Redundancy of Class III POU Proteins in the Oligodendrocyte Lineage*

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Class III POU proteins are prominent regulators of neural development. Tst-1/Oct6/SCIP, for instance, is essential for terminal differentiation of myelinating Schwann cells in the peripheral nervous system. Although Tst-1/Oct6/SCIP is also expressed in the myelin forming oligodendrocytes of the central nervous system, targeted deletion of Tst-1/Oct6/SCIP failed to reveal a gross alteration of myelination in the central nervous system. To better understand this apparent discrepancy, we examined the expression of POU proteins in both cultured primary oligodendrocytes and in the oligodendrocyte-like CG-4 cell line. These cells expressed Tst-1/Oct6/SCIP, Brn-1, and Brn-2 in significant amounts, indicating that Brn-1 and Brn-2 might have the capacity to compensate loss of Tst-1/Oct6/SCIP. We show that Tst-1/Oct6/SCIP, Brn-1, and Brn-2 were all down-regulated during the early phases of oligodendrocyte development both on RNA and protein level. All three POU proteins exhibited similar DNA binding characteristics. When promoters consisting of a single POU protein-binding site adjacent to a TATA box were used as reporters in transient transfections, Brn-1 proved to be a weaker transcriptional activator than Tst-1/Oct6/SCIP. In agreement with this, we found the transactivation domain of Brn-1, which we mapped between amino acids 119 and 237, significantly weaker than the transactivation domain of Tst-1/Oct6/SCIP. Taken together, our data imply a partial, but not complete redundancy between POU proteins in oligodendrocytes.

Glial cells of the mammalian nervous system can be grouped into various classes depending on their function and location. The two major classes of glia in the central nervous system are the myelin-forming oligodendrocytes and the non-myelinating astrocytes. Glial cells in the peripheral nervous system are similarly classified either as myelinating Schwann cells or non-myelinating Schwann cells. Progress has been made over recent years in understanding the contribution of transcription factors to Schwann cell development and differentiation (1, 2). Thus, it was convincingly shown that the paired domain protein Pax3, the POU protein Tst-1/Oct6/SCIP, and the zinc-finger protein Krox-20 are essential for different phases of Schwann cell development and myelination (3–6).

Less is known about the role of transcription factors during oligodendrocyte development. Preliminary data show, however, that some of the transcription factors identified as crucial regulators in the Schwann lineage such as Krox-20 are not expressed in the oligodendrocyte lineage (6, 7). Others seem to be expressed both in Schwann cells and in oligodendrocytes (8, 9). Such a transcription factor is Tst-1/Oct6/SCIP (9–11). Originally defined as the prominent POU protein in Schwann cells, it has later also been detected in oligodendrocytes (12, 13).

Targeted deletion of Tst-1/Oct6/SCIP in mice revealed a myelination defect in the peripheral nervous system, thus corroborating the role of Tst-1/Oct6/SCIP in Schwann cell development (3, 4). Unexpectedly, however, in the central nervous system myelination was apparently normal in these mice.

Recent years have seen the identification of an ever growing list of POU proteins which have been grouped into six different classes (14). Unlike the mammalian founding members of this family, which show a pituitary-specific (Pit-1), lymphocyte-specific (Oct-2), and ubiquitous (Oct-1) expression, respectively, most of the other members are widely expressed throughout the developing nervous system and become restricted to defined neural populations in the adult (15, 16). Such an expression pattern is also characteristic of all class III POU proteins including Tst-1/Oct6/SCIP (17). Their overlapping expression in the developing nervous system indicates that these POU proteins might perform partially redundant functions in cells in which they are co-expressed. Here we identify Brn-1 and Brn-2 as two class III POU proteins which are co-expressed with Tst-1/Oct6/SCIP in the oligodendrocyte lineage. By analyzing their expression during oligodendrocyte differentiation and by investigating the functional characteristics of Brn-1, we try to address the question of functional redundancy between class III POU proteins during oligodendrocyte differentiation.

EXPERIMENTAL PROCEDURES

Library Screening—A 324-bp° fragment corresponding to the POU domain of Brn-1 was derived from RT-PCR, radiolabeled and used to screen an oligo(dT)-primed cDNA library constructed from poly(A)° RNA of undifferentiated CG-4 cells in a-Ziplox (Life Technologies, Inc.). Plasmids were excised from three independent positive λ phages according to the manufacturer's instructions. All were found to contain Brn-1 sequences. Plasmid pIL1/Brn-1 contained the longest insert with a size of 3.3 kb, including the complete open reading frame. Its sequence was determined over 3,050 bp and submitted to EMBL/GenBank (accession number AJ001641).

Plasmids—The open reading frame for rat Brn-1 was retrieved as a 2.2-kb EcoRI/NsiI fragment from pIL1/Brn-1 and inserted between EcoRI and PstI sites of pCMV5, yielding pCMV/Brn1. Analogous mammalian expression plasmids for Brn-2, Brn-4, and Tst-1/Oct6/SCIP, and

° The abbreviations used are: bp, base pair(s); RT-PCR, reverse transcriptase-polymerase chain reaction; kb, kilobase(s); DBD, DNA-binding domain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CMV, cytomegalovirus.

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the POU-responsive luciferase reporters HSVoct-luc, and siteA-luc have been described (18–20).
PcMVGal4 contained coding sequences for the DNA-binding domain (DBD) of the yeast Gal4 protein (21). Insertion of Brn-1 sequences led to an in-frame fusion of both open reading frames, with Brn-1 following Gal4 in frame each fragment corresponding to the carboxyterminal region (amino acids 461–497) of Brn-1 was generated by PCR with flanking EcoRI and BamHI sites and inserted into pCMVGal4, yielding pCMVGal4/461–497. A fusion between Gal4-DBD and the amino-terminal region of Brn-1 (amino acids 3–315) was generated by inserting an MscI/AatIII fragment into pCMVGal4, thus creating pCMVGal4/3–315. Shortened versions of the amino-terminal part of Brn-1 were generated by using naturally occurring Ala1, NaeI, Nor1, NdeI, NsiI, SmaI, and SstII restriction sites (see Fig. 8A). Plasmid pCMVGal4/Tst-1N expressed a fusion between Gal4-DBD and amino acids 1–240 of Tst-1/Oct6/SCIP and will be described elsewhere.2 The Gal4-responsive luciferase reporter (3xUAS luc) contained three tandem copies of a Gal4-binding site in front of the rat prolactin minimal promoter (22). Bacterial expression of Brn-1 holoprotein was achieved by cloning a 1.7-kb NotI fragment from pZL1-Brn1 into PET25b (Novagen). Insertion of an SphI fragment from rat Brn-2 cDNA (18) into pET28b allowed bacterial expression of a protein corresponding to amino acids 27–202 of Brn-2.

Preparation of Primary Glial Cells—Glial cultures were prepared from brains of 1-day-old Wistar rats as described (23, 24). Schwann cells were isolated from 3-day-old rats by the method of Brookes (23), as modified by Porter et al. (24).

Tissue Culture—Rat CG-4 cells were grown on poly-on-ornithine-coated plates in Dulbecco’s modified Eagle’s medium, containing 1% fetal calf serum, supplements containing 2 mM L-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 1% (v/v) sulfoxide. Media were supplemented with 50 μM ascorbic acid, 10 μg/ml transferrin, 16 μg/ml insulin, 0.2 mM dithiothreitol, and 1% Nonidet P-40. Immediately after extraction for 15 min under constant rotation, cell debris was removed from the extract by centrifugation.

Electrophoretic Mobility Shift Assay—In general, 0.5 ng of 32P-labeled probe were incubated with 1 μg of nuclear or 2.5 μg of whole cell extract for 20 min on ice in a 20-μl reaction mixture containing 10 mM Hepes pH 7.9, 0.2 mM EDTA, 2 mM dithiothreitol, and 5% Nonidet P-40. Immediately after isolation of cell nuclei was added to the final concentration of 400 μM. After extraction for 15 min under constant rotation, cell debris was removed from the extract by centrifugation.

RESULTS
As a first step in identifying the POU proteins present in the oligodendrocyte lineage, we performed PCR with a pair of degenerate primers. Because the DNA recognition helices of the POU-specific domain and the POU homeodomain are strongly conserved between all known POU proteins (14), we designed primers within these two regions. Situated between these two primers is the linker of the POU domain, which allows unambiguous identification of the respective POU protein. Using cDNA from the rat oligodendrocyte progenitor cell line CG-4 (25) and from primary oligodendrocytes, we obtained significant amounts of PCR product specific for Tst-1/Oct6/SCIP, Brn-1, and Brn-2. Brn-4 (19, 30) was the only of the known class III POU proteins not represented in the PCR products (data not shown).

tified DNA sequence for rat Brn-1 revealed a high homology with the previously described genomic sequence for mouse Brn-1 (36). Both sequences overlapped over 2,069 bp which included the complete open reading frame of this intronless gene. When compared over this region, both sequences were found to exhibit an identity of 95%. On the protein level, only 4 amino acid substitutions (residues 10, 17, 137, and 152) and an insertion of two additional amino acids (residues 81 and 82) were detected in the rat as compared with the mouse. A similarly high degree of identity was observed between rat and human Brn-1 protein (37) with 2 amino acid substitutions (residues 137 and 155) and an insertion of three glycines (1 glycine following residue 42, 2 glycines following residue 297) in the human protein.

Using published sequences for class III POU genes (11, 18, 19, 36) and the newly identified rat Brn-1 sequence, we next designed specific primer pairs for each of these genes (see "Experimental Procedures"). We confirmed the specificity of each of these primer pairs in PCR with cloned POU cDNAs as templates (data not shown), and then applied the same primers to study the expression of class III POU genes during oligodendrocyte differentiation.

For this purpose we made use of the CG-4 cell line which in the past has been shown to function as a reliable model system for the differentiation of oligodendrocytes from their O2-A precursors (7, 25, 38). mRNA was prepared from CG-4 cells before the onset of differentiation as well as 3 days after induction, and, as a control, from forskolin-treated Schwann cells. After reverse transcription, the resulting cDNA served as template for semi-quantitative PCR. Reaction conditions were chosen such that amplification was in the linear range. Amplification of a GAPDH-specific product from each cDNA, furthermore, indicated that comparable amounts of cDNA were present in each sample (Fig. 2A). As expected, cDNA from Schwann cells contained significant amounts of Tst-1/Oct6/SCIP, whereas Brn-1 and Brn-2 were absent. Undifferentiated CG-4 cells, which are comparable to oligodendrocyte progenitors, on the other hand, expressed Brn-1, Brn-2, and Tst-1/Oct6/SCIP. Interestingly, the expression of all three POU proteins was down-regulated after differentiation as judged from the amounts of PCR products in samples from differentiated and undifferentiated CG-4 cells. In agreement with data from our degenerate PCR we could not detect Brn-4 in CG-4 cells at any stage of differentiation. Similarly, Brn-4 was absent from Schwann cell cDNA.

RT-PCR analysis was also performed on cDNA samples derived from primary oligodendroglial cells, which were kept in culture for 3 days or 2 weeks (Fig. 2B). Because differentiation proceeds with time in culture, 2-week-old cultures contained a lower proportion of oligodendrocyte progenitor cells and a correspondingly higher proportion of mature oligodendrocytes as 3-day-old cultures. As already observed for CG-4 cells, all class III POU proteins were expressed in the oligodendrocyte lineage. Expression levels for Brn-1, Brn-2, and Tst-1/Oct6/SCIP were significantly higher in 3-day-old cultures than in 2-week-old cultures, indicating that expression of all three POU genes is down-regulated during oligodendrocyte differentiation. Amplification of a GAPDH-specific product
again confirmed the presence of similar amounts of cDNA in both samples.

To assess whether this expression pattern was reflected on the protein level, we performed gel shift analysis on extracts of undifferentiated CG-4 cells using the octamer site from the Herpes simplex virus ICP0 gene (HSVoct) as a probe (39). As shown in Fig. 3A, two complexes were obtained with CG-4 cell nuclear extract. To identify the proteins present in these two complexes, we expressed each of the four known class III POU proteins ectopically in transfected COS cells. By using nuclear extracts from transfected COS cells, we then compared the mobility of complexes formed between class III POU proteins and the HSVoct probe with the mobility of the two CG-4 cell complexes. The upper complex from CG-4 cell extracts exhibited a mobility characteristic of Brn-1. The lower complex from CG-4 cell extracts, on the other hand, migrated at a position similar to Brn-2 or Tst-1/Oct6/SCIP. In agreement with RNA analyses, no complex was observed at a position corresponding to Brn-4.

Whole cell extracts of primary oligodendrocyte progenitor cells exhibited a similar pattern in electrophoretic mobility shifts as undifferentiated CG-4 cells (Fig. 3B), again pointing to the fact that CG-4 cells are a good model system for the oligodendrocyte lineage. Extracts from several other cells were also tested. Under identical assay conditions only the lower of the two complexes was observed with nuclear extracts of primary Schwann cells, C6 glioblastoma cells, or OLN-93 oligodendroglioma cells (data not shown).

To further corroborate the presence of Brn-1, Brn-2, and Tst-1/Oct6/SCIP in the respective complexes, we next performed antibody perturbation experiments (Fig. 3C). Using longer gels to obtain a higher resolution, we attempted to separate the Brn-2 and Tst-1/Oct6/SCIP containing complexes. On these gels, the lower complex indeed split into a more prominent upper and a less intense lower band. When antibodies specific to Brn-1 were incubated with CG-4 cell extracts and the HSVoct, the complex with the lowest mobility disappeared. Incubation with a Brn-2 specific antisera led to the selective disappearance of the middle complex, whereas an antisera against Tst-1/Oct6/SCIP abolished the complex with the highest mobility. Thus, all class III POU proteins except Brn-4 could be detected in CG-4 cell extracts. Assuming similar DNA binding characteristics (see below), Tst-1/Oct6/SCIP seemed to be less abundant in CG-4 cells than either Brn-1 or Brn-2.

Next we compared the amounts of class III POU proteins in extracts of differentiating CG-4 cells with the levels present in the undifferentiated state (Fig. 4A). Both the complex with the higher mobility and the complex with the lower mobility decreased in strength during CG-4 cell differentiation. A steep decline was observed after the first day of differentiation, followed by a more gradual decline over the next several days in culture. When extracts from primary oligodendrocytes were used instead of CG-4 cell extracts, we made very similar observations (Fig. 4B). Both the high and the low mobility complex were abundant in whole cell extracts from cultures which contained a high percentage of oligodendrocyte progenitors, and decreased to background levels as these progenitors differentiated in culture to mature oligodendrocytes.

The presence of three class III POU proteins in oligodendrocytes and the similarity of their expression pattern during oligodendrocyte differentiation indicated that these proteins might have at least partially redundant function. To address this question we compared the DNA binding characteristics of Brn-1 and Brn-2 with Tst-1/Oct6/SCIP. Oligonucleotides containing binding sites for POU proteins or for the fushi tarazu (ftz) homeodomain protein were assessed for their ability to interact with class III POU proteins (Fig. 5). Brn-1 efficiently bound octamer sequences, the classical binding sites for POU proteins (Fig. 5A). In addition, Brn-1 also interacted with a number of AT-rich non-canonical POU-binding sites, including CRH (18) and siteA (20). Other AT-rich DNA sequences such as the ftz recognition sequence (FTZ in Fig. 5) were not significantly bound by Brn-1. As first observed for the lymphoid-specific POU protein Oct-2 (40), Brn-1 exhibited cooperative binding to the combination of octamer and adjacent heptamer site from the immunoglobulin heavy chain enhancer (O’H’ in Fig. 5). Whereas Brn-1 still bound efficiently to an oligonucleotide with mutated heptamer site (O’H’ in Fig. 5), binding was severely diminished after destruction of the octamer site (O’H’ in Fig. 5). Tst-1/Oct6/SCIP and Brn-2 each exhibited binding characteristics almost indistinguishable from Brn-1 (Fig. 5, B and C).

We next asked whether Brn-1, Brn-2, and Tst-1/Oct6/SCIP similarly functioned in promoter activation assays. We performed a series of transient transfection experiments in U138...
giblastoma cells using promoters that had been constructed from POU domain-binding sites and a TATA box (Fig. 6). Plasmid HSVOct-luc contained a canonical POU domain-binding site, whereas siteA-luc contained an AT-rich DNA sequence which is recognized by class III POU proteins despite its divergence from classical octamer sequences. Tst-1/Oct6/SCIP activated both artificial promoters approximately 10-fold in most experiments, but on occasion up to 30-fold. Similar activation rates were also obtained with Brn-2 (data not shown). Brn-1, on the other hand, activated both simple promoter constructs to a lesser extent. Activation rates for HSVOct-luc were on average only 26% of the rates obtained for Tst-1/Oct6/SCIP. In case of siteA-luc, activation rates were only 16% of the rates obtained for Tst-1/Oct6/SCIP. Thus, reporter activation by Brn-1 was barely above baseline in transfections, in which we observed a 10-fold stimulation by Tst-1/Oct6/SCIP, but was readily visible in those transfections which exhibited maximal Tst-1/Oct6/SCIP stimulation.

As Brn-1 showed a weaker transactivation potential on simple promoter constructs than either Brn-2 or Tst-1/Oct6/SCIP, we analyzed the transactivation capacity of Brn-1 in more detail. To screen for the presence of a modular transactivation domain within Brn-1, the amino acid-terminal to the POU domain (amino acids 3–315) and the region carboxy-terminal to the POU domain (amino acids 461–497) were separately fused to the DNA-binding domain of Gal4 (Gal4-DBD, Fig. 7A). Transfer of a heterologous transactivation domain to Gal4-DBD has been shown to lead to the reconstitution of a functional transcription factor, and results in the activation of promoters with Gal4-binding sites in transient transfections. Gal4-DBD fusions were analyzed both in U138 and T98G human glioma cells with similar results. Fusion of the amino-terminal part of Brn-1 to Gal4-DBD resulted in a 9.8-fold stimulation, whereas transfer of the carboxy-terminal region did not result in a significant promoter stimulation (Fig. 8A), despite the fact that the carboxy-terminal fusion was synthesized in amounts comparable to the amino-terminal fusion (Fig. 7B). Thus, the amino terminus contained the transactivation domain of Brn-1. In direct comparisons, this domain exhibited approximately one-third of the activity of the transactivation domain of Tst-1/Oct6/SCIP (Fig. 8B).

To map the transactivation domain of Brn-1 with greater precision, we used restriction sites present in the Brn-1 coding sequence to construct several sets of Gal4-DBD fusion proteins (Fig. 7A). Their expression in transfected cells was confirmed in electrophoretic mobility shift analyses using the Gal4-binding site as a probe, and found to be in the same range as the expression of the isolated Gal4-DBD or the fusions of the amino-terminal and carboxy-terminal Brn-1 regions to Gal4-DBD (Fig. 7B).

First we successively removed amino acid residues from the hind end of the Brn-1 amino terminus. A fusion between Gal4-DBD and the first 265 amino acids of Brn-1 (Gal4/3–265) activated a Gal4-responsive promoter as efficiently as a fusion which carried the complete amino-terminal region (Fig. 9A). The first 237 amino acids of Brn-1 (Gal4/3–237) were still sufficient to obtain near-maximal activation of the Gal4-responsive reporter. However, when only the first 165 residues of Brn-1 were present in the Gal4 fusion protein (Gal4/3–165), transactivation was obliterated. Removal of additional amino acids did not lead to a recovery of the transactivation potential. These results indicated that the transactivation function of Brn-1 is contained within the first 237 amino acids of the protein.

To further corroborate the importance of this domain, we fused it to the Gal4 DNA-binding domain. As shown in Fig. 9C, a fusion between amino acids 119 and 237 of Brn-1 and Gal4-DBD yielded an 8.6-fold stimulation of a Gal4-responsive promoter, almost identical to the stimulation observed for the fusion between the complete Brn-1 amino terminus and Gal4-DBD.

In a reverse experiment, we deleted an internal segment from the amino-terminal region of Brn-1 corresponding to...
The role of POU proteins as regulators of neural development and differentiation has become increasingly clear with the availability of gene targeting techniques in mice. Especially POU proteins belonging to class III and class IV have been proven by this technology to be involved in various aspects of neural development (3, 4, 34, 35, 41–44). The class III POU proteins, Brn-1 and Brn-2, follow an expression pattern similar to Tst-1/Oct6/SCIP. These results were not only obtained with the CG-4 cell line as a model system (25). PCR on CG-4 cell cDNA with degenerate primers directed against conserved regions of the POU domain pointed to the expression of two other POU proteins most likely are other POU proteins related to Brn-1 and Brn-2. Assuming linkage between structure and function, these substituting proteins most likely are other POU proteins related to Tst-1/Oct6/SCIP. Therefore, we started to look for Tst-1/Oct6/SCIP-related POU proteins in the oligodendrocyte lineage, using the CG-4 cell line as a model system (25). PCR on CG-4 cell cDNA with degenerate primers directed against conserved regions of the POU domain pointed to the expression of two other class III POU proteins in the oligodendrocyte lineage in addition to Tst-1/Oct6/SCIP. The presence of these two class III POU proteins, namely Brn-1 and Brn-2, was subsequently confirmed in PCRs with gene-specific primers. As PCR products were analyzed in the linear range of this assay, an approximation of the expression of each of these POU proteins during CG-4 cell differentiation was possible. We found that expression of all three POU proteins decreased during differentiation. Thus, Brn-1 and Brn-2 follow an expression pattern similar to Tst-1/Oct6/SCIP. These results were not only obtained with the oligodendrocyte precursor cell line CG-4, but also with primary oligodendrocyte cultures, thus confirming the validity of the CG-4 cell line as a model system for oligodendrocyte differentiation.

In addition to the respective transcripts, we also detected protein for each of the three POU proteins both in CG-4 cells expressing the CG-4 cell line as a model system (25). PCR on CG-4 cell cDNA with degenerate primers directed against conserved regions of the POU domain pointed to the expression of two other class III POU proteins in the oligodendrocyte lineage in addition to Tst-1/Oct6/SCIP. The presence of these two class III POU proteins, namely Brn-1 and Brn-2, was subsequently confirmed in PCRs with gene-specific primers. As PCR products were analyzed in the linear range of this assay, an approximation of the expression of each of these POU proteins during CG-4 cell differentiation was possible. We found that expression of all three POU proteins decreased during differentiation. Thus, Brn-1 and Brn-2 follow an expression pattern similar to Tst-1/Oct6/SCIP. These results were not only obtained with the oligodendrocyte precursor cell line CG-4, but also with primary oligodendrocyte cultures, thus confirming the validity of the CG-4 cell line as a model system for oligodendrocyte differentiation.
and primary oligodendrocyte cultures. Confirming the results from semi-quantitative PCR, all POU proteins were found to decrease in amount during differentiation. Currently, we cannot distinguish whether the residual amounts detected after differentiation stem from a basal expression in differentiated cells or from a low percentage of cells which have remained in the undifferentiated state and continue to express high levels of all three POU proteins.

Our results are in excellent agreement with a previous study on the expression of Tst-1/Oct6/SCIP during oligodendrocyte differentiation (12). In addition to finding a comparable expression profile for Tst-1/Oct6/SCIP, the authors also detected a second protein in oligodendrogial extracts that cross-reacted with antibodies against Tst-1/Oct6/SCIP and exhibited a similar expression pattern during differentiation. Judged from the apparent mobility of this cross-reactive protein on denaturing polyacrylamide gels, it is very likely that this protein is identical to the Brn-1 protein described in this report.

As expression of Brn-1 and Brn-2 closely paralleled Tst-1/Oct6/SCIP expression in oligodendrocytes, it seemed possible that these two class III POU proteins perform functions during oligodendrocyte development similar to Tst-1/Oct6/SCIP, and could therefore functionally replace Tst-1/Oct6/SCIP in its absence. To address functional redundancy between class III POU proteins, we isolated the cDNA for rat Brn-1. A consecutive survey of its expression in various cells revealed that Brn-1 is absent from most cell lines. With the exception of the CG-4 cell line, none of the other glial cell lines routinely used in our laboratory expressed Brn-1. This included C6 and OLN-93 cells which are both thought to be of oligodendrocytic origin (48, 49). There might be two reasons for this: for one, C6 and OLN-93 cells might represent later stages of oligodendrocyte development than CG-4 cells, during which Brn-1 is no longer expressed. Second, Brn-1 expression might be rapidly extinguished upon prolonged culture in serum-containing medium. In support of the latter assumption, Brn-1 was readily detected in a number of primary brain tumors, but was mostly absent from long-term cultured gliomas (50).

Sequencing revealed that there are only minor differences between the amino acid sequences of rat, mouse, and human Brn-1 (36, 37). This strong conservation of Brn-1 during mammalian evolution points to an important developmental function of Brn-1. To analyze the DNA binding behavior of Brn-1, we expressed it ectopically in COS cells. Electrophoretic mobility shift analysis with a panel of oligonucleotides revealed that the DNA binding characteristics of Brn-1 were in fact indistinguishable from Brn-2 and Tst-1/Oct6/SCIP, with all class III POU proteins recognizing a number of AT-rich binding elements in addition to classical octamer sites.

In addition to DNA binding, we also compared the ability to activate selected promoters. Brn-1 was clearly a weaker transcriptional activator than either Brn-2 or Tst-1/Oct6/SCIP on promoter constructs that consisted of only a TATA box and a binding site for a POU protein. As binding of Brn-1 and Tst-1/Oct6/SCIP to these sites is essentially identical, the observed differences are most likely due to differences in the respective transactivation domains. The transactivation domain of Tst-1/Oct6/SCIP had been previously mapped to a region in the amino-terminal part of the protein that is rich in alanine and glycine residues (20, 32, 33). Here we show that the transactivation domain of Brn-1 is also localized to the amino-terminal part of the protein. It is contained within a domain of 119 amino acids between residue 119 and 237, which is rich in proline residues, contains a homopolymeric alanine stretch, but has a conspicuously low proportion of glycine residues. Thus, the transactivation domain of Brn-1 might not only be weaker in strength than the transactivation domain of Tst-1/Oct6/SCIP, but might also have a different quality, which might allow interaction with the transcription machinery in a manner different from Tst-1/Oct6/SCIP.

In conclusion, our results indicate that Brn-1, Brn-2, and Tst-1/Oct6/SCIP have many features in common. This might allow them to functionally replace each other in oligodendrocyte development during which they are co-expressed. In this case, deletion of all three POU proteins in mice might be necessary to severely disrupt central nervous system myelination. On the other hand, subtle differences in function could be detected between single POU proteins, as shown here for Tst-1/Oct6/SCIP and Brn-1. This might indicate that in addition to redundant functions, each of these proteins might carry out some tasks which cannot be taken over by the other two. Such a scenario would predict the existence of subtle deficits in...
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