cells, in contrast to control cells (Fig. 4E). The expression pattern of IκBβ correlated well with that of Foxj1, diminishing particularly in response to anti-CD3 or IL-2 stimulation (fig. S1B). In addition, transduction of primary Th cells by Foxj1 increased IκBβ expression (fig. S1C), and Foxj1 could transactivate the IκBβ promoter (fig. S1D). Finally, Foxj1$^{-/-}$ T cells contained diminished levels of IκBβ mRNA (Fig. 4F). These findings strongly suggest that Foxj1 antagonizes NF-κB activity at least in part by inducing and/or maintaining IκB activity. As a consequence of this, deficiency in Foxj1 leads to spontaneous NF-κB activation and subsequent immune dysregulation.

We suggest that Foxj1 regulates early Th activation, enforcing T cell quiescence by regulating NF-κB activity, in part via IκB (fig. S6). However, recent studies suggest that NF-κB may preferentially play a role in later, postcommitment phases of Th1 development and proliferation (*14*), suggesting that Foxj1 may also enforce quiescence by modulating the activity of another class(es) of transcriptional regulators and/or may have as-yet undefined direct effects on T cell differentiation genes, such as LKLF (*18*) or Tob (*19*). Regardless, the present findings are consistent with prior observations demonstrating dysregulated NF-κB activity in both human (*20*) and murine (*21*, *22*) lupus and inflammatory phenotypes of animals deficient in IκB activity (*23*) and furthermore are consistent with the significantly reduced expression of Foxj1 in murine lupus Th cells (fig. S1). Thus, studies to define further the target genes regulated by Foxj1 as well as other Fox transcription factors will undoubtedly shed insight into the regulation of Th differentiation and immune tolerance.

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Instructive Role of Wnt/β-Catenin in Sensory Fate Specification in Neural Crest Stem Cells

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Wnt signaling has recently emerged as a key factor in controlling stem cell expansion. In contrast, we show here that Wnt/β-catenin signal activation in emigrating neural crest stem cells (NCSCs) has little effect on the population size and instead regulates fate decisions. Sustained β-catenin activity in neural crest cells promotes the formation of sensory neural cells in vivo at the expense of virtually all other neural crest derivatives. Moreover, Wnt1 is able to instruct early NCSCs (eNCSCs) to adopt a sensory neuronal fate in a β-catenin–dependent manner. Thus, the role of Wnt/β-catenin in stem cells is cell-type dependent.

Wnt proteins are able to induce proliferation in different types of stem cells (*1*–*3*). In the central nervous system, Wnts act mitogenically on progenitor cells, and activation of β-catenin, a component of the canonical Wnt signaling pathway (*4*), leads to amplification of the neural progenitor pool (*5*, *6*).

The question arises whether canonical Wnt signaling regulates stem cell self-renewal in general or whether this function is cell type–dependent. To address this issue, we analyzed the role of Wnt/β-catenin in NCSCs. Neural crest cells generate most of the vertebrate peripheral nervous system (PNS) and several non-neural derivatives (*7*). Wnt signaling has previously been implicated in early stages of neural crest development, such as neural crest induction and melanocyte formation (*8*–*10*). In NCSCs, specific ablation of the β-*catenin* gene results in lack of melanocytes and sensory neural cells in dorsal root ganglia (DRG) (*11*). NCSCs without β-catenin emigrate and proliferate normally but are unable to acquire a sensory neuronal fate.

These results are consistent with a role of β-catenin signaling in inducing a sensory fate. To address this hypothesis, we used the *cre/loxP* system to generate mice expressing a constitutively active form of β-catenin specifically in neural crest cells (fig. S1) (*12*). We first assessed the developmental potential of control and mutant neural crest cells by performing in vivo fate mapping experiments (*13*). In the control (Fig. 1A), neural crest cells emanating from the anterior neural tube populated the nasofrontal and periocular region, where they become mesenchyme that later generates bones, connective components, and vascular structures of the head (*14*–*16*). Neural crest cells also migrated into branchial arches that contribute to craniofacial skeletal tissue and the major arteries (*14*, *17*, *18*). In the mutant (Fig. 1B), however,

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cranial neural crest–derived cells hardly spread over the forebrain area and were more restricted to ventral head structures. Only a vestigial first branchial arch was present; all other branchial arches were absent. Instead, mutant neural crest cells aggregated in prominent cranial ganglion-like structures dorsal to the normal site of branchial arch formation.

Transverse sections revealed strongly reduced numbers of both neural crest cells in the cardiac outflow tract and neural crest–derived melanocytes (Fig. 1, C to F). In vivo fate mapping also revealed a complete absence of the enteric nervous system (Fig. 1, G and H). Moreover, mutant cells were unable to associate with peripheral axons and, thus, to contribute to the Schwann cell lineage in peripheral nerves (Fig. 1, I and J). The markers sox10 and erbB3 were barely detectable in mutant peripheral nerves (*19*), and cad6-positive cranial nerves were missing (Fig. 2, A and B), confirming the lack of presumptive Schwann cells. Absence of neural crest–derivatives in the mutant is not attributable to cell death, because no increased apoptosis was observed in regions normally composed of neural crest targets (*19*).

Normally, cad6 expression at embryonic day 10.5 (E10.5) is confined to neural structures and absent in mesenchymal neural crest derivatives (Fig. 2, A, C, and E). In the mutant, however, the entire area of β-galactosidase–positive cranial neural crest cells also displayed cad6 expression (Fig. 2, B, D, and F). Within the ectopic domain of cad6 expression, neurofilament (nf) was readily detectable, as were several transcription factors characteristic of the sensory lineage (Fig. 2, H, K, and L) (*19*). Thus, upon sustained activation of β-catenin, sensory neurons are generated in anterior regions of the embryo that are usually devoid of neural derivatives of the neural crest. Similarly, the prominent cranial ganglia (Fig. 1B, arrowheads) contained many cells positive for sensory markers (fig. S2). Evidently, neural crest cells populating these structures have adopted a sensory neural fate.

In the trunk, ngn2 is normally expressed in emigrating neural crest cells and marks cells fated for sensory neural lineages (*20*). Its transient expression in cells aggregating in early DRG is followed by expression of the sensory markers ngn1 and neuroD. Whereas lack of β-catenin abolishes ngn2-expression (*11*), sustained β-catenin activity resulted in increased numbers of ngn2-positive cells, both in the trunk and at ectopic cranial locations (Fig. 2, M to P). Moreover, ngn2 expression was not restricted to the DRG anlage. Rather, ngn2-positive cells appeared to migrate on ventral routes and accumulated lateral to the dorsal aorta at sites of normal sympathetic ganglion formation (Fig. 2, P and R). Ectopic expression of ngn1 and neuroD was also found in these structures (Fig. 2, S and T; fig. S2). In contrast, the autonomic neuronal markers mash1 and ehand were virtually absent in the mutant PNS (Fig. 2, U and V; fig. S2), indicating that sensory neurons were forming at the expense of sympathetic neurons. The data also reveal that, unlike some other neural crest target structures (Fig. 1, C to J), sites of normal sympathetic ganglia development can be populated by sensory neurons.

Thus, β-catenin signaling not only appears to be required for sensory neurogenesis (*11*) but also to promote this fate. To evaluate this further, we investigated the developmental potential of isolated NCSCs expressing stabilized β-catenin. In culture, both control and mutant migratory neural crest expressed the NCSC-markers p75 and Sox10 (Fig. 3, A and B) (*21*). Moreover, migration, proliferation, and cell death was not significantly altered between the explants (Fig. 3, A to F; table S1), indicating that the loss of nonsensory lineages in the mutant is not due to selective mechanisms before or during neural crest emigration. In conditions permissive for sensory neurogenesis, control neural crest cells produced few Brn-3A–positive sensory neurons, and many cells maintained Sox10 expression (Fig. 3; table S1). In contrast, virtually all mutant cells lost Sox10 immunoreactivity and the majority became Brn-3A–positive, demonstrating that β-catenin signaling is able to specify a sensory neuronal fate in most neural crest cells. Because Sox10 has been associated with NCSC maintenance (*22*, *23*), the data also suggest that overexpression of β-catenin promotes the loss of multipotency in NCSCs.

We next investigated whether the effect of activated β-catenin in neural crest cells reflects a role of Wnt signaling. Unlike in control conditions, wild-type NCSCs exposed to Wnt1 efficiently generated Brn-3A–positive sensory neurons (Fig. 4). When we similarly challenged β-catenin–deficient NCSCs with Wnt1, we were unable to detect any sensory neurons (Fig. 4D), in agreement with our previous finding that β-catenin is required for sensory neurogenesis (*11*, *24*). Thus, Wnt1 promotes sensory neurogenesis in a β-catenin–dependent manner.

The effect of Wnt/β-catenin signaling could, in principle, be explained by two distinct mod-

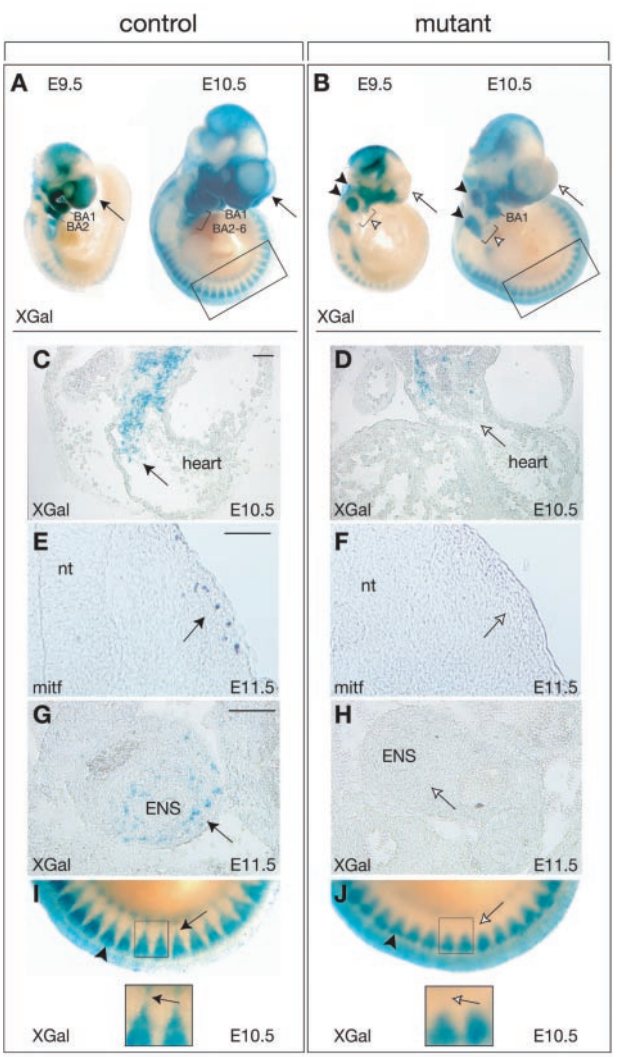

Fig. 1. In vivo fate mapping of NCSCs expressing stabilized β-catenin. (**A** and **B**) Neural crest cells and their progeny expressing β-galactosidase were revealed by whole mount XGal stainings (*13*). Mesenchymal neural crest in the nasofrontal region is present in control (arrows) and absent in mutant embryos (open arrows). Arrowheads point to prominent ganglion-like structures; open arrowheads indicate missing branchial arches (BA) in mutants. (**C** to **J**) XGal stainings and in situ hybridization on transverse sections show absence or reduction of cardiac neural crest, mitf-positive melanoblasts, and the enteric nervous system (ENS) in the mutant. Peripheral nerves [(I) and (J), enlarged areas marked by boxes in (A) and (B), respectively] were also missing. Arrows illustrate presence of and open arrows absence of neural crest derivatives. Arrowheads in (I) and (J) denote presence of DRG in control and mutant, respectively. nt, neural tube. Scale bars, 50 μm.

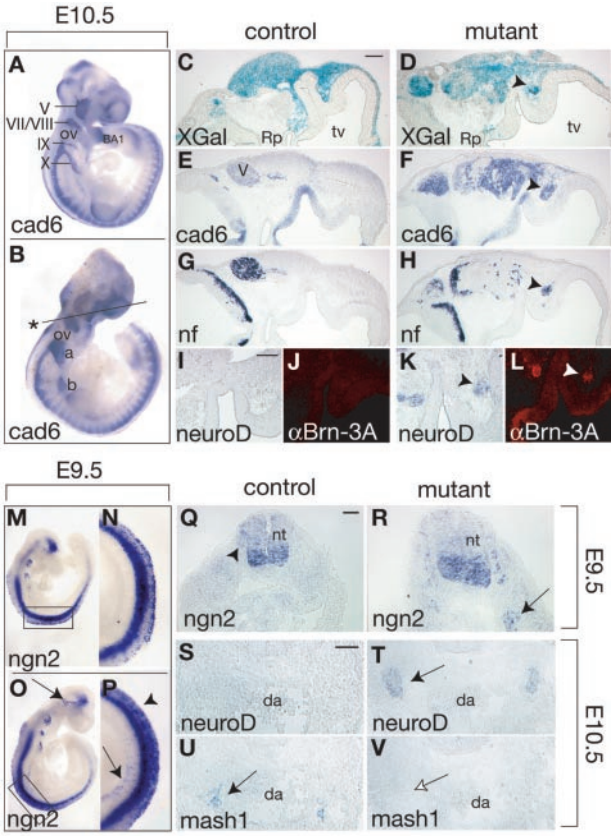

Fig. 2. Sensory neurogenesis at ectopic locations in mutant embryo. (**A** and **B**) cad6 expression at E10.5 reveals ganglia with sensory neuronal features in the mutant (B) formed instead of cranial nerves IX and X (a) and at the location of normal superior cervical ganglia (b) (see also fig. S2). V, VII/VIII, XI, and X designate cranial ganglia and nerves in the control (A). Ov, otic vesicle; BA, branchial arch. (**C** to **L**) Transverse sections cut at level (*) in (B). Note persistent expression of cad6 (F) in area of XGal-positive neural crest cells (D) and ectopic expression of nf in mutant (H). Ectopic neuronal cells (arrowheads) express the sensory markers neuroD (K) and Brn-3A (L). Rp, Rathke's pouch; tv, telencephalic vesicle. Scale bars, 100 μm. (**M** to **P**) In situ hybridization experiments show ectopic ngn2 expression in cranial [arrow in (O)] and ventral [arrow in (P)] regions in the mutant and increased expression in mutant migratory crest [arrowhead in (P)]. Control, (M) and (N); mutant, (O) and (P). (**Q** and **R**) On transverse sections at E9.5, ngn2-positive neural crest cells are restricted to the DRG anlage in control embryos [arrowhead in (Q)], whereas they spread ventrally in the mutant [arrow in (R)]. (**S** to **V**) At E10.5 in the mutant, neuroD-positive sensory neural cells [arrow in (T)] are found at the expense of mash1-positive autonomic neuronal cells [arrows in (U) and (V)]. da, dorsal aorta. Scale bars, 50 μm.

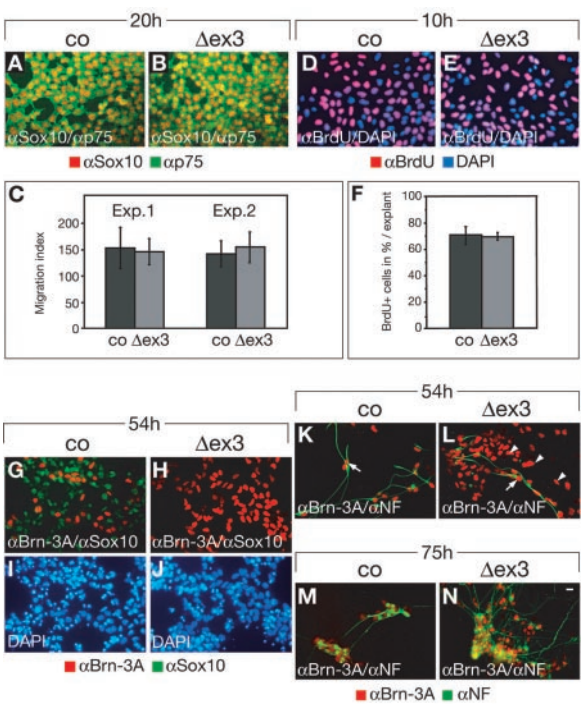

Fig. 3. Stabilized β-catenin does not affect emigration and proliferation of neural crest cells but promotes sensory neurogenesis. (**A** to **C**) Quantification of the migration index (*13*) did not reveal differences in migration of p75/Sox10-positive neural crest cells in control (co) and mutant (Δex3) explant cultures ($P > 0.2$). (C) Each bar represents the migration index (mean ± SD) of three different explants. (**D** to **F**) The number of proliferating, bromodeoxyuridine (BrdU)–incorporating cells was not significantly different between control and mutant cultures ($P > 0.75$). Three explants per genotype were analyzed, scoring 500 to 1000 cells per explant (F). Each bar represents the mean ± SD (**G** to **J**) Upon differentiation, mutant neural crest cells lose Sox10 expression and stain for the sensory marker Brn-3A, whereas many control NCSCs maintain Sox10 expression (table S1). (**K** to **N**) Differentiation is delayed in the mutant, because many Brn-3A–positive sensory neuronal cells (arrowheads) do not express NF (arrows) in the mutant at 54 hours (table S1). After prolonged incubation for 75 hours, however, most mutant cells are able to undergo full differentiation. Scale bar, 10 μm.

els: Wnt/β-catenin might promote the expansion of a sensory progenitor that has segregated from a neural crest cell with autonomic and other potentials (*20*, *25*, *26*). Alternatively, Wnt/β-catenin might have an instructive influence on fate decisions in an early NCSC able to generate sensory and autonomic neurons, glia, smooth muscle, and possibly other neural crest derivatives (*27*–*30*). The latter model is supported by the fact that in vivo sensory cells are not just expanded but rather generated at the expense of virtually all other neural crest lineages upon sustained β-catenin activation (Figs. 1 and 2). To rigorously distinguish between these models, we challenged early neural crest cells at clonal density with Wnt1 and, on sister plates, with different growth factors previously shown to promote specific fates in NCSCs (*31*) (Fig. 4, E to J; table S2). In control cultures without instructive growth factors, 88 ± 6.7% of all prospectively identified NCSCs generated mixed clones containing autonomic neurons and glia (*19*). In agreement with previous studies (*31*), BMP2 induced Mash-1–dependent autonomic neurogenesis in 90.5 ± 3.7% of all NCSCs, whereas upon transforming growth factor–β (TGF-β) treatment, about 70% of the cells adopted a smooth muscle–like fate. In the presence of Wnt1, 79.6 ± 2.5% of all NCSCs generated clones containing Brn-3A–positive sensory neurons. Most of these (95.4 ± 3.4%) were sensory neuron–only clones, which were not associated with Sox10 staining. The clone size of Wnt1-treated NCSCs was small, with many NCSCs giving rise to a single sensory neuron (Fig. 4, G and K). Furthermore, cell death was minimal in all clonal experiments (table S2), excluding selective effects of the factors added. The combined data indicate that Wnt signaling does not induce proliferation of a restricted sensory progenitor but rather promotes sensory fate decision in multipotent eNCSCs.

In vivo, members of the Wnt family specify neural crest from early dorsal neuroepithelial cells (*9*). Furthermore, the effects of ablation of *wnt1* and *wnt3* suggest a role of Wnt signaling in expansion of dorsal neural tube cells, including the premigratory neural crest (*10*). Our present data, together with the β-catenin loss-of-function analysis (*11*), indicate a subsequent function of Wnts in neural crest cells as they emigrate. Although the sensory lineage segregates early in PNS development (*20*, *25*, *26*), emigrating neural crest cells initially represent a population of stem cells (eNCSCs) that are homogeneous with respect to many developmental potentials, including sensory, autonomic, glial, smooth muscle–like, and possibly mesenchymal and other lineage formation (Fig. 2) (*27*). Similar to other growth factors that promote alternative fates (*31*), Wnt/β-catenin induces sensory neurogenesis by acting instructively on these eNCSCs. The molecular context that allows Wnt signaling to regulate cell cycle progression in certain stem

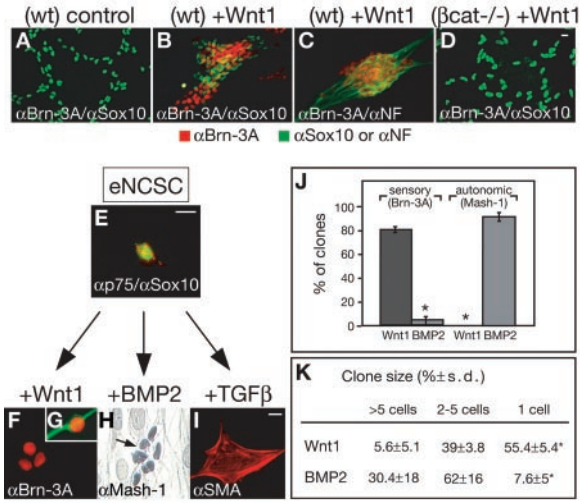

Fig. 4. Wnt1/β-catenin signaling instructs eNCSCs to adopt a sensory fate. (**A** to **D**) The effect of Wnt1 is β-catenin dependent. Wild-type (wt) NCSCs exposed to control monolayers fail to generate Brn-3A–positive sensory neurons (A), whereas wt NCSCs exposed to Wnt1 monolayers form ganglion-like cell aggregates containing Brn-3A/NF-positive sensory neurons [(B) and (C)]. β-catenin–deficient (β-cat$^{-/-}$) NCSCs are unable to generate sensory neurons, despite the presence of Wnt1 (D). Scale bar, 10 μm. (**E** to **J**) Clonal analysis demonstrates responsiveness of wt eNCSCs to different instructive growth factors including Wnt1. Of the p75-positive (red) founder cells, 90.3 ± 0.7% coexpressed Sox10 (green) (E). In the presence of Wnt1, founder cells generated clones of Brn-3A–positive sensory neurons (F) expressing NF (G). BMP2 instructed eNCSCs to generate Mash-1–expressing autonomic cells [arrow in (H)]. TGF-β induced a smooth muscle–like fate in most of the eNCSCs (I). Scale bars, 10 μm. (J) Quantification of clone composition (see also , fig. S2). (*) $P < 0.001$. The data are expressed as the mean ± SD of three independent experiments. Fifty to 150 clones were scored per experiment. (**K**) The cell number within individual Wnt1- and BMP2-treated clones was analyzed. Numbers (percentage of all clones per condition) are shown as the mean ± SD of three independent experiments, scoring 50 to 150 clones per experiment. (*) $P < 0.01$.

cells (*1*–*3*, *5*, *6*) and fate decision processes in NCSCs awaits investigation.

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Anterior Cingulate Conflict Monitoring and Adjustments in Control

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Conflict monitoring by the anterior cingulate cortex (ACC) has been posited to signal a need for greater cognitive control, producing neural and behavioral adjustments. However, the very occurrence of behavioral adjustments after conflict has been questioned, along with suggestions that there is no direct evidence of ACC conflict-related activity predicting subsequent neural or behavioral adjustments in control. Using the Stroop color-naming task and controlling for repetition effects, we demonstrate that ACC conflict-related activity predicts both greater prefrontal cortex activity and adjustments in behavior, supporting a role of ACC conflict monitoring in the engagement of cognitive control.

A major goal of cognitive neuroscience is to understand the precise neural mechanisms that underlie cognitive control (*1*). An important question about the nature of cognitive control is how do the processes involved in implementing control become engaged, or in other words, what controls control (*2*)? One partial answer comes from the conflict hypothesis, which posits that monitoring of response conflict acts as a signal that engages control processes that are needed to overcome conflict and to perform effectively (*3*, *4*). Two brain regions that have been associated with cognitive control processes are the ACC and the prefrontal cortex (PFC). Although it is commonly accepted that the PFC is involved in implementing control (*5*–*7*), there have been differing hypotheses regarding the contribution made by the ACC (*8*–*10*). One of these, the conflict hypothesis, contends that a function of ACC is the monitoring of processing conflict (*3*, *11*). However, this has recently been challenged on two grounds: first, failure to find behavioral evidence for trial-to-trial adjustments in control following conflict when stimulus repetitions

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