American Society
for Neurochemistry

Effects of Intron 1 Sequences on Human *PLP1* Expression: Implications for *PLP1*-Related Disorders

Patricia A. Wight[1]

Abstract

Alterations in the myelin proteolipid protein gene (*PLP1*) may result in rare X-linked disorders in humans such as Pelizaeus–Merzbacher disease and spastic paraplegia type 2. *PLP1* expression must be tightly regulated since null mutations, as well as elevated *PLP1* copy number, both lead to disease. Previous studies with *Plp1-lacZ* transgenic mice have demonstrated that mouse *Plp1* (*mPlp1*) intron 1 DNA (which accounts for slightly more than half of the gene) is required for the *mPlp1* promoter to drive significant levels of reporter gene expression in brain. However not much is known about the mechanisms that control expression of the human *PLP1* gene (*hPLP1*). Therefore this review will focus on sequences in *hPLP1* intron 1 DNA deemed important for *hPLP1* gene activity as well as a couple of "human-specific" supplementary exons within the first intron which are utilized to generate novel splice variants, and the potential role that these sequences may play in *PLP1*-linked disorders.

Introduction

Myelin proteolipid protein (PLP) is the most abundant protein found in myelin from the central nervous system (CNS; Eng et al., 1968; Norton and Poduslo, 1973; Jahn et al., 2009). The tetraspan protein plays a major role in the structure and function of myelin (Klugmann et al., 1997) and is thought to be a possible immunogen for multiple sclerosis (MS; Pender and Greer, 2007; Greer and Pender, 2008). PLP is important for communication between oligodendrocytes and axons (Gruenenfelder et al., 2011). The protein serves a neuroprotective role; it is required for proper sequestration of proteins into the myelin compartment, allegedly important for axo-glial metabolism and long-term support of axons (Werner et al., 2007). Expression of the *PLP1* gene is regulated in a spatiotemporal manner (Wight and Dobretsova, 2004). High levels are produced in oligodendrocytes concurrent with the active myelination period of development. Other select cells in the CNS and periphery express the gene albeit to lower levels, except for olfactory ensheathing cells (OECs) where levels are high (Dickinson et al., 1997). Its expression must be tightly regulated as evidenced by X-linked genetic diseases associated with *PLP1*. In humans, both segmental deletion (Raskind et al., 1991; Inoue et al., 2002) and duplication (Sistermans et al., 1998; Inoue et al., 1999; Mimault et al., 1999; Hodes et al., 2000) or higher copy number (Wolf et al., 2005) of the chromosomal region containing the *PLP1* gene can lead to the dysmyelinating disorder Pelizaeus–Merzbacher disease (PMD) or the related allelic disorder, spastic paraplegia type 2 (SPG2; Inoue, 2005; Werner et al., 2007; Hobson and Kamholz, 2013). Genetically engineered mice in which the gene has been

[1]Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Corresponding Author:
Patricia A. Wight, Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 4301 W. Markham Street, Slot 750, Little Rock, AR 72205, USA.
Email: pwight@uams.edu

deleted, or contain added copies of *PLP1* (or cDNA transgenes), demonstrate a variety of conditions ranging from late onset demyelination and axonopathy, to severe early onset demyelination (Kagawa et al., 1994; Readhead et al., 1994; Klugmann et al., 1997; Rosenbluth et al., 2006; Karim et al., 2007, 2010; Clark et al., 2013). Thus, *PLP1* expression is akin to a "Goldilocks" scenario—just the right amount of protein must be produced for normal function. However, very little is known about the mechanisms that regulate expression of the human *PLP1* gene (*hPLP1*). Previously our group has shown that the first intron of the mouse *Plp1* gene (*mPlp1*) contains regulatory element(s), crucial for its expression (Li et al., 2002). Thus this review will focus on sequences in intron 1 that have a bearing on *hPLP1* expression, and their possible significance in *PLP1*-related disorders.

PLP1 Gene

The *PLP1* gene is located on the X chromosome in a single copy (Figure 1). The gene contains seven major exons, distributed over nearly 16 kb of DNA in human and mouse (Diehl et al., 1986; Macklin et al., 1987; Ikenaka et al., 1988). The coding sequence from these species displays 95% identity at the nucleotide level, and at least 90% identity over the promoter (proximal 150 bp) and 5′- and 3′-untranslated regions (Macklin et al., 1987; Janz and Stoffel, 1993). The first intron is relatively large (>8 kb), accounting more than half of the gene, and is moderately conserved (Figure 2); 60% identity between human and mouse.

Classic *PLP1* Products (PLP/DM20) and Novel Splice Isoforms

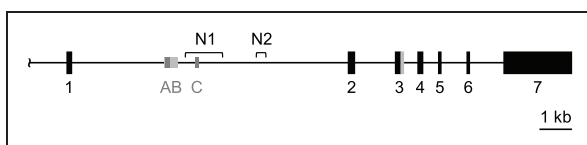The amino acid sequence of PLP is highly conserved in mammals; there is 100% identity among human and mouse. Two major (classic) protein products are generated due to alternative splicing in exon 3: PLP, a 276 amino acid polypeptide, and a similar protein called DM20, which lacks PLP residues 116 to 150 (Nave et al., 1987; Simons et al., 1987). DM20 is expressed prior to PLP early in development (Timsit et al., 1992), but as development proceeds, PLP becomes the predominant isoform (Kronquist et al., 1987; Gardinier and Macklin, 1988; LeVine et al., 1990; Schindler et al., 1990). In addition to the classic *PLP/DM20* transcripts, which are generated in all mammals, other splice variants are produced in some species through addition of supplementary exons present within intron 1. The first intron of *hPLP1* contains two such exons, exon AB and exon C, which get spliced independently of one another between *hPLP1* exons 1 and 2, in the human fetal and adult CNS (Figure 1). Utilization of these supplementary exons is for the most part restricted to the human species, although exon AB or A-specific portions of it have been found in transcripts from macaque, while exon C-containing transcripts are present in bovine (Sarret et al., 2010). These novel splice variants are expressed preferentially in neurons (Sarret et al., 2010), unlike their classic (*PLP/DM20*) counterparts. Splice variants that incorporate only A-specific regions of exon AB (A and A′) encode proteins containing an additional nine amino acids at the N-terminus of PLP or DM20. These isoforms are trafficked to the plasma membrane as shown by transfection analysis with applicable expression constructs (Sarret et al., 2010). Thus these novel proteins could pose as targets for immune-mediated degeneration of axons/neurons in MS patients. In contrast, it is not known whether the splice variants that contain exon AB (in its entirety) or exon C encode protein or function solely as noncoding RNAs. If they encode a protein product, the start of translation is predicted to be
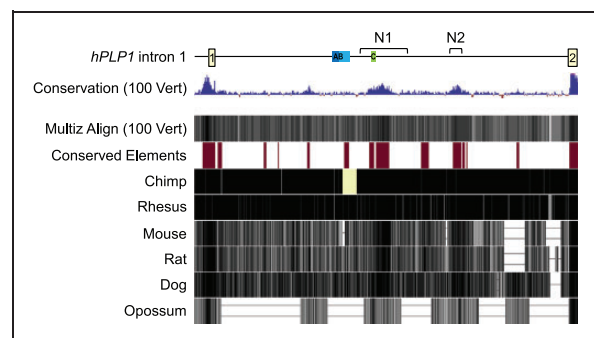

Figure 1. Schema of *hPLP1* gene. The *hPLP1* gene contains seven major exons represented by the numbered boxes; black lines indicate introns or upstream (5′-flanking) DNA. Exon 3 contains an internal splice donor site and, when utilized, results in the DM20 isoform (the PLP-specific portion of exon 3 is indicated in gray). As well, the first intron of *hPLP1* contains a couple of minor exons (AB and C; symbolized by smaller gray boxes) which get incorporated separately in splice variants. Exon AB may get incorporated in its entirety, or just as select portions of the A-specific region (dark gray area). N1 and N2 indicate areas of *hPLP1* intron 1 DNA orthologous to the wmN1 and wmN2 enhancer regions in *mPlp1*.

Figure 2. Comparison of *hPLP1* intron 1 sequence with other species using the UCSC Genome Brower (GRCh38/hg38 assembly). Sequence conservation for 100 vertebrates (including human) is indicated by vertical blue lines based on Multiz Alignments (100 Vert). Conserved elements between human and six other species (chimp, Rhesus, mouse, rat, dog, and opossum) are indicated by dark red blocks. Sequence conservation of human versus chimp, Rhesus, mouse, rat, dog, or opossum is shown at the bottom.

located near the end of *hPLP1* exon 4, due to introduction of multiple in-frame stop codons downstream of the traditional start site in exon 1 used to make the classic (PLP or DM20) products. This in turn would yield a peptide corresponding to the last 72 amino acids of PLP (Sarret et al., 2010). Interestingly, a secreted C-terminal PLP product has been shown to increase proliferation of oligodendrocyte lineage cells *in vitro* (Yamada et al., 1999). Thus, it is conceivable that exon AB- or C-containing mRNAs may encode a protein that acts as a growth factor.

Exons AB and C are not utilized in mouse even though the sequences are highly conserved (≥95% identity with human; Sarret et al., 2010). This is not due to a paucity of human-specific splicing components since the entire spectrum of splice variants were produced in a mouse cell line transfected with *hPLP1* constructs (Hamdan et al., 2015). Thus lack of these "human-specific" isoforms in *PLP1*-null patients may have unique consequences in man. An altogether different pair of supplementary exons are present in intron 1 from mouse; *mPlp1* exons 1.1 and 1.2 (Bongarzone et al., 1999; Li et al., 2009). Exon 1.1-containing splice variants encode an isoform with an additional 12 amino acid leader sequence (counting the initiator methionine residue), which causes the protein to be retained within the cell soma (Bongarzone et al., 1999).

PLP1-Related Disorders

As mentioned earlier, the essential role that *PLP1* plays is illustrated by mutations in the gene that lead to PMD or SPG2; conditions characterized by delayed motor and cognitive development, followed by a gradual decline, with variation in the age of onset and rate of deterioration between patients (Inoue, 2005; Gruenenfelder et al., 2011; Hobson and Kamholz, 2013). There is variable deficiency of myelin and reduced numbers of oligodendrocytes, and some axonal loss may occur. Underlying *PLP1* mutations include duplications (or higher copy number), deletions, and point (missense) mutations. More than 50% of PMD cases are caused by genomic duplications involving *hPLP1*, with point mutations and indels (small insertions and deletions) accounting for approximately 25% of PMD or SPG2 cases (Mimault et al., 1999). Increased *PLP1* gene dosage results in elevated amounts of PLP (Ikenaka et al., 1988; Regis et al., 2009). Overexpression of the gene has been shown to cause accumulation of cholesterol and PLP in endosomes or lysosomes and perturb myelin protein composition and myelination in transgenic mice (Simons et al., 2002; Karim et al., 2007, 2010). *Plp1* copy number in transgenic mouse models seems to roughly correlate with disease severity (Anderson et al., 1998, 1999; Karim et al., 2007). Hemizygous mice that harbor a *Plp1* transgene show late onset demyelination and axonal degeneration, whereas dysmyelination and early death occur in homozygous

mice (Readhead et al., 1994; Anderson et al., 1998, 1999). However, the cause of premature death in animals with significant levels of PLP overexpression (which is primarily evident during the early postnatal stage) remains unclear. Moreover, defects observed in *Plp1* overexpressing mice are not limited solely to the myelin compartment, or even to oligodendrocytes. *Plp1* overexpressing mice exhibit major oxidative phosphorylation deficits that include a 50% reduction in brain ATP levels and decreased mitochondrial membrane potentials in oligodendrocytes, as well as neurons (Hüttemann et al., 2009). Axonal damage has been observed in PMD patients with *hPLP1* duplications (Laukka et al., 2016). In addition, massive microglial cell activation and inflammation throughout the brain has been observed in *Plp1* overexpressing mice (Tatar et al., 2010).

In contrast, genomic deletion of *PLP1* leads to the formation of compact myelin devoid of PLP. The resulting myelin sheath is physically fragile, making it susceptible to subsequent demyelination. The lack of PLP/DM20 may lead to disruption of myelin-axon interactions, resulting in axonal degeneration (Inoue, 2005; Gruenenfelder et al., 2011). In addition, patients lacking *hPLP1* (as well as *Plp1* null mice) develop length-dependent axonal degeneration, in the absence of demyelination and inflammation (Garbern et al., 2002). All told, these results suggest that the level of *PLP1* gene expression must be tightly regulated; either too much or too little of the protein yield detrimental effects.

Transcriptional Regulatory Elements in PLP1 Intron 1

Enhancer trap studies in transgenic mice have identified a couple of conserved regions within *mPlp1* intron 1 that are capable of augmenting expression of a reporter gene driven by a minimal (heat shock protein) promoter (Tuason et al., 2008). The wmN1 region encompasses 1171 bp, which appears to be important for directing high levels of expression in myelinating oligodendrocytes and OECs. The other region, called wmN2 (313 bp), conferred high level expression in Schwann cells and their progenitors, dorsal root ganglia, and OECs, and weak expression in oligodendrocyte lineage cells. *In vitro* studies, with *hPLP1* constructs, indicate that the orthologous human wmN2 region is active in an immature oligodendrocyte (Oli-neu) cell line (Hamdan et al., 2015). Results from our laboratory indicate that the wmN1 region is essential for broad spatiotemporal expression of an *hPLP1-lacZ* transgene driven by its native (*hPLP1*) promoter (unpublished results by Hamdan et al.). Thus it is possible that mutations within these regions that disrupt enhancer function may be the underlying cause of PMD or SPG2 in patients with normal *hPLP1* gene dosage, whom lack mutations in their coding sequence.

Conclusions

In summary, the first intron of *hPLP1* contains several sequences important for its expression. Utilization of supplementary exons (exon AB and exon C), located in what is classically thought of as *hPLP1* intron 1, leads to generation of splice variants that are primarily restricted to the human species. Some of the splice variants contain only A-specific regions of exon AB and encode a novel isoform, having an additional nine amino acids at the N-terminus of PLP or DM20. Others are predicted to encode a truncated product that potentially could act as a growth factor or may function simply as noncoding RNAs. In addition, other regions (wmN1 and wmN2) within the intron seem to behave as enhancers necessary for *hPLP1* gene activity. These sequences may have special relevance for *hPLP1*-related disorders. For instance, mutations that disrupt enhancer function would lead to little or no expression of *hPLP1*, resulting in PMD or SPG2. Furthermore, splice variants generated through the addition of supplementary exons present in *hPLP1* intron 1 yield novel isoforms which are primarily restricted to the human species and preferentially expressed in neurons. A lack of these isoforms in *hPLP1* null patients or alternations in their levels may have unique implications in humans. Demyelinating peripheral neuropathy has been observed in humans (but not animals) that are null for *PLP1* (Garbern et al., 1997). Whereas mutations in *hPLP1* that alter the ratio of PLP to DM20 have been shown to cause the X-linked disorder, Hypomyelination of Early Myelinating Structures (HEMS; Kevelam et al., 2015). Thus it is conceivable that deviations from the conventional amounts of "human-specific" *hPLP1* splice variants may also lead to complications.

Declaration of Conflicting Interests

Funding

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