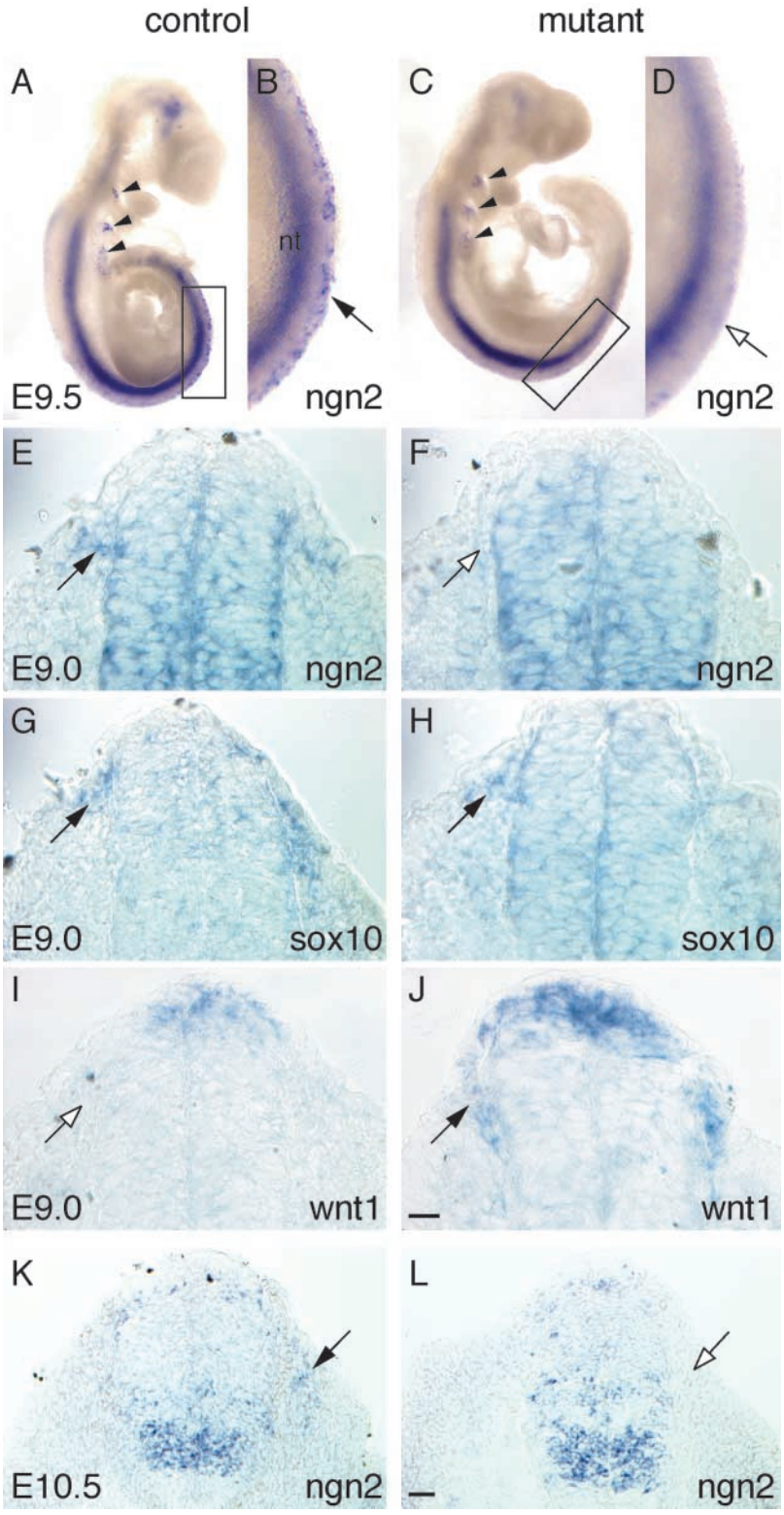
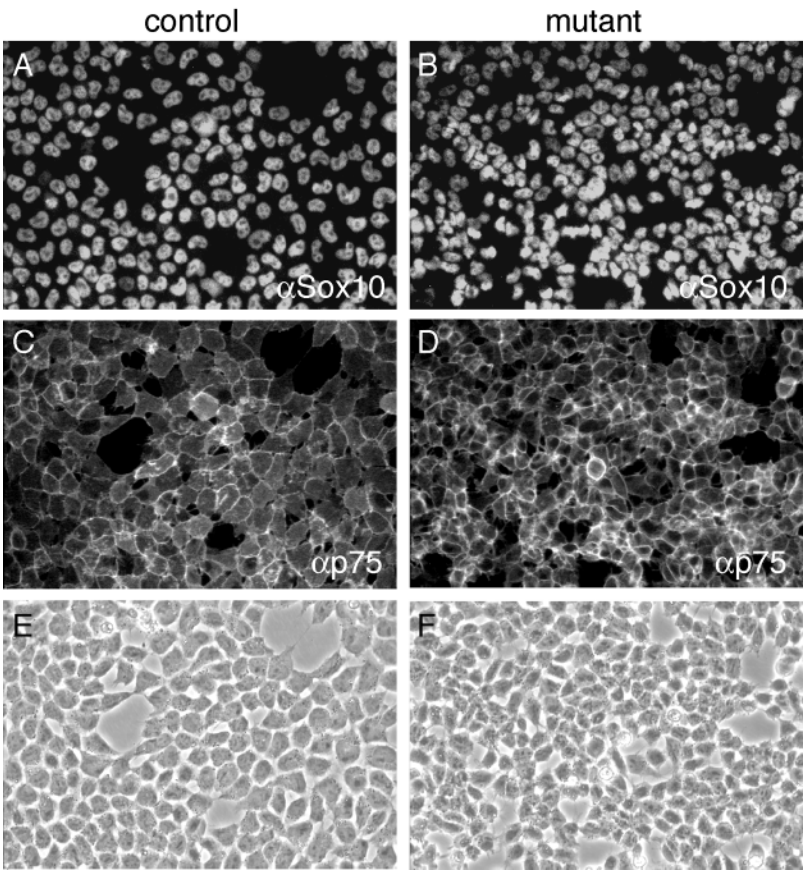


Figure 3. **Absence of the ngn2-expressing sensory sublineage in the emigrating crest of mutant embryos.** Whole mount in situ hybridization experiments showed ngn2 expression in the neural tube and in placodes (arrowheads) of control and mutant embryos at E9.5 (A–D). In migratory crest, ngn2 mRNA was only detectable in control (A and B, arrow) but not in mutant embryos (C and D, open arrow). The boxes in A and C indicate the areas enlarged in B and D, respectively. On transverse sections at E9.0, ngn2-positive neural crest cells were found in control (E, arrow) but not in mutant (F, open arrow) embryos. In contrast, on adjacent sections, neural crest emigrating from the dorsal neural tube was marked by sox10 mRNA both in control and mutant embryos (G and H, arrows). As at earlier stages, ngn2 mRNA was present in the DRG anlage of control (K, arrow) but not of mutant embryos (L, open arrow) at E10.5. Hybridization with a wnt1 riboprobe showed maintained wnt1 expression in the emerging crest of mutant embryos (J, arrow), whereas it was down-regulated in neural crest of control embryos (I, open arrow) at E9.0. Bars: (E–J) 10 μm; (K and L) 20 μm.

ment cell–specific genes such as *trp2* and is required for promoting melanoblast formation from precursor cells (Opdecamp et al., 1997; Yasumoto et al., 1997). In contrast to control embryos (Fig. 1, G and H), mitf-positive melanoblasts were not detectable in *β-catenin* mutant embryos (Fig. 1, I and J), indicating that melanoblasts never formed in the mutant.

Analysis of peripheral neural structures

Our initial analysis of embryos in which *β-catenin* had been eliminated by *wnt1-Cre*–mediated gene ablation previously revealed a reduction of neuronal cells in cranial ganglia and DRG at E10.5 (Brault et al., 2001), suggesting a role for *β-catenin* in the formation of peripheral neurons. To elucidate this further, we first analyzed the PNS of mutant ani-



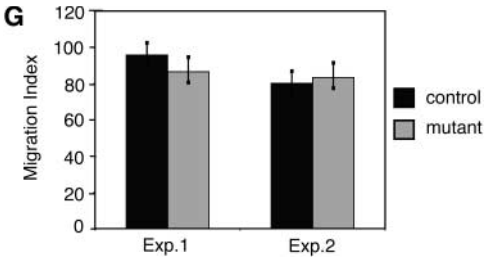


Figure 4. **Normal emigration of mutant neural crest cells.** Neural crest explants were obtained from neural tubes that had been isolated from control and *β-catenin* mutant mice at E9 and cultured for 20 h to allow emigration of neural crest cells. After emigration, neural crest cells were fixed and immunolabeled with anti-sox10 antibody (visualized by Cy3 fluorescence) (A and B) and anti-p75 antibody (visualized by FITC fluorescence) (C and D). Note that virtually all neural crest cells were double positive for the neural crest stem cell markers p75 and sox10. (E and F) Corresponding phase contrast pictures. (G) To compare and quantify the extent of control and mutant neural crest outgrowth after 20 h, the migration index was calculated using the NIH image 1.62 software (Materials and methods). Two independent experiments using nonsibling embryos were performed, scoring three explants of control and mutant embryos per experiment. Each bar represents the migration index (mean ± SD) of three different explants. Note that mutant explants were not significantly reduced in size and density.

mals at a late embryonic stage. Only a few occasional neurons marked by neurofilament (nf) 160 were detectable in the mutant as compared with the control in DRG of E16.5 embryos (Fig. 2, A and B). In contrast, sympathetic ganglia and the enteric nervous system were not affected (Fig. 2, E–H). To address whether the phenotype in mutant DRG was due to a specific requirement for *β-catenin* in neuronal differentiation, we analyzed the expression of the transcription factor sox10. In the PNS, sox10 is a marker for multipotent progenitors and glial cells whose expression is down-regulated as progenitors adopt a neuronal or nonneural fate (Paratore et al., 2001). Although control DRG and peripheral nerves were composed of many sox10-positive cells, we were unable to detect any progenitor cells or presumptive satellite glia lateral to the neural tube where DRG normally form (Fig. 2, C and D). Thus, only residual DRG were present in E16.5 mutant embryos, consisting of a few neuronal cells without associated progenitor cells or glia. As expected, given the near absence of sensory axons, mutant peripheral nerves were marked by sox10 but were reduced in size (Fig. 2, C and D). The absence of *β-catenin* apparently did not impair early Schwann cell differentiation, as Schwann cells in the mutant expressed P0 and MBP with a temporal expression profile comparable to the control (unpublished data).

Specification of neurogenin (ngn) 2–dependent sensory progenitor cells requires *β-catenin*

The neuronal subtypes in DRG are generated in two waves (Anderson, 1999). An early wave is dependent on the basic helix-loop-helix (bHLH) transcription factor neurogenin (ngn) 2 and gives rise to tyrosine receptor kinase (trk) B– and trkC-positive neurons (Ma et al., 1999). In contrast, a somewhat later wave of neurogenesis produces mostly trkA-positive neurons and is dependent on the bHLH factor ngn1. Ngn2 is expressed already in migratory crest cells, whereas ngn1 expression is induced only upon cellular association in the forming DRG (Gradwohl et al., 1996; Sommer et al., 1996). To analyze whether the residual neurons found in the mutant at later embryonic stages (Fig. 2) belonged to a particular sensory subtype, we performed in situ hybridization experiments with trkA, trkB, and trkC riboprobes. Although these markers were readily detectable in control DRG at E16.5, none of these neurotrophin receptors appeared to be expressed by the few nf-positive cells present in the mutant (unpublished data). Thus, these data left open whether the early, ngn2-dependent or the later, ngn1-dependent wave of neurogenesis, or both of these, was affected by *wnt1-Cre*–mediated *β-catenin* ablation. To address this issue, we examined the generation of ngn2-expressing sensory progenitor cells in *β-catenin* mutants. Whole mount in situ hybridization analysis performed at E9.5 revealed ngn2 expression in the neural tube, placodes, and neural crest of control embryos (Fig. 3, A and B). In contrast, ngn2-positive cells were virtually absent in the neural crest along the rostro-caudal axis of mutant embryos, whereas neural tube and placodal ngn2 expression was readily detectable (Fig. 3, C and D). Likewise, on transverse sections at E9.0, ngn2-expressing neural crest cells were

found in control, but not mutant, embryos (Fig. 3, E and F). On adjacent sections, sox10-positive neural crest cells were detected emigrating from the neural tube of both control and *β-catenin* mutant embryos, suggesting that neural crest emigration was not generally affected (Fig. 3, G and H). To monitor whether this phenotype reflected a delay in the generation of ngn2-positive cells, the expression of ngn2 was assayed at later developmental stages up to E12. Ngn2 expression in the PNS was also abolished in the mutant at these later stages (Fig. 3, K and L; unpublished data), indicating that the ngn2-dependent sensory sublineage was never generated in the absence of β-catenin activity.

The loss of ngn2-dependent sensory progenitors might be explained by a lineage-specific requirement of *β-catenin* in survival, proliferation, or fate specification of precursor cells. Both in mutant and control embryos at E8.5 and E9, TUNEL labeling, performed to assess cell death, revealed no or only minimal apoptosis in the dorsal trunk neural tube or the early migratory trunk neural crest (Brault et al., 2001; unpublished data). These data rule out the possibility that lineage-specific cell death could lead to the elimination of ngn2-expressing cells in the *β-catenin* mutant. Wnt signaling is involved in cell cycle regulation of neural tube cells and premigratory neural crest (Ikeya et al., 1997; Megason and McMahon, 2002). The drastic reduction of ngn2-positive cell numbers might thus be due to decreased proliferation. Given the apparently normal emigration of sox10-positive cells in mutant embryos (Fig. 3 H), the *β-catenin* mutation would have to specifically affect cell cycle progression of the ngn2-expressing neural crest lineage. A putative lineage-specific change in cell cycle progression might be difficult to detect in vivo. Immunostaining of mutant embryos with anti–phospho-histone H3 as well as BrdU labeling at E9.5 did not provide evidence for reduced proliferation in the dorsal neural tube or premigratory neural crest in the mutant (unpublished data). To further address this issue, we performed cell culture experiments in which the extent of neural crest outgrowth can be assessed. Neural tubes of control and mutant embryos were isolated at E9 and neural crest cells were allowed to emigrate in defined culture conditions permissive for the generation of ngn2-dependent sensory neurons from sox10-positive progenitor cells (Greenwood et al., 1999). In such a system, a reduction of ngn2-positive cell numbers by death or decreased proliferation rates would result in reduced outgrowth of neural crest cells with sensory neuronal potential. 20 h after plating the neural tubes, control and mutant explants formed by emigrating cells were highly similar and mainly composed of cells coexpressing the low-affinity neurotrophin receptor p75 and sox10 (Fig. 4, A–F), which are markers for neural crest stem cells (Stemple and Anderson, 1992; Paratore et al., 2001). Importantly, quantification of the outgrowth area (Huang et al., 1998) did not reveal any significant difference between control and *β-catenin* mutant neural crest explants (Fig. 4 G), demonstrating that emigration of mutant neural crest cells was normal, and the size of mutant and control explants was similar. Taken together, our data suggest that the early loss of ngn2-expressing cells was not due to a lineage-specific elimination of premigratory or migratory neural crest cells by increased cell death or decreased proliferation.

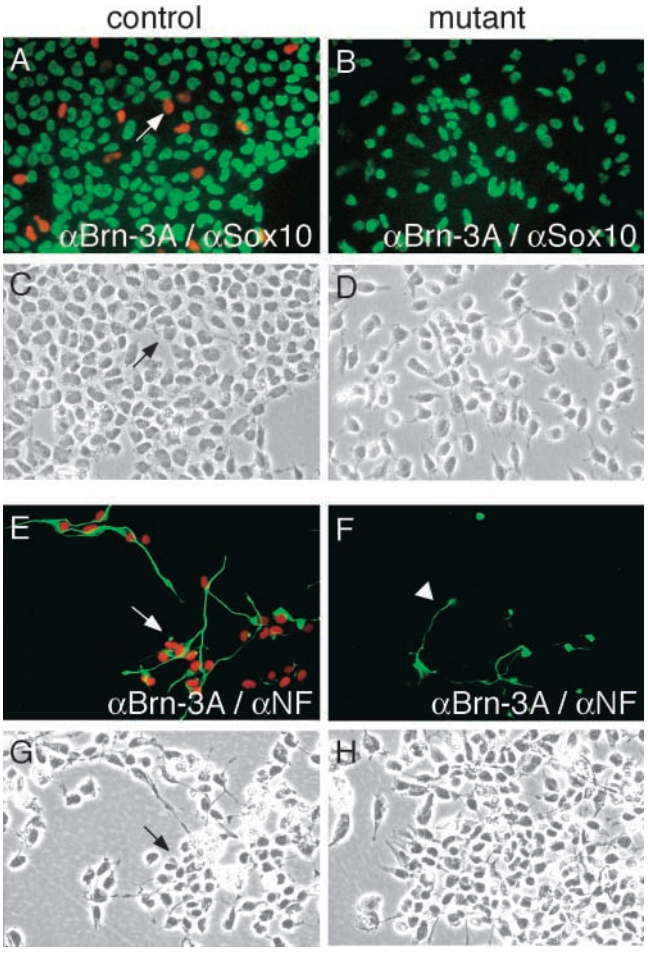


Figure 5. **Mutant neural crest cells are unable to generate sensory neurons.** Neural crest explants from control and mutant mice were prepared as described in the legend to Fig. 4. The cells were allowed to differentiate and were fixed after 36 h (A–D) or 48 h (E–H) in culture. The cultures were immunolabeled using anti–Brn-3A antibody (visualized by Cy3 fluorescence) (A, B, E, and F) and double stained either with anti-sox10 antibody (visualized by FITC fluorescence) (A and B) or anti–nf 160 (NF) (visualized by FITC fluorescence) (E and F). Sensory neuron precursors, defined by the expression of Brn-3A (A, arrow), and sensory neurons, defined by coexpression of Brn-3A and NF (E, arrow), were completely absent in mutant explants (B and F). Note that a few Brn-3A–negative nonsensory neurons were found in mutant explants (F, arrowhead). (C, D, G, and H) Corresponding phase contrast pictures.

A potential role of *β-catenin* in lineage specification might be reflected by altered gene expression in mutant cells. Among various markers tested, we found expression of wnt1 to be transiently maintained in emigrating neural crest cells, whereas usually wnt1 is rapidly down-regulated in wild-type neural crest cells as they emerge from the neural tube (Fig. 3, I and J). At later stages, this ectopic wnt1 expression was abolished (unpublished data). These data might be interpreted to mean that neural crest cells that would normally express ngn2 are unable to do so in *β-catenin* mutants and aberrantly express wnt1. To further support the hypothesis that *β-catenin* is involved in the specification of the early sensory neuronal lineage, a cell culture system was applied that allows monitoring of neuronal fate acquisition by neural crest cells on the cellular level. Neural crest stem cells

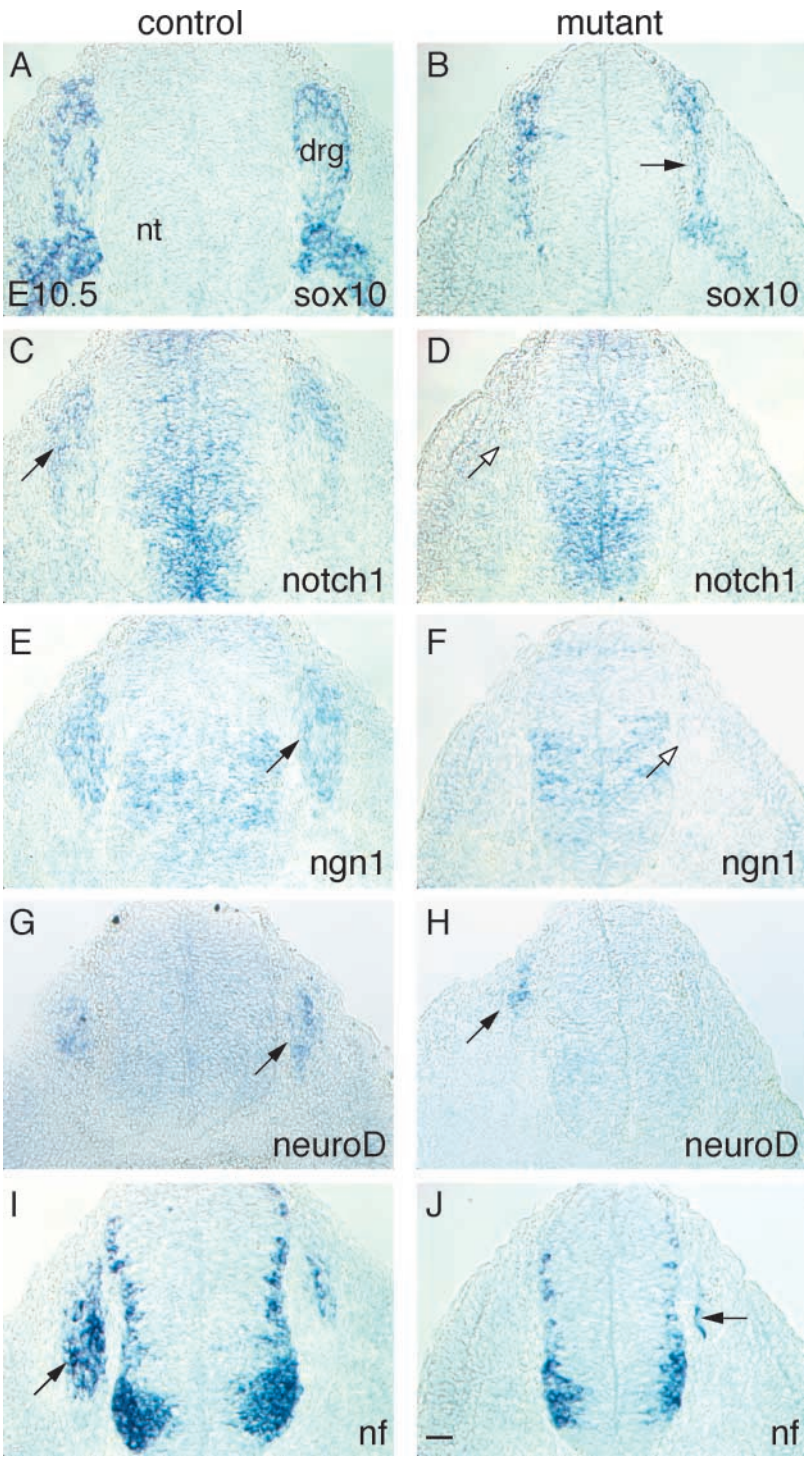


Figure 6. **Failure of DRG formation from sox10-expressing neural crest–derived progenitor cells.** Sox10-expressing cells were present in condensing DRG of control embryos (A) and lateral to the neural tube (nt) of mutant embryos (B, arrow) at E10.5. Near-adjacent sections hybridized with Notch1 riboprobes demonstrate lack of notch1 expression in sox10-positive progenitors of mutant embryos (D, open arrow), whereas Notch1 is expressed in progenitors of control embryos (C, arrow). Furthermore, ngn1-expressing cells were virtually absent lateral to the neural tube of mutant embryos (F, open arrow), whereas extensive ngn1 expression was found in the forming DRG of control embryos (E, arrow). In contrast to control embryos (G and I, arrows), only a few neuroD- and nf-expressing cells were present in mutant embryos (H and J, arrows). Bar, 20 μm.

were cultured in conditions that promote the generation of ngn2-dependent (but not ngn1-dependent) sensory neurons, as described before (Greenwood et al., 1999). As mentioned above, mutant cells emigrated normally from the neural tube in these conditions (Fig. 4). Upon continued culturing of such neural crest explants, many cells in control cultures adopted a sensory neuronal fate characterized by down-regulation of sox10 expression and induced expression of the POU domain transcription factor Brn-3A (Gerrero et al., 1993; Greenwood et al., 1999) (Fig. 5 A). In contrast, mutant neural crest cells maintained their sox10 expression and were unable to generate any Brn-3A–positive sensory neuronal precursors (Fig. 5 B). At a later time point, control explants had generated multiple sensory neurons, identified by coexpression of Brn-3A and NF, whereas in mutant cultures, we never observed sensory neuron formation (Fig. 5, E–H). Occasionally, NF-positive cells were found in the mutant cultures (Fig. 5 F, arrowhead). These were, however, Brn-3A negative, suggesting that some nonsensory neurons formed from mutant neural crest. Thus, signaling mediated by β-catenin is required for the specification of ngn2-dependent sensory neurons.

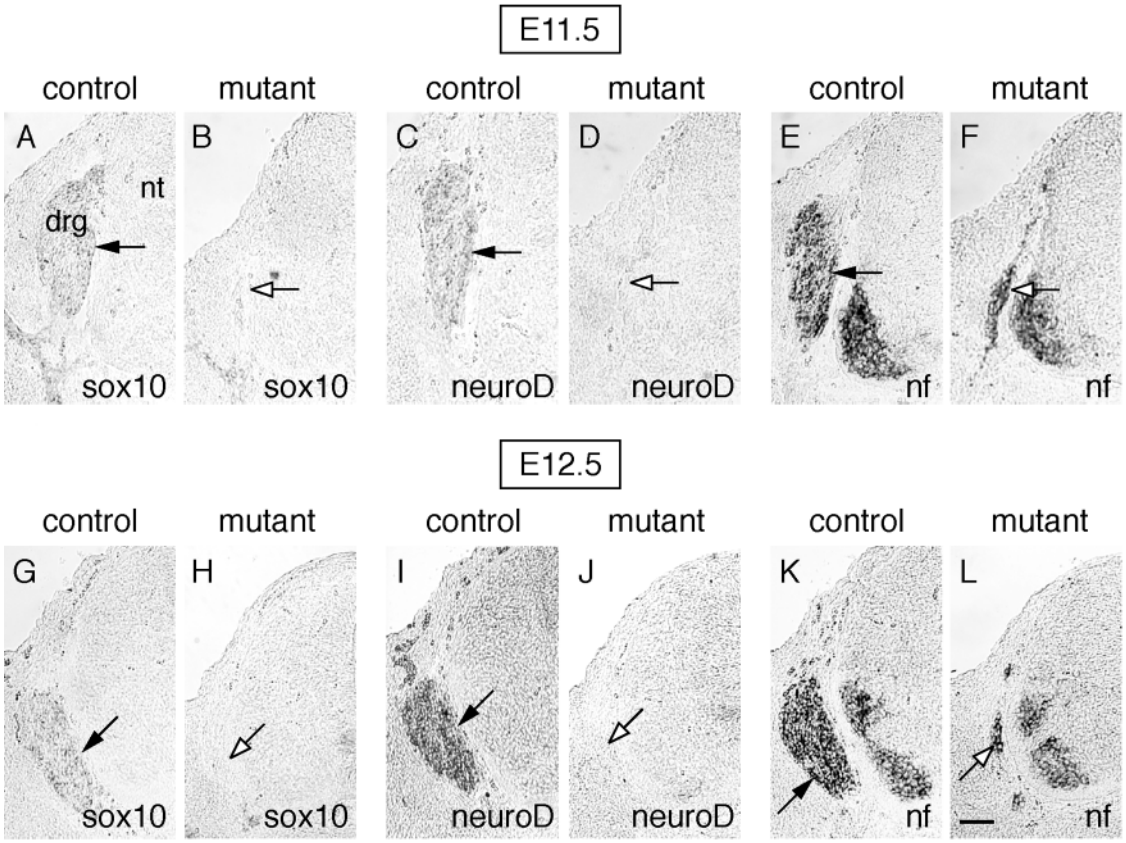


Figure 7. **Absence of de novo neurogenesis in the DRG anlage of mutant embryos at later stages.** At E11.5 (A–F) and E12.5 (G-L), progenitors and glial cells detected by hybridization with a sox10 riboprobe (A and G, arrows), as well as neurons and their precursors detected by expression of nf (E and K, arrows) and neuroD (C and I, arrows) mRNA, respectively, constituted the DRG in control embryos. In contrast, sox10- (B and H, open arrows) and neuroD- (D and J, open arrows) positive cells were completely missing in the mutant. Moreover, only a few nf-expressing neurons were present in mutant embryos (F and L, open arrows). Bar, 50 μm.

Impaired de novo neurogenesis in the DRG anlage

In the next set of experiments, we investigated whether *β-catenin* might play a role in the generation of the second, *ngn1*-dependent wave of sensory neurons. To this end, in situ hybridization experiments were performed at stages when neural crest–derived cells in aggregating DRG up-regulate ngn1 expression and contribute to extensive de novo neurogenesis. Sox10 and Notch1 were used as markers for undifferentiated progenitor cells, whereas ngn1, neuroD, and nf were used to mark the neuronal lineage. At E10.5, sox10-positive neural crest cells were found concomitantly with many ngn1-expressing progenitors in control DRG (Fig. 6, A and E). In control animals, neurogenesis was apparent by the expression of neuroD, a bHLH factor acting downstream of ngns (Ma et al., 1996), and by the presence of differentiated nf-positive neurons (Fig. 6, G and I). In the mutant, however, sox10-expressing cells were present in the area in which ganglia formation occurs in the wild type, but these cells did not appear to form proper ganglia (Fig. 6 B). Moreover, mutant neural crest cells did not express Notch1, unlike their control counterparts (Fig. 6, C and D). Neurogenesis was drastically reduced in the mutant, with virtually no detectable ngn1-expressing cells and little neuroD expression (Fig. 6, F and H). Consequently, few differentiated neurons marked by nf were detectable in the mutant in the region of normal DRG formation (Fig. 6 J).

Sensory neurogenesis was even more affected at E11.5 and E12.5. In control animals at these stages, sox10 expression in progenitors and presumptive satellite glia outlined the DRG, in which multiple neuroD-positive neuronal precursors coexisted with differentiated neurons (Fig. 7). In contrast, neuroD-expressing cells were missing in the mutant (Fig. 7, D and J), indicating that no new neurons were added to the few nf-positive cells that had been born at earlier stages (Fig. 7, F and L). Intriguingly, sox10 expression was also absent lateral to the neural tube and around the few sensory neurons of *β-catenin* mutant embryos (Fig. 7, B and H). Thus, progenitor cells that had been manifest at earlier stages in the area of normal DRG formation (Fig. 6) had disappeared in the mutant by E11.5.

In vivo fate mapping of neural crest cells in control and mutant embryos

Cell death might be an explanation for the loss of neural crest cells that fail to form DRG in mutant embryos. To address this, staining for activated caspase3 or TUNEL assays were performed at E10.5, E11.5, and E12.5 (Fig. 8 C; unpublished data). However, we were unable to detect increased cell death in the mutant embryos in locations where DRG cells normally aggregate in the wild type. Apart from cell death, there are alternative explanations for the disap-

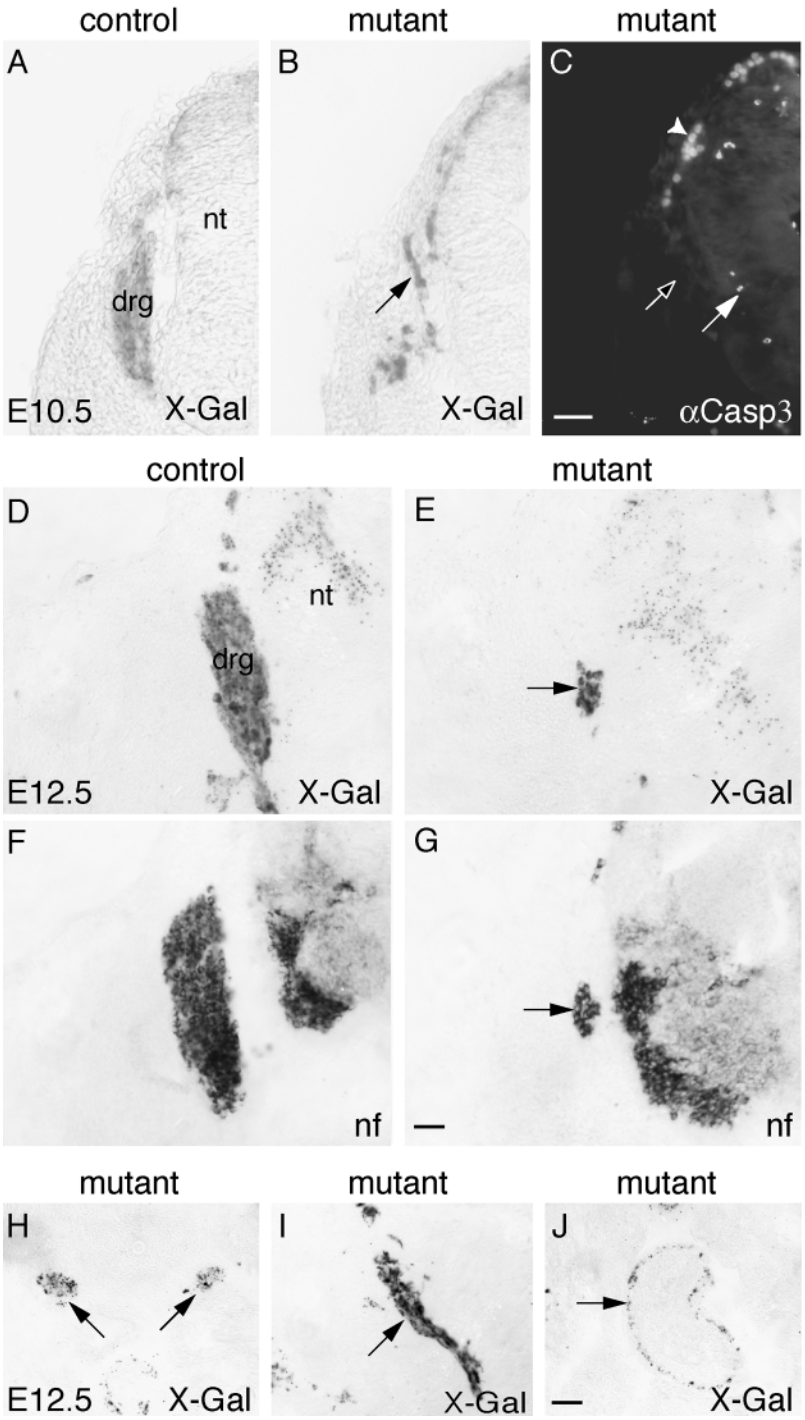


Figure 8. **Survival and in vivo fate mapping of mutant cells.** Control embryos carrying *wnt1-Cre* and the *R26R* allele displayed β-galactosidase activity in the developing DRG at E10.5 (A) and E12.5 (D). At E12.5, the control DRG was outlined by nf expression (F). Cre-expressing neural crest cells and their derivatives were also detectable by β-galactosidase expression in mutant embryos carrying *wnt1-Cre* and the *R26R* allele. At E10.5, mutant cells were localized lateral to the neural tube without forming proper DRG (B). No increased cell death was found in this area, as shown by immunostaining for the activated form of caspase3 (Casp3) (C, open arrow). B and C are adjacent sections. Note the Casp3-positive cells within the neural tube (arrow) and autofluorescent blood cells present at the dorsal margins of the embryo (arrowhead). At E12.5, neural crest–derived mutant cells expressing β-galactosidase (E, arrow) were confined to the domain of a few nf-positive cells present in the mutant (G, arrow). E and G represent adjacent sections. Mutant cells were able to normally colonize other neural crest–derived structures, such as sympathetic ganglia (H, arrows), peripheral nerves (I, arrow), and the enteric nervous system (J, arrow). Bars: (A–C) 50 μm; (D–J) 100 μm.

pearance of progenitor cells in the area of normal DRG formation. Mutant neural crest–derived cells might be able to localize to the site of normal DRG formation but might adopt an aberrant fate not detected by the neural markers tested so far. To investigate this issue, we performed in vivo fate mapping experiments using the ROSA26 Cre reporter allele *(R26R)* (Soriano, 1999). Upon Cre-mediated recombination, β-galactosidase is expressed from this allele in Cre-expressing cells and their progeny. Thus, the fate of control and *β-catenin* mutant neural crest cells can be followed in compound transgenic animals expressing *Cre* from the *wnt1* promoter. In animals wild type for *β-catenin*, β-galactosidase expression delineated all neural crest–derived structures at E10.5 and E12.5 (Fig. 8, A and D; Jiang et al., 2000). In *β-catenin* mutant embryos at E10.5, mutant cells expressing β-galactosidase were localized in streams lateral to the neural tube without forming proper DRG (Fig. 8 B). This expression pattern correlated with domains of sox10 expression on adjacent sections (unpublished data; Fig. 6). Most importantly, at E12.5, comparison of β-galactosidase and nf expression revealed that the only neural crest–derived cells that localized lateral to the neural tube were the few sensory neurons present in *β-catenin* mutants (Fig. 8, E and G). In contrast, similar to control cells, mutant β-galactosidase–

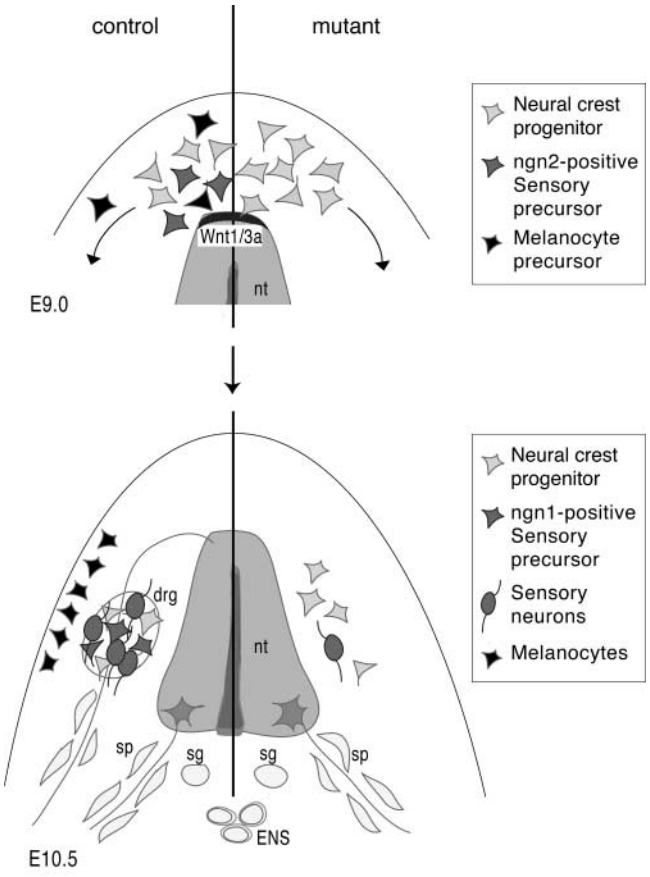


Figure 9. **Lineage-specific requirement of *β-catenin* in neural crest development.** (E9.0, control) Emigrating neural crest appears to be heterogeneous, consisting of multipotent neural crest progenitor cells, early sensory precursors (marked by ngn2 expression), and possibly melanoblasts. (E9.0, *β-catenin* mutant) Neural crest fails to generate sensory precursors and melanoblasts. The specification of sensory and melanocytic fates in premigratory or early migratory neural crest might depend on signaling by wnt1/wnt3a, which are expressed in the dorsal neural tube (nt) at the stage of crest emigration. (E10.5, control) Neural crest–derived progenitor cells aggregate in DRG and produce ngn1-dependent sensory precursors. (E10.5, mutant) Progenitors lateral to the neural tube fail to aggregate and to form proper DRG; virtually no ngn1-expressing sensory precursors and only very few differentiated sensory neurons are detectable. This might point to a role of β-catenin in mediating cell–cell interactions possibly involved in sensory neurogenesis, although other β-catenin functions cannot be excluded. Other crest derivatives such as sympathetic ganglia (sg), the enteric nervous system (ENS), and Schwann cell precursors along peripheral nerves (sp) form independently of β-catenin activity.

positive cells localized to sympathetic ganglia (Fig. 8 H), nerves (Fig. 8 I), and the enteric nervous system (Fig. 8 J). Hence, in vivo fate mapping did not reveal aberrant generation of nonneural cells from mutant progenitors in the area of normal DRG formation. Rather, mutant cells gave rise to multiple structures of the PNS while they failed to form proper DRG.

Discussion

In this study, we identify β-catenin as a crucial signal in neural crest development. Conditional *β-catenin* gene ablation in the dorsal neural tube and neural crest stem cells prevents the generation of melanoblasts and ngn2-dependent sensory neurons, presumably reflecting a role of *β-catenin* in premigratory crest or at early stages of emigration (Fig. 9). Moreover, *β-catenin* mutant neural crest cells fail to aggregate in DRG and to produce ngn1-dependent sensory neurons, whereas other neural crest derivatives, such as the enteric nervous system and sympathetic ganglia, form normally (Fig. 9). Analysis of mutant and control embryos, together with in vivo fate mapping and cell culture experiments, indicates a lineage-specific requirement of *β-catenin* for the specification of both melanocytes and sensory neuronal lineages from neural crest cells.

Role of β-catenin in melanocyte formation

Apart from its role in neural crest induction and expansion at early developmental stages (Ikeya et al., 1997; Saint-Jeannet et al., 1997; LaBonne and Bronner-Fraser, 1998), Wnt signaling has also been directly associated with the formation of the melanocyte lineage from neural crest. Both in avian cell cultures and in zebrafish in vivo, activation of the Wnt signaling pathway in neural crest cells promoted the formation of pigment cells (Dorsky et al., 1998; Jin et al., 2001). In agreement with a role of Wnt signaling in melanocyte formation, ablation of *β-catenin* in mouse neural crest cells not only leads to the loss of the melanocyte differentiation marker trp2 but also of mitf, a bHLH zipper transcription factor regulating melanoblast specification (Opdecamp et al., 1997; Yasumoto et al., 1997). This finding might reflect a direct relationship between β-catenin–dependent signaling and induction of *Mitf* gene expression, as the *Mitf* promoter harbors a binding site for a β-catenin–containing transcription factor complex and can be activated by Wnt (Dorsky et al., 2000b; Takeda et al., 2000). It has been reported that in compound mutant mice lacking both *wnt1* and *wnt3a*, late-emigrating neural crest lineages are missing because of a general reduction of the neural crest cell population, which would primarily affect later- rather than earlier-forming neural crest derivatives (Ikeya et al., 1997). This is an unlikely explanation for the lack of melanocytes in *β-catenin* mutant embryos because, unlike in chicken, melanocyte-forming neural crest cells in mouse embryos migrate along the dorsolateral pathway throughout the period of migration, including at early stages (Serbedzija et al., 1990). Taken together, the data indicate that β-catenin plays a role in specifying the melanocyte lineage from neural crest cells, most likely due to its function in mediating Wnt signaling (Fig. 9). Specification and early differentiation of melanocytes occurs in the so-called migration staging area, a space rich in extracellular matrix that is localized lateral to the dorsal neural tube (Wehrle-Haller and Weston, 1997; Dorsky et al., 2000a). In some, but not all, mutant embryos analyzed, we observed increased apoptosis in this area at E10.5 (but not at other stages) (unpublished data), consistent with the idea that at least some cells that fail to be specified as melanocytes are subsequently eliminated by cell death. If so, this effect might be lineage specific, because cell death in other neural crest derivatives, such as in the DRG anlage, was apparently not increased in the mutant.

Early specification of ngn2-dependent sensory neuronal cells by β-catenin

Neurogenic and melanogenic lineages segregate during the earliest stages of neural crest development (Henion and Weston, 1997). Furthermore, the promotion of melanocyte formation by Wnt signaling has been reported to occur at the expense of neural lineages (Dorsky et al., 1998; Jin et al., 2001). However, our analysis of neural structures in *β-catenin* mutant embryos showed that, similar to the melanocyte lineage, particular neural structures are reduced in size rather than increased. Thus, our data do not support a model in which β-catenin regulates a fate switch between neurogenic and melanogenic lineages. The discrepancy in results (Dorsky et al., 1998; Jin et al., 2001; this study) might be explained by distinct timing of interference with the Wnt signaling pathway, differential roles of Wnt and β-catenin, and species differences.

Although the reduction of peripheral nerves observed in *β-catenin* mutants is presumably secondary to the absence of sensory neurons, the lack of ngn2-dependent sensory neurons and their progenitors most likely reflects a primary role of β-catenin in the formation of this lineage. Normally, ngn2 expression is detectable in a subset of sox10-positive neural crest cells as they emerge from the neural tube (Sommer et al., 1996; Lo et al., 2002). In *β-catenin* mutants, sox10-positive cells emigrate in vivo but ngn2 expression is absent or restricted to a very few neural crest cells (Fig. 9). Similarly, sox10-positive neural crest cells emigrate in neural tube explants in vitro but these cells are unable to adopt a sensory neuronal fate, even in conditions promoting ngn2-dependent sensory neurogenesis. These data indicate that neural crest cells that would normally express ngn2 emigrate, but fail to acquire a sensory neuronal fate in the absence of *β-catenin*. Alternatively, ngn2-positive cells or their progenitors might be selectively eliminated in the mutant neural tube before or at emigration. However, at E8.5 and E9, we did not observe increased cell death in premigratory and migratory neural crest in *β-catenin* mutant embryos as compared with control embryos (this study; Brault et al., 2001). Moreover, the normal outgrowth of mutant neural crest explants in culture also speaks against a lineage-specific elimination of ngn2-positive cells.

In vivo, the increased expression of wnt1 in neural crest cells lacking *β-catenin* suggests that wnt1 expression is involved in a negative regulatory feedback loop with β-catenin. Moreover, this finding also allows the assumption that β-catenin controls the early specification of ngn2-dependent sensory neurons by mediating Wnt signaling rather than cadherin-dependent cell adhesion. Thus, β-catenin, presumably as a component of the Wnt signal transduction pathway, can be added to the list of signals, such as TGFβ family members, Notch, and NRG, that regulate cell fates in neural crest development (Anderson et al., 1997; Sommer, 2001). It remains to be determined whether this function is exerted by direct regulation of gene expression of the *ngn*s that encode bHLH factors specifying sensory neuron identity (Ma et al., 1999; Perez et al., 1999).

The absence of neural crest cells marked by ngn2 not only points to a role of *β-catenin* at earliest stages of neural crest development but also suggests that the ngn2-positive lineage might segregate from other neural crest lineages already before or shortly after delamination from the neural tube (Fig. 9). In agreement with this, in vivo fate mapping by dye injection revealed that most (but not all) neural crest cells were restricted to generate either sensory or autonomic neurons, but not both neuronal subtypes (Frank and Sanes, 1991; Fraser and Bronner-Fraser, 1991). Similarly, sensory precursors in neural crest explants turned out to be determined, unable to respond to factors inducing autonomic neurogenesis (Greenwood et al., 1999). Moreover, lineage-tracing experiments have recently demonstrated that ngn2-expressing cells preferentially contribute to sensory rather than autonomic ganglia (Zirlinger et al., 2002). Given the early function of β-catenin in sensory neurogenesis and given the expression of Wnts before neural crest emigration, the question arises of how undetermined cells with competence to generate derivatives other than sensory neurons are being maintained in the neural crest. β-Catenin signaling can be modulated by the activity of other signaling molecules, such as components of the Notch pathway and TGFβ family members (Hecht and Kemler, 2000; De Strooper and Annaert, 2001). Thus, the biological response of a neural crest cell to β-catenin might depend on its cellular context, which determines the signal combination and concentration as well as the timing of signaling that the cell is exposed to.

β-Catenin and neurogenesis during peripheral ganglia formation

Although the number of sensory neurons is drastically reduced in the DRG anlagen of *β-catenin* mutant embryos, a few sensory neurons are born at early developmental stages. From E11.5 onwards, however, sensory neurogenesis, as assayed by neuroD expression, was completely abolished in the mutant. The early generation of the residual neurons might suggest that they are derived from the early, ngn2-dependent rather than from the later, ngn1-dependent wave of neurogenesis. This issue was, however, difficult to assess because of the low or absent expression of markers that would distinguish sensory neuronal sublineages, such as ngn1 and ngn2 and trkA, B, and C (Anderson, 1999). The absence of neurotrophin receptor expression in the few neurons present in the mutant suggests that proper differentiation of sensory neurons either requires β-catenin activity in a cell-autonomous manner or depends on cellular interactions with neural cells missing in the mutant. In any case, however, our data clearly demonstrate a requirement of *β-catenin* in the formation of both ngn2- as well as ngn1-dependent sensory neurons (Fig. 9).

It is unclear whether the virtual absence of ngn1 expression in the mutant reflects a role of β-catenin in Wnt signaling or in mediating cadherin-dependent cellular adhesion. Interference with Wnt signaling in the neural crest does not just lead to a phenocopy of *β-catenin* mutants (Ikeya et al., 1997; Brault et al., 2001), suggesting Wnt-independent roles of *β-catenin* in neural crest development. However, the differences in phenotypes could reflect different stages at which the Wnt signaling pathway has been perturbed or could be due to the fact that *β-catenin* gene deletion affects signaling by different Wnt family members. Hence, we can-

not exclude, as proposed above for the ngn2-dependent early wave of sensory neurons, that later-forming sensory lineages would also be specified by Wnt signaling. Alternatively, interference with cadherin-dependent cell adhesion rather than Wnt signaling might lead to the absence of DRG formation in *β-catenin* mutants. In support of this idea, cell–cell adhesion is defective in *β-catenin* mutant embryos (Haegel et al., 1995; Brault et al., 2001). Moreover, there is circumstantial evidence that cellular interactions might be involved in sensory neurogenesis. Normally, ngn1 is induced as neural crest–derived cells aggregate to form DRG (Sommer et al., 1996), but it is not known whether there is a causal relationship between cellular association and ngn1 expression. Short-range cell–cell interactions, termed community effects, influence the response of multipotent progenitor cells to extracellular factors, thereby promoting neurogenesis at the expense of a nonneural fate (Hagedorn et al., 1999, 2000a; Paratore et al., 2001). Furthermore, it has been suggested that early differentiating neurons might interact with DRG progenitor cells and serve as a "scaffold" for later-born sensory neurons (Anderson, 1999). According to this idea, the strong reduction of early-born sensory neurons in *β-catenin* mutant embryos would impede ngn1-dependent sensory neurogenesis. Analysis of sox10 expression reveals that *β-catenin* gene ablation does not only affect the induction of neuronal traits in undifferentiated progenitor cells. Rather, ganglionic structures delineated by sox10-positive cells are not even formed, suggesting that undifferentiated progenitor cells fail to aggregate in DRG at any developmental stage (Fig. 9).

The absence of proper ganglia expressing sox10 is not just due to aberrant down-regulation of this marker, as demonstrated by in vivo fate mapping of mutant cells. *β-catenin* mutant neural crest cells expressing β-galactosidase from the recombined *R26R* allele emigrate normally, are then found for a transient period lateral to the neural tube without forming overt DRG, and are later confined to the few sensory neurons present in the mutant. Thus, mutant cells do not adopt an alternative, nonneural fate in the area of normal DRG formation. Moreover, the phenotype is unlikely to be explained by mutant cells that would aggregate but then die, as we found no evidence for increased cell death in the mutant DRG anlage at any stage analyzed. Hence, the most easily conceivable interpretation of our data is that mutant neural crest cells fail to aggregate in DRG and to generate sensory neurons and satellite glia, and instead populate other neural crest–derived structures. However, we did not observe an increase in nonsensory neural crest derivatives, suggesting that secondary mechanisms might regulate the generation of correct cell numbers in these tissues. Whether our data reflect the existence of migratory progenitors that in the wild type are able to generate both sensory as well as other neural crest–derived cell types remains to be addressed. In any case, however, the results are consistent with the hypothesis that cell–cell interactions are required for the promotion of neural fates in developing DRG. Signaling by Notch1, which is absent in the DRG anlage of *β-catenin* mutants, might be involved in this process. Moreover, such cellular interactions are likely to be mediated by N-cadherin–containing adhesion complexes, as progenitor cells aggregating in DRG express N-cadherin (unpublished data; Pla et al., 2001). Further experiments are required to elucidate the role of cell–cell interactions in promoting sensory neurogenesis.

Materials and methods

Mating scheme and genotyping

Breeding of mice and genotyping were performed as described in Brault et al. (2001). Embryos heterozygous for the *β-catenin* floxed and floxdel alleles and carrying the *wnt1-Cre* transgene were referred to as mutant embryos, whereas littermates that inherited the incomplete combination of the above alleles served as control animals (Brault et al., 2001).

In situ staining procedures

Nonradioactive in situ hybridization with digoxigenin-labeled riboprobes was performed on cryosections as previously described (Paratore et al., 1999). NBT/BCIP (Roche Diagnostics) were used as chromogens to visualize hybridization signals. Antisense riboprobes were labeled with digoxigenin according to the manufacturer's instructions (Roche Diagnostics). TUNEL staining and BrdU assays were performed according to the manufacturer's instructions (Roche Diagnostics). Immunostainings for the active form of caspase3 were performed on cryosections fixed for 15 min in 4% paraformaldehyde in PBS at RT. Sections were treated with blocking buffer (10% goat serum, 0.3% Triton, 0.1% BSA in PBS) for 30–60 min at RT before incubation with polyclonal rabbit anti-caspase3 antibody (1:100 dilution; BD Biosciences) for 2 h at RT. For anti–phospho-histone H3 stainings, the cryosections were fixed for 10 min in acetone at −20°C and incubated with a polyclonal rabbit antibody (diluted 1:500; Upstate Biotechnology) overnight at 4°C. Stainings were visualized by incubation for 1 h at RT using a Cy3-conjugated goat anti–rabbit IgG antibody (1:200 dilution; Jackson ImmunoResearch Laboratories). X-Gal staining on cryosections was done as previously described (Lickert et al., 2002).

Cell culture

Neural crest cultures in conditions permissive for sensory neurogenesis were essentially prepared as previously reported (Greenwood et al., 1999). After 20 h of neural crest emigration, the neural tubes were removed from the cultures and used for genotyping by PCR (Sommer et al., 1995). To quantify the extent of the neural crest outgrowth, digital images of the explant cultures at 20 h were acquired by video microscopy using 5× magnification. NIH image 1.62 software was used to measure the size of the explants. To this end, the outgrowth area (mm^2) was divided by the perimeter (mm) of the explant. This normalized number (in mm) is referred to as the migration index and provides an estimate of the total outgrowth of a neural crest explant (Huang et al., 1998).

Immunocytochemistry

After fixing the cells with 3.7% formaldehyde in PBS for 10 min, the cells were treated for 10 min at RT with blocking buffer (10% goat serum, 0.3% Triton X-100, 0.1% BSA in PBS). Neural crest cells were labeled with rabbit anti–mouse p75 (1:300 dilution; Chemicon International) for 1 h at RT and with monoclonal anti-sox10 antibody (1:10 dilution; Paratore et al., 2001) for 2 h at RT. Polyclonal rabbit anti–Brn-3A antibody (Fedtsova and Turner, 1995) and monoclonal anti-NF160 antibody NN18 (IgG) (Sigma-Aldrich) were diluted 1:300 in blocking buffer and stainings were performed at RT for 1 h. Immunostaining was visualized by incubation for 1 h at RT using the following secondary antibodies at 1:200 dilution: Cy3-conjugated goat anti–mouse IgG; Cy3-conjugated goat anti–rabbit IgG; FITC-coupled donkey anti–rabbit IgG (all from Jackson ImmunoResearch Laboratories); and FITC-coupled horse anti–mouse IgG (Vector Laboratories).

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References

Anderson, D.J. 1999. Lineages and transcription factors in the specification of vertebrate primary sensory neurons. *Curr. Opin. Neurobiol.* 9:517–524.

Anderson, D.J., A. Groves, L. Lo, Q. Ma, M. Rao, N.M. Shah, and L. Sommer. 1997. Cell lineage determination and the control of neuronal identity in the neural crest. *Cold Spring Harb. Symp. Quant. Biol.* 62:493–504.

Borchers, A., R. David, and D. Wedlich. 2001. *Xenopus* cadherin-11 restrains cranial neural crest migration and influences neural crest specification. *Development.* 128:3049–3060.

Brault, V., R. Moore, S. Kutsch, M. Ishibashi, D.H. Rowitch, A.P. McMahon, L. Sommer, O. Boussida, and R. Kemler. 2001. Inactivation of the *β-catenin* gene by *Wnt1-Cre*-mediated deletion results in dramatic brain malformation and failure of craniofacial development. *Development.* 128:1253–1264.

Cadigan, K.M., and R. Nusse. 1997. Wnt signaling: a common theme in animal development. *Genes Dev.* 11:3286–3305.

Danielian, P.S., D. Muccino, D.H. Rowitch, S.K. Michael, and A.P. McMahon. 1998. Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr. Biol.* 8:1323–1326.

De Strooper, B., and W. Annaert. 2001. Where Notch and Wnt signaling meet. The presenilin hub. *J. Cell Biol.* 152:F17–F20.

Dorsky, R.I., R.T. Moon, and D.W. Raible. 1998. Control of neural crest cell fate by the Wnt signalling pathway. *Nature.* 396:370–373.

Dorsky, R.I., R.T. Moon, and D.W. Raible. 2000a. Environmental signals and cell fate specification in premigratory neural crest. *Bioessays.* 22:708–716.

Dorsky, R.I., D.W. Raible, and R.T. Moon. 2000b. Direct regulation of nacre, a zebrafish MITF homolog required for pigment cell formation, by the Wnt pathway. *Genes Dev.* 14:158–162.

Dunn, K.J., B.O. Williams, Y. Li, and W.J. Pavan. 2000. Neural crest-directed gene transfer demonstrates Wnt1 role in melanocyte expansion and differentiation during mouse development. *Proc. Natl. Acad. Sci. USA.* 97:10050–10055.

Fedtsova, N.G., and E.E. Turner. 1995. Brn-3.0 expression identifies early postmitotic CNS neurons and sensory neural precursors. *Mech. Dev.* 53:291–304.

Frank, E., and J.R. Sanes. 1991. Lineage of neurons and glia in chick dorsal root ganglia: analysis in vivo with a recombinant retrovirus. *Development.* 111: 895–908.

Fraser, S.E., and M.E. Bronner-Fraser. 1991. Migrating neural crest cells in the trunk of the avian embryo are multipotent. *Development.* 112:913–920.

Gerrero, M.R., R.J. McEvilly, E. Turner, C.R. Lin, S. O'Connell, K.J. Jenne, M.V. Hobbs, and M.G. Rosenfeld. 1993. Brn-3.0: a POU-domain protein expressed in the sensory, immune, and endocrine systems that functions on elements distinct from known octamer motifs. *Proc. Natl. Acad. Sci. USA.* 90: 10841–10845.

Gradwohl, G., C. Fode, and F. Guillemot. 1996. Restricted expression of a novel murine *atonal*-related bHLH protein in undifferentiated neural precursors. *Dev. Biol.* 180:227–241.

Greenwood, A.L., E.E. Turner, and D.J. Anderson. 1999. Identification of dividing, determined sensory neuron precursors in the mammalian neural crest. *Development.* 126:3545–3559.

Gu, H., J.D. Marth, P.C. Orban, H. Mossmann, and K. Rajewsky. 1994. Deletion of a DNA polymerase β gene segment in T cells using cell type-specific gene targeting. *Science.* 265:103–106.

Gumbiner, B.M. 2000. Regulation of cadherin adhesive activity. *J. Cell Biol.* 148: 399–404.

Haegel, H., L. Larue, M. Ohsugi, L. Fedorov, K. Herrenknecht, and R. Kemler. 1995. Lack of β-catenin affects mouse development at gastrulation. *Development.* 121:3529–3537.

Hagedorn, L., U. Suter, and L. Sommer. 1999. P0 and PMP22 mark a multipotent neural crest-derived cell type that displays community effects in response to TGF-β family factors. *Development.* 126:3781–3794.

Hagedorn, L., J. Floris, U. Suter, and L. Sommer. 2000a. Autonomic neurogenesis and apoptosis are alternative fates of progenitor cell communities induced by TGFβ. *Dev. Biol.* 228:57–72.

Hagedorn, L., C. Paratore, G. Brugnoli, J.L. Baert, N. Mercader, U. Suter, and L. Sommer. 2000b. The Ets domain transcription factor Erm distinguishes rat satellite glia from Schwann cells and is regulated in satellite cells by neuregulin signaling. *Dev. Biol.* 219:44–58.

Hecht, A., and R. Kemler. 2000. Curbing the nuclear activities of β-catenin. Control over Wnt target gene expression. *EMBO Rep.* 1:24–28.

Henion, P.D., and J.A. Weston. 1997. Timing and pattern of cell fate restrictions in the neural crest lineage. *Development.* 124:4351–4359.

Huang, G.Y., E.S. Cooper, K. Waldo, M.L. Kirby, N.B. Gilula, and C.W. Lo. 1998. Gap junction–mediated cell–cell communication modulates mouse neural crest migration. *J. Cell Biol.* 143:1725–1734.

Huelsken, J., R. Vogel, V. Brinkmann, B. Erdmann, C. Birchmeier, and W. Birchmeier. 2000. Requirement for β-catenin in anterior–posterior axis formation in mice. *J. Cell Biol.* 148:567–578.

Huelsken, J., R. Vogel, B. Erdmann, G. Cotsarelis, and W. Birchmeier. 2001. β-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. *Cell.* 105:533–545.

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

Jiang, X., D.H. Rowitch, P. Soriano, A.P. McMahon, and H.M. Sucov. 2000. Fate of the mammalian cardiac neural crest. *Development.* 127:1607–1616.

Jin, E.J., C.A. Erickson, S. Takada, and L.W. Burrus. 2001. Wnt and BMP signaling govern lineage segregation of melanocytes in the avian embryo. *Dev. Biol.* 233:22–37.

Kubu, C.J., K. Orimoto, S.J. Morrison, G. Weinmaster, D.J. Anderson, and J.M. Verdi. 2002. Developmental changes in Notch1 and numb expression mediated by local cell-cell interactions underlie progressively increasing delta sensitivity in neural crest stem cells. *Dev. Biol.* 244:199–214.

LaBonne, C., and M. Bronner-Fraser. 1998. Neural crest induction in *Xenopus*: evidence for a two-signal model. *Development.* 125:2403–2414.

Leimeroth, R., C. Lobsiger, A. Lussi, V. Taylor, U. Suter, and L. Sommer. 2002. Membrane-bound neuregulin1 type III actively promotes Schwann cell differentiation of multipotent progenitor cells. *Dev. Biol.* 246:245–258.

Lickert, H., S. Kutsch, B. Kanzler, Y. Tamai, M.M. Taketo, and R. Kemler. 2002. Formation of multiple hearts in mice following deletion of *β-catenin* in the embryonic endoderm. *Dev. Cell.* 3:171–181.

Lo, L., E. Dormand, A. Greenwood, and D.J. Anderson. 2002. Comparison of the generic neuronal differentiation and neuron subtype specification functions of mammalian achaete-scute and atonal homologs in cultured neural progenitor cells. *Development.* 129:1553–1567.

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

Ma, Q., C. Fode, F. Guillemot, and D.J. Anderson. 1999. Neurogenin1 and neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. *Genes Dev.* 13:1717–1728.

Megason, S.G., and A.P. McMahon. 2002. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development.* 129:2087–2098.

Morrison, S.J., S.E. Perez, Z. Qiao, J.M. Verdi, C. Hicks, G. Weinmaster, and D.J. Anderson. 2000. Transient Notch activation initiates an irreversible switch from neurogenesis to gliogenesis by neural crest stem cells. *Cell.* 101:499–510.

Nakagawa, S., and M. Takeichi. 1998. Neural crest emigration from the neural tube depends on regulated cadherin expression. *Development.* 125:2963–2971.

Opdecamp, K., A. Nakayama, M.T. Nguyen, C.A. Hodgkinson, W.J. Pavan, and H. Arnheiter. 1997. Melanocyte development in vivo and in neural crest cell cultures: crucial dependence on the Mitf basic-helix-loop-helix-zipper transcription factor. *Development.* 124:2377–2386.

Paratore, C., U. Suter, and L. Sommer. 1999. Embryonic gene expression resolved at the cellular level by fluorescence in situ hybridization. *Histochem. Cell Biol.* 111:435–443.

Paratore, C., D.E. Goerich, U. Suter, M. Wegner, and L. Sommer. 2001. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. *Development.* 128:3949–3961.

Paratore, C., L. Hagedorn, J. Floris, L. Hari, M. Kleber, U. Suter, and L. Sommer. 2002. Cell-intrinsic and cell-extrinsic cues regulating lineage decisions in multipotent neural crest-derived progenitor cells. *Int. J. Dev. Biol.* 46:193–200.

Perez, S.E., S. Rebelo, and D.J. Anderson. 1999. Early specification of sensory neuron fate revealed by expression and function of neurogenins in the chick em-

bryo. *Development.* 126:1715–1728.

Pla, P., R. Moore, O.G. Morali, S. Grille, S. Martinozzi, V. Delmas, and L. Larue. 2001. Cadherins in neural crest cell development and transformation. *J. Cell. Physiol.* 189:121–132.

Reissmann, E., U. Ernsberger, P.H. Francis-West, D. Rueger, P.M. Brickell, and H. Rohrer. 1996. Involvement of bone morphogenetic protein-4 and bone morphogenetic protein-7 in the differentiation of the adrenergic phenotype in developing sympathetic neurons. *Development.* 122:2079–2088.

Saint-Jeannet, J.P., X. He, H.E. Varmus, and I.B. Dawid. 1997. Regulation of dorsal fate in the neuraxis by Wnt-1 and Wnt-3a. *Proc. Natl. Acad. Sci. USA.* 94:13713–13718.

Serbedzija, G.N., S.E. Fraser, and M. Bronner-Fraser. 1990. Pathways of trunk neural crest cell migration in the mouse embryo as revealed by vital dye labeling. *Development.* 108:605–612.

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

Shah, N.M., M.A. Marchionni, I. Isaacs, P. Stroobant, and D.J. Anderson. 1994. Glial growth factor restricts mammalian neural crest stem cells to a glial fate. *Cell.* 77:349–360.

Sommer, L. 2001. Context-dependent regulation of fate decisions in multipotent progenitor cells of the peripheral nervous system. *Cell Tissue Res.* 305:211–216.

Sommer, L., and M. Rao. 2002. Neural stem cells and regulation of cell number. *Prog. Neurobiol.* 66:1–18.

Sommer, L., N. Shah, M. Rao, and D.J. Anderson. 1995. The cellular function of MASH1 in autonomic neurogenesis. *Neuron.* 15:1245–1258.

Sommer, L., Q. Ma, and D.J. Anderson. 1996. Neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. *Mol. Cell. Neurosci.* 8:221–241.

Soriano, P. 1999. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* 21:70–71.

Stemple, D.L., and D.J. Anderson. 1992. Isolation of a stem cell for neurons and glia from the mammalian neural crest. *Cell.* 71:973–985.

Takeda, K., K. Yasumoto, R. Takada, S. Takada, K. Watanabe, T. Udono, H. Saito, K. Takahashi, and S. Shibahara. 2000. Induction of melanocyte-specific microphthalmia-associated transcription factor by Wnt-3a. *J. Biol. Chem.* 275:14013–14016.

Uusitalo, M., M. Heikkila, and S. Vainio. 1999. Molecular genetic studies of Wnt signaling in the mouse. *Exp. Cell Res.* 253:336–348.

Vleminckx, K., and R. Kemler. 1999. Cadherins and tissue formation: integrating adhesion and signaling. *Bioessays.* 21:211–220.

Wehrle-Haller, B., and J.A. Weston. 1997. Receptor tyrosine kinase-dependent neural crest migration in response to differentially localized growth factors. *Bioessays.* 19:337–345.

White, P.M., S.J. Morrison, K. Orimoto, C.J. Kubu, J.M. Verdi, and D.J. Anderson. 2001. Neural crest stem cells undergo cell-intrinsic developmental changes in sensitivity to instructive differentiation signals. *Neuron.* 29:57–71.

Yagi, T., and M. Takeichi. 2000. Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. *Genes Dev.* 14:1169–1180.

Yasumoto, K., K. Yokoyama, K. Takahashi, Y. Tomita, and S. Shibahara. 1997. Functional analysis of microphthalmia-associated transcription factor in pigment cell-specific transcription of the human tyrosinase family genes. *J. Biol. Chem.* 272:503–509.

Zirlinger, M., L. Lo, J. McMahon, A.P. McMahon, and D.J. Anderson. 2002. Transient expression of the bHLH factor neurogenin-2 marks a subpopulation of neural crest cells biased for a sensory but not a neuronal fate. *Proc. Natl. Acad. Sci. USA.* 99:8084–8089.