CMLS, Cell. Mol. Life Sci. 61 (2004) 810–821
1420-682X/04/080810-12
DOI 10.1007/s00018-003-3309-z

CMLS Cellular and Molecular Life Sciences

Review

Where, when and how much: regulation of myelin proteolipid protein gene expression

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Received 4 August 2003; received after revision 17 September 2003; accepted 24 September 2003

Abstract
The myelin proteolipid protein (PLP) gene (*Plp*) encodes the most abundant protein found in myelin from the central nervous system (CNS). Expression of the gene is regulated in a spatiotemporal manner with maximal levels of expression occurring in oligodendrocytes during the active myelination period of CNS development, although other cell types in the CNS as well as in the periphery can express the gene to a much lower degree. In oligodendrocytes, *Plp* gene expression is tightly regulated. Underexpression or overexpression of the gene has been shown to have adverse effects in humans and other vertebrates. In light of this strict control, this review provides an overview of the current knowledge of *Plp* gene regulation.

Key words. Myelin proteolipid protein gene; gene expression; spatiotemporal regulation.

Cloning the myelin *Plp* gene

The *Plp* gene encodes the most abundant protein present in central nervous system (CNS) myelin of higher vertebrates. Nearly 50% of the total protein found in adult CNS myelin results from *Plp* gene expression [1, 2]. Aided by the determination of the primary amino acid sequence [3–9], PLP complementary DNA (cDNA) clones have been isolated from a number of mammals including man [10–12], rat [13–16], mouse [17–19], cow [20], pig [21] and rabbit [22]. These clones contain an open reading frame that encodes the 276-amino acid protein, PLP, plus an N-terminal methionine residue that is not present in the final product. As well, cDNA clones have been isolated for the DM20 isoform [23, 24], which arises by alternative splicing of the primary transcript. DM20 transcripts are generated by use of an internal donor splice site within exon 3 and encode a protein identical to PLP except for deletion of 35 amino acids corresponding to PLP residues 116–150. PLP/DM20 cDNAs have also been isolated from more disparate species such as chicken [25], zebra finch [26], trout [27] and zebrafish [28]. The cDNA clones in turn have been used to help isolate *Plp* genomic clones, including those from man [29], rat [23, 30, 31], mouse [32–34], dog [35], cow [36] and pig [37]. These clones have been instrumental in deciphering the structure and function of the gene as well as providing tools to uncover how *Plp* gene expression is regulated.

Structure of the *Plp* gene

The *Plp* gene is present as a single copy in the genome [14, 15, 29], located on the X chromosome [18, 36–39]. It is a member of the lipophilin gene family [40], some of whose members may encode pore-forming polypeptides [41, 42]. The gene is now referred to as *Plp1* in the databases since the inclusion of an unrelated gene, A4, that is also known as *Plp2* [43, 44]. At the present time, the entire DNA sequence of the myelin *Plp* gene is known for man, mouse, cow [36] and pig [37], and determination of the rat sequence is nearly complete (Database accession

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no AJ006976, NT_039716, AJ009913, AJ009912 and NW_044423, respectively). The general structure of the gene (fig. 1A) is quite similar between these species, with seven exons distributed over 16–18 kb of DNA (reviewed in [45]). The coding sequence is spread across all seven exons with the first exon containing only the initiation methionine codon and the first base of the next codon. As mentioned above, exon 3 contains an internal 5′-splice site. Use of this alternative splice site generates transcripts encoding the DM20 isoform (fig. 1B).

Exon 7 is by far the longest of the exons (>2 kb) and contains multiple polyadenylation signals. The coding sequence is highly conserved between species. The amino acid sequence of PLP is 100% conserved between the human, mouse, rat and pig. In comparison to the human sequence, there is a single amino acid change in the protein from the dog (isoleucine instead of valine at residue 160) and rabbit (threonine instead of serine at residue 198) and two changes in the cow sequence (alanine instead of phenylalanine at residue 188 and threonine instead of serine at residue 198). Accordingly, there is greater than 95% identity of the coding sequence at the nucleotide level [46], and there is also a high degree of conservation (at least 90% identity between human and mouse) for the 5′- and 3′-untranslated regions (UTRs) and the proximal 150 bp of 5′-flanking DNA [32, 47–49]. There is more variability between the intervening sequences.

Intron 3 is the most conserved of the introns. When compared with the human sequence, intron 3 DNA shows greater than 80% identity to sequences from mouse (81%), rat (82%), cow (83%) and pig (84%). The intron contains a purine-rich sequence located near its 5′ end that functions as a splicing enhancer and is required for efficient PLP-specific splice site selection in oligodendrocytes, but is ineffective in nonglial cells [50].

The first intron is by far the longest, ranging in size from 8 kb in the mouse [51] to over 9 kb in the pig [37]. Curiously, the mouse gene contains an additional exon [52] that is sometimes incorporated into the mature messenger RNA (mRNA) through alternative splicing. This exon, designated as exon 1.1, lies within what is classically referred to as intron 1 DNA. It encompasses 109 bp and is situated 121 bp downstream from exon 1 DNA. Incorporation of this exon into transcripts results in additional isoforms being expressed in the mouse. These isoforms have been designated srPLP and srDM20 for soma-restricted (sr) since they are confined to the cell body of mature oligodendrocytes and not targeted to the plasma membrane and compact myelin as are the 'classic' PLP and DM20 proteins [53]. The sr proteins are also expressed in certain neuronal populations in the cerebel-

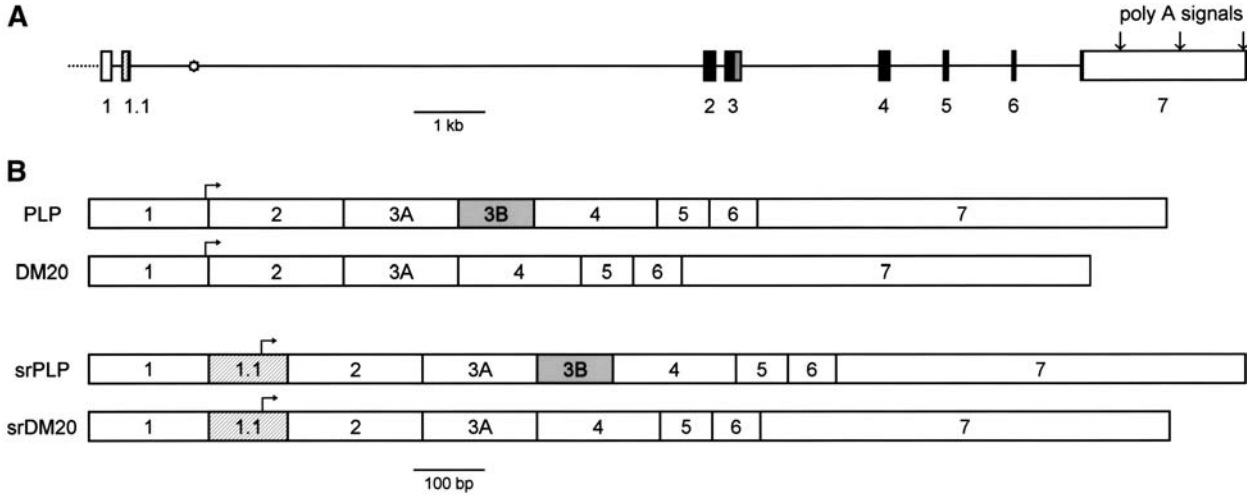

Figure 1. Schematic representation of the *Plp* gene and ensuing splice variants present in mice. (*A*) The mouse gene covers approximately 15.6 kb of DNA from the start of exon 1 to the end of exon 7. The promoter and upstream flanking sequences are depicted by a dotted line whose 5′ end is indeterminate. Exons are indicated by boxes with the translated sequences in black or gray. Exon 7 contains multiple polyadenylation (poly A) signals that can be selectively recognized to generate transcripts approximately 1.6, 2.4 and 3.2 kb in size. Introns are represented by solid thick lines, with the relative position of a positive regulatory region located in intron 1 denoted by a star. Exon 3 contains two donor splice sites. DM20 transcripts are produced if the internal splice site is used. PLP transcripts are generated if the donor splice site at the end of exon 3 is used (PLP-specific sequences are indicated by a gray box). Additionally, alternative RNA splicing produces some transcripts that incorporate exon 1.1, although their relative abundance is rather low in comparison to those transcripts, which lack exon 1.1 sequences. (*B*) Depiction of the splice variants that can be produced from *Plp* pre-mRNA. Transcripts are portrayed for those derived from the distal-most transcription start site and the first poly A signal; the poly A tail is not depicted. Incorporation of the latter half of exon 3 (exon 3B) results in the generation of PLP-specific transcripts. Incorporation of exon 1.1 produces sr splice variants. Bent arrows depict translation start sites. Translation is initiated from a site near the 3′ end of exon 1 sequences with 'classic' PLP and DM20 transcripts, while a downstream AUG codon within exon 1.1 sequences is used to initiate translation with srPLP and srDM20 mRNAs.

lum, hippocampus and olfactory system [53], and srDM20 transcripts have been detected in thymus and a mouse T cell line (LBRM) [52].

When compared with classic PLP and DM20 proteins, the analogous sr isoforms contain an additional 12-amino acid leader sequence (including the N-terminal methionine residue). Inclusion of exon 1.1 in the transcript causes a shift in the reading frame, bringing a stop codon (located in exon 2) into phase with the first AUG site near the end of exon 1. As a result, translation of sr products is initiated from an internal AUG codon within exon 1.1 that is positioned in the correct reading frame (fig. 1B). Although the sr products occur in relatively low abundance in the brains of mice when compared with classic PLP and DM20 proteins, exon 1.1 counterparts in man, rat, cow and pig probably do not exist due to lack of an invariant GT in the would-be donor splice site of the corresponding sequences.

Transcripts emanating from the *Plp* gene are heterogeneous in size not only because of alternative splicing, but also due to the use of multiple transcription initiation and polyadenylation sites. The vast majority of the size difference can be attributed to the particular polyadenylation site used. PLP transcript lengths have been estimated by Northern blot analysis to be approximately 1.6, 2.4 and 3.2 kb. However, the relative abundance of a particular class of message depends on the species. In the rat, PLP transcripts of 3.2 and 1.6 kb in size are much more prevalent than the 2.4 kb variety, with the 3.2 kb mRNA being the most abundant [14, 15, 20]. In the mouse, the situation is slightly altered, with the 2.4 kb species being more abundant than the 1.6 kb form [15, 17–19, 54]. An assortment of transcript sizes has also been detected in man analogous to the situation in the mouse [11]. Northern blot analysis and S1 nuclease studies have established that both PLP- and DM20-specific transcripts are present in all three of the major classes of mRNA sizes [55]. In addition, there is also a relatively stable nuclear pre-mRNA intermediate present in oligodendrocytes that retains intron 3 sequences. This intermediate, designated *Ppm-1* for *Plp*-specific pre-mRNA, gives rise only to the PLP isoform and is upregulated during the active myelination period in CNS development [56].

Minor variations in transcript size can also be attributed to the use of multiple transcription start sites, with the 5′-most start site slightly greater than 160 nucleotides (nt) upstream from the translation initiation site near the end of exon 1 [14, 32, 33, 37]. Although various methods used to map the sites yielded slightly different results, the transcription start sites have been grouped into three clusters and named according to their position relative to the translation start site near the end of exon 1, with the 5′-most cluster being designated as the distal cluster. During the peak of myelination in the CNS, transcription is initiated primarily at the distal cluster, while in Schwann cells a more proximal cluster (~40 nt downstream from the distal-most site) is preferred [57].

Spatial regulation of *Plp* gene expression

The *Plp* gene is abundantly expressed in oligodendrocytes, although much lower levels of expression have been detected in a variety of cell types, including Schwann cells [57–60], olfactory nerve ensheathing cells [61], cardiac myocytes [62], cells of the immune system [63, 64], certain types of neurons [52, 65], human amniotic epithelial cells [66], and in the testis [64], presumably in Leydig cells [67]. By reverse transciption-polymerase chain reaction (RT-PCR) analysis, DM20-specific products have been noted in even a wider range of tissues from the mouse [68]. As well, *Plp* gene expression has been noted in a several cell lines of nonglial origin [69] and even in benign smooth muscle tumors of the uterus [70].

Deletion-transfection approaches have been used to map transcription regulatory elements within the *Plp* promoter and upstream flanking DNA that govern cell type specificity. Although these studies identified elements that modulate *Plp* gene expression in both a positive and negative fashion, none of the elements identified were adequate to direct cell type-specific expression of the *Plp* gene entirely. Moreover, conflicting results were obtained between some of these investigations, which may have arisen due to differences in the particular cell types tested or to variable limits of detection of reporter gene expression. In studies utilizing the human gene, *Plp* DNA 204 bp upstream of the distal transcription start site was sufficient to promote substantial expression of chloramphenicol acetyltransferase (CAT) in both human glial (SVG) and rat brain tumor (B103) cells, but not in HeLa cells [71]. Since HeLa cells do not express the *Plp* gene, it was suggested that tissue-specific element(s) reside within this proximal promoter region.

However, in another study [49] using the human *Plp* promoter to drive luciferase reporter gene expression, constructs containing up to 1.4 kb of 5′-flanking DNA were expressed in either C6 glioma cells or CHO fibroblasts. Likewise, a construct containing only the proximal 184 bp of 5′-flanking DNA generated high levels of expression in both cell lines. In studies using mouse *Plp* sequences to drive luciferase reporter gene expression, constructs containing 1.4 or 5.4 kb of *Plp* 5′-flanking DNA demonstrated significant transcriptional activity in hamster glial (HJC) and mouse hepatoma (BW7756) cells [72]. Thus, sequences important in restricting cell type-specific expression were most likely absent from these constructs. A more deleted construct, which contained only 185 bp of 5′-flanking DNA, did not demonstrate activity above background in either the HJC- or

BW7756-transfected cells. However, the exact opposite result was obtained when the corresponding rat sequences were tested in rat C6 cells. In those experiments, a construct that contained 186 bp of rat 5′-flanking DNA demonstrated a 16-fold increase in CAT activity when compared with a construct containing 1 kb of *Plp* 5′-flanking DNA [31]. Virtually the same construct (although designated as – 225 PLP/CAT since a more proximal transcription start site was set at +1) also generated high levels of CAT activity when transfected into primary cultures of rat oligodendrocytes and were comparable to those generated by a construct containing 4.2 kb of 5′-flanking DNA, whereas transfected mouse L cells showed far less CAT activity [73]. When a battery of mouse PLP-luciferase constructs was tested in the mouse N20.1 oligodendrocytic cell line [74], a construct containing only the proximal 145 bp of 5′-flanking DNA generated the highest level of expression [75]. Furthermore, the pattern of expression was similar when the constructs were tested in mouse NIH 3T3 fibroblasts, suggesting that cell type-specific regulatory elements are missing from the proximal 1.4 kb of *Plp* 5′-flanking DNA. Together these studies suggest that the *Plp* basal promoter is contained within the first several hundred bp of 5′-flanking DNA, and that those elements which direct cell type-specific expression may reside outside of the proximal 5.4 kb of 5′-flanking DNA, although the inherent activity of the basal promoter is not the same for all cell types. Other deletion-transfection studies have suggested that several negative regulatory elements reside within *Plp* intron 1 DNA that are important for repressing expression in cells that do not express the gene [51, 76, 77].

Temporal regulation of *Plp* gene expression

Numerous studies have been conducted to evaluate the developmental profile of *Plp* gene expression in the CNS at both the mRNA [14, 15, 19, 20, 55, 69, 78–93] and protein [11, 55, 86, 94–98] levels. The consensus of these studies is that in oligodendrocytes, the peak of *Plp* gene expression is coincident with the active myelination period of CNS development, which occurs about 3 weeks postnatally in rodents. Early in CNS development, DM20 mRNA and protein precede those for PLP; however, as development progresses, PLP becomes the predominant isoform. A similar developmental pattern of expression was observed with the corresponding sr splice variants [52]. Nevertheless, there have been several reports suggesting that the PLP isoform can be expressed prior to or in the absence of DM20. Immunoblot analysis of proteins isolated from human fetal spinal cord detected PLP by gestational week 18 (GW18) while DM20 was first detected at GW20 [99], and a recent study with NG2 immunopositive oligodendrocyte precursor cells isolated from the cerebral cortex of both developing and adult mice showed that these cells express PLP mRNA, but not DM20 mRNA [100].

In contrast to the developmental switch observed in the CNS, DM20 predominates in the peripheral nervous system (PNS) [101]. Only myelinating Schwann cells express PLP, whereas both myelinating and nonmyelinating Schwann cells and satellite cells express DM20 [60]. The steady-state levels of PLP mRNA, but not DM20 mRNA, appear to be regulated at a posttranscriptional level in the PNS whereby axonal contact increases the stability of PLP-specific transcripts in myelinating Schwann cells [102]. Axonal contact has also been shown to have a positive effect on the overall levels of *Plp* gene expression in oligodendrocytes [103, 104], though expression of the gene can occur in the absence of neurons [105]. Axons also appear to influence transcription start site selection and are required to maintain the high proportion of transcripts initiated from the distal cluster of start sites during the peak of *Plp* gene expression in the CNS [104]. Transcription is initiated predominately from the distal start sites during the peak of *Plp* gene expression. After the peak, a more proximal start site is preferentially used [104].

Several studies have demonstrated that the developmental regulation of *Plp* gene expression in the brain is mediated, in part, at the level of transcription. Nuclear run-on experiments using rat nuclei isolated from whole brains showed a 2.4-fold increase in *Plp* gene activity from postnatal day 3 (P3) to P13 and an additional 3.2-fold increase out to P36, followed by a 2.0-fold decrease at P51 [72]. In other studies using nuclei isolated from rat forebrains, the transcription rate of the *Plp* gene was fairly constant from 3 to 29 months of age [89]. Nuclear run-on experiments have also been performed with nuclei isolated from the brains of mice at various ages. *Plp* gene activity increased ~2-fold from P7 to P20 [34] or from P12 to P18 in another study [83] and then declined by 20–30% at P25 or P30, respectively. The transcription rate continued to decline (~3-fold) out to P90 where it was roughly half the level seen at the initial time point (P12) tested [83]. These results demonstrate that *Plp* gene expression is regulated at the transcriptional level. However, other posttranscriptional mechanisms must exist since the increase in the transcription rate during development is less than the increase in mRNA levels; *Plp* gene activity increased only 2-fold in mouse brains from P7 to P14, while PLP mRNA levels increased over 10-fold during the same time frame [34].

PLP mRNA is relatively stable. The mRNA half-life has been estimated to be 25 h using primary cultures of mixed glia prepared from the brain [72], and well over 24 h in another study [106]. Similarly, the PLP mRNA half-life was estimated to be well over 24 h in enriched

oligodendrocyte cultures and in the C6 cell line [106]. It has been proposed that the 3′-UTR of PLP transcripts contain binding site(s) for RNA-stabilizing factors present in oligodendrocytes [106], which could help to explain why higher increases in PLP mRNA levels occur during development than can be accounted for solely by changes in the transcription rate. Studies with C6 cells have suggested that retinoic acid also upregulates *Plp* gene expression by increasing its mRNA half-life [107, 108].

Deletion-transfection analyses using *Plp-lacZ* fusion genes have suggested that mouse *Plp* intron 1 DNA contains a potent enhancer located slightly more than 1 kb downstream of exon 1 sequences. Originally this positive regulatory element was classified as an antisilencer since it seemed to override repression mediated by multiple negative elements located elsewhere within intron 1 DNA [76]. However, subsequent experiments showed that the positive regulatory element possessed enhancer-like properties; i.e. it functioned in an orientation-independent manner and multiple copies of the element resulted in higher levels of reporter gene expression in transfected N20.1 cells [109]. The positive regulatory element, probably better characterized as a region, is contained within mouse intron 1 DNA positions 1083–1777 (based on consecutively numbering the intron sequence with the first bp designated as 1). This was the only positive regulatory element to be mapped in the intron by deletion-transfection analysis. A plethora of putative transcription factor binding sites within this sequence has been noted [109]. Multiple factors appear bind to this region [76, 109], possibly forming an enhanceosome.

The sequence (fig. 2) of this positive regulatory region from the mouse gene has been compared to those from other species and shows extensive identity to the rat sequence (95%) and moderate identity to the human (71%), pig (56%) and cow (63%) sequences, although the latter three sequences show higher identity when compared among themselves. The positive regulatory region likely mediates the dramatic upswing of *Plp* gene activity in oligodendrocytes during the active myelination period since mice, which harbor a *Plp-lacZ* transgene containing *Plp* genomic sequences from the proximal 2.4 kb of 5′-flanking DNA downstream to an internal site in exon 2, express the reporter gene in a temporal (and spatial) manner consistent with that of the endogenous *Plp* gene in the brain with peak expression occurring during the myelination stage of CNS development [67, 110]. However, expression from a similar transgene, which does not contain any *Plp* intron 1 sequences, remained low and static throughout CNS development [67]. Both transgenes exhibited similar levels of expression in the brain early on in postnatal development (out to P9), and ectopic expression was not detected in other tissues [67]. When transgene expression was investigated in the testis, the presence or absence of intron 1 DNA was inconsequential [67]. These results coupled with those from the previously described transfection studies suggest that the positive regulatory region depicted in figure 2 is crucial for generating high levels of *Plp* gene expression in oligodendrocytes, but may not be important for its expression in other cell types. In another study, it was shown that 4.2 kb of human *Plp* 5′-flanking DNA was sufficient to drive expression of a human PLP cDNA in a tissue-specific manner in transgenic mice [111]. Together these transgenic studies suggest that some, but not all, cell types contain the requisite composite of transcription factors needed to activate the *Plp* promoter by itself; however, sequences located elsewhere within the gene are important for modulating expression, at least in oligodendrocytes.

The levels of *Plp* gene expression are strictly regulated in oligodendrocytes. Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia type 2 (SPG-2) are X-linked de-

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Mouse   1083 GATCTCAGCTCAGTCTCTAAGGAACTCTGCAAAATTAGGCATGTCACC
Rat     1083 ....................^T.....A...................
Human   1105 A...C...A..T.CTA..T.T.....A..TG.CC..G.A..G.....^
Pig     1109 A...C...A.TG.C.A..T.T.....TCTTG.TG..G..T.GT.TG.T
Cow     1097 A...C...A..T.CTA..T.T.....TC.TG.C...G..T.G..TG.T

Mouse   1131 ATGAATCACCATTTCATCATCTGGTAGACAGGGGCAACAATAACTGT
Rat     1130 .......G...C...........A.....................
Human   1152 ^.........C...C........A.A.TG...A..G........AC
Pig     1157 G.C...TT...CC..C........A.A.TG..AAT...C..TTT.C.
Cow     1145 ......T.TT.CG.........C.A.A.T....AT..TG....T..C
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Figure 2. Sequence alignment of the positive regulatory region present in *Plp* intron 1 DNA. By transfection analysis [109], the positive regulatory region in the mouse gene has been localized to intron 1 positions 1083–1177 (based on consecutively numbering the intron with the first base designated as position 1). The sequence of the sense strand is shown for the mouse gene and has been aligned to comparable sequences present within *Plp* intron 1 DNA from the rat, human, pig and cow (numbers indicate positions within the intron). Bases identical to those from the mouse sequence are indicated by a period (.). Bases not present are denoted by a carrot (^).

velopmental defects of myelin formation in humans with linkage to the *Plp* locus. A variety of *Plp* gene mutations, including point mutations, deletions and duplications, have been reported which elicit a range of clinical features in affected individuals, including nystagmus, psychomotor developmental delay, spasticity and ataxia [112–123]. Complete loss of the gene [112] or mutations leading to a PLP null phenotype [117, 118] result in fairly mild clinical manifestations, including moderate spastic quadriparesis, mild cognitive delay, ataxia and demyelinating peripheral neuropathy. Interestingly, the lack of PLP but not DM20 in Schwann cells has been shown to be the cause of the peripheral neuropathy in humans [124]. PMD patients with PLP null mutations have also been shown to develop length-dependent axonal degeneration in the CNS in the absence of demyelination and inflammation [125]. Thus the absence of *Plp* gene products is detrimental. On the other hand, overexpression of the gene can also result in PMD [126]. In fact, *Plp* gene duplications are the most common cause of the disease [127, 128], although the mechanism(s) by which increased gene dosage causes PMD in humans is not well understood. Transgenic animals have provided clues as to possible mechanisms. Transgenic animals expressing transgenes encoding either DM20 alone [129, 130] or in conjunction with PLP [131, 132] are characterized by severe hypomyelination and astrogliosis, seizures and premature death, even though only a 2- to 3.5-fold overexpression of the *Plp* gene is evident in them. Other transgenic studies have demonstrated that overexpression of the *Plp* gene leads to glial cell degeneration and hypomyelination [133, 134] or late-onset neurodegeneration [135]. This sensitivity to moderate overexpression of the *Plp* gene may help to explain why humans who have *Plp* gene duplications develop PMD [reviewed in 136].

Transcription factors that affect *Plp* gene activity

Transfection analysis as well as a variety of assays based on sequence-specific DNA binding have been important to help identify factors that may modulate *Plp* gene activity, and they have been the subject of several reviews [137–140]. Footprint analysis of the *Plp* proximal promoter, including the first half of exon 1 DNA, has been performed for the human [71], mouse [49] and rat [31] sequences and has revealed multiple protein binding sites. All three studies showed a footprint over a purine-rich element containing an 11-bp direct repeat between positions –75 and –54 based on the sequence alignment of Janz and Stoffel [49]. Using Southwestern blot analysis, a brain-enriched DNA-binding protein of ~60–66 kDa was shown to bind to this region [49, 71]. Although not Sp1 based on its size, the factor may be a related zinc finger protein [49]. A heterodimer composed of thyroid hormone receptor β (THRβ) and peroxisome proliferator-activated receptor (PPAR) binds to a novel thyroid hormone response element located at positions +18 to +31 in the human *Plp* gene [141]. In the presence of thyroid hormone, the THRβ/PPAR heterodimer was shown to increase the level of expression with a construct containing the human *Plp* promoter sequence (–312/+85) driving a CAT reporter gene [141]. These results are in line with the activation of *Plp* gene expression by thyroid hormone (reviewed in [137]).

Plp gene expression can also be activated by the zinc finger protein Yin Yang 1 (YY1) that binds positions –121 to –113 in the promoter [142]. Other potential binding sites for YY1 have been noted within *Plp* intron 1 DNA [51]. Whether these sites are important for modulating the activity of the gene is unknown. Myelin transcription factor 1 (MyT1) is a zinc finger protein capable of binding to the *Plp* promoter region (positions –271 to –262), which was cloned by screening a human fetal brain expression library with *Plp* DNA that included this site [142, 143]. MyT1 is expressed early on in the oligodendrocyte lineage and continues to be expressed as the cells differentiate, but diminishes in oligodendrocytes after *Plp* gene products begin to accumulate [144]. MyT1 may function as an architectural protein, bending the DNA in the promoter region, in order to facilitate the assembly of active transcription complexes [144]. The MyT1 binding site is adjacent to and possibly overlaps the binding site for MYT2, whose cDNA clone was also identified in the expression library screen [145]. The effect, if any, of MYT2 protein on *Plp* gene expression is currently unknown.

Gtx/Nkx6.2 is a homeodomain transcription factor that is capable of binding to at least four sites within the first 1.3 kb of the *Plp* promoter [146]. It is expressed in differentiated, postmitotic oligodendrocytes, and its expression pattern parallels that of several myelin-specific genes, including *Plp*. Although the protein has been shown to possess repressor activity, transfection of a Gtx/Nkx6.2 expression construct was unable to modulate the activity of a cotransfected PLP-CAT construct [147]. Furthermore, *Plp* gene expression in Gtx/Nkx6.2 null mice is unaltered, suggesting that the transcription factor might not play a role in *Plp* gene regulation, although compensation by a related transcription factor could explain the apparent lack of an effect in these animals [148].

Overexpression of the transcription factor Nkx2.2 has been shown to promote the expression of a reporter gene (green fluorescence protein [GFP]) driven by the mouse *Plp* promoter encompassing the first 2.4 kb of 5′-flanking DNA in cotransfected NIH 3T3 cells [149]. Although it was suggested the Nkx2.2 binding sites within the *Plp* promoter were responsible for mediating this response [149], it should be pointed out that *Plp* intron 1 DNA was also present in the PLP-GFP construct tested, and it too

contains an Nkx2.2 consensus target site [150] located 376 bp downstream of exon 1 sequences. Likewise, neurogenin 3 (Ngn3), a basic helix-loop-helix protein, was also shown to drive expression of GFP when the same PLP-GFP construct was cotransfected with a Ngn3 expression construct in NIH 3T3 cells [151]. These results suggest that Nkx2.2 and Ngn3 may be important in directly activating *Plp* gene expression. However, both proteins are also important for oligodendrogliogenesis [149, 151]. Since maximal activation of *Plp* gene expression is coincident with terminal differentiation of oligodendrocytes [105], discriminating between effects that directly impact *Plp* gene activity from those affecting oligodendrogliogenesis, and consequently the activity of the gene, is difficult.

Concluding remarks

Through the study of *Plp* gene regulation, new insight has been garnered regarding possible functions for *Plp* gene products. Expression of the gene is necessary for normal structure of CNS myelin as evidenced by defects in the intraperiod line of *Plp* knockout mice [152–155]. *Plp* null mice demonstrate axonal swelling, demyelination and outright failure of certain tracts to myelinate, suggesting that *Plp* gene products are important for interactions between oligodendrocytes and axons [156–158]. Yet expression of the gene occurs in pre-myelinating cells as well as in nonglial cells, suggesting that additional functions must exist beyond its structural role in myelin (reviewed in [159, 160]). Other proposed functions include roles in cell signaling and as a differentiation/survival factor [35, 161–166].

Although regulation of *Plp* gene expression is exerted primarily at the levels of transcription, RNA splicing and mRNA stability, the opportunity exists for control at subsequent stages. PLP/DM20 is synthesized on ribosomes bound to the endoplasmic reticulum (ER) and makes its way through the secretory pathway. Only the N-terminal methionine residue is removed from the nascent translated product [167]. The protein is subsequently acylated on several cysteine residues [168, 169] and has a fairly long half-life once incorporated into the myelin membrane [170–172]. Expression of the gene is tightly regulated in oligodendrocytes. Overexpression of PLP in primary cultures of oligodendrocytes by infection with a viral expression construct leads to mistrafficking of the protein to late endosomes/lysosomes instead of to the Golgi complex and causes sequestration of cholesterol in these compartments as well [173]. Similarly, transgenic mice that overexpress PLP also accumulate PLP and cholesterol in late endosomes/lysosomes. It has been proposed that the mistrafficking of these compounds, as well as lipid raft components, perturbs the myelination process and affects the viability of oligodendrocytes [173]. In addition, some PLP-overexpressing transgenic animals have been shown to accumulate excess amounts of the protein in the ER of oligodendrocytes, which impairs the normal flow of proteins through the secretory pathway, and if in sufficient quantity, can trigger apoptosis [174, 175]. Similar processes may also be operative in PMD patients with increased *Plp* gene dosage.

Overexpression of the native gene is of greater detriment to oligodendrocytes than the lack of expression [176]. Thus, *Plp* gene expression must be under very strict control in the average person. We propose that the positive regulatory region present in the first intron is crucial for mediating the precise levels of *Plp* gene products needed in oligodendrocytes. Mutations in the element itself or alterations in the composition of the cognate binding factors may be relevant to some cases of PMD wherein the *Plp* coding sequence is intact. Currently, we are in the process of trying to identify the factors that bind to this regulatory region and to delete the sequence from the endogenous gene in mice to test our hypothesis.

Acknowledgements. The authors wish to express their gratitude to all of the investigators who helped to elucidate the regulation of this gene. Work in our laboratory has been supported by grants from the National Multiple Sclerosis Society (RG 2705C3/1) and the National Institutes of Health (NS37821). We would also like to thank the UAMS Office of Grants and Scientific Publications for reviewing the manuscript.

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