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(Plp1b), and Mbp have been identified in zebrafish (Brosamle and Halpern, 2002). Zebrafish Olig2, like its mammalian counterpart, is required for primary motor neuron and OL development (Park et al., 2002). Sox10, Nkx2.2a, and Oct-6 have all been reported to function in OL differentiation in zebrafish (Levavasseur et al., 1998; Park et al., 2002; Kirby et al., 2006). In this study, we report that zebrafish and other teleosts also possess an *olig1* gene. Zebrafish Olig1 is expressed in the OL lineage and can form homodimers as well as heterodimers with Olig2 and the ubiquitous bHLH transcription factor E12. In zebrafish, Olig1 can interact with Sox10 directly, whereas Olig2 cannot. In mouse, both OLIG1 and OLIG2 bind to SOX10. *In vitro* and *in vivo* evidence indicates that an Olig1/Sox10 complex can activate *mbp* transcription by binding to elements in the 5′ region of the *mbp* gene. These findings cast new light on the role of Olig1 in CNS myelination.

Materials and Methods

Cloning and analysis of the zebrafish olig1 *gene.* A tBLASTn (Basic Local Alignment Search Tool) search with the mouse OLIG1 protein sequence (GenBank accession number NP_058664) on the Ensembl zebrafish database (h6tabasebin48(botmDanio_rd)-o/tin da-

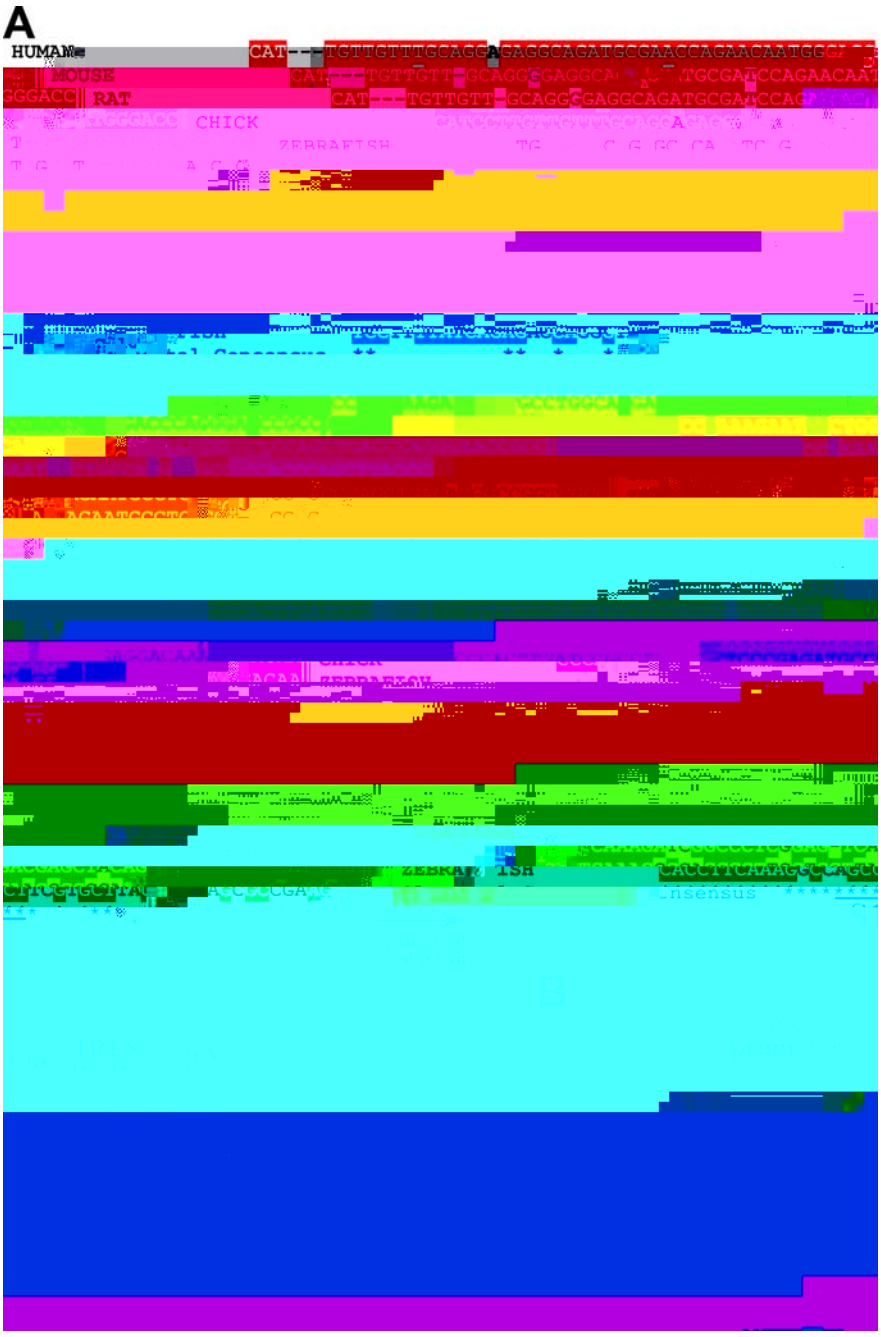
vitrogen) until the dye migrated off the end of the gel. The gels were dried and visualized by autoradiography.

Results

Zebrafish *lig1* is expressed in the OL lineage

Zebrafish *olig1* transcripts were first detected in the diencephalon at 48 h postfertilization (hpf) by *in situ* hybridization (Fig. 1*A*), whereas *olig2* and *olig3* appeared earlier (Park et al., 2002; Filippi et al., 2005). Subsequently, the *olig1*

Interestingly, we observed that zebrafish Olig1 could complex with Sox10, whereas Olig2 could not (Fig. 2



4. Transcription factor binding sites in the *mbp* promoter region. ***A***, Alignment of the *mbp* promoter sequences from human, mouse, rat, chick, and zebrafish. Conserved nucleotides are in red, and clustal consensus sequences are indicated by asterisks. Conserved Sox10 and Olig1 binding sites are underlined. ***B***, Schematic maps of a series of *mbp* reporter constructs. A 1.6 kbp *mb*

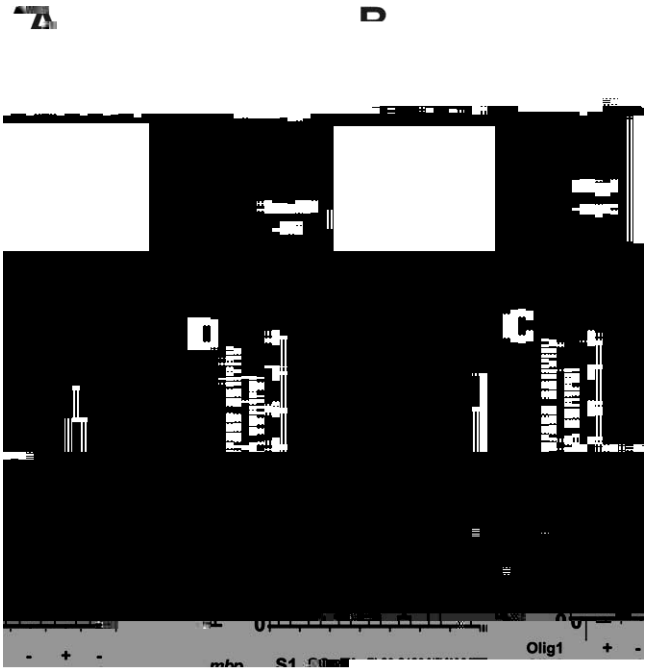
immunoprecipitated with anti-Myc or anti-V5 antibodies. The presence or absence of the *mbp* promoter sequence was then detected in the immunoprecipitate by PCR. Sox10 could bind to the *mbp* promoter on its own (Fig. 6*A*), whereas Olig1 or Olig2 failed to bind (Fig. 6*B*). However, the *mbp* promoter could be precipitated by anti-Myc antibodies after coinjection of *olig1-myc* and *sox10-V5* mRNAs. In contrast, a negative result was obtained with anti-Myc when *olig2-myc* and *sox10-V5* mRNAs were coinjected under the same conditions (Fig. 6*B*). These data indicate that Olig1 can form an *mbp* binding complex with Sox10 but that Olig2 cannot. To test whether the Olig1/Sox10 complex can activate *mbp* transcription, we injected *olig1-myc* mRNA and/or *sox10-V5* mRNA into one-cell-stage zebrafish embryos and collected embryos at 3 dpf to detect *mbp* mRNA expression by *in situ* hybridization on transverse spinal cord sections. Compared with *olig1* or *sox10* single injections, *olig1*/*sox10* double injection gave rise to a significant increase in the number and intensity of *mbp*-expressing cells in the spinal cord (Fig. 7*A*). In addition, some ectopically expressed *mbp* signal was detected in somites after *olig1/sox10* double injection.

The series of *mbp*-luciferase reporters referred to above also contain a downstream *IRES-DsRed* sequence, so that that they are, in effect, double reporters of luciferase and DsRed. We injected these reporter constructs into one-cell-stage embryos and subsequently performed *in situ* hybridization with a DsRed probe (Fig. 7*B*). There were many more *DsRed*-expressing cells in embryos injected with wild-type *mbp-DsRed* reporter DNA (five fish, 10 sections per fish) than in embryos injected with the reporter containing a mutated Eb (five fish, 10 sections per fish) (Fig. 7*B*). In embryos injected with *mbp-DsRed* reporter containing mutated S1, S2, Eb+S2, S1+S1, or S1+Eb+S2 binding sites, no DsRed signal whatsoever was detected (data not shown). Overall, our data provide strong evidence that a protein complex of Olig1 and Sox10 directly binds to and activates transcription from the *mbp* promoter.

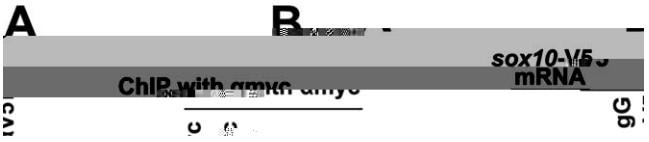
Discussion

lig1 is expressed by OL lineage cells during zebrafish CNS development

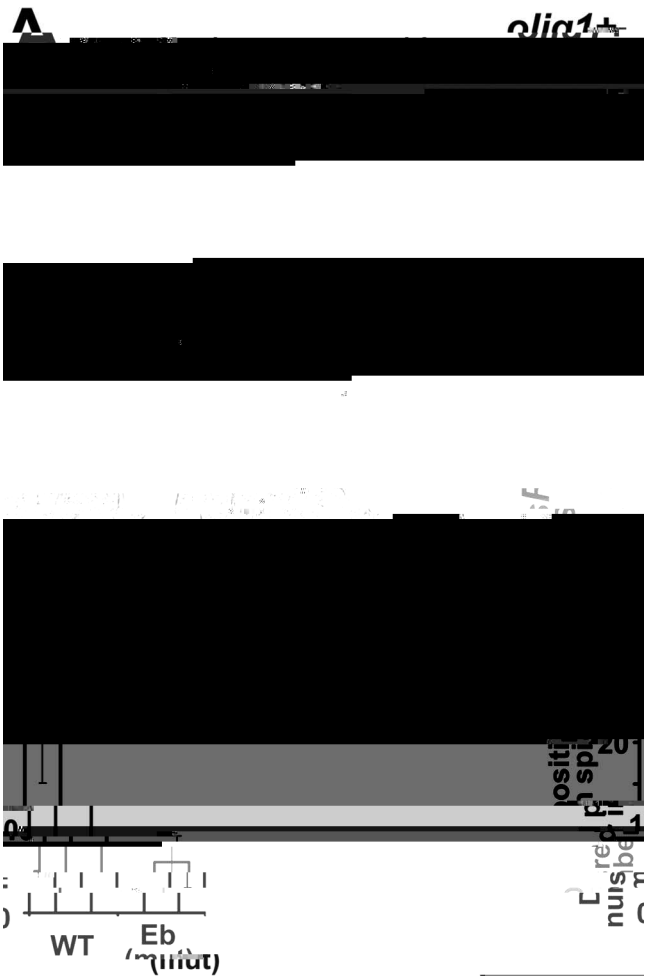
Zebrafish *olig1* and *olig2*, like their mammalian counterparts, were expressed strictly in the CNS (Fig. 1). In mouse spinal cord, both OLIG1 and OLIG2 are expressed in the pMN (progenitors of motor neurons) domain in the ventral neuroepithelium, which is known to give rise to both motor neurons and OLs (Lu et al., 2000; Zhou et al., 2000). OLIG1 and OLIG2 are rapidly downregulated in postmitotic motor neurons but continue to be expressed in OL lineage cells throughout development and in the adult. In zebrafish, *olig2* is expressed in a pMN-like region of neural plate cells at 9.5 hpf (Park et al., 2002) where *olig1* is not expressed (data not shown). After motor neuronogenesis, expression of *olig2* remains on in OLPs but is downregulated in differentiating OLs and motor neurons. *olig1* first emerges in zebrafish spinal cord at 72 hpf (Fig. 1*C*) and partly overlaps with *olig2* in white matter (Fig. 1*D–F*), suggesting that some migrating OLPs express *olig1*. Unlike *olig2*, *olig1* partly colocalizes with differentiated OL markers *mbp*,



5. Olig1 and Sox10 regulate *mbp* transcription *in vitro*. ***A***, ***B***, Analysis of Olig1 and Sox10 DNA binding activities by EMSA. Purified Olig1 and Sox10 could shift a ^{32}P-labeled 39 bp double-stranded oligonucleotide containing Eb and S2 sites. A 100-fold excess of unlabeled wild-type oligonucleotide, mutated Eb (for Olig1), or mutated S2 (for Sox10) oligonucleotide was used as the competitor. Mouse IgG, mouse anti-Myc IgG (for Olig1), or mouse anti-V5 IgG (for Sox10) was used for supershift. Parallel lanes contained 10-fold different amounts of supershifting antibodies (labeled 1/10 or +). ***C***, ***D***, Luciferase assay was performed with cell lysates from transfected Cos-7 cells. pCDNA control vector was used to normalize the amount of transfected DNA. The firefly luciferase activity was standardized by reference to the *Renilla* luciferase activity. The results are displayed as fold increase of luciferase activity compared with control transfection of pRenilla and pCDNA only. Results are the mean ± SE of three independent experiments. T, ild type.



6. Olig1 and Sox10 bind synergistically to the *mbp* promotor *in vivo*. ChIP assays were performed with 24 hpf, mRNA-injected embryos, fixed with formaldehyde. ***A***, ***B***, Mouse IgG and mouse anti-V5 IgG (***A***) or mouse anti-Myc IgG (***B***) were used for immunoprecipitation. PCR was used to detect the *mbp* promotor region. This fragment was also amplified from the lysate (input), genomic DNA (gDNA), and water control (H_2O). The immunoprecipitated DNA/protein complex was also detected by estern blotting (B; ***B***).



7. Olig1 and Sox10 regulate *mbp* transcription *in vivo*. Transverse sections through trunk; dorsal is to the top. ***A***, The *mbp* expression in *olig1*/*sox10* mRNA-injected embryos was revealed by *in situ* hybridization at 3 dpf. ***B***, Different *mbp* reporter constructs were injected into one-cell-stage embryos. *mbp* transcription was evaluated by *in situ* hybridization at 3 dpf with a *DsRed*-specific probe. No DsRed signal was detected in embryos injected with S1, S2, Eb+S2, S1+S1, or S1+Eb+S2 mutated constructs (data not shown). Quantification of DsRed-positive cells in spinal cord (10 sections) showed that there were many more *DsRed*-expressing cells in spinal cord of embryos injected with the wild-type (T) construct than in embryos injected with the Eb mutated (mut) construct. Error bars indicate SEM. Scale bar, 20

plp1b, and *mpz* (Fig. 1*J–R*) at 72 hpf, continuing into adulthood (data not shown). The differences between the expression patterns of zebrafish and mammalian *olig1* and between zebrafish *olig1* and *olig2* imply some functional differences between Olig1 and Olig2 and perhaps between Olig1 in fish versus mammals.

Functional differences between Olig1 and Olig2

As described originally, *Olig1* knock-out mice (with a *PGKneo* cassette at the *Olig1* locus) have delayed OL development but are otherwise normal (Lu et al., 2002). However, it was subsequently reported that after removal of the *PGKneo* cassette, the mice were dysmyelinating (they failed to activate myelin gene expression, developed tremor and seizures, and died in the third postnatal week) (Xin et al., 2005). This implied that the active *PGKneo* cassette might cause *cis* upregulation of the adjacent *Olig2* gene and hence rescue the "true" *Olig1* null phenotype. We performed *olig1* knock-down experiments with specific antisense morpholinos but did not observe any reduction in *mbp* expression. This might not be surprising given that *olig1* is not expressed until 72 hpf, which is close to the limit of the effectiveness of morpholinos. However, coinjection of *olig1*and *sox10* mRNAs strongly increased *mbp* expression in fish embryos compared with injection of *sox10* or *olig1* alone.

OLIG2 is necessary for motor neuron and OL development

both in rodents (Lu et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002) and zebrafish (Park et al., 2002). OLIG2 is reported to interact with a range of different transcription factors including E12, IDs (Samanta and Kessler, 2004), NKX2.2 (Sun et al., 2003), and NGN2 (Lee et al., 2005), whereas the interacting partners of OLIG1 have not yet been established. We analyzed binding partners of Olig1 and Olig2 in zebrafish by coimmunoprecipitation. Zebrafish Olig2, like mouse OLIG2, can form homodimers and can also bind to E12, Nkx2.2a, and Ngn3. Zebrafish Olig1, in contrast, can form homodimers and heterodimers with Olig2 or E12 but fails to interact with Nkx2.2a or Ngn3.

Another difference between zebrafish Olig1 and Olig2 is that Olig1 can bind to Sox10 whereas Olig2 cannot. This seemed to contradict the previous report that OLIG2 can bind to SOX10 in mouse (Wissmuller et al., 2006). However, we have shown that this is a true species difference: OLIG2/Olig2 from mice or zebrafish can both bind mouse SOX10 but not zebrafish Sox10. This probably reflects several key amino acid differences between the HMG domains of mouse SOX10 and zebrafish Sox10 (supplemental Fig. 1*E*, available at www.jneurosci.org as supplemental material), specifically in the H3 and C-terminal regions, which are known to be important for protein–protein interactions of SOX proteins (Remenyi et al., 2003; Wissmuller et al., 2006). In addition to the species divergence between SOX10 and Sox10, there is also divergence between the binding properties of OLIG1 and OLIG2 (and between Olig1 and Olig2), illustrated by the fact that OLIG1/Olig1 but not OLIG2/Olig2 can bind zebrafish Sox10 (summarized in Fig. 8). There is 22% amino acid sequence divergence between the bHLH domains of OLIG1 and OLIG2 (36% divergence between bHLH domains of zebrafish Olig1 and Olig2), but it is not yet possible to pinpoint which amino acids are responsible for their different Sox10 binding activities because the crystal structure of OLIG proteins has not yet been determined. It is also not known whether, or how, the different structural properties of OLIG1/Olig1 and OLIG2/Olig2 might be reflected in their interactions with other transcriptional cofactors in mice or fish.

The Olig1/Sox10 complex directly activates *mb* transcription

SOX10 is a key mediator of OL terminal differentiation (Stolt et al., 2002). In Sox10 null mouse embryos, expression of myelin genes

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and that