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., 2000). 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 Sox8lacZ allele (Sock et al., 2001) were kept as heterozygotes in the presence or absence of a Sox10lacZ allele on a mixed C3H/129SvJ background (Britsch et al., 2001). For the generation of embryos and pups during this study, Sox8lacZ/+ , Sox8−/− , Sox8lacZ/− , Sox8−/− ; lacZ/+ , Sox8−/− ; lacZ/− mice were crossed with Sox8lacZ/+ , Sox10lacZ/+ 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 to standard protocols as previously described (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.

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 32P-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 MgCl2, 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 (Sox8lacZ) matches closely that of our Sox10lacZ 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 Sox8lacZ 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,
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 Ca2+ -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.

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
additionally lacked one Sox10 allele (Sox8\textsuperscript{lacZ/lacZ}, Sox10\textsuperscript{+/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\textsuperscript{lacZ/lacZ}, Sox10\textsuperscript{+/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. 3J). 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\textsuperscript{+/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\textsuperscript{lacZ/lacZ}, Sox10\textsuperscript{+/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\textsuperscript{lacZ/lacZ}, Sox10\textsuperscript{+/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 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. 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 Sox8lacZ/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 Sox8lacZ/lacZ mice.

Postnatal mortality among Sox8lacZ/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 Sox8lacZ/lacZ, Sox10+/lacZ mice (see Fig. S1D,H,L,P, T,X at http://dev.biologists.org/supplemental). Some Sox8lacZ/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 Sox8lacZ/lacZ, Sox10+/lacZ mice. During this study, Sox8lacZ/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. 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 (+/+), Sox8lacZ/lacZ, Sox10+/lacZ and Sox8lacZ/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 means±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≤0.001).
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.

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 doxycycline-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...
Sox8 and oligodendrocytes

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 Sox8lacZ and Sox10lacZ alleles, it should be possible to compare expression levels of Sox8 and Sox10 in developing oligodendrocytes through measurements of β-galactosidase amounts in Sox8lacZ and Sox10lacZ 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 Sox8lacZ and Sox10lacZ 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
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 Sox10lacZ oli
godendrocytes 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 Sox10lacZ spinal cords was drastically increased by the absence of Sox8, and still prominent in Sox8lacZlacZ, Sox10lacZ 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 Sox8lacZlacZ, Sox10lacZ oligodendrocytes is as severe as in Sox10lacZlacZ 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 transcription 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 Sox8lacZ and Sox10lacZ 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 lacZSox10 allele. Although certainly oversimplified, Sox8 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 Sox8lacZlacZ, Sox10lacZ 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


