YY1 negatively regulates mouse myelin proteolipid protein (Plp1) gene expression in oligodendroglial cells

Olga E Zolova1 and Patricia A Wight2
Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, U.S.A.

Cite this article as: Zolova OE and Wight PA (2011) YY1 negatively regulates mouse myelin proteolipid protein (Plp1) gene expression in oligodendroglial cells. ASN NEURO 3(4):art:e00067.doi:10.1042/AN20110021

ABSTRACT

YY1 (Yin and Yang 1) is a multifunctional, ubiquitously expressed, zinc finger protein that can act as a transcriptional activator, repressor, or initiator element binding protein. Previous studies have shown that YY1 modulates the activity of reporter genes driven by the myelin PLP (proteolipid protein) (Plp1) promoter. However, it is known that Plp1 intron 1 DNA contains regulatory elements that are required for the dramatic increase in gene activity, coincident with the active myelination period of CNS (central nervous system) development. The intron in mouse contains multiple prospective YY1 target sites including one within a positive regulatory module called the ASE (anti-silencer/enhancer) element. Results presented here demonstrate that YY1 has a negative effect on the activity of a Plp1-lacZ fusion gene [Plp1+lacZ] in an immature oligodendroglial cell line (Oli-neu) that is mediated through sequences present in Plp1 intron 1 DNA. Yet YY1 does not bind to its alleged site in the ASE (even though the protein is capable of recognizing a target site in the promoter), indicating that the down-regulation of PLP+[+Z] activity by YY1 in Oli-neu cells does not occur through a direct interaction of YY1 with the ASE sequence. Previous studies with YY1 conditional knockout mice have demonstrated that YY1 is essential for the differentiation of oligodendrocyte progenitors. Nevertheless, the current study suggests that YY1 functions as a repressor (not an activator) of Plp1 gene expression in immature oligodendrocytes. Perhaps YY1 functions to keep the levels of PLP in check in immature oligodendrocytes before vast quantities of the protein are needed in mature myelinating oligodendrocytes.

Key words: gene expression, gene regulation, myelin proteolipid protein gene, Oli-neu cells, repression, YY1.

INTRODUCTION

YY1 (Yin and Yang 1) is a multifunctional, ubiquitously expressed, zinc finger protein that can act as a transcriptional activator, repressor, or initiator element binding protein (for reviews see Shi et al., 1997; Thomas and Seto, 1999; Gordon et al., 2006; He and Casaccia-Bonnefil, 2008). YY1 is present in oligodendrocytes where it is localized to the nucleus (Rylski et al., 2008). The protein has been shown to recognize a target motif in the promoter of the human myelin PLP (proteolipid protein) gene (PLP1) where it seemingly functions as a transcriptional activator (Berndt et al., 2001). PLP1/Plp1 gene expression is regulated in a spatiotemporal manner and, in oligodendrocytes, is responsible for nearly half the protein in CNS (central nervous system) myelin from adults (reviewed in Wight and Dobretsova, 2004). Categorization of YY1 as an activator of human PLP1 gene transcription stems from studies by Berndt et al. showing that: (i) it binds selectively to a YY1 target site located within a region of the PLP1 promoter referred to as site 3 [positions −130 to −104 relative to the transcription start point (+1)] (Berndt et al., 2001), which previously had been shown to bind nuclear protein(s) in a sequence-specific manner (Berndt et al., 1992); (ii) deletion–transfection analysis in glial cells using various PLP1 promoter driven reporter constructs revealed a dramatic decrease in reporter gene activity when site 3 was deleted along with another, unrelated, protein binding site (site 2; positions −76 to −50) (Berndt et al., 1992); (iii) the level of expression of a PLP1-reporter gene construct that contains PLP1 sequences from −1088 to +85 was increased when human (SVG) or rat (CG4) glial cell lines were co-transfected with a YY1 expression plasmid, but not with an analogous construct having a mutant site 3 that disrupts YY1 binding (Berndt et al., 2001). However, as noted by Berndt et al. (1992), the same (unmutated) PLP1 sequence was not
effective in driving expression of a reporter gene in transgenic mice (Gout et al., 1991), whereas a related transgene that contains nearly 4.2 kb of 5′-flanking DNA could do so (Nadon et al., 1989, 1994). Discrepancies between the outcomes with transfection and transgenic approaches have been noted for other genes as well (Zimmerman et al., 1990; Kitis and Leinwand, 1992; Donoviel et al., 1996), and may be due to differences in the chromatin status of the construct itself, or to inherent differences in the cells themselves. The current study was undertaken to evaluate the role of YY1 in regulating mouse Plp1 gene activity. The structure of the gene in mouse and man is quite similar (reviewed in Wight and Dobretsova, 2004). The 5′-flanking DNA is highly conserved between the two species, demonstrating 50% identity for the proximal 1.3 kb of sequence and 91% identity among the initial 135 nucleotides using the algorithm of Myers and Miller (1988). Moreover, the sequence orthologous to site 3 in the mouse Plp1 gene [positions −132 to −106 based on the numbering system by Macklin et al. (1987)] is highly conserved, having 93% identity with the human sequence. Within this sequence there is an exact match to a high affinity CCAT core YY1 target site (consensus = VDCCATNWY) (Yant et al., 1995) that is identical to the corresponding sequence in man. However, unlike the case when the human PLP1 promoter was used to drive a reporter gene in glial cell lines (Berndt et al., 1992), deletion of ‘site 3’ from the mouse Plp1 promoter did not cause a major diminution in the levels of reporter gene activity (Wight et al., 1997), suggesting that this sequence does not function as a positive regulatory element in mouse. More recently it has been shown that overexpression of YY1 in the immortalized mouse oligodendroglial cell line, Oli-neu, causes a dose-dependent decrease in mouse Plp1 promoter driven reporter gene activity when the cells were kept proliferating in growth medium (ODM) and 1% horse serum (He et al., 2007a). However, YY1 overexpression did not lead to a significant change in reporter gene activity when the cells were press to differentiate by dibutylr cAMP treatment the day following transfection, except perhaps for a slight decrease with the highest amount (2 μg/well) of YY1 plasmid (He et al., 2007a). Thus, if anything, the mouse counterpart to site 3 (termed prom 3) appears to function as a negative regulatory element.

While the aforementioned studies have focused on effects of YY1 via regulatory sequences within the promoter and adjoining 5′-flanking DNA, the first intron of the mouse Plp1 gene also contains many prospective YY1 binding sites (Wight and Dobretsova, 1997). Transgenic mouse studies (Li et al., 2002b) have shown that inclusion of Plp1 intron 1 DNA in Plp1-lacZ fusion genes is critical to attain high levels of expression in brain during the active myelination period of CNS development, in keeping with the temporal pattern exhibited by the endogenous Plp1 gene. Transfection analysis using a battery of Plp1-lacZ constructs containing partial deletion of Plp1 intron 1 DNA revealed the presence of a single positive-regulatory element within the intron that is active in N20.1 cells (Dobretsova and Wight, 1999). Besides being able to overcome (or counterbalance) the effects from negative-regulatory elements located elsewhere in the intron, the positive-regulatory element functions as an enhancer in N20.1 cells (Dobretsova et al., 2000; Meng et al., 2005). Thus, we designated the positive regulatory element ASE (antisilencer/enhancer). The ASE has been minimally mapped to mouse Plp1 intron 1 positions 1093–1177 (Dobretsova et al., 2004) and contains an exact match to a high affinity YY1 target site (consensus = VDCCATNWY; Yant et al., 1995) spanning intron 1 positions 1137–1145. The intron also contains nine other potential YY1 binding sites with exact matches to the CCAT core consensus sequence. To address the effects that these sites might play in modulating Plp1 gene expression, transfection analysis was performed in Oli-neu cells with Plp1-lacZ fusion genes containing all, some, or none of mouse Plp1 intron 1 DNA. Oli-neu cells were used for this study because they are slightly more mature than N20.1 cells, based on the relative amounts and types of splice variants expressed by several myelin genes including Plp1 (Pereira et al., 2011). In some experiments cells were co-transfected with an expression construct to increase the levels of YY1. Here we report that inclusion of Plp1 intron 1 DNA greatly enhances the levels of Plp1-driven lacZ expression in Oli-neu cells. The increase in expression is largely attributable to the ASE regulatory element. However, over-expression of YY1 mitigates this response, apparently through an indirect mechanism. YY1 did not bind to the alleged target motif in the ASE as determined by gel shift analysis, although it did to a target site in prom 3 from the promoter. Both the ASE and prom 3 contain an exact match to the YY1 consensus sequence (VDCCATNWY). Therefore, the YY1 consensus sequence can be refined further based on differences in the sequence between ASE and prom 3.

**MATERIALS AND METHODS**

**Cell culture**

Dr Patrizia Casaccia (Mount Sinai School of Medicine) generously provided the mouse Oli-neu cell line (Jung et al., 1995) with permission from Dr. Jacqueline Trotter (University of Mainz). The Oli-neu cell line was derived by immortalization of primary cultures of enriched oligodendrocytes with the t-neu oncogene. Oli-neu cells were grown at 37°C in SATO medium devoid of mitogens (ODM) according to the modifications by He et al. (2007a) and supplemented with 1% horse serum. Oli-neu cells were maintained in an atmosphere of 10% CO2. The mouse N20.1 cell line (Verity et al., 1993) was derived by immortalization of primary cultures of enriched oligodendrocytes with a temperature-sensitive form of SV40 large T antigen. N20.1 cells were grown at 34°C in DMEM/F-12 (Dulbecco’s modified Eagle medium/Ham’s F-12 nutrient mixture; Invitrogen) supplemented with 15 mM Hepes, 2.438 g/l of sodium bicarbonate, 4 g/l of glucose, 100
μg/ml of G-418 and 10% FBS (fetal bovine serum; HyClone), and maintained in an atmosphere of 5% CO₂. Monolayer cultures of HeLa cells were grown at 37°C in DMEM/F-12 supplemented with 15 mM Hepes, 2.5 mM l-glutamine, 5% FBS, 100 units/ml of penicillin G, 100 μg/ml of streptomycin and 0.25 μg/ml of amphotericin B, and maintained in an atmosphere of 5% CO₂.

Plasmids
Details regarding the construction of PLP(+)Z (Wight et al., 1993), PLP(−)Z (Wight and Dobretsova, 1997), and the Plp1 intron 1 partial deletion constructs, PLPA12–8068 and PLPA809–5807 (Dobretsova and Wight, 1999), have been described previously. The constructs utilize the mouse Plp1 promoter and associated sequences to drive expression of the reporter gene. Every Plp1-lacZ construct contains the proximal 2.4 kb of Plp1 5′-flanking DNA, all of exon 1 DNA, and the first 37 bp of exon 2. PLP(+)Z also contains all of Plp1 intron 1 DNA, whereas PLP(−)Z lacks the intron altogether. The corresponding partial deletion constructs contain some of Plp1 intron 1 DNA and were named according to the deleted intronic sequence. For instance, PLPA809–5807 is missing Plp1 intron 1 DNA from positions 809–5807 based upon numbering the entire intron from positions 1–8140 (Wight and Dobretsova, 1997). Plasmid PLPA809–5807 +F(AP4) (Dobretsova et al., 2004) contains the ASE sequence (Plp1 intron 1 positions 1093–1177) inserted into the PstI site at the deletion–junction site of PLPA809–5807 in the native (forward; F) orientation, and is referred to here as PLPA809–5807+ASE-F for purposes of clarity.

F-YY1 (FLAG-tagged YY1) was expressed from the plasmid pCEP4F-YY1 (Yao et al., 2001), which contains a cDNA for human YY1 immediately downstream of the CMV (cytomegalovirus) promoter in pCEP4F (Zhu et al., 1995). The tagged protein contains a single copy of the FLAG epitope at its N-terminus.

Transfection analysis
Oli-neu cells were seeded at a density of 1 × 10⁶ cells per 35-mm well (six-well dishes, Costar) the day prior to transfection. Cells were transfected with an equimolar amount of a given Plp1-lacZ construct and a fixed amount (0.35 μg/well) of a plasmid [RsvL (Rous sarcoma virus–luciferase)] containing the luciferase reporter gene under control of the LTR (long terminal repeat) promoter of Rsv (rous sarcoma virus) to monitor for differences in transfection efficiency. Empty vector (pBluescript SK+) lacks the intron altogether. The corresponding partial deletion constructs contain some of Plp1 intron 1 DNA and were named according to the deleted intronic sequence. For instance, PLPA809–5807 is missing Plp1 intron 1 DNA from positions 809–5807 based upon numbering the entire intron from positions 1–8140 (Wight and Dobretsova, 1997). Plasmid PLPA809–5807 +F(AP4) (Dobretsova et al., 2004) contains the ASE sequence (Plp1 intron 1 positions 1093–1177) inserted into the PstI site at the deletion–junction site of PLPA809–5807 in the native (forward; F) orientation, and is referred to here as PLPA809–5807+ASE-F for purposes of clarity.

F-YY1 (FLAG-tagged YY1) was expressed from the plasmid pCEP4F-YY1 (Yao et al., 2001), which contains a cDNA for human YY1 immediately downstream of the CMV (cytomegalovirus) promoter in pCEP4F (Zhu et al., 1995). The tagged protein contains a single copy of the FLAG epitope at its N-terminus.

Nuclear extracts
Nuclear extracts were prepared from cells (Oli-neu, N20.1, and HeLa) grown to ~80% confluence in 162 cm² flasks by the methods of Dignam et al. (1983). In some cases, nuclear extracts were prepared from 10 six-well plates of Oli-neu cells that had been transfected 48 h earlier with pCEP4F-YY1 (0.1 μg/well) and pBluescript SK+ (2.9 μg/well) and nuclear extracts prepared 48 h post-DNA addition for subsequent use in EMSA (electrophoretic mobility shift assay) and Western-blot analysis. Statistical analyses were performed using the ANOVA module from SigmaPlot 11 (Systat Software Inc.).

EMSA
EMSA analysis was performed as previously described (Dobretsova and Wight, 1999). EMSA probes were generated from synthetic double-stranded oligonucleotides (27–mers) that were radiolabelled using [γ-32P]ATP (PerkinElmer) and T4 polynucleotide kinase (New England BioLabs). The sequence for one strand of each oligonucleotide is shown in Table 1 relative to a prospective YY1 binding site (consensus=VDCCATNWY). YY1 consensus and mutant oligonucleotides were obtained from Santa Cruz Biotechnology. Mouse Plp1-specific oligonucleotides were obtained from Integrated DNA Technologies. Prom 3 is orthologous to the human site 3 oligonucleotide used in an earlier study (Berndt et al., 2001) and spans the mouse Plp1 promoter from positions −132 to −106 based on the numbering system utilized by Macklin et al. (1987). Prom 1 corresponds to Plp1 5′-flanking DNA positions −1593 to −1570 and contains two mismatches compared with the VDCCATNWY consensus, while still maintaining the CCAT core.
The 1128–1154 double-stranded oligonucleotide corresponds to Plp1 intron 1 DNA position 1128–1154 (Wight and Dobretsova, 1997). Binding reactions for EMSA were assembled at room temperature in a total volume of 20 µl and consisted of 1–4 µg protein [nuclear extract alone or 0.1 µg 68 kDa YY1 polyhistidine-tagged fusion protein (Santa Cruz Biotechnology) plus 3.9 µg BSA], 1–2 µg poly(dI-dC)•(dI-dC) and 2–4 × 10⁶ c.p.m. of labelled probe in a solution of 10 mM Tris/HCl (pH 8.0), 50 mM KCl, 0.5 mM EDTA, 5% glycerol and 1 mM DTT (dithiothreitol). Some reactions contained unlabelled ‘competitor’ DNA at 50-, 100- or 200-fold molar excess over the probe. DNA–protein complexes were resolved on non-denaturing 5% polyacrylamide gels and visualized by autoradiography.

Western– blot analysis

Proteins (10 µg from nuclear extracts) were denatured by heat (95°C for 5 min) in 50 mM Tris/HCl (pH 6.8), 2% SDS, 10% glycerol, 0.1% Bromophenol Blue and 100 mM DTT (loading buffer). Proteins were fractionated on an SDS/PAGE gel (7.5% or 10% polyacrylamide) and subsequently transferred to a nitrocellulose membrane (Optitran BA-S 85, Schleicher & Schuell) using the Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) for 2 h at 100 V. Proteins were visualized by staining with Ponceau Red [0.1% (w/v) of Ponceau S (Sigma–Aldrich) in 5% acetic acid]. Membranes were subsequently destained and then blocked with 10% (w/v) non-fat dried skimmed milk powder in TBST (Tris-buffered saline with Tween-20; 20 mM Tris/HCl, pH 7.6, 150 mM NaCl, 0.1% Tween 20) for 30 min at room temperature and washed three times (5 min each) in TBST. Membranes were incubated for 1 h with mouse monoclonal anti-YY1 (H-10) antibody (1:200 dilution; Santa Cruz Biotechnology) or anti-FLAG M2 antibody (10 µg/ml; Sigma–Aldrich) in TBST containing 3% BSA. Membranes were washed three times (5 min each) in TBST followed by incubation with secondary antibodies [HRP (horseradish peroxidase)-conjugated donkey anti-mouse IgG; Jackson ImmunoResearch Laboratories] diluted 1:5000 or 1:10000 in TBST with 5% non-fat dried skimmed milk powder. Membranes were washed three times (10 min each) in TBST and immunoreactive bands visualized using a chemiluminescence reagent kit (PerkinElmer).

RESULTS

The presence of Plp1 intron 1 DNA greatly increases the level of Plp1-lacZ expression in Oli-neu cells, which is mitigated by elevated levels of YY1

Previous studies with Plp1-lacZ transgenic mice (Wight et al., 1993; Li et al., 2002b) have shown that inclusion of Plp1 intron 1 DNA in the transgene is crucial to attain a robust developmental increase in β-gal activity in brain, concurrent with the active myelination period of CNS development. In particular, the PLP(+1Z transgene depicted in Figure 1, which utilizes Plp1 genomic DNA [proximal 2.4 kb of 5′-flanking DNA downstream to the first 37 bp of exon 2] to drive lacZ reporter gene expression, is temporally regulated in brain similar to that of the endogenous Plp1 gene. However, the developmental increase that is normally reached during the active myelination period is severely attenuated when Plp1 intron 1 DNA is omitted from the transgene [PLP(−1Z] construct depicted in Figure 1).

Deletion–transfection analysis with constructs equivalent to PLP(+1Z aside from missing a portion of Plp1 intron 1 has identified a single positive regulatory element (ASE) and multiple negative regulatory elements that are functional in N20.1 cells (Dobretsova and Wight, 1999; Li et al., 2002a; Dobretsova et al., 2004). The cumulative effects of these regulatory elements are counterbalanced in N20.1 cells, hence PLP(+1Z and PLP(−1Z yield relatively similar levels of β-gal activity in transfected cells. However, as shown in Figure 2, the relative levels of β-gal activity in transfected Oli-neu cells is much greater for PLP(+1Z (∼50-fold higher) than PLP(−1Z. To test the effects that increased levels of YY1 might have on this ratio, Oli-neu cells were co-transfected with a YY1 expression plasmid (pCEP4F-YY1). Elevated levels of YY1, via co-transfection with only 0.025 µg pCEP4F-YY1, caused a dramatic drop in the β-gal activity for PLP(+1Z, which was not significantly different from that obtained with PLP(−1Z (Figure 2). Increased amounts of YY1 plasmid led to a further decrease, in a dose-dependent manner. Because PLP(+1Z and PLP(−1Z are the same except for the presence or absence of Plp1 intron 1 DNA respectively the drop in β-gal
Oli-neu cells were transfected with equimolar amounts of a particular molecule in a manner similar to that depicted in Figure 2. Overexpression of YY1 in Oli-neu cells causes a decrease in the activity for PLP(+)Z in the midst of elevated YY1 levels must be mediated through regulatory element(s) in the intron.

YY1 overexpression impairs ASE function
As noted earlier, the intron contains multiple potential YY1 binding sites, one of which is situated within the ASE. It is possible that overexpression of YY1 may interfere with the binding of a requisite activator whose recognition site overlaps or abuts the putative YY1 target site in the ASE.

To test this supposition, Oli-neu cells were co-transfected with equimolar amounts of a given Plp1-lacZ construct in the presence or absence of YY1 expression plasmid. The fold change in the levels of β-gal activity was measured and compared between the transfected and control samples. The results showed a significant decrease in the levels of β-gal activity in PLP(+)Z transfected cells compared with those transfected with PLP(−)Z (Figure 3). Removal of the ASE sequences resulted in a dramatic decrease in the levels of β-gal activity compared with PLP(+)Z transfected cells containing endogenous levels of YY1 (i.e., not co-transfected with YY1 plasmid). Reinstatement of the ASE sequences into the deletion–junction site of PLP(−)Z restored the levels of β-gal activity back to those obtained with PLP(+)Z (Figure 3).

YY1 levels are similar in Oli-neu and N20.1 oligodendroglial cell lines
Because the ASE appears far more potent in Oli-neu cells compared with N20.1 cells, which could result in a higher amount of YY1 plasmid exceeding the endogenous level, and was discernible by an increase in molecular weight on account of the FLAG tag. Taken together, these data suggest that overexpression of YY1 has a negative effect on the action of the ASE in Oli-neu cells.
approximately 75-fold higher in Oli-neu cells (Figure 3), while only 2-fold higher in N20.1 cells as previously shown (Dobretsova et al., 2004) it is possible that the two cell lines have different amounts of YY1, which in turn leads to higher levels of Plp1 gene expression in Oli-neu cells compared with N20.1 cells (Pereira et al., 2011). However, as shown in Figure 5, endogenous levels of YY1 in the nucleus are similar between Oli-neu and N20.1 cells, and even HeLa cells. Therefore the difference between the apparent potency of the ASE in Oli-neu and N20.1 cells cannot be explained by disparate levels of nuclear YY1.

YY1 binds to a motif in the mouse Plp1 promoter, but not to the ASE

PCR assisted binding site selection has been used to define high-affinity YY1 binding motifs (Yant et al., 1995). The vast majority of high-affinity binding sites selected contained a core sequence of CCAT, although a few possessed a core sequence of ACAT. The consensus sequence, VDCCATNWY, was found to fit 89% of the selected CCAT-containing oligonucleotides (Yant et al., 1995). EMSA analysis was performed to test whether YY1 is capable of binding specifically to such recognition motifs present in the Plp1 gene. As shown in Figure 6, a prominent DNA–protein complex was formed when nuclear proteins from Oli-neu cells were incubated with a positive control probe containing a single YY1 (consensus) binding site (lane 7). The complex was diminished by the addition of unlabelled homologous DNA (lanes 8–9), but not with a related mutant oligonucleotide (mut YY1) whose YY1 target site is disrupted (lanes 10–11). Furthermore, unlabelled prom 3 oligonucleotide (lanes 12–13), which corresponds to mouse Plp1 promoter sequence from positions −132 to −106, could also compete for binding. Prom 3 is the mouse
equivalent of a human-based oligonucleotide that previously was shown to bind YY1 at site 3 in the PLP1 promoter (Berndt et al., 2001), and fulfils the YY1 consensus. Likewise, when prom 3 was used as a probe, a similarly sized EMSA complex was formed (lane 14), which could be competed for by the addition of unlabelled YY1 consensus oligonucleotide, suggesting that YY1 can bind to this region of the mouse Plp1 promoter. However, when an upstream oligonucleotide (prom 1) was tested that corresponds to mouse Plp1 5'-flanking DNA positions −1596 to −1570 and contains a CCAT core but two mismatches relative to the YY1 consensus, no DNA–protein complex was formed (lane 2). Hence, sequences flanking the CCAT core are also critical for YY1 binding.

EMSA analysis was also performed with nuclear extracts from Oli-neu cells that had been transfected with pCEP4F-YY1 two days earlier. As shown in Figure 7, there was enrichment of the EMSA sequence-specific complex formed by the YY1 consensus and prom 3 probes with nuclear extracts prepared from transfected cells (compare lanes 2 with 3, and 8 with 9), which were effectively diminished by addition of the YY1 consensus oligonucleotide as an unlabelled (cold) competitor (lanes 4 and 11). Incubation of the probes with 100 μg full-length human YY1 protein (produced in Escherichia coli as a 68 kDa polyhistidine tagged–fusion protein) led to an EMSA complex of nearly similar mobility whose level was greatly diminished by addition of unlabelled 'homologous' DNA (compare lanes 5 with 6, and 12 with 13). (The mobility of the EMSA complex formed with the purified YY1 protein was slightly retarded presumably due to the addition of the polyhistidine tag.) However, an EMSA complex was not formed between the purified YY1 protein and the 1128–1154 probe (lane 19) that contains a potential YY1 binding motif within the ASE sequence that fits the YY1 consensus exactly. Furthermore, even though some nuclear proteins from Oli-neu cells seem to recognize the 1128–1154 probe in a sequence-specific manner (compare lane 16 with lanes 17 and 18), it is not due to YY1 since the level of EMSA complexes remained unchanged when nuclear extracts from pCEP4F-YY1 transfected cells were tested (compare lanes 15 and 16), which was not diminished by the addition of unlabelled YY1 consensus oligonucleotide as a possible competitor (lane 18). Taken together, these results indicate that YY1 does not bind directly to the ASE sequence.

DISCUSSION

Past transfection studies (Berndt et al., 2001; He et al., 2007a) that tested the consequences of YY1 overexpression on the
regulation of PLP1/Plp1 gene expression have focused largely on effects mediated through sequences in the promoter and upstream 5'-flanking DNA. Yet YY1 has been shown to bind to target sites within the first intron of (other) genes and either activate (Kim et al., 2003, 2006, Foti and Reichardt, 2004) or repress (Yan et al., 2001; Zabel et al., 2002) their expression. Because mouse Plp1 intron 1 DNA contains multiple putative YY1 binding sites (Wight and Dobretsova, 1997) and is essential for the generation of high levels of Plp1-lacZ transgene expression in brain during the active myelination period of CNS development (Li et al., 2002b), effects from elevated levels of YY1 on Plp1-lacZ gene activity via Plp1 intron 1 sequences were investigated in the current study.

Previously it has been shown that targeted ablation of Yy1 in oligodendrocyte progenitor cells through the use of Cnp-cre mice results in hypomyelination and cells being arrested at an immature stage (He et al., 2007a). The impediment in development is likely due to an inability to transcriptionally down-regulate the expression of oligodendrocyte differentiation inhibitors such as Id4, Tcf4 and Hes5 (He et al., 2007a; Shen et al., 2008); YY1 recruits HDAC1 (histone deacetylase 1) to the promoters of these inhibitors during oligodendrocyte progenitor differentiation causing 'repression of repressors', which in turn is required for differentiation to progress (He et al., 2007b). Concomitantly, there is an 80% reduction in mRNA levels for Plp1, MAG (myelin-associated glycoprotein), and UDP-galactose ceramide galactosyltransferase (Ugt8) in the brains of YY1 conditional knockout mice at postnatal day 18 (He et al., 2007a), presumably due to a failure in oligodendrocyte terminal differentiation.

On the other hand, YY1 overexpression in glial cells via transfection has produced a variety of effects, some of which are contradictory. Endogenous levels of Plp1 gene transcripts in primary cultures of oligodendrocyte progenitors were largely unchanged when transfected with different amounts (0.5, 1 and 2 μg) of a plasmid (pCX-yy1) which expresses human YY1 (He et al., 2007a). However, it is unclear exactly what the transfection efficiency was in those experiments. If the efficiency was relatively low, then any changes in Plp1 gene expression due to the elevation in YY1 levels would likely have been obscured by the high background of untransfected cells. Consequently, most studies have utilized Plp1-reporter gene constructs co-transfected with YY1 expression plasmids to determine how YY1 modulates Plp1 gene activity. Ideally, another reporter plasmid such as RSVL would also be included in order to correct for differences in transfection efficiency between samples.

In the present study, where differences in transfection efficiency have been accounted for, overexpression of YY1 in Oli-neu cells caused a decrease in PLP(+Z) expression relative to that for PLP(−Z) (Figure 2). Given that both Plp1-lacZ constructs are equivalent except for the addition of Plp1 intron 1 DNA in PLP(+Z), the down-regulation of PLP(+Z) expression must have been mediated through regulatory
element(s) from the intron. Indeed, activity of the ASE (a very potent enhancer in Oli-neu cells) was thwarted by elevated levels of YY1 (compare the relative β-gal activities for PLPA809–5807+Ase-F in Oli-neu cells transfected with or without the YY1 expression plasmid in Figure 3). However, even though the ASE contains a potential YY1 binding motif that complies exactly with the VDCCATNWY consensus, YY1 did not bind to this sequence, although it could to another fit situated in the promoter (prom 3; Figure 6) analogous to the circumstances with site 3 in the human PLP1 promoter (Berndt et al., 2001). [Analysis of the mouse Plp1 sequence using MatInspector (Cartharius et al., 2005) revealed a YY1 motif in the sequence for prom 3, but not the ASE, using the consensus sequence CGCCATNTT as defined by Kim et al. (2007). Because both the prom 3 and 1128–1154 oligonucleotides contain a mismatch at position 2 compared with the CGCCATNTT consensus, but only the 1128–1154 oligonucleotide contains an additional mismatch (T→C) at the last position, it is likely that the terminal thymidine residue is important for YY1 binding.] Hence elevated levels of YY1 appear to counter ASE function (albeit not through direct binding to the ASE sequence itself), and consequently down-regulate Plp1-lacZ gene activity in Oli-neu cells. As well, YY1 overexpression in Oli-neu cells has been shown to have a negative effect on the activity of a reporter gene whose expression is driven wholly by the mouse Plp1 promoter (and associated 5′-flanking DNA) (He et al., 2007a). Thus, expression of the mouse Plp1 gene appears to be negatively affected by YY1. Curiously, the human promoter was positively regulated by YY1 in CG4 cells (rat oligodendroglial cell line), although increasing the amounts of YY1 plasmid transfected was inversely correlated with reporter gene activity (Plp1-CAT activity/μg protein) that had not been corrected for any deviations in transfection efficiency (Berndt et al., 2001). Whether these seemingly incongruent findings are the result of differences in the host cell (Oli-neu vs. CG4) and/or the relative amount of YY1 plasmid transfected to Plp1-reporter construct, or simply misleading because variations in transfection efficiency had not been accounted for in some studies, or truly due to a species-specific difference in the gene’s regulation is presently unknown. Nonetheless, down-regulation of mouse Plp1 driven reporter gene expression by YY1 is in line with the results obtained from mice with conditional ablation of Yy1 in oligodendrocyte lineage cells. He et al. (2007a) demonstrated that YY1 expression is necessary for oligodendrocyte progenitor differentiation. Presumably, YY1 is required to repress expression of terminal differentiation inhibitors. When oligodendrocyte progenitors isolated from the cortex of neonatal Yy1fllox/flox mice were transduced with a CMV-Cre adenoviral vector and subsequently encouraged to differentiate by mitogen withdrawal, PLP immunoreactivity was much decreased in the YY1 ablated cells because they failed to differentiate. However, this does not mean, a priori, that YY1 is an activator of mouse Plp1 gene expression. In fact, our results and those of He et al., (2007a) suggest that YY1 negatively influences its expression through sequences in Plp1 intron 1 and the promoter, respectively. Yet, when Oli-neu cells are pressed to differentiate, elevated levels of YY1 did not significantly alter the activity of a mouse Plp1 promoter-luciferase construct except for possibly a slight decrease with the highest amount (2 μg/well) of YY1 plasmid tested (He et al., 2007a). Thus the repressive effect of YY1 on Plp1 gene expression appears to lessen (or be lost) as the cells move through the latter stages of differentiation. Perhaps YY1 helps to keep Plp1 gene activity levels in check in immature cells before copious quantities of PLP are needed in myelinating oligodendrocytes.

Taken together, our results and those of others (He et al., 2007a) suggest that YY1 has a negative effect on mouse Plp1 gene expression in oligodendrocyte progenitor cells and immature oligodendrocytes. The decrease in Plp1 gene activity caused by YY1 is mediated in part through sequences present in the first intron, and with respect to the ASE does not occur via direct recognition of a YY1 target site. Perhaps elevated levels of YY1 cause a decrease in the expression of one or more of the activators that form a complex on the ASE. Alternatively, YY1 overexpression could diminish ASE activity by having an effect on the posttranslational modification of an ASE constituent, or bind via a protein–protein interaction and subsequently recruit a negative regulatory factor to the ASE complex. No matter what, this is above and beyond any effect mediated through the promoter (e.g. prom 3) since all of the Plp1-lacZ constructs used in the present study contain the same Plp1 5′-flanking DNA. It is worth mentioning that this effect was observed in the face of proportionately much less transfected YY1 plasmid compared with studies (Berndt et al., 2001; He et al., 2007a) that focused solely on the PLP1/Plp1 promoter (and adjacent 5′-flanking DNA). In summary, YY1 appears to function as a negative regulator of mouse Plp1 gene activity via multiple regions of the gene, including the first intron.

ACKNOWLEDGEMENTS

We thank Dr Sergey Zolov for help with Western blottings and Dr Timothy Chambers for the anti-FLAG antibody.

FUNDING

This work was supported by the National Multiple Sclerosis Society [grant number RG 2705] and the National Institutes of Health [grant numbers R01 NS37821, P30 NS047546].

REFERENCES

Berndt JA, Kim JG, Hudson LD (1992) Identification of cis-regulatory elements in the myelin proteolipid protein (PLP) gene. J Biol Chem 267:14730–14737.

Berndt JA, Kim JG, Tosic M, Kim C, Hudson LD (2001) The transcriptional regulator Yin Yang 1 activates the myelin PLP gene. J Neurochem 77:935–942.

Cartharius K, Frech K, Grote K, Kloczke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics 21:2933–2942.
Dignam JD, Lebovitz RM, Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11:1475–1489.

Dobretsova A, Wight PA (1999) Antisilencing: myelin proteolipid protein gene expression in oligodendrocytes is regulated via derepression. J Neurochem 72:2227–2237.

Dobretsova A, Kokorina NA, Wight PA (2000) Functional characterization of a cis-acting DNA antisilencer region that modulates myelin proteolipid protein gene expression. J Neurochem 75:1368–1376.

Dobretsova A, Kokorina NA, Wight PA (2004) Potentiation of myelin proteolipid protein (Plp) gene expression is mediated through AP-1-like binding sites. J Neurochem 90:1500–1510.

Donoviel DB, Shield MA, Buskin JN, Haugen HS, Clegg CH, Haushka SD (1996) Analysis of muscle creatine kinase gene regulatory elements in skeletal and cardiac muscles of transgenic mice. Mol Cell Biol 16:1649–1658.

Foti DM, Reichardt JK (2004) YY1 binding within the human HS3D82 gene intron 1 is required for maximal basal promoter activity: identification of YY1 as the 3’fl-A factor. J Mol Endocrinol 33:99–119.

Gordon S, Akopyan G, Garban H, Bonavida B (2006) Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. Oncogene 25:1126–1142.

Gout O, de Santo R, Arnheiter H, Hudson L, Dubois-Dalcq M (1991) A transgenic tag for tracking transplanted glial cells. Soc Neurosci Abstr 17:375.

He Y, Casaccia-Bonnefil P (2008) The Yin and Yang of YY1 in the nervous system. J Neurochem 106:1493–1502.

He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, Shi Y, Nave KA, Casaccia-Bonnefil P (2007a) The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. Neuron 55:217–230.

He Y, Sandoval J, Casaccia-Bonnefil P (2007b) Events at the transition between cell cycle exit and oligodendrocyte progenitor differentiation: the role of HDAC and YY1. Neuron Glia Biol 3:221–231.

Jung M, Krämer E, Grzenkowski M, Tang K, Blakemore W, Aguzzi A, Khazaie K, He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, Shi Y, Nave KA, Casaccia-Bonnefil P (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci 11:1024–1034.

Shi Y, Lee JS, Galvin KM (1997) Everything you have ever wanted to know about Yin Yang 1. Biochim Biophys Acta 1332:F49–F66.

Thomas MJ, Seto E (1999) Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? Gene 236:197–208.

Verity AN, Bredesen D, Vanderschueren C, Handley WW, Campagnoni AT (1993) Expression of myelin protein genes and other myelin components in an oligodendrocyte cell line conditionally immortalized with a temperature-sensitive retrovirus. J Neurochem 60:577–587.

Wight PA, Dobretsova A (1997) The first intron of the myelin proteolipid protein gene confers cell type-specific expression by a transcriptional repression mechanism in non-expressing cell types. Gene 201:111–117.

Wight PA, Dobretsova A (2004) Where, when and how much: regulation of myelin proteolipid protein gene expression. Cell Mol Life Sci 61:810–821.

Wight PA, Duchala CS, Readhead C, Macklin WB (1993) A myelin proteolipid protein-lacZ fusion protein is developmentally regulated and targeted to the myelin membrane in transgenic mice. J Cell Biol 123:443–454.

Wight PA, Dobretsova A, Macklin WB (1997) Regulation of murine myelin proteolipid protein gene expression. J Neurosci Res 50:917–927.

Yan B, Heus J, Lu N, Nichols RC, Raben N, Plotz PH (2001) Transcriptional regulation of the human acid alpha-glucosidase gene. Identification of a repressor element and its transcription factors Hes-1 and YY1. J Biol Chem 276:1789–1793.

Yant SR, Zhu W, Millinoff D, Slichtom JL, Goodman M, Gumucio DL (1995) High affinity YY1 binding motifs: identification of two core types (ACAT and CCAT) and distribution of potential binding sites within the human beta globin cluster. Nucleic Acids Res 23:3452–3462.

Yao YL, Yang WM, Seto E (2001) Regulation of transcription factor YY1 by acetylation and deacetylation. Mol Cell Biol 21:5979–5991.

Zabel MD, Wheeler W, Weis JJ, Weis JH (2002) Yin Yang 1, Oct1, and NFAT-4 form repeating, cyclosporin-sensitive regulatory modules within the murine Cd21 intronic control region. J Immunol 168:3341–3350.

Zhu X, Mancini MA, Chang KH, Liu CY, Chen CF, Shan B, Jones D, Yang-Feng TL, Lee WH (1995) Characterization of a novel 350-kilodalton nuclear phosphoprotein that is specifically involved in mitotic-phase progression. Mol Cell Biol 15:5017–5029.

Zimmerman K, Legouy E, Stewart V, Depinho R, Alt FW (1990) Differential regulation of the N-myb gene in transfected cells and transgenic mice. Mol Cell Biol 10:2096–2103.