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Zebrafish *narrowminded* disrupts the transcription factor *prdm1* and is required for neural crest and sensory neuron specification

Laura Hernandez-Lagunas[a], **Irene F. Choi**[a], **Takao Kaji**[a], **Peter Simpson**[a], **Candice Hershey**[b], **Yi Zhou**[b], **Len Zon**[b], **Mark Mercola**[c], and **Kristin Bruk Artinger**[a,*]

Kristin Bruk Artinger: Kristin.Artinger@uchsc.edu

[a]Department of Craniofacial Biology, University of Colorado Health Sciences Center, Denver, CO 80262, USA

[b]Howard Hughes Medical Institute/Childrens Hospital, Division of Hematology/Oncology, Boston, MA, USA

[c]Stem Cell and Regeneration Program, The Burnham Institute, La Jolla, CA, USA

Abstract

Specification of both neural crest cells and Rohon–Beard (RB) sensory neurons involves a complex series of interactions between the neural and non-neural ectoderm. The molecular mechanisms directing this process are not well understood. The zebrafish *narrowminded* (*nrd*) mutation is unique, since it is one of two mutations in which defects are observed in both cell populations: it leads to a complete absence of RB neurons and a reduction in neural crest cells and their derivatives. Here, we show that *nrd* is a mutation in *prdm1*, a SET/zincfinger domain transcription factor. A Morpholino-mediated depletion of *prdm1* phenocopies the *nrd* mutation, and conversely overexpression of *prdm1* mRNA rescues the *nrd* RB sensory neuron and neural crest phenotype. *prdm1* is expressed at the border of the neural plate within the domain where neural crest cells and RB sensory neurons form. Analysis of *prdm1* function by overexpression indicates that *prdm1* functions to promote the cell fate specification of both neural crest cells and RB sensory neurons, most likely as a downstream effector of the BMP signaling pathway.

Keywords

Narrowminded; Neural crest; Rohon–Beard sensory neurons; Blimp-1

Introduction

Neural crest cells, RB sensory neurons and cranial placodes form at the border between the neural and nonneural ectoderm in response to signaling mechanisms along the mediolateral axis of the embryo, including factors such as BMPs, Wnts, and FGFs. Although these proteins are required to specify a narrow region that is permissive for formation of the cell

*Corresponding author. Fax: +1 303 315 3013.

types at the neural plate border (Garcia-Castro, 2002; Garcia-Castro and Bronner-Fraser, 1999; Lewis et al., 2004; Liem et al., 1995; Mayor et al., 1997; Nguyen et al., 1998, 2000); for review, see Knecht and Bronner-Fraser, 2002), it is not clear how these signals are integrated to specify these cell types or to define their stereotypic position in the embryo. Neural crest cells are specified early in embryogenesis and migrate extensively to form the peripheral nervous system (PNS), craniofacial structures as well as other derivatives (Le Douarin, 1982). RB sensory neurons transduce mechanosensory stimuli in response to touch and remain within the central nervous system (CNS; Lamborghini, 1980). How these two populations are specified within a specific domain at the lateral neural plate remains an interesting question. Evidence from lineage experiments suggest that both neural crest cells and RB sensory neurons can arise from a common progenitor population but typically do not share a common precursor (Cornell and Eisen, 2000; Jacobson and Moody, 1984). Because these two cell types can arise from one precursor, it remains an unanswered question if the characteristic mechanism for neural crest and RB sensory neuron formation is to be generated from one precursor or two separate precursors within the same domain. But if they can share a common precursor, it is interesting to consider how a precursor cell is capable of giving rise to very different populations of neurons. One possibility is that a similar set of combinatorial transcription factors provides the information for the differentiation of neural crest and RB sensory neurons. We have determined that the *nrd* mutation is an excellent model for the study of questions related to cell fate specification between progenitors for RB sensory neurons and neural crest cells, since these populations are absent or reduced in mutant embryos. *nrd* was isolated as part of a small scale ENU-based mutagenesis screen in which mutations that affect neurogenesis and neural crest formation were isolated (Artinger et al., 1999; Kim et al., 2000). We have determined that *nrd* acts cell-autonomously to specify RB sensory neurons (Artinger et al., 1999), suggesting that this molecule integrates several signals that specify cell fate at the border of the neural plate. While RB sensory neurons fail to form at all, neural crest cells do arise in *nrd* mutant embryos but at reduced numbers as compared to wildtype in all neural crest derivatives studied including PNS neurons, melanocytes and craniofacial cartilage. Thus, knowledge of *nrd* will shed light on the specification of both of these very different cell types that may share a common precursor.

The cell fate apportionment within the region involves the combinatory expression of several transcription factors, such as *neurogenins*, components of the *notch* pathway, *dlx* and *msx*, that refine the initial pattern established by the inductive signals and restrict the region competent to form the cell types at the neural plate border (Cornell and Eisen, 2000, 2002; Tribulo et al., 2003; Woda et al., 2003). *prdm1* transcription factors have been shown to be involved in cell fate specification in both the mammalian and zebrafish embryonic development. In the mouse embryo, *prdm1* appears to function primarily in the immune system by promoting B-cell differentiation (Shaffer et al., 2002; Shapiro-Shelef et al., 2003; Turner et al., 1994). Expression is observed within the embryonic epidermis, not the CNS (Chang et al., 2002). In *Xenopus*, the *prdm1* orthologue is expressed within the anterior endomesodermal cells and lateral stripes in the neural plate (de Souza et al., 1999) and functions in the formation of the head structures. In zebrafish, *prdm1* functions by regulating cell fate between fast and slow twitch muscle fibers (Baxendale et al., 2004). However, the

function of *prdm1* in the nervous system has not been specifically described. Here, we have shown that zebrafish *nrd* is a mutation in *prdm1*. *prdm1* is required for the specification of neural crest cells and RB sensory neurons, acting cell autonomously in RB sensory neuron and most likely in neural crest specification, downstream of initial BMP inductive signal.

Materials and methods

Zebrafish strain and maintenance

The zebrafish were maintained according to Westerfield (1993). *nrd*^{m805} was isolated from a small scale in situ hybridization based screen described in our previous publication (Artinger et al., 1999), and is in the EKK background. To produce a polymorphic strain for genetic mapping, fish with the *nrd* mutation were crossed into WIK.

Cloning of nrd(prdm1)

Genomic DNA was isolated from a large number of homozygous *nrd*^{m805} embryos. Bulk segregant analysis with SSLP markers was performed to identify the linkage group. Analysis of SSLP markers was used to fine map the mutation. We identified linkage with *nrd*^{m805} in a 3-cM interval between z21155 and z6365 on linkage group 16. Technical difficulties required us to regenerate the line, using both a new cross of EKK *nrd*^{m805} into WIK and EKK *nrd*^{m805}:HuC:GFP (generated by A. Chitnis). This allowed us to position *nrd*^{m805} closer to z19602. From the Sanger Institute Zebrafish Sequencing Project (http://www.sanger.ac.uk/Projects/D_rerio/), we were able to identify zebrafish *prdm1* as a candidate in the region between z6365 and z19602. cDNA was isolated from *nrd*^{m805} and wildtype siblings at 48 hpf and *prdm1* cDNA was cloned by PCR using Platinum Taq High Fidelity polymerase (Invitrogen) and primers: forward 5′-GGATCCATGTGTGGCTGGGACCAG-3′ and reverse 5′-CTCGAGCTAGGTATCCATGGCCTCCT-3′. Both a wildtype and *nrd*^{m805} 2.4 kb cDNA fragment was inserted into pCS2+ between *Bam*HI and *Xho*I and sequenced by ABI-prism (Barbara Davis Center Sequencing Core, UCHSC) for direct comparison. A stop codon denoting a truncation was observed in *nrd*^{m805} at amino acid 154 was confirmed in multiple PCR reactions within the two *nrd*^{m805} strains.

Whole-mount in situ hybridization and immunohistochemistry

Whole-mount in situ hybridization was performed as described by Thisse (1998). The following DIG-labeled antisense RNA probes (Roche Diagnostics) and Alkaline phosphatase using NBT/BCIP color reaction were used for in situ hybridization: *prdm1*, *HuC* (Kim et al., 1996), *foxd3* (Odenthal and Nusslein-Volhard, 1998), *crestin* (Rubinstein et al., 2000). Additionally, fluorescent-labeled probes utilizing Alkaline phosphatase and fast red were used in double in situ hybridization. The fast red reaction was performed first and inactivated with 0.1 M glycine–HCL, followed by the second color reaction. The HNK-1 antibody (Sigma) was used at the dilution 1:1000. Immunohistochemistry was performed as described by Solnica-Krezel and Driever (1994).

Antisense morpholino oligonucleotide and RNA injections

An antisense Morpholino Oligonucleotide (MO) was designed against exon2–intron2 splice site (E2I2) of *prdm1* and has the sequence of 5′-TGGTGTCATACCTCTTTGGAGTCTG-3′. The control MO has a sequence of 5′-CCTCTTACCTCAGTTACAATTTATA-3′. The oligonucleotides were dissolved in distilled water. Three to 24 ng *prdm1*-MO were injected into the yolk of 1- to 8-cell-stage embryos together with 10 kDa lysinated Fluorecein dextran (LFD; Molecular Probes) for a total volume of 6–14 nl. Sense *prdm1* RNA was synthesized with Ambion Message Machine kit from $PCS2^{+}$-*prdm1* for rescue experiments. One hundred to 700 pg was injected along with a fluorescent tracer, as for the MO injections.

Nomenclature note

According to the Zfin zebrafish nomenclature guide, *narrowminded* will now be referred to as *prdm1* or *U-boot* (*prdm1*).

Results

narrowminded is a mutation in prdm1

To clone the *nrd* mutation, we first established the position on the genetic map using bulk segregant analysis with single-strand length polymorphic (SSLP) markers (Fig. 1). This analysis initially placed *nrd* on linkage group 16 between SSLP markers z21155 and z6365 (Fig. 1A). Subsequent analysis revealed that *nrd* was more closely linked to z19602. Further examination of candidate genes within this interval drew our attention to *prdm1*, which had recently been positioned by the Sanger Institute Zebrafish Sequencing Project (Zv3 ensembl). *prdm1* is a SET/zinc finger transcription factor that is known to be critical for cell fate determination in the mammalian immune system where the protein is called Blimp-1. In the case of mammals, *prdm1* acts as a repressor of genes such as beta-interferon (Gyory et al., 2004; Tamura et al., 2003), c-*myc* (Lin et al., 1997) and pax-5 (Klein et al., 2003), to drive differentiation of a B-cell to a plasma cell. Analysis of *prdm1* cDNA from homozygote *nrd* zebrafish revealed a point mutation that results in a premature stop codon, consistent with ENU-based mutagenesis, and causes a truncation of the protein at amino acid 154 within exon 4 (Figs. 1B, C). The predicted *nrd* (*prdm1*) protein contains only part of the SET domain and none of the five zinc finger DNA binding domains. Another alteration in the *nrd* allele (698 V to M) encodes a missense mutation at the 3′ end of the protein. Since this change is downstream of the mutation that created the stop codon, it would not be represented in the *nrd* protein. In addition to these mutations, we identified two substitutions in the wildtype EK/WIK strain *prdm1* sequence that differ from the published sequence (95 amino acid H to R; and 260 D to G). These differences are seen within the EK/WIK wildtype or heterozygote EK/WIK strain, suggesting that these are polymorphisms between this strain and the published strain (Tubingen). Although we believe that a truncation at position 154 would eliminate DNA binding, and thus create a complete null allele, we cannot rule out the possibility that *nrd* retains residual activity or harbors neo- or antimorphic activity, perhaps because of cryptic splicing or transcriptional reinitiation from a downstream start codon.

prdm1 is required for neural crest and Rohon–Beard sensory neuron development

We confirmed that loss of *prdm1* is responsible for *nrd* phenotype by phenocopying the defect by antisense Morpholino (MO) depletion. The Morpholino is predicted to disrupt normal splicing of the *prdm1* RNA at the exon2–intron2 boundary (E2I2-MO; Gene Tools, LLC; Draper et al., 2001). The *nrd* phenotype is characterized by the complete absence of RB sensory neurons, and a reduction in the number of neural crest cells and their derivatives. As visualized with the same panel of markers used to analyze the *nrd* mutants, injection of *prdm1* MO replicated this phenotype: RB sensory neurons were absent and neural crest cell numbers were reduced (*prdm1* MO in Figs. 2C, I as compared to wildtype in Figs. 2 A, G and *nrd* in Figs. 2B, H; Table 1). Embryos injected with standard control MO at the same concentration displayed the wildtype RB sensory neuron and neural crest cell phenotype (data not shown). Similarly, HNK-1 and *crestin* expression are identical in *nrd* and *prdm1*-depleted embryos (Figs. 2F, L as compared to wildtype in Figs. 2D, J and *nrd* in Figs. 2E, K). The large HNK-1 expressing RB sensory neurons are absent from both *nrd* and *prdm1* MO injected embryos. Often, the *nrd* mutant embryos maintain projections along dorsal lateral fasciculas (DLF), as well as interneurons cell bodies, which are not observed to the same extent in the *prdm1* MO injected embryos (smaller stained neurons in Fig. 2E as compared to 2F). Trunk neural crest cell migration is reduced and disorganized in homozygous *nrd* and *prdm1* MO-injected embryos. Cranial neural crest numbers in *nrd* embryos are comparable to wild-type levels by 14 hpf of development (Artinger et al., 1999). However, the trunk region remains depleted of neural crest at 24 hpf, suggesting that the mechanism by which neural crest cells are induced in the absence of *prdm1* is different along the anterior–posterior axis or that *prdm1* is involved in the maintenance of one population over the other (Figs. 2K, L as compared to wildtype in 2J; for cranial regions see (Artinger et al., 1999). In addition to the migrating neural crest cells, we observed a reduction in neural crest derivates, including PNS neurons, melanocytes, and craniofacial cartilage, in the *prdm1* MO-injected embryos (similar to the *nrd* phenotype; data not shown). Interestingly, a higher dose of MO (16–18 ng) had to be injected to generate the RB sensory neuron phenotype than was required for the neural crest phenotype (12 ng), suggesting differential sensitivity to *prdm1* dosage (Table 1). Low concentrations of *prdm1* may suffice to specify the RB sensory neurons while a higher concentration, more readily reduced by the MO, might be needed to specify the neural crest cells.

To provide further support for the hypothesis that *nrd* is a mutation in *prdm1*, we injected *nrd* embryos with *prdm1* mRNA. This rescued both the RB sensory neuron and the neural crest phenotype observed in *nrd* embryos. HNK-1-positive RB sensory neurons appeared in *nrd* mutant embryos injected with wildtype *prdm1* mRNA (Fig. 3E; compared to wildtype in A and *nrd* in C), and neural crest cells expressing *crestin* were similar to wildtype levels (Fig. 3F; compared to wildtype in B and *nrd* in D). Additionally, in the *nrd* background following injection of *prdm1* RNA, we also observed neurons were positioned more ventrally that have the morphology of a RB sensory neuron, which peripheral processes, except they were smaller in size. Thus, *prdm1* mRNA is sufficient to drive RB sensory neuron and neural crest cell differentiation in an *nrd (prdm1)*$^{-/-}$ background. In both cell types, however, we were unable to drive a complete rescue of the *nrd* mutant phenotype with injection of *prdm1* mRNA. This may be due to inadequate levels of *prdm1* at the

appropriate time in development. We did not observe any difference in dosage sensitivity in regard to the *prdm1* RNA injections as with the MO experiments.

prdm1 is expressed at the neural plate border

Given the phenotype of the *nrd* mutation, we sought to determine if expression of *prdm1* is consistent with a role in cell fate specification in the nervous system. At 90% epiboly and continuing to tail bud stage, we find that *prdm1* RNA is expressed within a region that spans the border of the neural plate, with more pronounced expression anteriorly (Figs. 4A–C; arrows). By 11 hpf (2–3 somite stage), *prdm1* is expressed in a broad domain spanning the neural plate border and also at the midline in the adaxial cells (Fig. 4D; higher magnification in E). Adaxial cells (labeled “A”) form adjacent to the axial hypochord and contain muscle pioneer cells (Thisse et al., 1993). At 13 hpf, expression is decreased within the neural plate border region (Fig. 4F; arrow), but later at 24 hpf, expression is strong in the median fin fold, somites, and posterior fin bud (not shown). An RNA probe to the 3′end of *prdm1* revealed reduced *prdm1* expression in homozygous *nrd* (*prdm1*) mutant embryos (Figs. 4G, H; arrowheads show neural plate border). The observed decrease in *prdm1* mRNA could occur for several reasons including absence of *prdm1*-expressing neural crest cells and RB sensory neurons, non-sense-mediated decay of the RNA, or feedback auto-regulation of *prdm1* gene in the wildtype.

To confirm that the expression of *prdm1* is within the domain where neural crest and RB sensory neurons arise, we performed double in situ hybridization analysis with *Foxd3* and *HuC*. *prdm1* is expressed in a broad domain at the lateral edge of the neural plate, which includes neural crest, RB sensory neurons, and cranial placodes. At 12 hpf, HuC is expressed in all primary neurons, including the RB sensory neurons (Figs. 5A, B; blue), and overlaps with the *prdm1* expression (Fig. 5B; shown overexposed at higher magnification in red). *prdm1* is also expressed within the same domain as *Foxd3*-positive neural crest cells (blue with *prdm1* in red; Figs. 5C, D). Based on this co-expression analysis, *prdm1* is expressed within the same domain as forming neural crest cells and RB sensory neurons.

prdm1 functions downstream of the initial BMP inducing signal

Previous studies have implicated the BMP signaling pathway in the initial induction of neural crest cells. To determine how *prdm1* fits into the hierarchy of the BMP pathway during specification of cells at the neural plate border, we examined its expression in several BMP mutations: *swirl* (*swr*) which encodes zebrafish *BMP2b* (Kishimoto et al., 1997), *somitabun* (*sbn*) which encodes the BMP mediator *Smad5* (Hild et al., 1999), and *snailhouse* (*snh*), which encodes *BMP7* (Dick et al., 2000). Each of these BMP signaling components have previously been shown to be required for specification of neural crest cells and dorsal and intermediate neurons in the spinal cord in zebrafish (Nguyen et al., 1998, 2000), and their homologues also function in the induction of neural crest cells in chick and *Xenopus* (Liem et al., 1995; Marchant et al., 1998). *prdm1* expression is eliminated at the neural plate border in *swr* (*Bmp2b*), expanded in *sbn* (*Smad5*) and reduced in *snh* (*BMP7*) mutant embryos (Figs. 6B–D, ventral views; higher magnification in Figs. 6F–H as compared to wildtype in Figs. 6A, E). This is consistent with the reduction of the domain of neural crest and RB sensory neurons in *swr* (*Bmp2b*) and *snh* (*BMP7*), and an expansion in *sbn* (*Smad5*),

shown schematically below the images in Fig. 6 (as observed by Nguyen et al., 1998, 2000). Dorsal views are shown in Figs. 6J–L, as compared to wildtype in Fig. 6I, indicating the maintenance of adaxial *prdm1* expression (A) in all BMP pathway mutants. Thus, the neural plate border corresponds to the *prdm1* expression domain: In *swr*, the neural plate border region does not form, and in *sbn* and *snh*, the border is shifted to the ventral side (arrows; Figs. 6F–H). These data suggest that *prdm1* acts downstream of the initial BMP inducing signal or is dependent on BMP signaling for its expression.

prdm1 functions to promote both neural crest and Rohon–Beard sensory neurons

To determine how *prdm1* functions in the specification and differentiation of neural crest cells and RB sensory neurons, we performed ectopic expression of *prdm1*. If neural crest cells and RB sensory neurons derive from a common precursor, *prdm1* might mediate a switch between these two cell types within the precursor population. To examine this possibility, we overexpressed *prdm1* RNA in wildtype embryos. Overexpression of *prdm1* increased the number of RB sensory neurons as shown by the increased number of HNK-1-positive cell bodies at 24 hpf (Fig. 7B as compared to wildtype in 7A.) Wildtype embryos averaged 54 HNK+ cell bodies/20 somite length compared to 58–60 in *prdm1* overexpressing embryos (Table 2). A noticeable difference observed between wildtype and embryos overexpressing *prdm1* is the spacing between the cell bodies. In wildtype embryos, there is one cell body in each segment per side, with a space between the neurons (Fig. 7A arrow). The embryos overexpressing *prdm1* have more cell bodies per segment and the spacing between the cell bodies is reduced or absent (Table 2; Fig. 7B arrows). Although the wildtype cell bodies are close together, they typically do not touch each other as seen in the embryos overexpressing *prdm1*. In addition to the increased number of cell bodies, the processes that intervate the skin appear to have increased, with a thicker diameter of the axon in *prdm1*-overexpressing embryos (Fig. 7B as compared to wildtype in 7A; arrowheads). The increase in the number of RB sensory neurons was often observed to be one sided, which can be explained by a sidedness of the injection. Analysis of *crestin* expression at 24 hpf shows that neural crest cell numbers also increased after ectopic expression of *prdm1* (Fig. 7C as compared to D). In wildtype embryos, neural crest cells begin their migration from the dorsal aspect of the neural keel and migrate along defined pathways through the somite, with a characteristic single line of cells in each pathway at one time (Fig. 7C arrows). After ectopic expression of *prdm1*, the numbers of migrating cells appear to have increased such that many more cells are exiting the neural keel (Fig. 7D, arrowheads), with the appearance of more than one cell diameter in the somite at one time (Fig. 7D, arrows). Interestingly, in the injected embryos, the normal migration seems to be accelerated to a more ventral location as compared to wildtype. These data suggest that *prdm1* can promote the formation of both Rohon–Beard sensory neurons and neural crest cells.

Discussion

Taken together, our analysis indicates that zebrafish *prdm1* is required for RB sensory neuron and neural crest cell specification. Several studies have shown that *prdm1* functions in cell fate specification during vertebrate embryogenesis. Mammalian *prdm1* is necessary

and sufficient to drive differentiation of plasma cells from B-cells (Shaffer et al., 2002; Shapiro-Shelef et al., 2003; Turner et al., 1994). The targeted disruption of *prdm1* in mouse results in an early embryonic lethal suggesting that *prdm1* functions in multiple cells types during development (Shapiro-Shelef et al., 2003). A nervous system defect has not specifically been noted, but the possibility remains that a phenotype exists but is partially masked by redundant or compensatory mechanisms. Thus, it would be interesting to determine in detail the nervous system phenotype in the *prdm1* null mice. With respect to the zebrafish, we have not examined *nrd* (*prdm1*) mutant embryos for immune deficits. Since *nrd* mutant zebrafish die during early embryogenesis, they might not survive long enough to visualize defects in B-cell development. It is known that, in addition to the neural phenotype described here, zebrafish and *Xenopus prdm1* functions during gastrulation in the formation of the head structures and to control cell movements (de Souza et al., 1999; Bischof and Driever, personal communication; Solnica–Krezel, personal communication).

Another zebrafish mutation, *U-boot*, has recently been shown to alter the *prdm1* coding region. *U-boot* (*prdm1*) mutant embryos display a U-shaped somite phenotype and lack slow twitch muscle fibers (Baxendale et al., 2004). The sequence differences between *nrd* and *U-boot* may explain the differing phenotypes. As shown here, *nrd* truncates *prdm1* at amino acid 154 within the SET domain, whereas *U-boot* is a missense mutation within two sites, one within the second zinc finger domain and the other between the SET domain and the first zinc finger (Baxendale et al., 2004). Whereas *nrd* appears to be a null allele, *U-boot* is predicted to be a hypomorphic allele that would elicit a milder phenotype. Consistent with this possibility, neural crest cell, RB sensory neuron or cranial placode deficits have not been described in *U-boot* embryos, although pigment and fin defects exist (Kelsh et al., 1996). Therefore, it would be interesting to determine if similar, but subtler, nervous system defects exist in *U-boot* (*prdm1*) mutant embryos. We have primarily focused on the nervous system defects in *nrd* (*prdm1*). However, we have observed a U-shaped somite phenotype in *nrd* (data not shown), consistent with those observed in the *U-boot* mutation, suggesting that similar muscle defects exist.

We have shown that *prdm1* RNA expression is altered in mutants that affect BMP signaling, indicating that *prdm1* likely functions downstream of BMPs to establish mediolateral pattern at the border of the neural plate. If neural crest cells and RB sensory neurons derive from a common precursor, *prdm1* might promote the differentiation of these two cell types within the precursor population. Consistent with this possibility, ectopic expression of *prdm1* increased the number of RB sensory neurons and neural crest cells, suggesting that *prdm1* function may be more complex than a simple switch between these cells types. The increase in RB sensory neurons after ectopic expression of *prdm1* is subtle and shows only a trend towards an increase in the number of neurons. Thus, *prdm1* may promote the formation of RB sensory neurons but is not sufficient to override Notch-Delta signaling, which regulates the final cell number via lateral inhibition (Chitnis, 1995; Cornell and Eisen, 2000). Conversely, since both neural crest and RB sensory neurons are deficient in *nrd* (*prdm1*) mutants, *prdm1* might be required for RB sensory neuron fate and one of several signals that can specify neural crest fates. Previous mosaic analysis suggests that *prdm1* functions cell autonomously in the specification of RB sensory neurons (Artinger et al., 1999). Results

from coexpression experiments suggest that *prdm1* also functions cell autonomously for neural crest cells, since the expression is observed within the neural crest forming domain. Although we cannot rule out the possibility that *prdm1* also has a non-cell autonomous role, this suggests that within the precursor cell population, cell intrinsic *prdm1*-dependent signals instruct precursors to become both neural crest cells and RB sensory neurons, rather then promoting one fate versus another. Our hypothesis is that the precursor population persists in *nrd* since some neural crest cells still form in mutant embryos. This suggests that these precursors are capable of responding to *prdm1*-independent signals that also promote the neural crest lineage. Detailed lineage labeling of the precursor populations at the nascent lateral border of the neural plate in wildtype and mutant embryos is needed to resolve this issue. The use of *nrd* as a probe of *prdm1* function will provide a powerful method for investigating the diversification of neurogenic cell fates at the lateral border of the neural plate.

Note added in proof

While this paper was in revision, the identification of a neural crest and Rohon-Beard sensory neuron phenotype in the *U-boot* mutation, a *narrowminded* allele, was published by Roy and Ng in *Current Biology*, Blimp-1 specifies neural crest and sensory neuron progenitors in the zebrafish embryo, *Current Biology* 2004 (14), 1772–1777. *Ubo* does seem slightly less severe then *nrd* in regards to the neural crest, but very similar in RB sensory neuron phenotype. Interestingly, using a heat shock construct to overexpress *Ubo* mRNA beginning at 80% epiboly, an increase in RB sensory neurons is demonstrated, suggesting that a high level of expression at the appropriate time may be critical for *prmd1*/Blimp-1 function.

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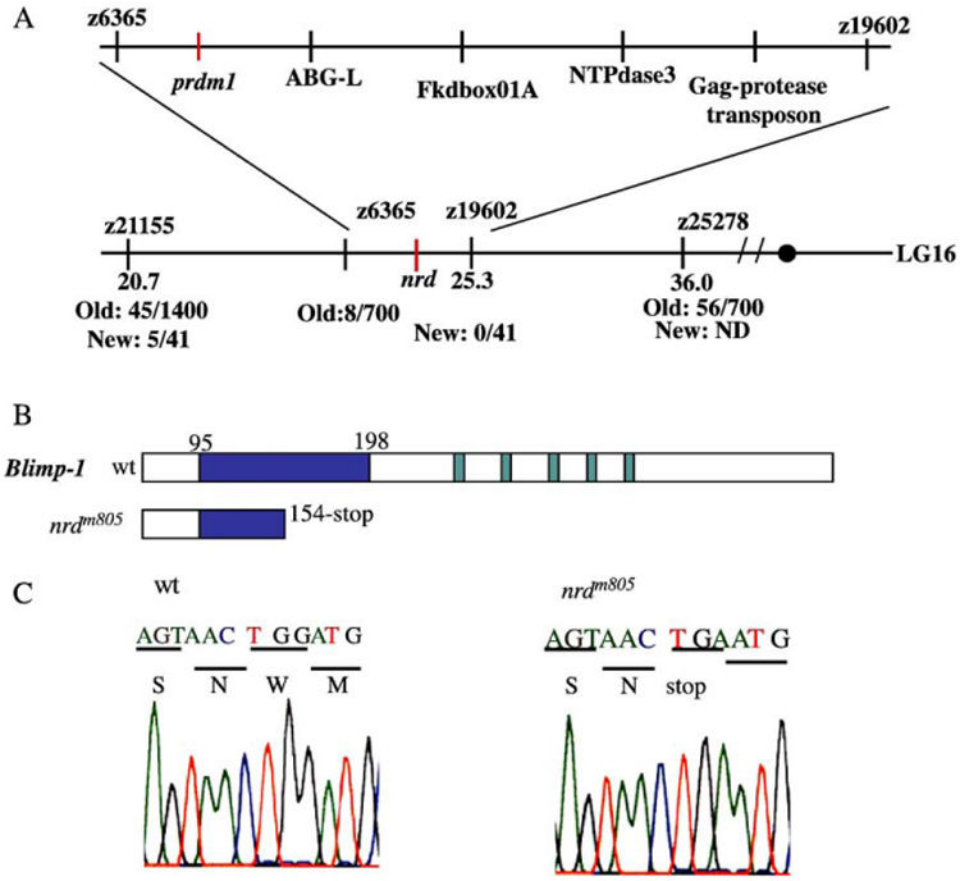

Fig. 1.

Linkage between *nrd* and *prdm1*. (A) Physical and genetic maps of zebrafish linkage group (LG) 16. The genomic interval that contains *nrd* was determined using bulk segregant analysis with single strand length polymorphic (SSLP) markers ("z" positioned before the number). We positioned *nrd* between z6365 and z19602. Recombination frequency is listed as old and new. Old is from the original *nrd* strain *nrd*^{m805}EK/WIK and New is *nrd*^{m805}*HuC*:GFP. Several genes are located in this interval, including *prdm1*, which we identified as a candidate (Sanger Zv3 ensembl; other genes in the region include *ABG-L*, *Fkdbox01A*, *NTPdase3*, *Gag-protease transposon*). (B, C) Sequence comparison of wildtype (wt) and *nrd*^{m805} *prdm1*. (B) Diagrams illustrate conceptual wildtype and *nrd*^{m805} Blimp-1 (prdm1) protein products. The wildtype sequence contains a SET domain (blue) between amino acid 95–198 and 5 zinc finger DNA binding domains (green). The *nrd*^{m805} sequence has a stop codon (TGA) at amino acid 154 (exon 4) within the SET domain and before the zinc finger domains. (C) Sequence comparison between wildtype (wt; left) and *nrd*^{m805} (right). Sequence was confirmed in multiple PCR reactions within the two *nrd*^{m805} strains, and both contained the stop at position 154.

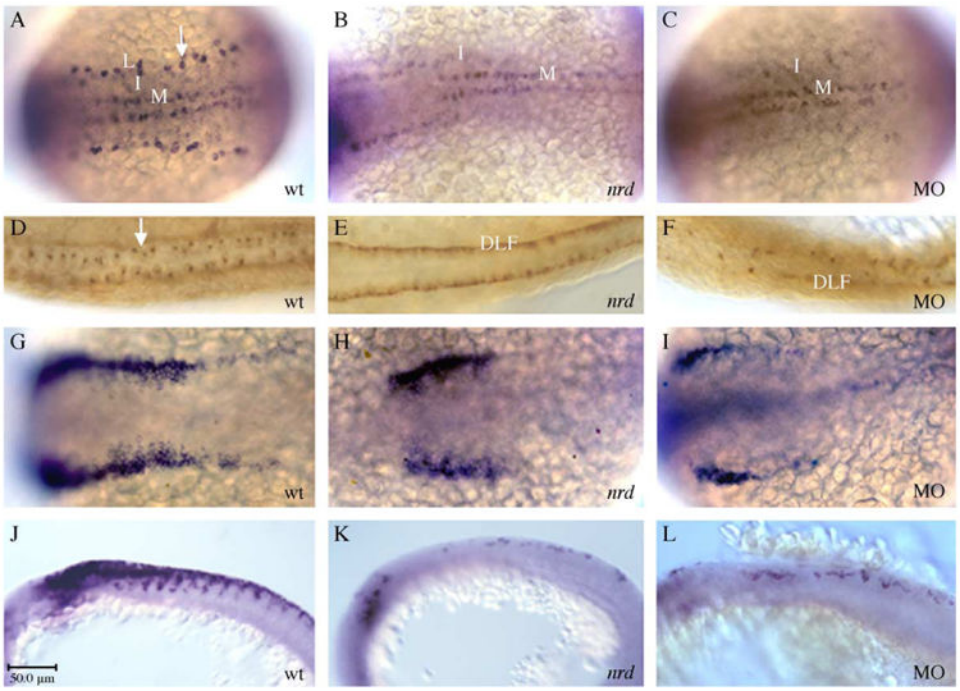

Fig. 2.

prdm1 MO injection reduces neural crest and eliminates Rohon–Beard sensory neurons, reproducing the *nrd* phenotype. (A–C, G–I) 11 hpf dorsal views, anterior is to the left. (D–F) Twenty-four hpf dorsal views, anterior to the left. (J–L) Eighteen hpf lateral views, anterior is to the left. (A–C) In situ hybridization with *Hu*C showing the three lateral stripes of primary neurons: lateral (L), intermediate (I) and medial (M). The neurons in the lateral stripe form RB sensory neurons (arrow in A) and are completely absent in *nrd* (B). Following *prdm1* MO injection (C), there is a loss of RB sensory neurons, reproducing the *nrd* phenotype (B). (D–F) HNK-1 immunohistochemistry at 24 hpf, showing the characteristically large RB sensory neurons in a wildtype embryo (arrow in D). Both *nrd* (E) and *prdm1* MO-injected embryos (F) lack RB sensory neurons. Consistent with a loss of RB sensory neurons, both *nrd* embryos (H) and *prdm1* MO embryos (I) display a reduction in *foxd3* expression at 11 hpf as compared to wildtype (G). This early reduction of neural crest remains in older embryos shown with *crestin* (J–L). Abbreviations: wt, wildtype; *nrd*, *narrowminded* mutant embryos; MO, *prdm1* MO; DLF, dorsal lateral fasciculas; Scale bar is 50 µm.

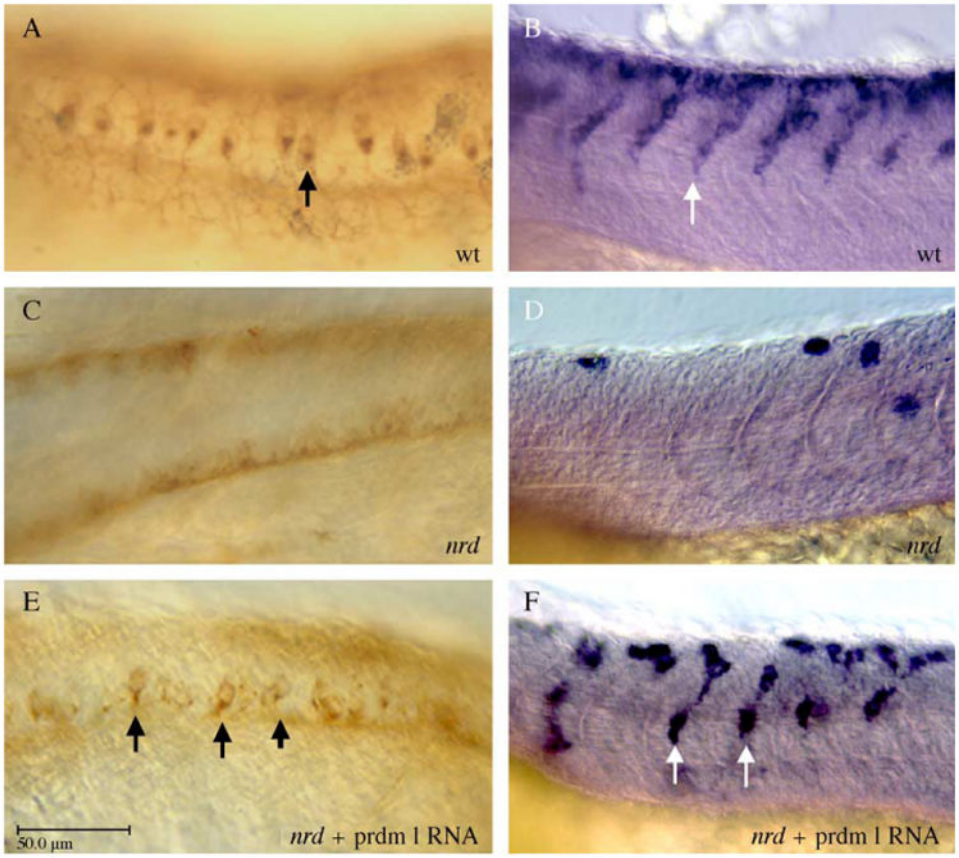

Fig. 3.

prdm1 mRNA injection can rescue Rohon–Beard sensory neurons and neural crest cells in *nrd* mutant embryos. HNK-1 immunohistochemistry, dorsolateral views; anterior is to the left. (A) Expression pattern of HNK-1 in RB sensory neurons in a wildtype embryo. (C) *nrd*^{m805} embryos lack RB sensory neurons and do not express HNK-1 in the dorsal neural tube. (E) RB sensory neurons are induced to form in *nrd*^{m805} mutant embryos after injection of *prdm1* mRNA (arrows point to several RB sensory neurons). *Crestin* expression in lateral views, anterior to the left. (B) Wildtype expression of *crestin* at 24 hpf. (D) *nrd* embryos have very little trunk neural crest migration (F) After injection of *prdm1* mRNA, numerous *crestin*-positive neural crest cells can be seen in the migration pathway (arrows). Abbreviations: wt, wildtype; *nrd*, *narrowminded* mutant embryos. Scale bar is 50 µm.

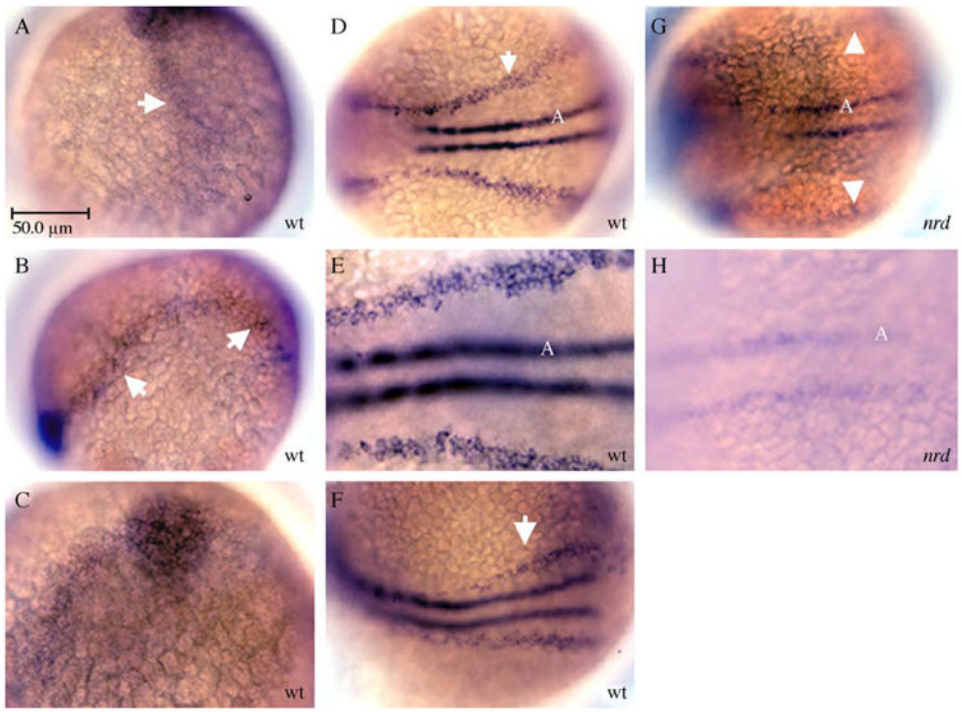

Fig. 4.

Expression of *prdm1* in the nervous system of wildtype and mutant *nrd* zebrafish embryos. Lateral views (A, B) and dorsal view(C–H) where anterior is to the left, except in A and C where anterior is to the top. (A) At 90% epiboly, expression is observed along the border of the neural plate where neural crest cells and RB sensory neurons are being specified (arrow), more strongly at the anterior-most border. At tailbud stages (B), expression remains high at the neural plate border (arrows). The expression is particularly strong at the anterior border at tail bud stages (C). (D) At 11 hpf, expression is high at the lateral edge of the neural plate (arrow; higher magnification in E), and also is expressed ventrally in the adaxial cells (A) adjacent to the midline. (F) By 13 hpf, the expression at the border of the neural plate remains only in the most posterior region (arrow). (G, H) Analysis of *prdm1* expression in *nrd* mutant embryos at 11 hpf. Low (G) and high (H) magnification images of *nrd*^{m805} embryos showing reduced expression of *prdm1*, especially at the neural plate border (arrowheads in G). A slight reduction in expression is also observed in the ventral adaxial cells (A). Abbreviations: wt, wildtype; *nrd*, *narrowminded* mutant embryos; A, adaxial cells. Scale bar is 50 µm.

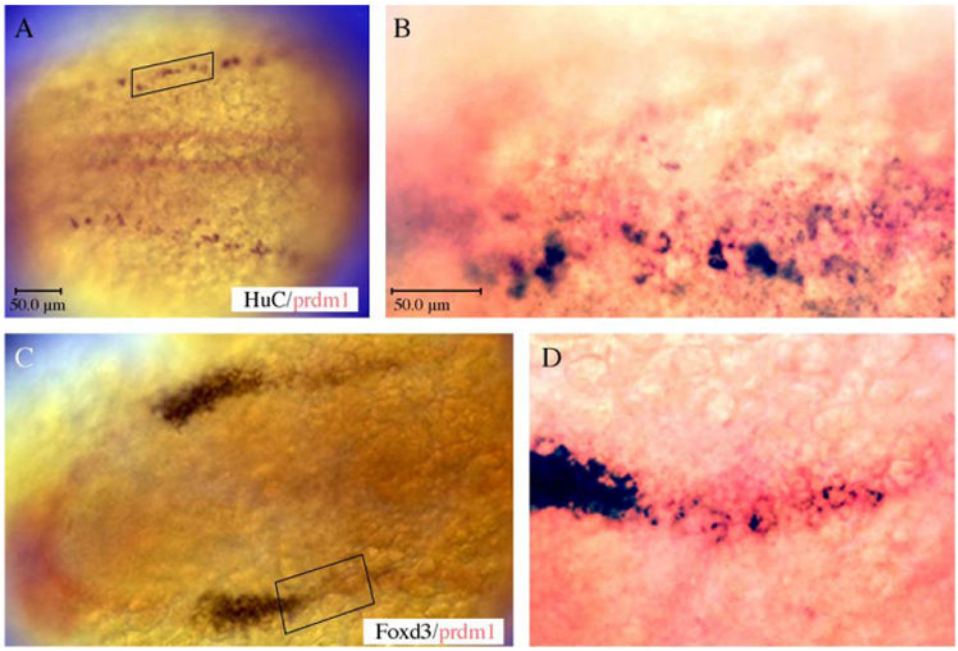

Fig. 5.

prdm1 is expressed in the domain where neural crest and Rohon–Beard sensory neurons form. Double in situ hybridization of *prdm1* with *Foxd3 and HuC* at 12 hpf; dorsal views, anterior to the left. (A, B) Low and high magnification views of *prdm1* (red) and *HuC* (blue) expression. *HuC*-positive cells are co-expressed in the *prdm1* expression domain. Boxes indicate region viewed under higher magnification in (B, D). (C, D) *prdm1* (red) and *Foxd3* (blue) expression indicate that neural crest cells are formed within the *prdm1*-expressing domain. Scale bar is 50 µm.

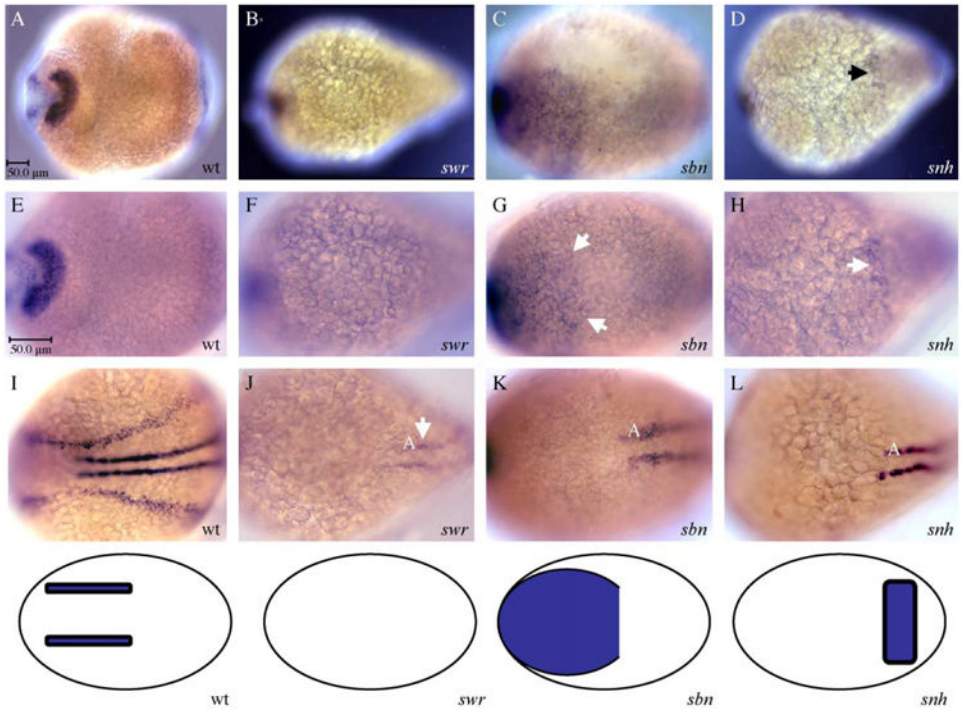

Fig. 6.

Expression of *prdm1* in the nervous system of BMP mutant zebrafish embryos. BMP mutants are visualized from the ventral side, where the reduction or expansion of the neural crest/RB sensory neuron domain is observed. Ventral (A–H) views of 12 hpf wildtype and BMP mutant embryos. *swr* (*Bmp2b*) mutant embryos (B, F) show an absence of *prdm1* mRNA at the lateral border of the neural plate (compare to wildtype in A, E). The domain of expression of *prdm1* is expanded in *sbn* (*Smad5*) embryos at 12 hpf (Low (C) and high (G) magnification; ventral views, compare to ventral view in wildtype in E), consistent with an increase of the neural crest/RB sensory neuron domain. Low (D) and high (H) magnification views of 12 hpf *snh* (*Bmp7*) showing a reduction in expression in the neural crest/RB sensory neuron domain of these embryos (arrows in D, H; compare to wildtype in A, E). Dorsal view of wildtype embryo in (I) as compared to dorsal views of *swr* (J), *sbn* (K), and *snh* (L) show that the adaxial expression (arrow in J) remains. Below, images are schematic representations of the neural crest domain in each of the mutant embryos as compared to wildtype. Since the BMP mutants are ventralized and the neural plate domain is expanded, the neural plate border form on the ventral side in *sbn* and *snh* and is completely absent in *swr*. Abbreviations: wt, wildtype; *swr*, *swirl*; *sbn*, *somitabun*; *snh*, *snailhouse;* A, adaxial cells. For (A–D), scale bar is shown in A and is 50 µm; for (E–L), scale bar is in E and is 50 µm.

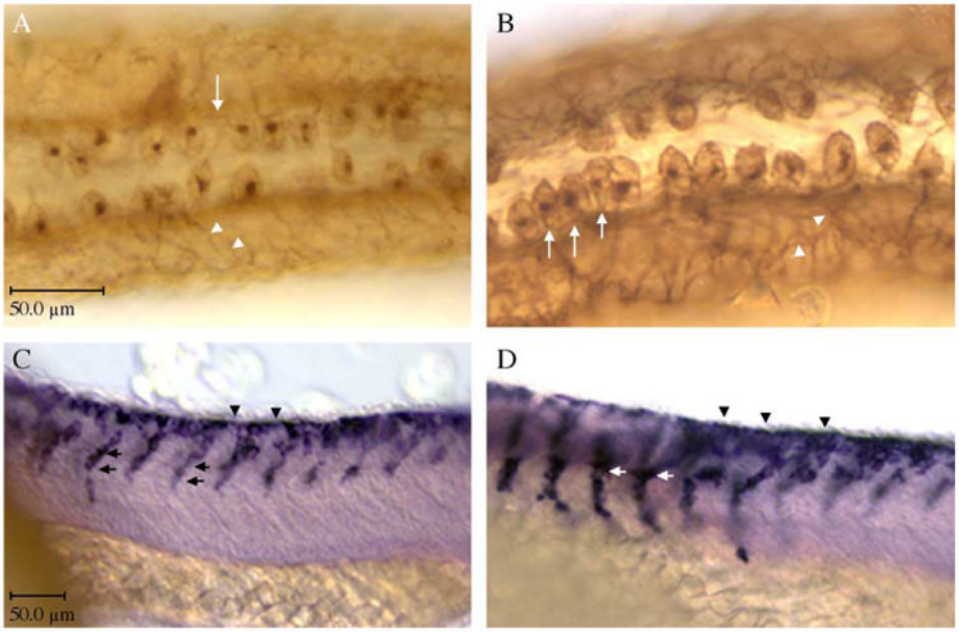

Fig. 7.

Ectopic expression of *prdm1* increases neural crest and Rohon–Beard sensory neurons. (A, B) HNK-1 immunohistochemistry at 24 hpf; dorsal views, anterior to the left. (A) Wildtype expression of HNK-1, (B) Subsequent to *prdm1* mRNA injections, RB sensory neurons are increased (arrows). (C, D) *Crestin* expression at 24 hpf, lateral views, anterior to the left. (C) Wildtype expression of *crestin*, (D) Following injection of *prdm1* mRNA, the neural crest emigration and migration streams appear thick with cells (arrows) as compared with the same region in wildtype embryos. The migration of the cells also appears premature in the *prdm1* expressing embryos. At the same time point in development, cells in *prdm1*-expressing embryos have migrated down to the yolk, whereas wildtype cells are only half way down their migration path. Scale bar for A, B is in A and is 50 µm; for C, D the scale bar is in C and is 50 µm.

Table 1

***prdm1* MO and rescue effects in zebrafish[a]**

Injection	Amounts (ng/embryo)	*N*	*N nrd −/−*	Phenotype[b] (% Reduced or absent)		Phenotype[b] (% Rescued)	
				NC	RBSN[c]	NC	RBSN[c]
Control MO	20	22		0	0		
prdm1-MO	6	15			13		
	12	47		56	40		
	16–18	37		57	100		
	24	8			100		
nrd + *prdm1*-RNA rescue	0.1–0.3	145	32				35
	0.3–0.6	103	25			20	49

[a] All sets of injection experiments were done at least three times.

[b] Analyzed at the 24 hpf.

[c] RBSN = Rohon–Beard sensory neuron, NC = neural crest.

Table 2

Overexpression effect of *prdm1* on the numbers of RB sensory neurons[a]

Injection	Amounts (ng/embryo)	*N*	Average number of RB sensory neurons[b]
Uninjected	0	12	54.8 ± 1.3
prdm1 RNA	0.25	10	59.2 ± 2.4
	0.7	22	58.7 ± 2.0
	0.91	6	60 ± 4.8

[a] All sets of injection experiments were done at least three times.

[b] The number of Rohon–Beard sensory neurons (RBSN) were counted on each side of a 24-hpf embryo, visualized with HNK-1 immunohistochemistry.