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Sox10 Is Necessary for Oligodendrocyte Survival Following Axon Wrapping

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Abstract

Cells of the oligodendrocyte lineage, which form the myelinating glia of the vertebrate central nervous system, undergo a stepwise developmental progression entailing specification from neuroepithelial precursors, proliferation, migration to expand and distribute the population, and differentiation to ensheath axons with myelin. Understanding the genetic mechanisms that regulate each of these steps during development is important, because this might lead to therapies to promote remyelination following neural injury or disease. Genetic studies in mice indicated that the Sox10 transcription factor is required during the differentiation stage to promote myelin gene expression. However, whether Sox10 also promotes other features of oligodendrocyte differentiation remained unknown. In this study, we used time-lapse imaging to investigate the behavior and fates of oligodendrocyte lineage cells in zebrafish embryos and larvae that lacked Sox10 function. This revealed that the myelinating subset of oligodendrocyte progenitor cells (OPCs) migrates, divides, and wraps axons normally, but then dies. Nonmyelinating oligodendrocyte progenitors divided more frequently, maintaining a normal population size. New oligodendrocytes produced by these progenitors wrapped axons and survived, but did not express myelin genes at high levels. We conclude that, in addition to promoting myelin gene expression, Sox10 function is necessary for the survival of myelinating oligodendrocytes subsequent to axon wrapping but is not required for the survival of nonmyelinating OPCs.

Keywords

Glia; myelin; CNS development; zebrafish; glia-neuron interaction; glia differentiation; Nkx2.2; Olig2

INTRODUCTION

Oligodendrocytes, the myelinating glial cells of vertebrate central nervous systems, are produced by specific subsets of neural precursors during development. In the spinal cord, the majority of oligodendrocytes arise from ventral precursors called pMN cells, which express the Olig2 bHLH transcription factor (Lu et al., 2000; Masahira et al., 2006; Park et al., 2004; Takebayashi et al., 2000; Zhou et al., 2000), although a small subset of oligodendrocytes arises from dorsal spinal cord (Cai et al., 2005; Fogarty et al., 2005; Vallstedt et al., 2005). Initially, oligodendrocyte lineage cells pass through a progenitor phase. These cells, called

oligodendrocyte progenitor cells (OPCs), divide to expand the OPC population and migrate to become dispersed throughout the spinal cord (Baumann and Pham-Dinh, 2001; Miller, 2002; Pfeiffer et al., 1993). Near the end of embryogenesis and during early postnatal stage, many OPCs stop dividing and extend long-membrane processes that wrap and myelinate axons. The stepwise progression of oligodendrocytes development implies the action of mechanisms that regulate their specification from neural precursors, migration from their origins to their target axons, proliferation, axon recognition, and wrapping and formation of myelin.

Among factors necessary for the development of oligodendrocyte are similar HMG-domain proteins of the SoxE family, Sox8, Sox9, and Sox10 (Wegner, 2008). Although genetic analyses in mice have revealed that these proteins have partially overlapping or redundant functions, they also play distinct roles in the oligodendrocyte cell lineage. For example, in the absence of Sox9 function, mouse embryos initially have a severe deficit of OPCs (Stolt et al., 2003). Therefore, Sox9 appears to be particularly important for OPC specification. On the other hand, Sox10-deficient embryos have normal number of OPCs, but deficits of myelin gene-expressing cells, indicating that Sox10 is required for oligodendrocyte differentiation (Stolt et al., 2002).

Analysis of mutant mice left unresolved whether Sox10 function is required early during oligodendrocyte differentiation to promote axon interaction or subsequent to axon wrapping to promote myelin formation. To address this, we investigated *sox10* function and oligodendrocyte development in zebrafish. Similar to mice, *sox10* mutant zebrafish embryos have normal numbers of OPCs, but a deficit of cells that expressed myelin genes. Using *in vivo* time-lapse imaging, we then learned that the myelinating subpopulation of oligodendrocytes initiate axon wrapping, but die soon after in *sox10* mutant embryos. Notably, mutant OPCs from a nonmyelinating subpopulation divide more frequently and wrap axons following oligodendrocyte death, but do not express myelin genes. These data therefore reveal a specific requirement for Sox10 in promoting survival of cells fated to form myelinating oligodendrocytes subsequent to axon wrapping.

METHODS

Animals

Embryos were obtained by pair-wise matings and kept at 28.5°C in egg water or embryo medium (15 mM NaCl, 0.5 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 0.15 mM KH₂PO₄, 0.05 mM NH₂PO₄, and 0.7 mM NaHCO₃). Embryos were staged to hours postfertilization (hpf) or days postfertilization (dpf) according to Kimmel et al. (1995). To prevent pigmentation, embryos were raised in egg water containing 0.003% phenylthiourea from 2 dpf. The following zebrafish lines were used: *Tg(olig2:egfp)* (Shin et al., 2003), *Tg(nkx2.2a:megfp)* (Kirby et al., 2006), *Tg(sox10(7.2):mrfp)* (Kucenas et al., 2008), and *colorless^{tw11} (cIs^{tw11})* (Dutton et al., 2001).

Whole-Mount RNA *In Situ* Hybridization, Immunocytochemistry, and BrdU Labeling

Embryos and larvae were fixed in 4% paraformaldehyde in 1× PBS overnight at 4°C and stored in methanol at −20°C at least for 1 day. *In situ* hybridization was performed essentially as described previously (Hauptmann and Gerster, 2000) using DIG-labeling RNA probes with the following modification: 4 dpf larvae were permeabilized with 10 mg/ml Proteinase K for 20 min at room temperature and additionally washed with 0.2× SSC twice for 15 min and then 0.1× SSC twice for 15 min following hybridization. *cldnk* (<http://zfin.org/>; NCBI accession no. NM_001003464) was identified in a microarray screen for oligodendrocyte-specific genes (N. Takada, unpublished). Other RNA probes used for this

study were *plp1a* (Park et al., 2002) and *mbp* (Brosamle and Halpern, 2002). Images were collected using an Olympus AX70 or Zeiss Axio Observer microscope equipped with DIC optics and a Retiga Exi 1300 color digital camera. Once captured, images were imported into Adobe Photoshop, and adjustments were limited to contrast, levels, color-matching settings, and cropping.

For immunocytochemistry, embryos and larvae were fixed in 4% AB fix (4% paraformaldehyde, 8% sucrose, and 1× PBS) overnight at 4°C. Embryos and larvae for sectioning were embedded in 1.5% agar/5% sucrose and placed in 30% sucrose/PBS solution to equilibrate. The blocks were frozen in 2-methyl-butane chilled by immersion in liquid nitrogen. The sections (12 µm) were obtained by using a cryostat microtome. A rabbit antibody against a MBP peptide (CSRSRSPPKRWSTIF) was raised commercially (Open Biosystems). Other primary antibodies used were rabbit anti-Sox10 (1:500) (Park et al., 2005), rabbit anti-cleaved Caspase 3 (PC697, Calbiochem, 1:100), mouse anti-acetylated Tubulin (6-11B-1, Sigma Aldrich, 1:100), and mouse anti-BrdU (G3G4, Developmental Studies Hybridoma Bank, 1:1,000). For fluorescent detection of antibody labeling, we used Alexa Fluor 568 and Alexa Fluor 647 goat anti-mouse or goat anti-rabbit conjugates (1:200, Invitrogen). Images were collected using a Zeiss LSM 510 laser scanning confocal microscope and exported and analyzed using Volocity software (Improvision) and Adobe Photoshop. Image adjustments were limited to level settings, contrast, and cropping.

For BrdU labeling, larvae were incubated in 0.5% solution of BrdU (Roche) in embryo medium for 24 h at the indicated stages. The embryos were then fixed using 4% pfa in PBS and sectioned as described earlier. Before anti-BrdU immunocytochemistry, the sections were treated for 30 min with 2 M HCl.

***In Vivo* Time-Lapse Imaging**

Embryos and larvae were anesthetized with 3-aminobenzoic acid ethyl ester (Tricaine), mounted laterally in 0.8% low-melting temperature agarose on 35-mm glass-bottomed dishes, and incubated in embryo medium. Confocal time-lapse movies were obtained by using a 40× oil immersion objective mounted on a Zeiss Axiovert 200 microscope equipped with a PerkinElmer spinning disk confocal system. Z-stack images were obtained and compiled into a QuickTime movie using Volocity software (Improvision). During time lapse, embryos and larvae were maintained at 28.5°C using a heated stage chamber.

RESULTS

Sox10 Is Required for Oligodendrocyte Differentiation

We began our investigation of zebrafish Sox10 by analyzing the number and distribution of OPCs in *colourless^{tw11}* (*cls*) mutant embryos, which carry a nonsense mutation that terminates translation immediately before the Sox10 transcription activation domain (Dutton et al., 2001). Embryos and larvae heterozygous for the *tw11* allele appear identical to wild type; consequently, this allele produces no appreciable dominant effect. To visualize OPCs, we used the transgenic reporter *Tg(olig2:egfp)*, which expresses EGFP in pMN precursors, motor neurons, and OPCs under control of *olig2* regulatory DNA (Shin et al., 2003). OPCs migrated normally in *cls^{-/-};Tg(olig2:egfp)* embryos and were indistinguishable in number from wild type through 6 dpf (Fig. 1A–H). To quantify OPCs, we cut transverse sections and counted EGFP⁺ cells that occupied the dorsal spinal cord. Consistent with our observations of living embryos, we found no difference in the number of dorsal OPCs at 80 hpf or 5.5 dpf (Fig. 1A'–H',I). To confirm identification of OPCs, we examined expression of Sox10, which specifically marks oligodendrocyte lineage cells in zebrafish (Park et al., 2002), using immunocytochemistry. Both dorsal and ventral EGFP⁺ cells expressed Sox10

in wild-type embryos and larvae (Fig. 1A'–D'). However, *cls* mutant embryos and larvae were not labeled by anti-Sox10 antibody (Fig. 1E'–H'), because the *cls^{tw11}* allele eliminates the peptide sequence used for immunization. Nevertheless, both ventral and dorsal EGFP⁺ cells had morphologies and occupied positions characteristic of OPCs.

We next used gene expression to assess oligodendrocyte differentiation. At 4.5 dpf, oligodendrocytes of wild-type larvae expressed RNA encoded by *plp1a*, *mbp*, and *cldnk* (Fig. 2A–C). On the other hand, very few cells expressed the same genes in similarly staged *cls*^{−/−} larvae (Fig. 2D–F,I). We also examined MBP expression using immunocytochemistry. By 6 dpf, some MBP accumulation became evident in wild-type larvae, particularly surrounding the large Mauthner axons in ventral spinal cord (Fig. 2G), whereas *cls*^{−/−} larvae had very little MBP expression (Fig. 2H). Together, these data show that *cls* mutant larvae have normal numbers of oligodendrocyte lineage cells, but a deficit of cells that express myelin genes. Therefore, Sox10 function is necessary for oligodendrocyte differentiation but not specification, indicating that Sox10 has similar functions in zebrafish and mice.

Sox10 Function Is Required for Oligodendrocyte Survival Following Axon Wrapping

Oligodendrocyte differentiation proceeds through a series of steps including membrane extension, axon contact, and wrapping, formation of paranodal and juxtaparanodal specializations, and expression of myelin genes and myelin compaction. To further define the requirement for Sox10 function in oligodendrocyte differentiation, we used the *Tg(nkx2.2a:megfp)* transgenic reporter, which expresses membrane-tethered EGFP in a myelinating subset of oligodendrocyte lineage cells (Kirby et al., 2006; Kucenas et al., 2008). As we have shown previously (Kirby et al., 2006), premyelinating OPCs extended long-membrane processes in wild-type embryos (Fig. 3A). By 3 dpf, oligodendrocytes began to wrap axons, forming longitudinally arrayed internodes (Fig. 3B). At 60 hpf, OPC morphology in *cls*^{−/−} embryos was similar to that of OPCs in wild type (Fig. 3C). However, at 3 dpf, *cls*^{−/−} larvae had a deficit of *nkx2.2a*⁺ cells and showed little evidence of axon wrapping (Fig. 3D).

To learn if OPCs can initiate axon wrapping in the absence of Sox10 function, we examined sectioned material obtained from 64 hpf embryos and labeled with anti-acetylated tubulin to mark axons. In transverse sections of wild-type *Tg(nkx2.2a:megfp)* embryos, green OPC processes encircle axons, indicative of wrapping (Fig. 3E–G). Wrapped axons were also evident in *cls*^{−/−}:*Tg(nkx2.2a:megfp)* embryos, although at lower frequency (Fig. 3K–M). Sagittal sections also revealed axon wrapping by OPCs in both wild-type and *cls*^{−/−} embryos (Fig. 3H–J,N–P). These data raise the possibility that Sox10 function is not necessary to initiate axon wrapping by OPCs, but is required for the maintenance of wrapping.

To learn the fate of *nkx2.2a*⁺ OPCs, we performed *in vivo* time-lapse imaging. This confirmed our observations made by examining embryos and larvae at fixed time points that OPC migration and membrane process activity were normal (Fig. 4; Supp. Info. Movie 1). However, soon after the initiation of axon wrapping, oligodendrocytes fragmented and died (Fig. 4; Supp. Info. Movie 1). We also labeled wild-type and *cls*^{−/−} larvae, carrying the *Tg(olig2:egfp)* reporter with antibody specific to activated Caspase-3, which serves as a marker of cells undergoing apoptotic death. *olig2*⁺ cells were rarely labeled by activated Caspase-3 antibody (Fig. 5A–C), whereas mutant larvae had numerous *olig2*⁺ oligodendrocyte lineage cells that expressed activated Caspase-3 (Fig. 5D–J). Therefore, Sox10 is required for the survival of myelinating oligodendrocytes following initiation of axon wrapping.

Because OPC number, marked by *olig2* reporter gene expression, was normal in *cls*^{-/-} larvae, the deficit of *nkx2.2a*⁺ oligodendrocytes at 3 dpf from cell death was unexpected. We therefore examined the *nkx2.2a*⁻ subpopulation of OPCs. To do so, we used *Tg(sox10(7.2):mrfp)*, which expresses a membrane-tethered RFP in all oligodendrocyte lineage cells under the control of *sox10* regulatory DNA (Kirby et al., 2006; Kucenas et al., 2008). At 4 and 6 dpf, the majority of oligodendrocytes with wrapping morphologies in wild-type *Tg(nkx2.2a:megfp); Tg(sox10(7.2):mrfp)* larvae were *nkx2.2a*⁺ *sox10*⁺ (Fig. 6A–F,M–O). A smaller number of wrapping oligodendrocytes and all nonmyelinating OPCs were *nkx2.2a*⁻ *sox10*⁺ (Fig. 6A–F and data not shown) (Kucenas et al., 2008). On the other hand, the majority of oligodendrocyte lineage cells in *cls*^{-/-}; *Tg(nkx2.2a:megfp); Tg(sox10(7.2):mrfp)* larvae were *nkx2.2a*⁻ *sox10*⁺, and, as shown earlier, there were fewer wrapping oligodendrocytes (Fig. 6G–L,P–R). Furthermore, *cls*^{-/-} larvae appeared to have more *nkx2.2a*⁻ *sox10*⁺ OPCs than wild type. Therefore, loss of Sox10 function results in a deficit of myelinating oligodendrocytes and an apparent expansion in the nonmyelinating OPC population.

To examine directly the behaviors of myelinating and nonmyelinating subpopulations of oligodendrocyte lineage cells in the absence of Sox10 function, we performed time-lapse imaging of *cls*^{-/-}; *Tg(nkx2.2a:megfp); Tg(sox10(7.2):mrfp)* larvae. Notably, we observed frequent divisions of both *nkx2.2a*⁺ and *nkx2.2a*⁻ OPCs (Fig. 7A and Supp. Info. Movie 2). OPC divisions during similar periods in wild-type larvae are more rare (Kirby et al., 2006). Both *nkx2.2a*⁺ and *nkx2.2a*⁻ OPCs initiated axon wrapping. However, *nkx2.2a*⁺ oligodendrocytes died within a few hours after wrapping, whereas *nkx2.2a*⁻ OPCs persisted (Table 1). To further investigate OPC division, we exposed wild-type and mutant larvae to BrdU, a thymidine analog that is incorporated into the DNA of S-phase cells. *cls*^{-/-} larvae had significantly more BrdU⁺ *olig2*⁺ OPCs than wild type, whereas the number of BrdU⁺ *olig2*⁺ ventral spinal cord precursors was unchanged (Fig. 7B). We conclude that, in the absence of *sox10* function, non-myelinating OPCs divide more frequently, consequently maintaining normal numbers of oligodendrocyte lineage cells, but are unable to generate myelinating oligodendrocytes.

DISCUSSION

In the zebrafish spinal cord, OPCs initiate expression of *sox10* as they are formed by ventral neural precursors (Kucenas et al., 2008; Park et al., 2002). Many OPCs migrate dorsally as they constantly extend and retract long-membrane processes until, at early larval stage, a subset begins to wrap and myelinate axons (Kirby et al., 2006). Most myelinating oligodendrocytes express *nkx2.2a*, whereas nonmyelinating OPCs do not (Kucenas et al., 2008). Very few spinal cord oligodendrocyte lineage cells die during normal development in zebrafish, but cells ablated with a laser are rapidly replaced by nearby OPCs (Kirby et al., 2006). Therefore, zebrafish oligodendrocyte lineage cells consist of dynamic and interacting populations of myelinating and nonmyelinating cells.

The structurally similar SoxE transcription factors Sox8, Sox9, and Sox10 appear to contribute both comparable and distinct functions in the development of oligodendrocyte lineage cells in mice (Wegner, 2008). Sox9 is expressed widely in neuroepithelial precursors, including pMN cells, during neurogenic and gliogenic periods (Stolt et al., 2003). Sox8 is initiated later and apparently restricted to pMN precursors and their descendent oligodendrocyte lineage cells, whereas Sox10 expression is limited to OPCs and oligodendrocytes (Stolt et al., 2002, 2005). Therefore, although expression of each Sox protein is initiated at a different time in development, there is a substantial overlap of expression in cells of the oligodendrocyte lineage. The results of various gene inactivation tests are consistent with the possibility that coexpressed Sox proteins provide at least

partially overlapping functions. *Sox9* mutant embryos initially have substantial deficits of OPCs, but these are later restored to near normal number (Stolt et al., 2003). Loss of *Sox8* function, which, on its own, has little impact on OPC formation, in combination with *Sox9* mutation, eliminates nearly all OPCs (Stolt et al., 2005). In *Sox10* mutant mice, OPCs are formed in apparently normal number, but fail to differentiate into myelinating oligodendrocytes (Stolt et al., 2002). Oligodendrocyte differentiation is only slightly and transiently delayed in *Sox8* homozygous mutant mice, but the additional inactivation of one copy of *Sox10* greatly delays differentiation (Stolt et al., 2004), indicating that both *Sox8* and *Sox10* can promote oligodendrocyte maturation and myelination. The combined loss of *Sox9* and *Sox10* functions following OPC formation causes a reduction of oligodendrocyte lineage cells by apoptosis, which results, at least in part, from reduced expression of the PDGF receptor alpha (PDGFR α) in OPCs (Finzsch et al., 2008). Therefore, *Sox8*, *Sox9*, and *Sox10* together promote formation, differentiation, and survival of oligodendrocyte lineage cells.

Thus far, published evidence indicates that *Sox10* promotes oligodendrocyte differentiation by promoting expression of a subset of myelin genes. *Sox10* binds to *MBP* genomic DNA *in vitro* and *in vivo* and can promote reporter gene expression via *MBP* sequence in cell culture (Li et al., 2007; Stolt et al., 2002; Wei et al., 2004). *Sox10* also binds to genomic DNA from the *Myelin Protein Zero* gene to promote its expression in Schwann cells (Peirano and Wegner, 2000; Peirano et al., 2000). The reduction of myelin gene expression, we observed in *cls/sox10* mutant zebrafish embryos, is consistent with the direct action of *Sox10* at regulatory sequences associated with myelin genes. However, *Sox10* alone or in combination with *Olig1* did not promote expression of zebrafish *plp1b* or *mpz*, consistent with an apparent absence of *Sox10*-binding sites within genomic sequence upstream of the *plp1b* and *mpz* transcription start sites (Li et al., 2007). Nevertheless, *Plp* gene expression is reduced in both mouse and zebrafish *Sox10* mutants (Stolt et al., 2002) (this study). Therefore, reduced *Plp* gene expression might be an indirect consequence of *Sox10* deficiency.

Our time-lapse imaging now adds an additional level of insight to the role of *Sox10* in oligodendrocyte development. These studies revealed that in embryos and larvae homozygous for the *cls^{tw11}* allele, the myelinating subset of OPCs, which expresses *olig2*, *sox10*, and *nkx2.2a*, migrates, extends membrane processes, and wraps axons normally, but then rapidly dies. Therefore, *Sox10* is required for the survival of myelinating oligodendrocytes, and the absence of myelin gene expression reflects loss of this cell population. This observation raises the possibility that *Sox10* also promotes expression of genes that encode factors that mediate interactions between axons and oligodendrocytes necessary for oligodendrocyte survival, although we cannot rule out the possibility that oligodendrocyte death is an indirect consequence of their failure to fully differentiate. A potential direct requirement is probably distinct from the requirement of *Sox10* in combination with *Sox8* to maintain high levels of PDGFR α expression in OPCs, because oligodendrocytes downregulate PDGFR α expression as they differentiate (Butt et al., 1997; Chittajallu et al., 2005; Ellison and de Vellis, 1994; Oumesmar et al., 1997).

Several previous observations have implicated the importance of axons for oligodendrocyte survival. For example, transection of rat optic nerves resulted in loss of oligodendrocytes by programmed cell death and oligodendrocytes survived longer in culture in the presence of neurons (Barres et al., 1993). In the rodent brain, many oligodendrocyte lineage cells that were not in apparent contact with axons died, whereas oligodendrocytes that wrapped axons were spared (Trapp et al., 1997). One class of molecule implicated in mediating axon-dependent oligodendrocyte survival and is therefore a candidate target for *Sox10* regulation is the integrins, which are receptors for extracellular matrix components such as laminin.

Neurons express laminins (Liesi et al., 2001), and some axons are surrounded by a laminin-rich extracellular matrix at the time of myelination (Colognato et al., 2002; Farwell and Dubord-Tomasetti, 1999). Laminin-2 and the laminin receptor $\alpha 6 \beta 1$ increase oligodendrocyte survival in response to growth factors in culture (Frost et al., 1999). Consistent with this, mice lacking the $\alpha 6$ integrin subunit have an elevated number of dying oligodendrocytes (Colognato et al., 2002). Mice lacking the $\beta 1$ subunit similarly had elevated levels of apoptotic oligodendrocytes, although myelination appeared normal (Benninger et al., 2006). Therefore, expression of genes encoding the $\alpha 6$ and $\beta 1$ subunits is conceivably controlled by Sox10.

Despite the loss of myelinating oligodendrocytes from *cls/sox10* mutants, the overall number of oligodendrocyte lineage cells was normal. Similarly, *Sox10* mutant mice have normal numbers of oligodendrocyte lineage cells but a deficit of cells that express myelin genes (Stolt et al., 2002). Our time-lapse imaging and BrdU studies provide evidence that oligodendrocyte death is accompanied by more frequent divisions of nonmyelinating OPCs. Previously, we showed that following ablation of oligodendrocytes and OPCs using a laser, neighboring OPCs could divide more frequently, migrate into the ablated region, and wrap axons (Kirby et al., 2006). OPCs continuously extend long-membrane processes, and we interpreted such protrusive activity as a surveillance mechanism that helps determine oligodendrocyte spacing. We speculate that the replacement of dying oligodendrocytes in *cls/sox10* mutant larvae also results from the surveillance of oligodendrocyte distribution by nonmyelinating OPCs, which express the transgenic *sox10* reporter (but are genetically deficient for *sox10* function) but do not express *nkx2.2a*. Notably, these newly produced OPCs wrapped axons, but did not express myelin genes at high levels. Consequently, neither *sox10* nor *nkx2.2a* functions are necessary for axon-wrapping behavior by oligodendrocyte lineage cells. Additionally, these cells persisted until the end of our imaging experiments, longer than the first wave of myelinating oligodendrocytes. Although we do not know the fate of these secondary wrapping OPCs, because *cls* mutant larvae die by about 6 dpf, this observation suggests that nonmyelinating OPCs do not become dependent on axonal signals for survival, even when they come in physical contact with axons. During normal development and homeostasis, different survival mechanisms for OPCs and oligodendrocytes likely help match the number of myelinating cells to target axons while maintaining a large population of OPCs. Coordinate action of Sox10 and Nkx2.2 transcription factors therefore might couple expression of myelin genes with the expression of factors that mediate axon-dependent survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Loss of *sox10* function does not alter oligodendrocyte lineage cell number or distribution.

A–H: Lateral views of *Tg(olig2:egfp)* reporter expression at the level of the trunk spinal cord in wild-type and *cls*^{-/-} embryos and larvae. Dorsal is up. Asterisks in (A) indicate examples of dorsally migrated OPCs. Bracket marks the spinal cord (SC). **A'–G' :** Transverse sections, dorsal up, showing *Tg(olig2:egfp)* reporter expression (green) and Sox10 protein expression (red). Arrows indicate *olig2*⁺ *Sox10*⁺ OPCs in wild-type embryos and larvae (A'–C') and *olig2*⁺ *Sox10*⁻ OPCs in *cls*^{-/-} embryos and larvae (E'–G'). **I:** Quantification of dorsally migrated *olig2*⁺ OPCs reveals no difference between wild type and mutant. Error bars represent SEM. Scale bars, 24 μM.

Fig. 2. *sox10* function is required for oligodendrocyte differentiation. **A–F:** Lateral views of spinal cords, dorsal up, showing *plp1a*, *cldnk*, and *mbp* RNA expression in 4.5-dpf larvae. Brackets mark spinal cord (SC) and notochord (NC). **G, H:** Transverse sections showing MBP expression (red) and anti-acetylated tubulin labeling (blue) at 6 dpf detected by immunocytochemistry. Arrows indicate prominent MBP deposition surrounding Mauthner axons in wild-type larva. **I:** Quantification of *plp1a*⁺ and *cldnk*⁺ cells. Graphs show dorsal, ventral, and total spinal cord cells that express these genes. Error bars represent the SEM. Scale bar, 24 μ M.

Fig. 3. OPCs initiate axon wrapping in *cls* mutant embryos. **A–D:** Lateral views of spinal cords, dorsal up, of wild-type and *cls*^{−/−} embryos and larvae carrying the *Tg(nkx2.2a:megfp)* reporter to mark the myelinating subset of oligodendrocyte lineage cells. Asterisks mark dorsally migrated OPCs and arrows indicate axon wrapping. OPC number and morphology are similar in wild-type and *cls*^{−/−} embryos at 60 hpf (A, C), but mutants have a deficit of oligodendrocytes at 3 dpf (B, D). Transverse (**E–G**, **K–M**) and sagittal (**H–J**, **N–P**) sections of 64 hpf wild-type and *cls*^{−/−} spinal cords expressing the *Tg(nkx2.2a:megfp)* reporter (green) and labeled with anti-acetylated Tubulin (red). OPC processes ensheath axons in both wild-type (**E–J**) and mutant (**K–P**) embryos. Scale bars, 24 μM (A–D), 4.5 μM (E–G, K–M), and 9 μM (H–J, N–P).

Fig. 4.

Sox10 is required for oligodendrocyte survival following axon wrapping. Frames captured from time-lapse movie of a *cls*^{-/-}; *Tg(nkx2.2a:megfp)* embryo beginning at about 60 hpf. Panels show lateral images of the spinal cord, with dorsal up. Numbers indicate time elapsed since the beginning of the sequence. Large arrow and arrowhead point to the soma of oligodendrocytes, and small arrows and arrowheads indicate initiation of axon wrapping. Oligodendrocytes begin to fragment and die following axon wrapping. Asterisk marks an OPC that migrated into the field of view following oligodendrocyte death. Scale bar, 24 μ M.

Fig. 5. Oligodendrocytes undergo apoptotic death in *c/s* mutant embryos. Transverse sections of 64-hpf wild-type (A–C) and *c/s* mutant (D–J) spinal cords expressing the *Tg(olig2:egfp)* reporter (green) and labeled with antiactivated Caspase-3 antibody (red). Arrows indicate *olig2*⁺ activated Caspase-3⁺ cells with fragmented appearance. Scale bar, 15 μ M.

Fig. 6.

nkx2.2a⁻ oligodendrocytes wrap axons in *cls*^{-/-} larvae. All images show lateral views of spinal cords, dorsal up, of wild-type and *cls*^{-/-} larvae carrying *Tg(sox10(7.2):mrfp)* and *Tg(nkx2.2a:megfp)* reporters. In wild type (A–F), most myelinating oligodendrocytes express both *nkx2.2* and *sox10* reporters (arrowheads). In *cls* mutants (G–L), some axon wrapping by cells marked by *sox10* reporter expression is evident, but these cells appear to express the *nkx2.2* reporter at low or nonexistent levels (arrowheads). (M–R) Higher magnification views showing axon wrapping by *sox10*⁺ *nkx2.2a*⁺ oligodendrocytes in wild type (M–O; arrowheads) but that in *cls* mutants oligodendrocytes that wrap axons express only the *sox10* reporter (P–R; arrows). Some axons in wild type are also wrapped by oligodendrocytes that express only the *sox10* reporter (N,O; arrows). Brackets mark *nkx2.2a*⁺ axons that descend from the hindbrain through the dorsal longitudinal fasciculus (DLF). Scale bars, 24 μ M.

Fig. 7.

OPCs divide and wrap axons following oligodendrocyte death in *cls^{-/-}* larvae. **A:** Frames captured from a time-lapse movie of a *cls^{-/-}; Tg(nkx2.2a:megfp); Tg(sox10(7.2):mrfp)* larva beginning at 4.5 dpf. Numbers indicate time elapsed since the beginning of the sequence. Panels show lateral views of the spinal cord, with dorsal up. Arrowheads and arrows indicate OPCs. Most OPCs within the field of view divided during the 14-h period of the time-lapse imaging. Dotted box outlines axon wrapping by an oligodendrocyte expressing only the *sox10* transgenic reporter. **B:** Quantification of OPC division. Wild-type and *cls^{-/-}* larvae carrying the *Tg(olig2:egfp)* transgenic reporter were incubated in a BrdU solution for 24 h from 5 to 6 dpf. Graphs show number of BrdU⁺ *olig2⁺* dorsal OPCs and BrdU⁺ *olig2⁺* pMN precursors. Error bars indicate the SEM. Statistical significance was determined using the unpaired *t*-test.

TABLE 1Survival Time of Oligodendrocytes After Initiating the Axon Wrapping in *cls*^{-/-}

Nkx2.2a ⁺		Nkx2.2a ⁻	
Case 1	9 h	Case 1	>14
Case 2	11 h	Case 2	>14
Case 3	8 h	Case 3	>20
Case 4	11 h		
Case 5	3 h		
Case 6	4 h		
Case 7	12 h		