Transcription factors Sox8 and Sox10 perform non-equivalent roles during oligodendrocyte development despite functional redundancy

C. Claus Stolt, Petra Lommes, Ralf P. Friedrich and Michael Wegner*

Institut für Biochemie, Universität Erlangen, Fahrstrasse 17, D-91054 Erlangen, Germany
*Author for correspondence (e-mail: m.wegner@biochem.uni-erlangen.de)

Accepted 9 February 2004

Development 131, 2349-2358
Published by The Company of Biologists 2004
doi:10.1242/dev.01114

Summary

Development of myelin-forming oligodendrocytes in the central nervous system is dependent on at least two members of the Sox family of high-mobility-group-containing transcription factors. Sox9 is involved in oligodendrocyte specification, whereas Sox10 is required for terminal differentiation. We show that oligodendrocytes in the spinal cord additionally express the highly related Sox8. In Sox8-deficient mice, oligodendrocyte development proceeded normally until birth. However, terminal differentiation of oligodendrocytes was transiently delayed at early postnatal times. Sox8-deficient mice thus exhibited a similar, but less severe phenotype than did Sox10-deficient mice. Terminal oligodendrocyte differentiation was dramatically delayed in Sox8-deficient mice with only a single functional *Sox10* allele hinting at redundancy between both Sox proteins. This redundancy was also evident from the fact that Sox8 bound to naturally occurring Sox10 response elements, was able to form DNA-dependent heterodimers with Sox10 and activated Sox10-specific oligodendrocytic target genes in a manner similar to Sox10. However, Sox8 expression levels were significantly lower than those for Sox10. Resulting differences in protein amounts might be a main reason for the weaker impact of Sox8 on oligodendrocyte development and for unidirectional compensation of the Sox8 loss by Sox10.

Supplemental data available online

Key words: Sry, High-mobility-group, Sox10, Redundancy, Gene dosage, Oligodendrocyte

Introduction

Transcription factors of the Sox protein family represent important developmental regulators in vertebrates as well as invertebrates (Wegner, 1999). With sequencing of their genomes completed, it is now evident that there are 20 Sox proteins in mice and humans (Schepers et al., 2002). Sox proteins can be divided into 10 groups numbered A-J, with groups B-F being represented by at least one member in *Drosophila* and in all vertebrate model organisms (Bowles et al., 2000; Wegner, 1999).

Sox8 has been recently identified as a member of Sox group E (Pfeifer et al., 2000; Schepers et al., 2000) and shares extensive sequence homology with Sox9 and Sox10, the other two members of this group of Sox proteins (Wegner, 1999). Sox9 and Sox10 have been characterized extensively for their involvement in chondrogenesis, male sex determination, neural crest development and gliogenesis, respectively (Bi et al., 1999; Britsch et al., 2001; Foster et al., 1994; Herbarth et al., 1998b; Pingault et al., 1998; Southard-Smith et al., 1998; Stolt et al., 2003; Stolt et al., 2002; Wagner et al., 1994).

Although expression pattern and behaviour in cell culture systems are indicative of Sox8 functions in the male gonad and in skeletal muscle (Schepers et al., 2003; Schmidt et al., 2003), analysis of Sox8-deficient mice has so far failed to reveal severe defects in the development of major organ systems (Sock et al., 2001). Redundancy and functional compensation by related factors is one of the most plausible explanations for the lack of a severe phenotype in Sox8-deficient mice. Supporting such an assumption, most Sox8-expressing cells are also positive for either Sox9 or Sox10 (Sock et al., 2001). In case of the male gonad, for example, Sertoli cells express Sox8 as well as Sox9 (Schepers et al., 2003). If functional compensation indeed exists between the three Sox E proteins, it must be non-reciprocal as both Sox9- and Sox10-deficient mice exhibit severe developmental defects despite the continued presence of Sox8.

We have previously noted that Sox8 expression in the developing spinal cord is indicative of an expression in the oligodendrocyte lineage (Sock et al., 2001). These glial cells of the central nervous system arise from neural stem cells in a limited domain of the ventral ventricular zone (Pringle and Richardson, 1993), which earlier gives rise to motoneurons (Briscoe et al., 1999). From this pMN domain, oligodendrocyte progenitors spread throughout the whole spinal cord as embryogenesis proceeds. At the end of embryogenesis, they accumulate in the marginal zone and start to differentiate. Terminal differentiation of oligodendrocytes peaks during the first postnatal weeks, and is characterized by the production of large amounts of lipids and a limited set of myelin proteins such as myelin-associated glycoprotein (Mag), myelin basic protein (Mbp) and proteolipid protein (Plp) (Lemke, 1988). The resulting formation of myelin sheaths around axons allows

rapid saltatory conductance in the central nervous system (CNS).

Recently, several transcription factors have been identified as cell-intrinsic regulators of oligodendrocyte development. These include the Olig1 and Olig2 bHLH proteins, neurogenins and Nkx2.2 (Lu et al., 2002; Nieto et al., 2001; Qi et al., 2001; Takebayashi et al., 2002; Zhou and Anderson, 2002; Zhou et al., 2001). Sox proteins also feature prominently during development of these cells. Early specification of oligodendrocyte progenitors requires Sox9 (Stolt et al., 2003). Once specified, oligodendrocyte progenitors express both Sox9 and Sox10, and can cope with loss of either protein (Stolt et al., 2003; Stolt et al., 2002). A role for Sox10 becomes evident at the onset of terminal differentiation when Sox9 expression is naturally extinguished in oligodendrocytes. In Sox10-deficient spinal cords, only few oligodendrocyte progenitors start to express myelin proteins with delayed kinetics and in amounts insufficient for myelination (Stolt et al., 2002). The residual myelin gene expression in Sox10-deficient spinal cords is compatible with the existence of a third activity besides Sox9 and Sox10 with overlapping expression and function. In view of its possible expression in the oligodendrocyte lineage (Sock et al., 2001), Sox8 is an attractive candidate. Its role during oligodendrocyte development in the spinal cord is addressed in the present study.

Materials and methods

Animal husbandry and genotyping

Mice with a *Sox8*^{lacZ} allele (Sock et al., 2001) were kept as heterozygotes in the presence or absence of a *Sox10*^{lacZ} allele on a mixed C3H/C57Bl6J background (Britsch et al., 2001). For the generation of embryos and pups during this study, *Sox8*$^{lacZ/+}$ mice were crossed with *Sox8*$^{lacZ/+}$, *Sox10*$^{lacZ/+}$ mice. Genotyping was performed by PCR as described (Britsch et al., 2001; Sock et al., 2001).

Tissue preparation, immunohistochemistry, X-Gal staining, in situ hybridization and quantification of β-galactosidase

Embryos (from 12.5 dpc to 18.5 dpc) and pups (at postnatal days 1, 3 and 7) were obtained from staged pregnancies. Spinal cords were additionally dissected from adult animals. After fixation in paraformaldehyde, genotyped, age-matched tissues were sectioned on a cryotome. Sections (10 μm) were used for immunohistochemistry, 20 μm sections for X-Gal staining and in situ hybridization according to standard protocols as previously described (Stolt et al., 2003; Stolt et al., 2002). For better comparison, all shown spinal cord sections are from the forelimb level. For immunohistochemistry, the following primary antibodies were used in various combinations: anti-Mbp mouse monoclonal (1:100 dilution, Chemicon), anti-GFAP mouse monoclonal (1:100 dilution, Chemicon), anti-NeuN mouse monoclonal (1:500 dilution, Chemicon), anti-Mag mouse monoclonal (undiluted, gift of U. Bartsch, Hamburg University), anti-Olig2 rabbit antiserum (1:2000 dilution, gift of H. Takebayashi, Kyoto University), anti-Sox10 rabbit antiserum (1:100 dilution) (Stolt et al., 2003), anti-β-galactosidase rabbit antiserum (1:500 dilution, ICN) or anti-β-galactosidase goat antiserum (1:500 dilution, Biotrend). Secondary antibodies conjugated to Cy2 and Cy3 immunofluorescent dyes (Dianova) were used for detection. In situ hybridization was performed with DIG-labeled antisense riboprobes for Mbp and Plp (Stolt et al., 2002). Samples were analyzed and documented using either a Leica TCS SL confocal microscope or a Leica inverted microscope (DMIRB) equipped with a cooled MicroMax CCD camera (Princeton Instruments, Stanford, CA). Quantification of β-galactosidase was performed on extracts prepared by homogenization of freshly dissected spinal cords using a chemiluminescent β-galactosidase detection assay (Roche Biochemicals).

Cell culture, RT-PCR and luciferase assays

N2A neuroblastoma cells were maintained in Dulbecco's Modified Eagle's Medium containing 5% fetal calf serum, and transfected using Superfect reagent (Qiagen). Stable N2A cell clones capable of doxycycline-dependent, inducible Sox8 expression were generated as previously described for Sox10 (Peirano et al., 2000). RNA from these cells was prepared in both the induced (Sox8 positive) and uninduced (Sox8 negative) state. After reverse transcription to cDNA, semi-quantitative PCR was performed to detect products specific for Sox8, Plp, Mbp and β-actin. For luciferase assays, N2A cells were transfected transiently in duplicates in 24-well plates with 200 ng of luciferase reporter plasmid and 200 ng of effector plasmids per well. Luciferase reporters containing a long version (positions –656 to +31) and a short version (positions –256 to +31) of the *Mbp* promoter were used (Stolt et al., 2002). Effector plasmids corresponded to pCMV5-based expression plasmids for Sox8 (Schmidt et al., 2003) and Sox10 (Kuhlbrodt et al., 1998a). Cells were harvested 48 hours post-transfection, and extracts were assayed for luciferase activity (Stolt et al., 2002).

Protein extracts and electrophoretic mobility shift assay

Extracts from transfected N2A cells (10 cm dishes) ectopically expressing full-length or shortened Sox8 and Sox10 proteins, were prepared as described (Kuhlbrodt et al., 1998b). For electrophoretic mobility shift assays, protein extracts were incubated with 0.5 ng of ^{32}P-labeled oligonucleotide probes for 20 minutes on ice in a 20 μl reaction mixture containing 10 mM Hepes (pH 8.0), 5% glycerol, 50 mM NaCl, 5 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 4 μg of bovine serum albumin, and 2 μg of poly(dGdC) as unspecific competitor. The following oligonucleotide probes were used: sites 1-3 from the *Mbp* promoter (Stolt et al., 2002) and the prototypic dimeric binding site C/C′ from the Protein zero promoter (Peirano et al., 2000; Peirano and Wegner, 2000; Schlierf et al., 2002). For supershift experiments, 0.1 μl of antisera were additionally added after 10 minutes, and incubation was continued for a further 10 minutes. Samples were loaded onto native 4% polyacrylamide gels and electrophoresed in 0.5×TBE (45 mM Tris/45 mM boric acid/1 mM EDTA, pH 8.3) at 120 V for 1.5 hours. Gels were dried and exposed for autoradiography.

Results

Spinal cord expression of Sox8 is restricted to the oligodendrocyte lineage

We have generated a mouse model in which the complete open reading frame of the *Sox8* gene has been replaced by a *lacZ* marker gene (Sock et al., 2001). The design of this mutant *Sox8* allele (*Sox8*^{lacZ}) matches closely that of our *Sox10*^{lacZ} allele (Britsch et al., 2001). In both mouse strains, β-galactosidase expression faithfully recapitulates expression of the corresponding Sox gene as evident from comparison of in situ hybridization with gene-specific riboprobes and X-gal staining. We exploited the β-galactosidase marker to visualize Sox8 expression in the developing spinal cord of *Sox8*$^{+/lacZ}$ mice which have previously been shown to be viable, fertile and without severe CNS phenotype (Sock et al., 2001).

At 12.5 days post coitum (dpc), X-gal staining was restricted within the spinal cord to a limited domain in the ventral part of the ventricular zone whose position is identical to or strongly overlapping with the pMN domain (Fig. 1A). Two days later,

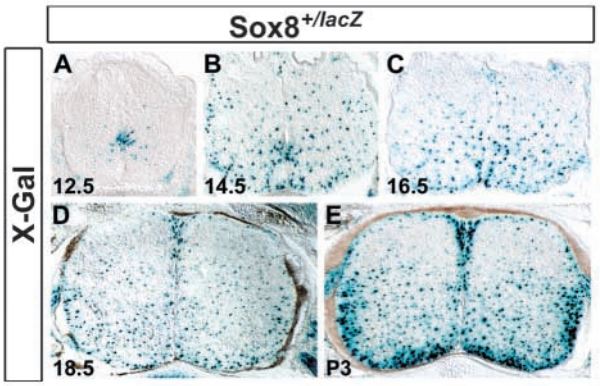

Fig. 1. Sox8 expression in the embryonic spinal cord. Sox8-specific β-galactosidase activity was detected colorimetrically using X-gal substrate in transverse spinal cord sections from the forelimb region of *Sox8*$^{+/lacZ}$ mice at 12.5 dpc (A), 14.5 dpc (B), 16.5 dpc (C), 18.5 dpc (D) and postnatal day 3 (E).

β-galactosidase-positive cells were no longer restricted to this domain, but had dispersed throughout the parenchyma of the spinal cord in a pattern typical of glial progenitors (Fig. 1B). The number of these mantle zone cells increased through 16.5 dpc (Fig. 1C). At 18.5 dpc, β-galactosidase-positive cells had started to accumulate in the marginal zone (Fig. 1D) and continued to do so postnatally (Fig. 1E) as expected if at least some belonged to the oligodendrocyte lineage.

The identity of the Sox8-expressing cells in the perinatal and early postnatal spinal cord was determined by co-immunohistochemistry using β-galactosidase as a marker for Sox8 expression in combination with antibodies known to identify the major cell types of the CNS. In these experiments, we failed to detect β-galactosidase expression in cells labeled by the pan-neuronal marker NeuN indicating that Sox8 is not expressed in neurons (Fig. 2A). Similarly, β-galactosidase expression was absent from cells positive for the glial fibrillary acidic protein (GFAP) which marks the majority of white matter astrocytes (Fig. 2B). The Ca^{2+}-binding protein S100β labels grey matter astrocytes as well as some oligodendrocyte progenitors (Richter-Landsberg and Heinrich, 1995). Perinatally, only few of the S100β-expressing cells were also positive for β-galactosidase (Fig. 2C). These double-labeled cells did not exhibit an astrocyte-like morphology, but rather resembled oligodendrocyte progenitors. In agreement, expression of β-galactosidase colocalized strongly with expression of both Olig2 and Sox10 (Fig. 2D,E), which at this time exclusively label cells of the oligodendrocyte lineage independent of their differentiation status. Mbp and Plp selectively mark the fraction of differentiating and differentiated oligodendrocytes. Again, nearly all Mbp- or Plp-positive cell somata were co-labeled with antibodies against β-galactosidase (Fig. 2F; data not shown), indicating that Sox8 expression not only occurs in developing oligodendrocytes, but is maintained throughout and after differentiation. Processes of differentiated oligodendrocytes, by contrast, contained much lower, but readily detectable amounts of β-galactosidase whose presence is, however, masked in merged pictures by the strong Mbp immunoreactivity (Fig. 2F). A comparable β-galactosidase expression was also observed in the adult spinal cord (data not shown). From late embryogenesis onwards, oligodendrocytes thus constitute the main Sox8-expressing cell type in the spinal cord, whereas there is no evidence for significant Sox8 expression in either neurons or astrocytes.

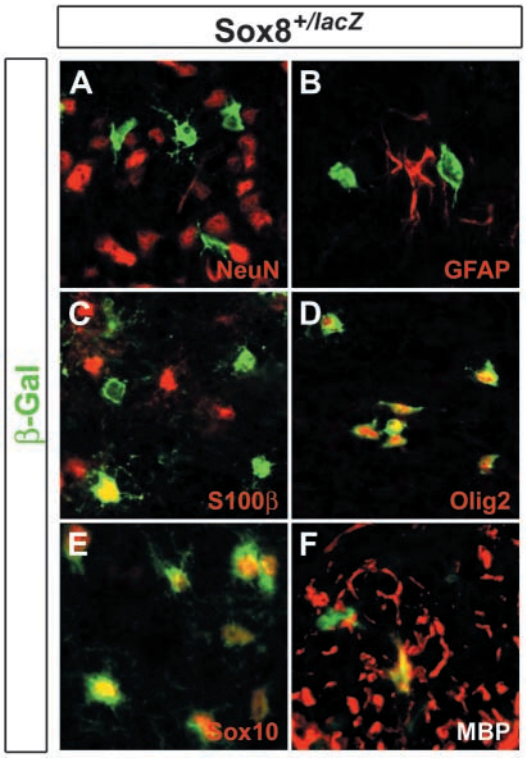

Fig. 2. Cell type-restricted expression of Sox8 in the postnatal spinal cord. Immunohistological analysis of spinal cord from *Sox8*$^{+/lacZ}$ mice at postnatal day 1 using antibodies against β-galactosidase (green) in combination with cell-type specific antibodies (red) NeuN (A), GFAP (B), S100β (C), Olig2 (D), Sox10 (E) and Mbp (F).

Sox8 is dispensable for progression of oligodendrocyte development in the embryonic spinal cord

Using Olig2 as a marker for cells of the oligodendrocyte lineage, we followed development of oligodendrocyte progenitors in the spinal cord of Sox8-deficient mice. By visual inspection, we failed to detect significant differences between spinal cords of Sox8-deficient mice and wild-type littermates both with regards to the overall number and the distribution of oligodendrocyte progenitors. Olig2 expression levels were also comparable. These observations were corroborated at various developmental stages from 14.5-18.5 dpc (Fig. 3A,B; data not shown) and confirmed using Sox10 as a second marker for the oligodendrocyte lineage (data not shown). Thus, progression of oligodendrocytes through embryonic development is normal in the absence of Sox8.

We have previously shown, that oligodendrocyte development is also normal in Sox10-deficient spinal cords during these developmental phases (Stolt et al., 2002). Accordingly, no significant differences were detected in this study between *Sox10*$^{+/lacZ}$ and either wild-type or Sox8-deficient spinal cords (Fig. 3C).

We also generated and analyzed Sox8-deficient mice which

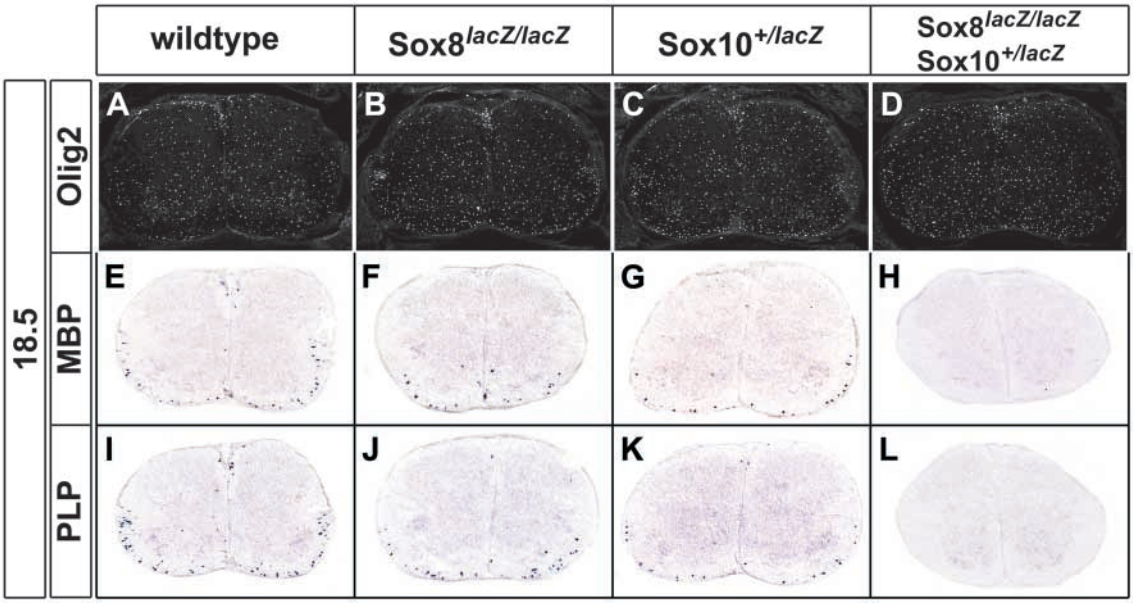

Fig. 3. Oligodendroglial development in the embryonic spinal cord of mice carrying *Sox8* and *Sox10* null alleles. Immunohistochemistry with antibodies specific for the oligodendrocyte marker Olig2 (A-D), and in situ hybridization with probes specific for *Mbp* (E-H) and *Plp* (I-L) were performed on transverse sections from the forelimb region of embryos at 18.5 dpc. (A,E,I) Wild-type spinal cords; (B,F,J), *Sox8*$^{lacZ/lacZ}$ spinal cords; (C,G,K), *Sox10*$^{+/lacZ}$ spinal cords; (D,H,L), *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ spinal cords.

additionally lacked one *Sox10* allele (*Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$). Mice deficient for both Sox8 and Sox10 could not be obtained for the stages analyzed in this study due to early embryonic lethality (C.C.S., P.L. and M.W., unpublished data). Embryonic spinal cords from *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice exhibited a normal number of oligodendrocytes at 16.5 dpc and 18.5 dpc as judged from Olig2 and Sox10 expression (Fig. 3D; data not shown). Additionally, the distribution of oligodendrocyte progenitors throughout the spinal cord was indistinguishable from those in control genotypes. Direct quantification of Olig2-positive cells in spinal cord sections of the various genotypes from comparable axial levels confirmed that there is no significant difference between genotypes in the number of Olig2-positive cells at 18.5 dpc (Fig. 4A).

Sox8 influences terminal differentiation of oligodendrocytes in cooperation with the related Sox10

Sox8-deficient mice are viable (Sock et al., 2001) and thus allow terminal differentiation of oligodendrocytes to be followed from late embryonic stages throughout postnatal development by in situ hybridization using probes specific for myelin genes such as Mbp. At 18.5 dpc, beginning Mbp expression in marginal zone cells marked the onset of terminal differentiation in wild-type embryos (Fig. 3E). Mbp-expressing cells were also present in Sox8-deficient spinal cords, but in reduced numbers (Fig. 3F). Similar results were obtained by in situ hybridization with a Plp-specific probe (Fig. 3I,J). Direct quantification on spinal cord sections confirmed a 42-50% reduction of Plp- and Mbp-positive cells in Sox8-deficient mice (Fig. 4C). Thus, terminal differentiation of oligodendrocytes is influenced by the presence of Sox8, but not strictly dependent on it. By comparison, previous analyses of Sox10-deficient mice had noted a near complete absence of terminally differentiating oligodendrocytes at this stage of spinal cord development (Stolt et al., 2002).

Loss of a single *Sox10* allele also led to a decrease in both Mbp- and Plp-expressing cells (Fig. 3G,K). According to our quantifications, *Sox10*$^{+/lacZ}$ spinal cords exhibited a 53-60% reduction at 18.5 dpc. Terminal differentiation of oligodendrocytes is thus slightly stronger affected in Sox10 heterozygous than in Sox8-deficient mice (Fig. 4C).

When both mutations were combined in *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice, expression of Mbp and Plp was severely reduced. Almost no terminally differentiating oligodendrocytes were detected at 18.5 dpc by these markers in spinal cords of *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice (Fig. 3H,L; Fig. 4C), arguing that both Sox proteins cooperate during this process.

To analyze whether terminal oligodendrocyte differentiation is permanently or transiently affected, in situ hybridization studies of myelin gene expression were continued in the postnatal spinal cord. At postnatal day 3, we still observed a reduction in Mbp- and Plp-expressing cells in both Sox8-deficient and Sox10 heterozygous spinal cords relative to the wild type (Fig. 5A-C,E-G). Despite Olig2-positive cell numbers comparable with the wild type (Fig. 4B), Mbp- and Plp-expressing cells were similarly reduced in Sox8-deficient and Sox10 heterozygous mice by ~30% (Fig. 4D). Decreased expression of Mbp and Plp was confirmed on the protein level for both genotypes by immunohistochemistry (see Fig. S1A-C,E-G at http://dev.biologists.org/supplemental/). Because of the preferential occurrence of both myelin proteins in oligodendrocyte processes and forming myelin sheaths, it is impossible to tell from immunohistochemical analyses whether signal reduction is due to reduced numbers of expressing cells or also due to reduced cellular expression levels. At postnatal day 3, both Sox8-deficient and Sox10 heterozygous spinal cords also exhibited weakened immunoreactivity for Mag (see

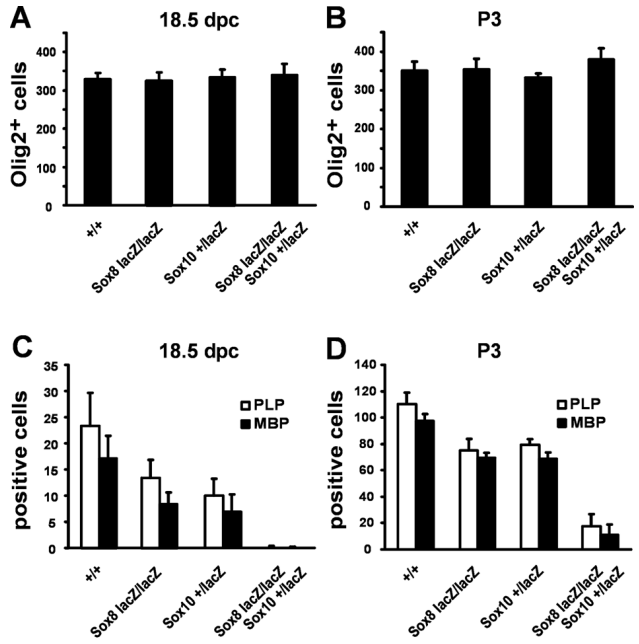

Fig. 4. Quantification of oligodendrocyte marker gene expression in the spinal cord of mice carrying *Sox8* and *Sox10* null alleles. The number of Olig2-positive cells (A,B), Plp-positive (white bars) or Mbp-positive (black bars) cells (C,D) was determined at 18.5 dpc (A,C) and postnatal day 3 (B,D) in wild-type (+/+), *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ and *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ spinal cords, as indicated below the bars. At least 15 separate 20 μm sections from the forelimb region of two independent embryos were counted for each genotype. Data are presented as mean±s.e.m. Differences from the wild type were statistically significant for all mutant genotypes in C,D, as determined by Student's *t*-test ($P \leq 0.001$).

Fig. S1I-K at http://dev.biologists.org/supplemental/), which unlike Mbp and Plp is not under direct Sox10 control (Stolt et al., 2002).

At postnatal day 7, however, terminal oligodendrocyte differentiation in spinal cords of *Sox8*$^{lacZ/lacZ}$ and *Sox10*$^{+/lacZ}$ mice became comparable with the wild type as judged both by in situ hybridization analysis (Fig. 5I-K,M-O) and immunohistochemistry (see Fig. S1M-O,Q-S,U-W at http://dev.biologists.org/supplemental/) arguing that the reduced rate of terminal oligodendrocyte differentiation observed in these genotypes at late embryonic stages and during the first postnatal days is a transient feature. The transient character of the terminal differentiation defect also explains why myelin abnormalities or ensuing neurological problems have not been observed for *Sox10*$^{+/lacZ}$ or *Sox8*$^{lacZ/lacZ}$ mice.

Postnatal mortality among *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice is high. Nevertheless, some mice survive for several days so that oligodendrocyte development can be followed in this genotype throughout the first postnatal week. Although spinal cord cells positive for Mbp or Plp transcripts appeared during postnatal development, their number remained strongly reduced at both 3 and 7 days after birth (Fig. 5D,H,L,P). At postnatal day 3, the number of Plp- or Mbp-expressing cells was only 10-15% compared with the wild type (Fig. 4D). Co-immunohistochemistry with antibodies directed against Mbp, Plp or Mag all supported the conclusion that terminal differentiation of oligodendrocytes is severely affected in *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice (see Fig. S1D,H,L,P,T,X at http://dev.biologists.org/supplemental). Some *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ exhibited unsteady movements and action tremor, pointing to hypomyelination of the CNS. Although our results point to a severe delay rather than a complete block in oligodendrocyte differentiation, we do not know whether oligodendrocyte differentiation would ever be robust enough for significant myelin formation to occur in the CNS of *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice. During this study, *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice did not survive past postnatal day 8 (data not shown). In fact, the oligodendrocyte defect and ensuing hypomyelination might be one of the reasons for the high postnatal mortality of these mice.

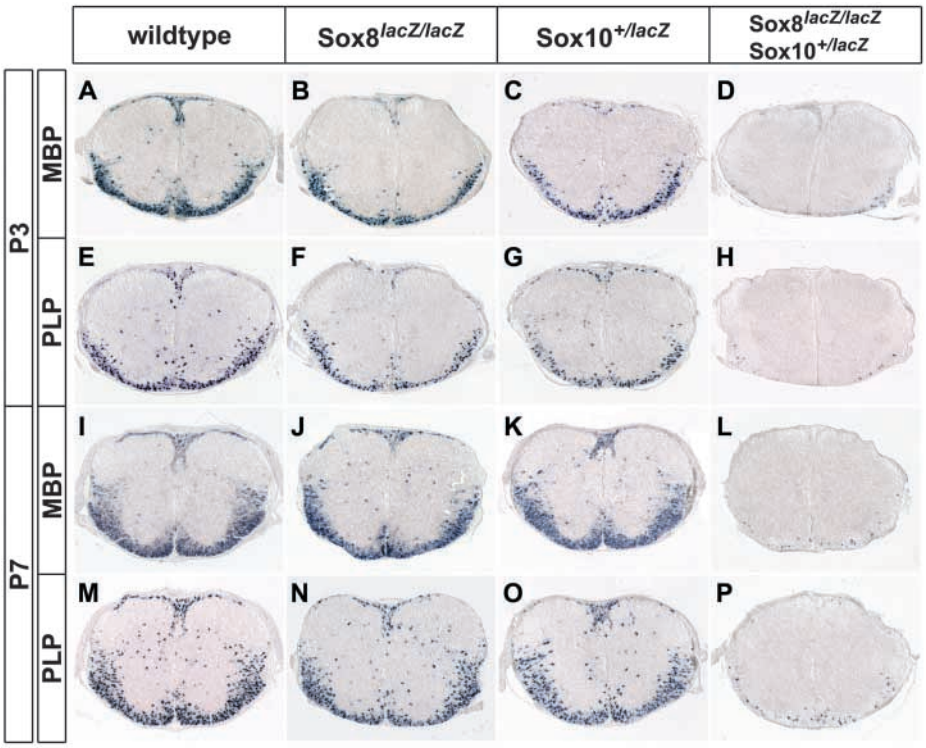

Fig. 5. In situ hybridization studies of terminal oligodendrocyte differentiation in early postnatal spinal cords of mice carrying *Sox8*- and *Sox10*-null alleles. In situ hybridization with probes specific for *Mbp* (A-D,I-L) and *Plp* (E-H,M-P) were performed on transverse spinal cord sections from the forelimb region at postnatal day 3 (A-H) and postnatal day 7 (I-P). (A,E,I,M) Wild-type spinal cords; (B,F,J,N), *Sox8*$^{lacZ/lacZ}$ spinal cords; (C,G,K,O), *Sox10*$^{+/lacZ}$ spinal cords; (D,H,L,P), *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ spinal cords.

Sox8 is capable both of binding to myelin gene promoters and interacting with Sox10

One of the mechanisms by which Sox10 is able to influence terminal differentiation of oligodendrocytes is through direct binding to myelin gene promoters and transcriptional regulation of myelin gene expression (Stolt et al., 2002). Sox10 interacts with three binding sites in the proximal part of the *Mbp* promoter which together mediate its Sox10-dependent activation (Stolt et al., 2002). Two of these sites (sites 2 and 3) bind a single Sox10 molecule, whereas site 1 is cooperatively bound by two Sox10 molecules (Stolt et al., 2002). When gel retardation assays were performed with these sites and extracts that contain either full-length Sox10 or full-length Sox8, we obtained characteristic protein-DNA complexes on all three sites (Fig. 6A). Addition of antibodies directed against Sox10 selectively supershifted the Sox10-containing complex. In a reciprocal manner, antibodies specific for Sox8 led to a selective supershift of the Sox8-specific complex and left the Sox10 complex unaffected, indicating that Sox8 was indeed contained within the complex. The mobility obtained for the Sox8-specific complexes was very similar to that obtained for the corresponding Sox10 complexes in good agreement with the fact, that both proteins have very similar sizes. It is also evident that the mobility of those complexes obtained with site 1 was lower than those obtained with either site 2 or site 3 (Fig. 6A). In case of Sox10, this lower mobility is due to cooperative binding of two molecules (Stolt et al., 2002). We conclude from the nearly identical mobility of the Sox8-containing complex that Sox8 also binds to site 1 as a dimer.

With Sox8 and Sox10 being co-expressed in oligodendrocytes (Stolt et al., 2003), these Sox proteins are expected to encounter each other on target gene promoters. At low Sox protein levels, both Sox8 and Sox10 independently bound to monomeric sites as paradigmatically shown in an electrophoretic mobility shift assay with both full-length Sox8 and a shortened Sox10 protein on site 2 (Fig. 6B). Increasing the amount of one Sox protein (Sox10 in Fig. 6B) led to increased displacement of the other Sox protein (Sox8 in Fig. 6B), showing that Sox8 and Sox10 compete for binding on monomeric sites.

On dimeric sites, options are different (Fig. 6C). When incubated alone with a dimer site, both a shortened Sox8 and a long Sox10 version yielded one predominant complex with a mobility characteristic of the respective homodimer. When mixed, a new complex with intermediate mobility appeared, showing that heterodimerization between Sox8 and Sox10 had occurred. Thus, dimeric sites in target gene promoters allow Sox8 and Sox10 to functionally interact with each other in DNA-dependent heterodimers.

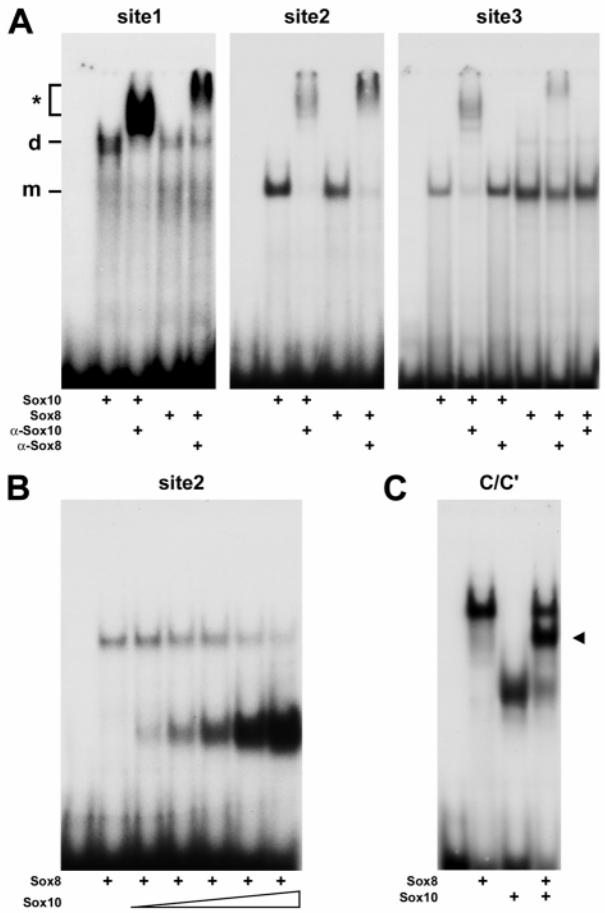

Fig. 6. Competition and interaction of Sox8 and Sox10 on bona fide binding sites from target gene promoters. (A) Electrophoretic mobility shift assays with the Sox10-binding sites 1-3 from the *Mbp* promoter (Stolt et al., 2002) as probes, and extracts from transfected N2A cells-expressing full-length Sox8 and Sox10 proteins as indicated below the lanes. Antibodies directed against Sox8 (α-Sox8) or Sox10 (α-Sox10) were added to some reactions during the incubation period as indicated. m, bound monomer; d, bound homodimer. Supershifted complexes are marked with an asterisk. (B) Electrophoretic mobility shift assay with site 2 from the *Mbp* promoter as probe and Sox8-containing N2A cell extract as protein source. Increasing amounts of a truncated Sox10 (MIC variant) (see Kuhlbrodt et al., 1998b; Schlierf et al., 2002) were added to the reactions as indicated. (C) Electrophoretic mobility shift assay with the prototypic dimeric Sox10-binding site C/C′ (Peirano and Wegner, 2000) and extracts from transfected N2A cells expressing a short version of Sox8 and a long version of Sox10 either alone or in combination as indicated below the lanes. The heterodimer is indicated by an arrowhead.

Sox8 directly activates expression of oligodendroglial myelin genes

Given the fact that Sox8 recognizes bona fide Sox10 target sites by itself and as heterodimers with Sox10, we analyzed whether Sox8 would be able to activate the *Mbp* promoter which has previously been shown to be responsive in transient transfections to Sox10 (Stolt et al., 2002). Luciferase expression was stimulated approximately eightfold by Sox10 when placed under the control of a long version or a short version of the *Mbp* promoter (Fig. 7A). Luciferase expression from the *Mbp* promoter was also increased by Sox8. Although the approximately five- to sevenfold activation rates were below those obtained for Sox10, this difference was not statistically significant. Similar activation rates were obtained in the presence of both Sox8 and Sox10 (data not shown).

Using a stable N2A cell line with doxcycline-dependent inducible Sox8 expression, we analyzed whether Sox8 would be able to activate endogenous expression of myelin genes in a heterologous cell line as previously shown for Sox10 (Stolt et al., 2002). As evident from semi-quantitative RT-PCR

analyses, expression of both Plp and Mbp was elevated in the stable N2A cell line upon induction of Sox8, whereas expression of the β-actin control remained unaffected (Fig. 7B). Endogenous Plp expression was activated stronger than Mbp expression as similarly observed for Sox10 (Stolt et al., 2002). Thus, we conclude that Sox10 target genes can also be activated by Sox8. Side-by-side comparison of induction rates in stable N2A cell lines expressing either Sox8 or Sox10 revealed somewhat lower induction rates for Sox8 (data not shown). This could point to the fact that Sox8 is a slightly weaker transcriptional activator than Sox10. However, it is difficult to quantitatively compare effects obtained in different stable cell lines, especially considering that induced Sox8 levels appeared lower than induced Sox10 levels in the respective stable lines (data not shown).

Sox8 is expressed at lower levels than Sox10 in terminally differentiating oligodendrocytes

Given the fact that the same *lacZ*-coding sequence was inserted in almost identical manner in both the *Sox8* and the *Sox10* locus to yield the *Sox8*^{lacZ} and *Sox10*^{lacZ} alleles, it should be possible to compare expression levels of Sox8 and Sox10 in developing oligodendrocytes through measurements of β-galactosidase amounts in *Sox8*$^{+/lacZ}$ and *Sox10*$^{+/lacZ}$ spinal cords, provided expression levels do not undergo abrupt changes, that might not be reproducible by β-galactosidase with its long half-life. However, no such drastic changes in expression levels have been observed for Sox8 or Sox10 in oligodendrocytes (Sock et al., 2001; Stolt et al., 2002) (this study).

At 14.5 dpc, β-galactosidase was produced at four-fold higher amounts from the *Sox10* locus than from the *Sox8* locus (Fig. 7C). As development proceeded, this difference became less pronounced. At 16.5 dpc and 18.5 dpc, β-galactosidase expression from the *Sox8* locus reached 50-60% of the levels obtained for *Sox10* (Fig. 7C). At the onset of terminal differentiation, Sox10 expression levels were thus approximately twice as high as Sox8 levels. This difference vanished further in adult animals, where 85% of Sox10 levels were achieved for Sox8 (Fig. 7C). From 18.5 dpc onwards, β-galactosidase levels in spinal cords from mice with various combinations of *lacZ* alleles corresponded to the additive value obtained for the single *Sox8*^{lacZ} and *Sox10*^{lacZ} alleles (data not shown), arguing that at these times Sox8 and Sox10 expression in oligodendrocytes are largely independent of each other.

Discussion

Despite expression in many different cell types at important points of development, it has been difficult to determine the function of Sox8 (Sock et al., 2001). Many of the expression sites of Sox8 overlap with expression sites for either Sox9, Sox10 or both (Schepers et al., 2003; Schmidt et al., 2003) pointing to compensatory mechanisms between these proteins.

We show that in the late embryonic, early postnatal and adult spinal cord, Sox8 is selectively expressed in the oligodendrocyte lineage. At these times, we failed to detect expression in either astrocytes or neurons. In agreement with previous observations about the general expression pattern of Sox8 (Sock et al., 2001), oligodendrocytic expression was already present before terminal differentiation, and maintained throughout and thereafter. This expression pattern is strongly

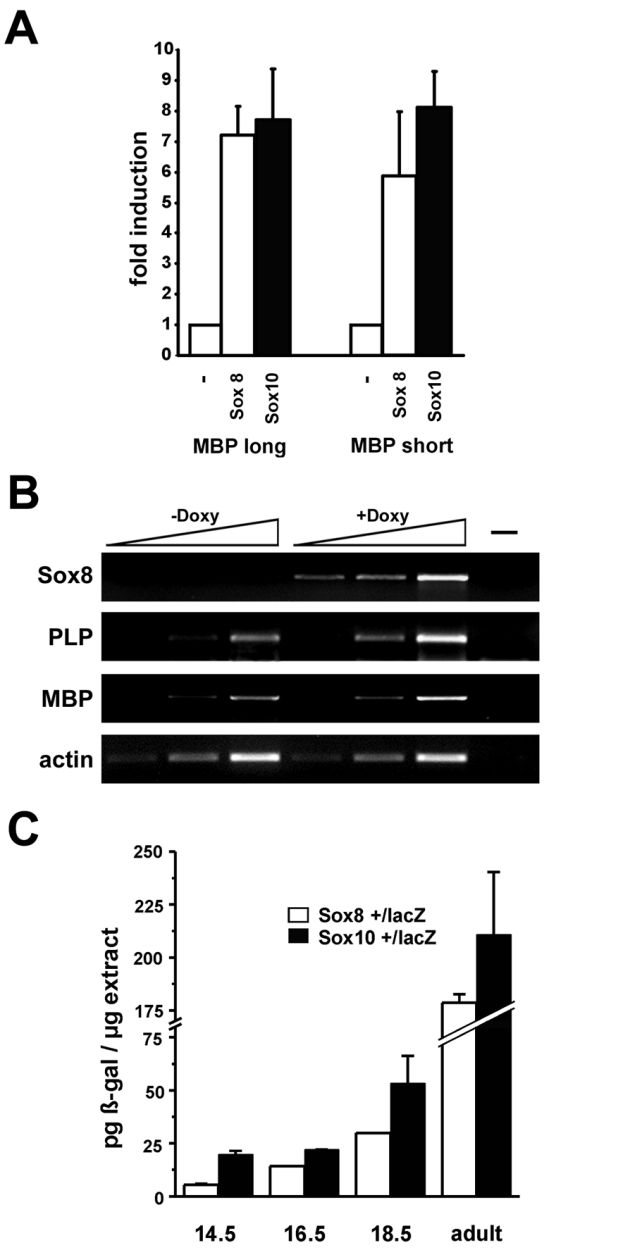

Fig. 7. Activation of myelin gene expression by Sox8, and comparison of β-galactosidase activities in *Sox8*$^{+/lacZ}$ and *Sox10*$^{+/lacZ}$ spinal cords. (A) N2A cells were transfected with reporter plasmids in which the luciferase gene was under control of a long (positions –656 to +31) or a short (positions –256 to +31) version of the rat *Mbp* promoter. Expression plasmids for Sox10 and Sox8 were co-transfected as indicated below the bars. Luciferase activities in extracts from transfected cells were determined in three independent experiments each performed in duplicates. Data are presented as fold inductions ±s.e.m. with the activity of the promoter in the absence of co-transfected Sox protein (–) arbitrarily set to 1. (B) RT-PCR analysis on cDNA obtained from N2A cells inducibly expressing Sox8. – Doxy, no doxycycline (Sox8 absent); + Doxy, doxycycline added (Sox8 present). Transcript levels of *Sox8*, *Plp*, *Mbp* and β-actin were compared semi-quantitatively using increasing numbers (n, n+3, n+6) of amplification cycles. –, no cDNA added. (C) The amount of β-galactosidase present per µg extract from *Sox8*$^{+/lacZ}$ (white bars) and *Sox10*$^{+/lacZ}$ (black bars) spinal cords was determined at 14.5 dpc, 16.5 dpc, 18.5 dpc and in adult mice as indicated below the bars.

reminiscent of the related Sox10 (Stolt et al., 2002) and additionally shows a strong overlap with Sox9 (Stolt et al., 2003). Oligodendrocytes thus express all three group E Sox proteins before terminal differentiation, and a combination of Sox8 and Sox10 from thereon. Despite this significant overlap in *Sox* gene expression, there are defined defects in oligodendrocyte development in Sox9- and Sox10-deficient spinal cords, with Sox9 playing a role in oligodendrocyte specification (Stolt et al., 2003) and Sox10 being required for terminal differentiation (Stolt et al., 2002).

Sox8-deficient mice are viable and reach a normal lifespan without exhibiting overt neurological symptoms (Sock et al., 2001). Therefore, severe oligodendrocyte defects were not expected. There was, however, a transient delay in terminal differentiation and myelination of spinal cord oligodendrocytes. Delayed terminal differentiation has previously been observed in Nkx2.2-deficient mice and in Olig1-deficient mice for oligodendrocytes (Lu et al., 2002; Qi et al., 2001), and in Oct6-deficient mice for Schwann cells, the oligodendrocyte counterparts of the peripheral nervous system (Bermingham et al., 1996; Jaegle et al., 1996). Interestingly, we also found a differentiation delay in *Sox10*$^{+/lacZ}$ oligodendrocytes arguing that this process is as sensitive to *Sox10* gene dosage as the development of melanocytes and the enteric nervous system (Britsch et al., 2001; Herbarth et al., 1998a; Pingault et al., 1998; Southard-Smith et al., 1998).

The delay of terminal oligodendrocyte differentiation in *Sox10*$^{+/lacZ}$ spinal cords was drastically increased by the absence of Sox8, and still prominent in *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice at the end of the first postnatal week. Owing to lethality, we were unable to determine whether terminal differentiation of oligodendrocytes would ever be sufficient for sustained CNS myelination in this genotype. Retinal transplantation of neural stem cells indicated that this is not the case for Sox10-deficient oligodendrocytes (Stolt et al., 2002). Thus, it will be interesting to see in future experiments whether the terminal differentiation defect in *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ oligodendrocytes is as severe as in *Sox10*$^{lacZ/lacZ}$ oligodendrocytes.

Our data argue that Sox8 and Sox10 perform redundant functions during terminal differentiation of oligodendrocytes. We have shown here that Sox8 is able to bind to a number of bona fide Sox10 response elements in a manner indistinguishable from Sox10. Additionally, Sox8 and Sox10 bind cooperatively to some response elements as heterodimers in a manner similar to the respective homodimers. Thus, there is no evidence for differential DNA binding or promoter recognition between both Sox proteins. In good agreement, Sox8 activated expression from the *Mbp* promoter in transient transfections and induced expression from the endogenous *Mbp* and *Plp* genes in N2A cells in a manner similar to Sox10 (Bondurand et al., 2001; Peirano et al., 2000; Stolt et al., 2002). Interestingly, we have previously observed in vivo a weak residual Mbp expression in some Sox10-deficient oligodendrocytes (Stolt et al., 2002). As Sox8 was the only remaining Sox E protein in the cells, the residual Mbp expression might be attributed to the activity of Sox8.

Redundancy of structurally related transcription factors has been frequently observed. Usually, the redundant proteins can fully compensate each other's loss so that their function is unmasked only after combined deletion. This is not the case for Sox8 and Sox10, as Sox10 compensates loss of Sox8 much more effectively than vice versa.

One possible explanation for this non-reciprocal compensation is that Sox8 can only substitute for Sox10 in specific functions, so that Sox8 turns on only a subset of Sox10 target genes. The failure to activate the full complement of Sox10 target genes would then be causative for the severe terminal differentiation defect in Sox10-deficient oligodendrocytes. Although it is impossible to discount such a model in the absence of the full list of oligodendrocytic target genes for both proteins, there is little evidence so far that would favor this model as the main cause for the observed effects.

If Sox8 activates more or less the same transcriptional program in oligodendrocytes as Sox10, it might do so less efficiently. One reason for a reduced efficiency could be a reduced intrinsic transactivation capacity of the Sox8 protein relative to Sox10. Sequence conservation between Sox8 and Sox10 proteins are significant within the respective transactivation domains of both proteins but not as high as in the DNA-binding domain (Kuhlbrodt et al., 1998a; Schepers et al., 2000). Thus, it is at least conceivable that interaction with the transcription machinery, chromatin remodeling activities or transcriptional co-factors is qualitatively or quantitatively different between both Sox proteins, as suggested for Sox8 and Sox9 (Schepers et al., 2003). Our transient transfection data would not exclude such a model as maximal induction rates obtained for saturating amounts of Sox8 were lower than those obtained for Sox10. However, induction rates did not differ enough to identify the intrinsic transactivation capacities of the proteins as the main difference between Sox8 and Sox10 in oligodendrocytes.

Another explanation focuses on the lower expression levels of Sox8 relative to Sox10 in oligodendrocytes as determined by comparison of β-galactosidase activities in *Sox8*$^{+/lacZ}$ and *Sox10*$^{+/lacZ}$ mice. The difference is most pronounced during early oligodendrocyte development. However, even at the time of terminal oligodendrocyte differentiation, we still detected a twofold higher expression level of the *lacZ*^{Sox10} allele. Although certainly oversimplified, *Sox* gene function in the oligodendrocyte lineage might be explained at first approximation by a model in which the different expression levels of the respective Sox proteins are taken into account and functions are regarded as approximately equal. Thus during terminal differentiation, one *Sox10* allele contributes roughly one-third to the overall *Sox* gene activity, whereas one *Sox8* allele accounts for one-sixth. If the total amount of Sox protein drops to approximately two-thirds (as is the case for the Sox10 heterozygote and the Sox8-deficient mouse), minor disturbances become evident in the form of a transient delay of terminal differentiation. Severity of this defect increases with decreasing amounts of residual Sox protein as is the case after combined losses of *Sox8* and *Sox10* alleles. This leads to the dramatically extended terminal differentiation delay in *Sox8*$^{lacZ/lacZ}$, *Sox10*$^{+/lacZ}$ mice, in which the total amount of remaining Sox protein has dropped to approximately one-third.

Although differences in expression levels are thus capable of explaining the different role of Sox8 and Sox10 during terminal differentiation of oligodendrocytes, it is almost certain that there will be other modulating factors such as differences in protein stability or, as already discussed, differences in transactivation capacities between both Sox proteins. Their

contribution will be most easily revealed in mouse models in which *Sox8* is inserted into the *Sox10* locus and therefore expressed at levels characteristic of Sox10.

Sox8 and Sox10 are not the only structurally related transcription factors that are co-expressed during development of myelinating glia. The bHLH transcription factors Olig1 and Olig2 are both found in cells of the oligodendrocyte lineage and behave similarly in gain-of-function analyses. Nevertheless, their deletion leads to different phenotypes in the mouse with loss of Olig2 leading to an early specification defect and loss of Olig1 affecting maturation of oligodendrocytes (Lu et al., 2002). This argues that loss of Olig1 is compensated unidirectionally by Olig2 during early phases of oligodendrocyte development.

Myelinating Schwann cells express Oct6 and the closely related POU protein Brn2 with a similar developmental profile. Analysis of mice deficient for both Oct6 and Brn2 and the phenotypic rescue of Oct6-deficiency by ectopic Brn2 expression both support the notion that the two POU proteins perform redundant functions (Jaegle et al., 2003). Nevertheless, the delay in Schwann cell differentiation is more pronounced in Oct6-deficient mice than in mice that lack Brn2, indicating that both proteins have a different importance during Schwann cell development. There is also some indication that Brn2 is present in lower amounts than Oct6. The relationship between Oct6 and Brn2 in Schwann cells is thus strongly reminiscent of the one between Sox10 and Sox8 in oligodendrocytes. It remains to be seen whether these unidirectional redundancies are a more general phenomenon for important developmental regulators of myelinating glia. Because of their unidirectionality, it is tempting to speculate that they do not primarily present a fail-safe mechanism to ensure proper myelination but rather are an expression of the relatively young evolutionary history of vertebrate glia.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to M.W. (We1326/7-2).

References

Bermingham, J. R., Scherer, S. S., O'Connell, S., Arroyo, E., Kalla, K. A., Powell, F. L. and Rosenfeld, M. G. (1996). Tst-1/Oct-6/SCIP regulates a unique step in peripheral myelination and is required for normal respiration. *Genes Dev.* **10**, 1751-1762.

Bi, W., Deng, J. M., Zhang, Z., Behringer, R. R. and de Crombrugghe, B. (1999). Sox9 is required for cartilage formation. *Nat. Genet.* **22**, 85-89.

Bondurand, N., Girard, M., Pingault, V., Lemort, N., Dubourg, O. and Goossens, M. (2001). Human Connexin 32, a gap junction protein altered in the X-linked form of Charcot-Marie-Tooth disease, is directly regulated by the transcription factor SOX10. *Hum. Mol. Genet.* **10**, 2783-2795.

Bowles, J., Schepers, G. and Koopman, P. (2000). Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. *Dev. Biol.* **227**, 239-255.

Briscoe, J., Sussel, L., Serup, P., Hartigan-O'Connor, D., Jessell, T. M., Rubenstein, J. L. and Ericson, J. (1999). Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. *Nature* **398**, 622-627.

Britsch, S., Goerich, D. E., Riethmacher, D., Peirano, R. I., Rossner, M., Nave, K. A., Birchmeier, C. and Wegner, M. (2001). The transcription factor Sox10 is a key regulator of peripheral glial development. *Genes Dev.* **15**, 66-78.

Foster, J. W., Dominguez-Steglich, M. A., Guioli, S., Kwok, C., Weller, P. A., Stevanovic, M., Weissenbach, J., Mansour, S., Young, I. D., Goodfellow, P. N. et al. (1994). Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature* **372**, 525-530.

Herbarth, B., Meissner, H., Westphal, M. and Wegner, M. (1998a). Absence of polyomavirus JC in glial brain tumors and glioma-derived cell lines. *Glia* **22**, 415-420.

Herbarth, B., Pingault, V., Bondurand, N., Kuhlbrodt, K., Hermans-Borgmeyer, I., Puliti, A., Lemort, N., Goossens, M. and Wegner, M. (1998b). Mutation of the Sry-related Sox10 gene in *Dominant megacolon*, a mouse model for human Hirschsprung disease. *Proc. Natl. Acad. Sci. USA* **95**, 5161-5165.

Jaegle, M., Mandemakers, W., Broos, L., Zwart, R., Karis, A., Visser, P., Grosveld, F. and Meijer, D. (1996). The POU factor Oct-6 and Schwann cell differentiation. *Science* **273**, 507-510.

Jaegle, M., Ghazvini, M., Mandemakers, W., Piirsoo, M., Driegen, S., Levavasseur, F., Raghoenath, S., Grosveld, F. and Meijer, D. (2003). The POU proteins Brn-2 and Oct-6 share important functions in Schwann cell development. *Genes Dev.* **17**, 1380-1391.

Kuhlbrodt, K., Herbarth, B., Sock, E., Hermans-Borgmeyer, I. and Wegner, M. (1998a). Sox10, a novel transcriptional modulator in glial cells. *J. Neurosci.* **18**, 237-250.

Kuhlbrodt, K., Schmidt, C., Sock, E., Pingault, V., Bondurand, N., Goossens, M. and Wegner, M. (1998b). Functional analysis of Sox10 Mutations found in human Waardenburg-Hirschsprung patients. *J. Biol. Chem.* **273**, 23033-23038.

Lemke, G. (1988). Unwrapping the Genes of Myelin. *Neuron* **1**, 535-543.

Lu, Q. R., Sun, T., Zhu, Z., Ma, N., Garcia, M., Stiles, C. D. and Rowitch, D. H. (2002). Common developmental requirement for olig function indicates a motor neuron/oligodendrocyte connection. *Cell* **109**, 75-86.

Nieto, M., Schuurmans, C., Britz, O. and Guillemot, F. (2001). Neural bHLH genes control the neuronal versus glial fate decision in cortical progenitors. *Neuron* **29**, 401-413.

Peirano, R. I., Goerich, D. E., Riethmacher, D. and Wegner, M. (2000). Protein zero expression is regulated by the glial transcription factor Sox10. *Mol. Cell. Biol.* **20**, 3198-3209.

Peirano, R. I. and Wegner, M. (2000). The glial transcription factor Sox10 binds to DNA both as monomer and dimer with different functional consequences. *Nucleic Acids Res.* **28**, 3047-3055.

Pfeifer, D., Poulat, F., Holinski-Feder, E., Kooy, F. and Scherer, G. (2000). The SOX8 gene is located within 700 kb of the tip of chromosome 16p and is deleted in a patient with ATR-16 syndrome. *Genomics* **63**, 108-116.

Pingault, V., Bondurand, N., Kuhlbrodt, K., Goerich, D. E., Prehu, M.-O., Puliti, A., Herbarth, B., Hermans-Borgmeyer, I., Legius, E., Matthijs, G. et al. (1998). Sox10 mutations in patients with Waardenburg-Hirschsprung disease. *Nat. Genet.* **18**, 171-173.

Pringle, N. P. and Richardson, W. D. (1993). A singularity of PDGF alpha receptor in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. *Development* **117**, 525-533.

Qi, Y., Cai, J., Wu, Y., Wu, R., Lee, J., Fu, H., Rao, M., Sussel, L., Rubenstein, J. and Qiu, M. (2001). Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. *Development* **128**, 2723-2733.

Richter-Landsberg, C. and Heinrich, M. (1995). S-100 immunoreactivity in rat brain glial cultures is associated with both astrocytes and oligodendrocytes. *J. Neurosci. Res.* **42**, 657-665.

Schepers, G. E., Bullejos, M., Hosking, B. M. and Koopman, P. (2000). Cloning and characterisation of the sry-related transcription factor gene sox8. *Nucleic Acids Res.* **28**, 1473-1480.

Schepers, G. E., Taesdale, R. D. and Koopman, P. (2002). Twenty pairs of Sox: extent, homology, and nomenclature of the mouse and human Sox transcription factor families. *Dev. Cell* **3**, 167-170.

Schepers, G., Wilson, M., Wilhelm, D. and Koopman, P. (2003). Sox8 is expressed during testis differentiation in mice and synergizes with SF1 to activate the AMH promoter in vitro. *J. Biol. Chem.* **278**, 28101-28108.

Schlierf, B., Ludwig, A., Klenovsek, K. and Wegner, M. (2002). Cooperative binding of Sox10 to DNA: requirements and consequences. *Nucleic Acids Res.* **30**, 5509-5516.

Schmidt, K., Glaser, G., Wernig, A., Wegner, M. and Rosorius, O. (2003). Sox8 is a specific marker for muscle satellite cells and inhibits myogenesis. *J. Biol. Chem.* **278**, 29769-29775.

Sock, E., Schmidt, K., Hermanns-Borgmeyer, I., Bösl, M. R. and Wegner, M. (2001). Idiopathic weight reduction in mice deficient in the high-mobility-group transcription factor Sox8. *Mol. Cell. Biol.* **21**, 6951-6959.

Southard-Smith, E. M., Kos, L. and Pavan, W. J. (1998). Sox10 mutation disrupts neural crest development in *Dom* Hirschsprung mouse model. *Nat. Genet.* **18**, 60-64.

Stolt, C. C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U. and Wegner, M. (2002). Terminal

differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. *Genes Dev.* **16**, 165-170.

Stolt, C. C., Lommes, P., Sock, E., Chaboissier, M.-C., Schedl, A. and Wegner, M. (2003). The Sox9 transcription factor determines glial fate choice in the developing spinal cord. *Genes Dev.* **17**, 1677-1689.

Takebayashi, H., Nabeshima, Y., Yoshida, S., Chisaka, O. and Ikenaka, K. (2002). The basic helix-loop-helix factor olig2 is essential for the development of motoneuron and oligodendrocyte lineages. *Curr. Biol.* **12**, 1157-1163.

Wagner, T., Wirth, J., Meyer, J., Zabel, B., Held, M., Zimmer, J., Pasantes, J., Bricarelli, F. D., Keutel, J., Hustert, E. et al. (1994). Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene Sox9. *Cell* **79**, 1111-1120.

Wegner, M. (1999). From head to toes: the multiple facets of Sox proteins. *Nucleic Acids Res.* **27**, 1409-1420.

Zhou, Q. and Anderson, D. J. (2002). The bHLH transcription factors olig2 and olig1 couple neuronal and glial subtype specification. *Cell* **109**, 61-73.

Zhou, Q., Choi, G. and Anderson, D. J. (2001). The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2. *Neuron* **31**, 791-807.