

# **Distinct Phenotypes Associated with Increasing Dosage of the PLP Gene: Implications for CMT1A Due to *PMP22* Gene Duplication**

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**ABSTRACT:** Increased dosage of the proteolipid protein (*Plp*) gene causes CNS disease (Pelizaeus-Merzbacher disease [PMD]), which has many similarities to disorders of the PNS associated with duplication of the peripheral myelin protein-22 (*PMP22*) gene locus. Transgenic mice carrying extra copies of the wild-type *Plp* gene provide a valid model of PMD. Variations in gene dosage can cause a wide range of phenotypes from severe, lethal dysmyelination through late-onset demyelination. A predilection for different fiber diameters may occur within the various phenotypes with dysmyelination being more obvious in large fibers and late-onset degeneration predominantly affecting small fibers. Although the frequency of apoptotic oligodendrocytes is increased with high gene dosage, the number of mature oligodendrocytes appears adequate. Oligodendrocytes in the dysmyelinated CNS express a range of genes typical of mature cells, yet are unable to assemble sufficient myelin. Oligodendrocytes contain abnormal vacuoles and stain intensely for PLP and other proteins such as MAG. The findings suggest that with high gene dosage much of the PLP, and possibly other proteins, is missorted and degraded in the lysosomal system.

## **INTRODUCTION**

What relevance has proteolipid protein (PLP), the major protein of CNS myelin, to the understanding of Charcot-Marie-Tooth (CMT) disorders? The most prevalent form of the neuropathy, CMT1A, is associated with duplications and mutations of the peripheral myelin protein-22 (*Pmp22*) gene. Although there is no sequence identity between PLP and PMP22, there are useful similarities and comparisons, particularly in relation to the effects of disease. The *Plp* and *Pmp22* genes are both members of larger families<sup>1-3</sup>. Each is represented by two isoforms that show different developmental and spatial profiles<sup>4,54</sup>; the smaller isoform of PLP, termed DM20, is generated by the deletion of 35 amino acids as a result of

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alternative splicing in exon 3.<sup>4</sup> The major isoforms of PLP and PMP22 are present in compact CNS and PNS myelin, respectively. The minor isoforms are expressed not only in the myelinating glia but other cell types and are postulated to be involved in cell development. PLP/DM20 and PMP22 are highly hydrophobic tetraspan molecules, translated at the rough endoplasmic reticulum (RER) with subsequent passage via the Golgi apparatus to the cell membrane. PMP22 is glycosylated, whereas PLP is acylated. (For recent reviews on PLP and PMP22, see Naef and Suter<sup>5</sup> and Girffiths *et al.*<sup>7</sup>

The most interesting comparisons are in relation to gene mutations and the consequential disease. A range of mutations of the X-linked *Plp* gene cause Pelizaeus-Merzbacher disease (PMD) and its allelic disorder, spastic paraplegia type 2 (SPG2)<sup>8,9</sup>. Although a wide range of mutations are reported in PMD, the most commonly described are missense mutations and gene dosage effects. Duplication of the *Plp* locus is now thought to represent the most common cause of PMD,<sup>10,11</sup> although the boundaries of the duplicated region have not been defined. Duplication of a 1.5-Mb region of chromosome 17, containing the *Pmp22* gene, is the most frequent basis of CMT1A, with missense mutations as a less common cause. Convincing evidence that the increased dosage of the two myelin genes was indeed the basis of the myelin pathology was provided when transgenic animals carrying extra copies of the wild-type genes developed disease phenotypes similar to the human disorders.<sup>12-15</sup> Spontaneous mouse models with missense mutations of the *Pmp22* and *Plp* genes are represented by *Trembler* (*Tr*) and its alleles in the PNS and the alleles of *jimpy* (*Plp<sup>jp</sup>*) in the CNS. (*jimpy*, is a splicing defect with subsequent deletion of exon 5; its various alleles, such as *rumpshaker*, are missense mutations resulting in amino acid substitutions.) This presentation will concentrate primarily on the comparative effects of increased dosage of the respective genes.

#### ***Transgenic Mice with Increased Copies of the Plp Gene***

The transgenic mice studies are based primarily on two lines of mice previously reported by us and termed transgenic lines #66 and #72.<sup>13</sup> The autosomally integrated transgene of ~26 kb contains the transcriptional unit of the wild-type murine *Plp* gene. Homozygous inheritance of the #66 transgene produces the highest gene dosage and most severe phenotype, whereas #72, inherited homozygously, produces an intermediate dosage and phenotype. Hemizygous inheritance of either #66 or #72 transgenes results in the lowest increase of gene dosage producing a late-onset phenotype. Another line of mice (termed 4e) carrying a genomic transgene was generated by Ikenaka's research group.<sup>12</sup> Lines of mice carrying various copy numbers of a cDNA transgene for *Dm20* have also been generated.<sup>16</sup> The mice with the genomic transgenes, which represent valid models of PMD, show phenotypes related to the gene dosage.

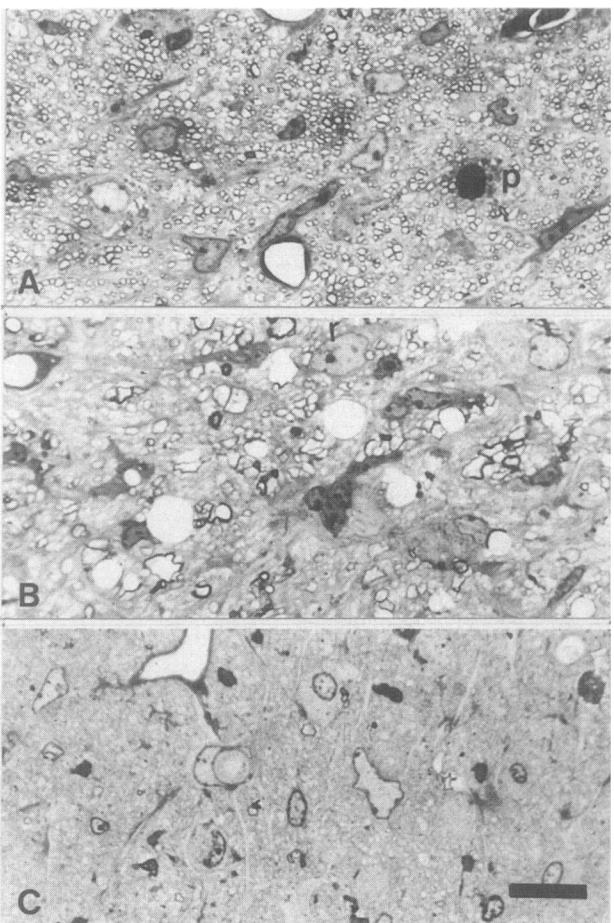
### **INCREASED GENE DOSAGE OF MYELIN GENES AS A CAUSE OF DISEASE**

#### ***Increased Plp Gene Dosage Produces a Wide Range of Phenotypes in Mice***

The evidence from human disease and the transgenic models leaves little doubt that increased *Plp* or *Pmp22* gene dosage causes myelin abnormalities in CNS and PNS, respectively. What has been surprising is the range of phenotypes associated with increased dosage of the *Plp* gene. The highest gene dosage is associated with clinical signs of tremor, ataxia, and seizures and premature death due to severe dysmyelination.<sup>17</sup> At the other extreme, low gene dosage is compatible with normal development and absence of clinical signs until late in life. The animals then develop a neurodegenerative disorder associated with demyelination and axonal degeneration (see below).<sup>18</sup> Intermediate levels

of gene dosage result in less severe dysmyelination, which may cause no observable clinical deficits initially but at older ages a superimposed neurodegeneration may occur causing clinical signs (FIG. 1) (TABLE 1).

It is therefore evident that increased gene dosage does not result in a stereotyped phenotype but rather can demonstrate marked variation in age of onset, severity of signs, eventual outcome and underlying pathology. However, these observations are based on animals that carry various copy numbers of transgenes, with probable different integration sites, compared to man where a duplication of the endogenous gene locus occurs. It is quite possible that the level of expression of the duplicated locus varies between patients or between



**FIGURE 1. The temporal alteration in optic nerve pathology associated with an “intermediate” increase in transgene dosage.** The mice are homozygous for the #72 transgene (three extra copies of the wild-type *Plp* gene per transgene) at (A), P20; (B), P60; (C), P120. At P20 (A) the nerve is still myelinating, and many axons are surrounded by a myelin sheath that is thinner than the corresponding wild-type; the stage of dysmyelination. One cell (P), probably an oligodendrocyte, has a pyknotic nucleus. (B) By P60, degenerative changes are evident. Many axons have lost their myelin sheaths, and other sheaths are vacuolated. (C) By P120, the nerve is completely demyelinated. Bar = 20  $\mu$ m.

TABLE 1. Summary of the major phenotypic effects of increased *Pip* gene dosage in transgenic mice<sup>a</sup>

Dosage	Clinical	Myelin Formation	Demyelination and/or Degeneration	Tract Predilection for Degeneration	Oligo Apoptosis Staining	Abnormal Oligo	Microglia	Astrocytes
High	Ataxia Tremor Seizure Death	Dys	Yes	No	++	++	++	++
Medium (early life)	Normal	Dys	No	No	+	+	+	No
Medium (later life)	Ataxia Tremor Seizure Death	Dys	Yes	Yes	No	Not examined	+	+
							More in affected tracts	
Low (early life)	Normal	Normal	No	No	No	No	No	No
Low (later life)	Ataxia	Normal	Yes	Yes	No	No	+++	+++
		Tremor Seizure Death	initially					

<sup>a</sup>Dosage has been arbitrarily assigned to high, medium, and low depending on transgene copy number and homozygous or hemizygous inheritance. Oligo = oligodendrocyte. Dys = dysmyelination.

affected families. Expression of the endogenous gene locus could be influenced by the presence of, as yet unidentified, modifying genes. At least three spontaneous animal myelin mutants (*rumpshaker*, *md-rat*, *hindshaker*) show marked variation in phenotype, depending on the genetic background<sup>7</sup>; and in one of these, *hindshaker*, a chromosomal region has been identified that probably harbors putative modifying genes.<sup>19,20</sup> Similar regions are bound to occur in man, and their influence on the mutant locus may well have a major effect on phenotype. Variations of *Pmp22* gene dosage in transgenic mice and rats also produce a range of phenotypes.<sup>15,20</sup>

### ***Selective Effects of Increased Plp Gene Dosage on Different Fibers***

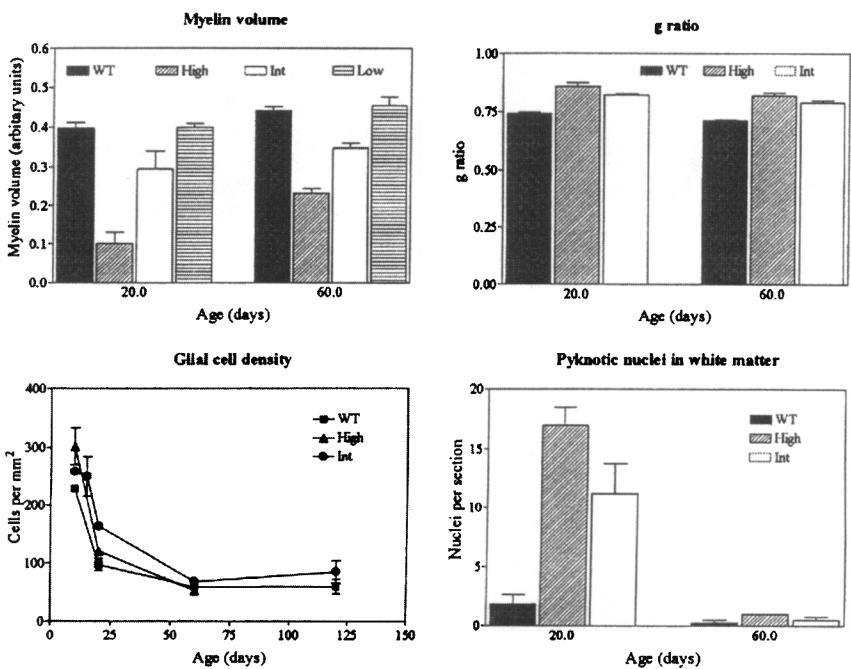
The pathology associated with increased *Plp* gene dosage shows predilection for certain fiber sizes. The dysmyelination, although generalized, tends to be more marked in the areas of the CNS that myelinate later during development.<sup>13</sup> However, within the earlier myelinating regions, such as the ventral spinal cord, the myelin deficit is most obvious in larger diameter fibers. In contrast, the late-onset demyelination and degeneration shows a striking predilection for small-diameter axons such as the optic nerve, fasciculus gracilis, and corticospinal tracts.<sup>18</sup> Interestingly, a similar pattern of selectivity is seen in the axonopathy associated with a null *Plp* gene.<sup>22</sup> The reason for these different selectivities is unknown. Increased *Pmp22* expression also has a selective effect; the myelin deficit is more severe in ventral compared to dorsal nerve roots, and larger fibers are more vulnerable.<sup>15</sup>

### ***Axonal Involvement in Altered Plp Gene Dosage***

The oligodendrocyte represents the main cell type in which the *Plp* gene is expressed. Lower levels of expression are present in various other glia, such as Schwann cells, but no convincing evidence of neuronal manifestation has been provided. However, dosage disturbances of the *Plp* gene are associated with axonal lesions. A low increase in gene dosage causes late-onset neurodegeneration of small-diameter fibers.<sup>18</sup> The most striking effect is found with a null *Plp* gene, generated by gene targeting. Despite assembling and maintaining normal amounts of myelin, the PLP-deficient mice develop an axonopathy<sup>22,23</sup>. The mechanism of this change is unknown but suggests a disturbance in the interrelationship of oligodendrocyte and axon. A similar close interaction occurs in the PNS between axon and Schwann cell. It is established that the dysmyelination caused by the *Tr* mutation of the *Pmp22* gene perturbs the structure and function of the axon,<sup>24,25</sup> and axonal damage is also seen in dosage disorders of the gene. These changes have been related to the reduction or absence of the myelin sheath. In the light of the *Plp*-knockout mouse, it could also be considered that specific myelin proteins might be more directly involved in the intercommunication of axon and glia.

### ***Relationship of Glial Cells and Gene Dosage***

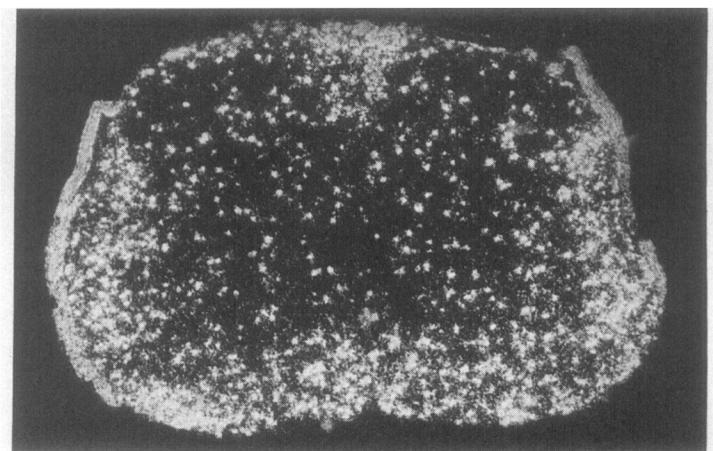
Although increased dosage of the *Plp* gene clearly perturbs the oligodendrocytes' ability to assemble and/or maintain myelin, the mechanisms underlying this are uncertain. One possibility is a decreased number of mature oligodendrocytes, due either to a developmental failure or premature cell death. We have quantified the numbers of glial cells and pyknotic nuclei in the cervical white matter in mice with different copy numbers of *Plp* genomic transgenes (FIG. 2). At postnatal day 20 (P20), during the phase of myelination, the glial cell density is elevated in the dysmyelinated mice with the higher gene dosages.



**FIGURE 2.** Graphs indicating the effect of *Plp* transgene dosage on myelin and glial cells in the spinal cord white matter at P20 during the period of myelin formation and at P60 during the period of myelin maintenance. For clarity, the transgene dosage is presented as high (homozygous #66 transgene), intermediate (Int) (homozygous #72 transgene), or low (hemizygous #66 or #72 transgene). The effect of low transgene dosage has been shown only for the myelin volume, and for this—and all the other parameters—the results were identical to wild-type (WT). With the high and intermediate transgene dosage, the amount of myelin is reduced, the myelin sheaths are disproportionately thin (as shown by increased g ratio), and the glial cell density and the number of pyknotic nuclei are increased in proportion to the dosage. The effects are most marked during the period of myelination.

At least some of this elevation in glial numbers is due to an increase in microglia; GFAP staining is also increased.<sup>13</sup> The number of pyknotic nuclei is also increased in these animals. By P60, a time when spinal cord myelination is normally complete, there is less difference between affected and wild-type mice. Animals with a low transgene copy number that develop the late-onset neurodegeneration have normal cell counts and no elevation of pyknotic nuclei during myelination. However, during the late-onset neurodegeneration there is a marked increase in microglia and an increase in GFAP staining.<sup>18</sup> Ultrastructurally, myelinating oligodendrocytes appear normal in mice with a low copy number of transgenes that develop late-onset disease. In contrast, many oligodendrocytes of animals with higher transgene dosage and dysmyelination contain numerous autophagic vacuoles and disrupted RER.<sup>12,13,17</sup> However, not all cells are affected, and the frequency of abnormal cells appears to decrease at older ages. At least some of the abnormal oligodendrocytes are capable of assembling myelin sheaths.

*In situ* hybridization with a *Plp* probe was performed in order to provide an estimate of oligodendrocyte numbers. At P20, an age when myelination of the spinal cord is well



**FIGURE 3.** Cervical cord from mouse with high transgene dosage (homozygous, #66 transgene) aged P20. The tissue has been hybridized with an  $^{35}\text{S}$ -labeled riboprobe to *Plp/Dm20* and viewed in darkfield illumination. The number of expressing oligodendrocytes and signals per cell appears similar to wild-type.

advanced, the numbers of *Plp*<sup>+</sup> cells in dysmyelinated mice due to high transgene dosage appear similar to wild-type, with a strong signal per cell (FIG. 3).

No reliable data is available on the absolute number or proportion of mature oligodendrocytes that are necessary for myelination to occur in a region. Our overall impression from the morphological, *in situ* hybridization and immunostaining studies is that the dysmyelination is not due to a lack of mature cells *per se*, but rather that those present cannot assemble sufficient myelin. There is, however, a positive relationship between the severity of dysmyelination and the number of dead (pyknotic) cells. The reason for the increased cell death is unknown but, as stated, we do not think it is sufficient to be the primary cause of the hypomyelination.

PMP22 was identified as the rat homologue of a growth arrest gene (*gas-3*)<sup>26</sup> raising the possibility that perturbations of Schwann cell division and survival might underlie CMT disorders. Some evidence has been provided that increasing the level of PMP22 in transfected cells is associated with alterations in the cell cycle or apoptosis<sup>27–29</sup>. A detailed study of Schwann cell survival has not been undertaken in *Pmp22* transgenic mouse and rat models of CMT. However, from morphological studies there is no obvious evidence of increased apoptosis and the myelin-forming Schwann cells and axons have segregated in a 1 : 1 manner suggesting that a deficiency of Schwann cells is not the primary reason for dysmyelination or demyelination.<sup>15,21,30,31</sup>

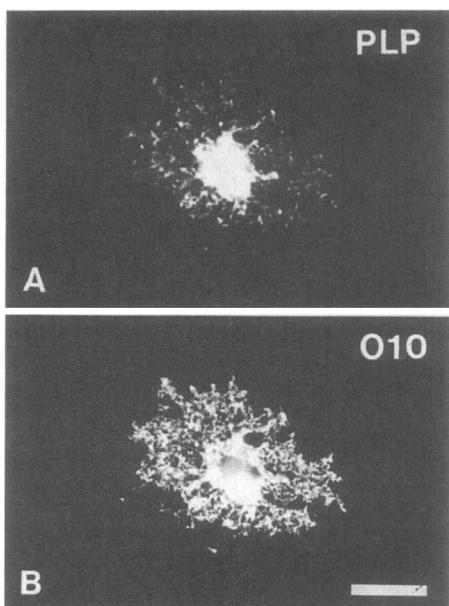
#### *Molecular Differentiation of Oligodendrocytes with Increased Plp Gene Dosage*

A modest increase in *Plp* gene dosage, which is associated with late-onset disease, does not perturb myelination. Immunostaining for a number of myelin antigens produces results identical to wild-type.<sup>18</sup> Because higher and intermediate increases in *Plp* gene dosage cause dysmyelination, we examined the molecular differentiation in oligodendrocytes during myelination. Resin sections of spinal cords of affected mice, aged P3, contained a reduced number of myelin sheaths, although those present immunostained strongly for PLP/DM20 and MBP (data not shown). Immunostaining of cryosections from mice aged

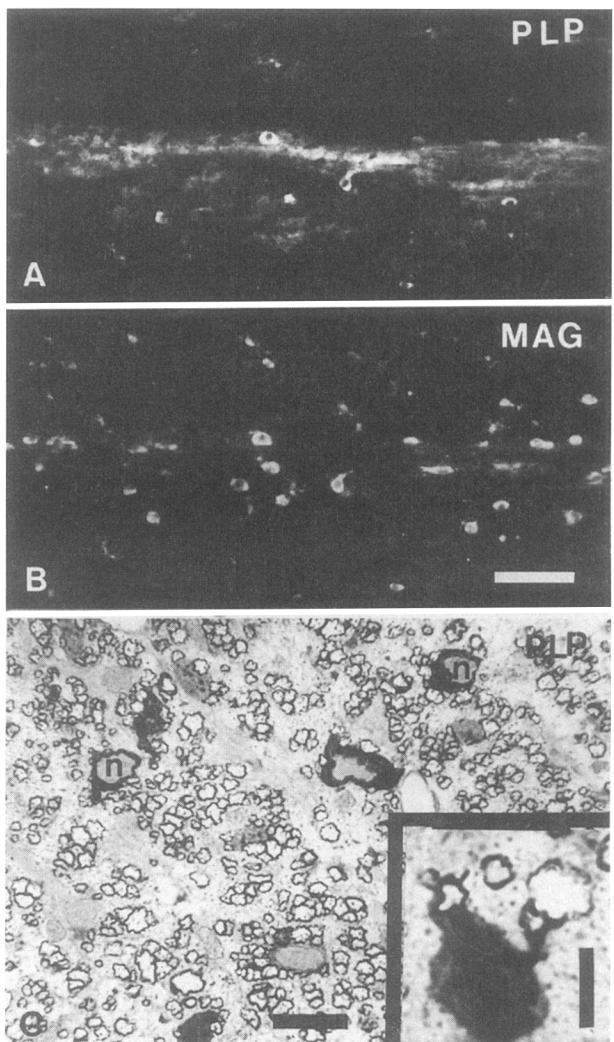
P17 to P21 for MAG, PLP, MOG, MBP, MOBP, and MOSP showed that each was present and capable of being incorporated into myelin sheaths or the periaxonal space in the case of MAG (data not shown). To determine whether PLP was correctly inserted into the cell membrane, we cultured oligodendrocytes from brains of P12 mice with a high transgene copy number and wild-type littermates for 24 hours before surface immunostaining with the O10 antibody.<sup>32</sup> Oligodendrocytes from affected (Fig. 4) and wild-type mice stained identically. We conclude that oligodendrocytes with a high dosage of the *Plp* gene show a molecular maturity similar or identical to wild-type in terms of the range of genes expressed and that the proteins are targeted to their correct destinations. Despite this, they are unable to assemble myelin sheaths of appropriate thickness. A similar situation occurs in rats homozygous for a *Pmp22* transgene (representing a high gene dosage effect) whose Schwann cells produce little if any myelin yet demonstrate a considerable degree of molecular differentiation, expressing a range of myelin antigens.<sup>30</sup>

#### ***Abnormalities in the Distribution and Localization of Myelin Antigens***

Although mice carrying the highest *Plp* transgene copy number express a range of mature myelin antigens, the distribution of some of these is abnormal. The cell bodies and processes from many oligodendrocytes stain intensely for PLP/DM20; the PLP-specific isoform, MAG; MOSP; and, to a lesser degree, MOG (FIG. 5A, B). A similar intense staining of the cell body for MBP is not seen. During normal early myelination, before the formation of myelin sheaths, similar staining of the cell body occurs.<sup>33,34</sup> In the normal mouse spinal cord, such cell body staining is visible until about P5, after which the products are



**FIGURE 4. Oligodendrocyte from a mouse with high transgene dosage.** Cells were dissociated from forebrain of P12 mouse and cultured for 24 hours before double immunostaining with surface O10 and permeabilized for PLP/DM20. The PLP/DM20 has been correctly inserted into the plasmalemma, as shown by the O10 staining. Bar = 30  $\mu$ m.



**FIGURE 5. Many oligodendrocytes in the CNS of mice with high transgene dosage stain hyperintensely for certain myelin proteins.** (A,B) Cryosection of saggital brain from area of corpus callosum double immunostained for PLP/DM20 and for MAG. A small amount of myelin is present in the corpus callosum, but many oligodendrocyte cell bodies stain intensely for the two proteins, particularly MAG. Bar = 100  $\mu$ m. (C) Resin section of P20 spinal cord immunostained for PLP/DM20. Myelin sheaths are positive, showing that some protein is incorporated into myelin. The cell bodies and processes of oligodendrocytes stain abnormally strongly for the antigen. Ultrastructural examination of adjacent thin sections showed that such cells contained numerous autophagic vacuoles and, often, disrupted RER. Their nuclei (n) appear normal. Bar = 20  $\mu$ m. *Inset:* Area from P10 spinal cord to show one of the hyperstained oligodendrocytes extending processes to myelinate axons. Bar = 10  $\mu$ m.

detected in the myelin sheaths. In these dysmyelinated, transgenic mice, intense staining of cell bodies and processes persists up to at least P60. However, not all oligodendrocytes are affected. Using adjacent PLP-immunostained resin sections (FIG. 5C) and thin sections for electron microscopy, it appears that most, if not all, intensely stained cells are those with cytoplasmic vacuoles and disrupted RER. Immature oligodendrocytes are not immunostained. The reactivity is fairly uniform throughout the cell body with occasional focal intensities. The subcellular localization of the product has not been completed, but the diffuse appearance may reflect PLP in the RER, whereas the focal intensities may indicate its presence in autophagic vacuoles. The nuclei of such cells is normal with no evidence of pyknosis (FIG. 5C). Despite the abnormal distribution of various myelin membrane proteins and the cytological abnormalities, many oligodendrocytes are able to assemble a thin myelin sheath (FIG. 5C, *inset*) of normal periodicity.

### MECHANISMS OF DISEASE

In normal animals during development, there is a phase of rapid myelin synthesis followed by a sustained period of maintenance. However, at all stages there is a "turnover" of myelin constituents, and in the mature animal any degradation is matched by synthesis to maintain the status quo. During development, sheaths are remodeled until the internodal myelin volume is appropriate for the axon diameter. Increased gene dosage can affect the stages of synthesis and/or maintenance. The higher and intermediate increases in gene dosage interfere with the ability to produce myelin and are eventually associated with increased breakdown of some of the preformed myelin. As discussed above, we suggest this is not due to a lack of mature oligodendrocytes, even though there is some increase in apoptosis. Rather, we suggest that various myelin components such as PLP are targeted for degradation. Whether the majority of the myelin components reach the cell surface before endosomal re-uptake has yet to be established. However, we have shown that even in the most severe dysmyelination seen in our mice (homozygous line #66) the various myelin constituents are, in principle, capable of being inserted into the sheath; whether the correct absolute or relative amount of each is inserted is unknown. Future metabolic studies will determine the dynamics of PLP, and other myelin components, in dysmyelination and answer some of the current uncertainties.

With the smallest dosage increase, the phase of myelin assembly and early maintenance appears unperturbed. At some later stage, perhaps over halfway through the lifespan of the mouse, some myelin sheaths break down. This is associated with a microglial response, but the oligodendrocyte autophagic vacuoles, found in the dysmyelinated CNS, are not present. The myelin sheath appears to unravel from the axon<sup>18</sup> and is not removed by macrophages, as in inflammatory demyelination. There is no obvious attempt at remyelination, although this response tends to be muted in older animals.<sup>35</sup> The expression of several myelin genes (in addition to *Pip*) is upregulated throughout the life of these mice, although the actual amount of myelin is not increased relative to wild-type.<sup>18</sup> It is possible that the demands of constantly maintaining a state of heightened activity eventually compromises the oligodendrocyte's ability to maintain the myelin; a form of "metabolic burn-out."

### CONCLUSIONS

Many of the features found with increased dosage of the *Pip* gene in the CNS find a counterpart with the *Pmp22* gene in the PNS. Overexpression of either gene can impair myelin formation and/or myelin maintenance, depending on dosage. The cytological

features associated with high levels of *Plp* and *Pmp22* gene expression in oligodendrocytes and Schwann cells, respectively, suggest that much of the myelin protein is degraded, rather than incorporated into a stable myelin sheath. The available evidence suggests that common pathogenic mechanisms operate in both cell types.

### ACKNOWLEDGMENTS

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### REFERENCES

1. SINOWAY, M.P., K. KITAGAWA, L. FIDLER, R.M. GOULD & D.R. COLMAN. 1994. Tissue lipoproteins revisited: new proteolipid protein gene family members in elasmobranchs. *Neurochem. Res.* **19**: 1047–1054.
2. KITAGAWA, K., M.P. SINOWAY, C. YANG, R.M. GOULD & D.R. COLMAN. 1993. A proteolipid protein gene family: expression in sharks and rays and possible evolution from an ancestral gene encoding a pore-forming polypeptide. *Neuron* **11**: 433–448.
3. TAYLOR, V., A.A. WELCHER & U. SUTER. 1995. Epithelial membrane protein-1, peripheral myelin protein 22, and lens membrane protein 20 define a novel gene family. *J. Biol. Chem.* **270**: 28824–28833.
4. NAVE, K.-A., C. LAI, F.E. BLOOM & R.J. MILNER. 1987. Splice site selection in the proteolipid protein (PLP) gene transcript and primary structure of the DM-20 protein of central nervous system myelin. *Proc. Natl. Acad. Sci. USA* **84**: 5665–5669.
5. SUTER, U., G.J. SNIPES, R. SCHOENER-SCOTT, A.A. WELCHER, S. PAREEK, J.R. LUPSKI, R.A. MURPHY, E. SHOOTER & P.I. PATEL. 1994. Regulation of tissue-specific expression of alternative peripheral myelin protein-22 (PMP22) gene transcripts by two promoters. *J. Biol. Chem.* **269**: 25795–25808.
6. NAEF, R. & U. SUTER. 1998. Many facets of the peripheral myelin protein PMP22 in myelination and disease. *Microsc. Res. Tech.* **41**: 359–371.
7. GRIFFITHS, I.R., M. KLUGMANN, T.J. ANDERSON, C.E. THOMSON, D.A. VOYIOUKLIS & K.-A. NAVE. 1998. Current concepts of PLP and its role in the nervous system. *Microsc. Res. Tech.* **41**: 344–358.
8. SEITELBERGER, F., S. URBANITS & K.-A. NAVE. 1996. Pelizaeus-Merzbacher disease. In *Neurodystrophies and Neurolipidoses*. H.W. Moser, Ed.: 559–579. Elsevier Science. Amsterdam.
9. SEITELBERGER, F. 1995. Neuropathology and genetics of Pelizaeus-Merzbacher disease. *Brain Pathol.* **5**: 267–273.
10. SISTERMANS, E.A., R.F. DE COO, I.J. DE WIJS & B.A. VAN OOST. 1998. Duplication of the proteolipid protein gene is the major cause of Pelizaeus-Merzbacher disease. *Neurology* **50**: 1749–1754.
11. HODES, M.E. & S.R. DLOUHY. 1996. The proteolipid protein gene: double, double, . . . and trouble. *Am. J. Hum. Genet.* **59**: 12–15.
12. KAGAWA, T., K. IKENAKA, Y. INOUE, S. KURIYAMA, T. TSUJII, J. NAKAO, K. NAKAJIMA, J. ARUGA, H. OKANO & K. MIKOSHIBA. 1994. Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. *Neuron* **13**: 427–442.
13. READHEAD, C., A. SCHNEIDER, I.R. GRIFFITHS & K.-A. NAVE. 1994. Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. *Neuron* **12**: 583–595.
14. HUXLEY, C., E. PASSAGE, A. MANSON, G. PUTZU, D. FIGARELLA-BRANGER, J.F. PELLISSIER & M. FONTÉS. 1996. Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum. Mol. Genet.* **5**: 566–569.

15. SEREDA, M., I.R. GRIFFITHS, A. PÜHLHOFER, H. STEWART, M.J. ROSSNER, F. ZIMMERMANN, J.P. MAGYAR, A. SCHNEIDER, E. HUND, H.M. MEINCK, U. SUTER & K.-A. NAVE. 1996. A transgenic rat model of Charcot-Marie-Tooth disease. *Neuron* **16**: 1049–1060.
16. MASTRONARDI, F.G., C.A. ACKERLEY, L. ARSENAULT, B.I. ROOTS & M.A. MOSCARELLO. 1993. Demyelination in a transgenic mouse: a model for multiple sclerosis. *J. Neurosci. Res.* **36**: 315–324.
17. INOUE, Y., T. KAGAWA, Y. MATSUMURA, K. IKENAKA & K. MIKOSHIBA. 1996. Cell death of oligodendrocytes or demyelination induced by overexpression of proteolipid protein depending on expressed gene dosage. *Neurosci. Res.* **25**: 161–172.
18. ANDERSON, T.J., A. SCHNEIDER, J.A. BARRIE, M. KLUGMANN, M.C. MCCULLOCH, D. KIRKHAM, E. KYRIAKIDES, K.-A. NAVE & I.R. GRIFFITHS. 1998. Late-onset neurodegeneration in mice with increased dosage of the proteolipid protein gene. *J. Comp. Neurol.* **394**: 506–519.
19. KING, H. 1998. Phenotypic and genetic analysis of the *hindshaker* mutation. Ph.D. thesis/dissertation. University of Glasgow, Glasgow, Scotland.
20. KING, H., M.C. MCCULLOCH, J.A. BARRIE, E. KYRIAKIDES, B.M. CATTANACH, C.V. BEECHEY & I.R. GRIFFITHS. 1997. *Hindshaker*, a novel mutant showing hypomyelination preferentially affecting the spinal cord. *J. Neurocytol.* **26**: 557–566.
21. HUXLEY, C., E. PASSAGE, A.M. ROBERTSON, B. YOUL, S. HUSTON, A. MANSON, D. SABÉRAN-DJONIEDI, D. FIGARELLA-BRANGER, J.F. PELLISIER, P.K. THOMAS & M. FONTÉS. 1998. Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. *Hum. Mol. Genet.* **7**: 449–458.
22. GRIFFITHS, I.R., M. KLUGMANN, T.J. ANDERSON, D. YOOL, C.E. THOMSON, M.H. SCHWAB, A. SCHNEIDER, F. ZIMMERMANN, M.C. MCCULLOCH, N.L. NADON & K.-A. NAVE. 1998. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* **280**: 1610–1613.
23. KLUGMANN, M., M.H. SCHWAB, A. PÜHLHOFER, A. SCHNEIDER, F. ZIMMERMANN, I.R. GRIFFITHS & K.-A. NAVE. 1997. Assembly of CNS myelin in the absence of proteolipid protein. *Neuron* **18**: 59–70.
24. DE WAEGH, S.M. & S.T. BRADY. 1991. Local control of axonal properties by Schwann cells: neurofilaments and axonal transport in homologous and heterologous nerve grafts. *J. Neurosci. Res.* **30**: 201–212.
25. DE WAEGH, S.M., V.M.Y. LEE & S.T. BRADY. 1992. Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. *Cell* **68**: 451–463.
26. WELCHER, A.A., U. SUTER, M. DE LEON, G.J. SNIPES & E.M. SHOOTER. 1991. A myelin protein is encoded by the homologue of a growth arrest-specific gene. *Proc. Natl. Acad. Sci. USA* **88**: 7195–7199.
27. ZOIDL, G., S. BLASS-KAMPMANN, D. D'URSO, C. SCHMALENBACH & H.W. MÜLLER. 1995. Retroviral-mediated gene transfer of the peripheral myelin protein PMP22 in Schwann cells: modulation of cell growth. *EMBO J.* **14**: 1122–1128.
28. ZOIDL, G., D. D'URSO, S. BLASS-KAMPMANN, C. SCHMALENBACH, R. KUHN & H.W. MÜLLER. 1997. Influence of elevated expression of rat wild-type PMP22 and its mutant PMP22<sup>trembler</sup> on cell growth of NIH3T3 fibroblasts. *Cell Tissue Res.* **287**: 459–470.
29. FABBRETTI, E., P. EDOMI, C. BRANCOLINI & C. SCHNEIDER. 1995. Apoptotic phenotype induced by overexpression of wild-type *gas3/PMP22*: its relation to the demyelinating peripheral neuropathy CMT1A. *Genes Dev.* **9**: 1846–1856.
30. NIEMANN, S., M. SEREDA, U. SUTER, I.R. GRIFFITHS & K.-A. NAVE. 1998. Uncoupling of myelin assembly and Schwann cell differentiation by transgenic overexpression of PMP22. Unpublished work.
31. MAGYAR, J.P., R. MARTINI, T. RUELICKE, A. AGUZZI, K. ADLKOFER, Z. DEMBIC, J. ZIELASEK, K.V. TOYKA & U. SUTER. 1996. Impaired differentiation of Schwann cells in transgenic mice with increased PMP22 gene dosage. *J. Neurosci.* **16**: 5351–5360.
32. JUNG, M., I. SOMMER, M. SCHACHNER & K.-A. NAVE. 1996. Monoclonal antibody O10 defines a conformationally sensitive cell-surface epitope of proteolipid protein (PLP): evidence that PLP misfolding underlies dysmyelination in mutant mice. *J. Neurosci.* **16**: 7920–7929.

33. STERNBERGER, N.H., Y. ITOYAMA, M.W. KIES & H.D. WEBSTER. 1978. Myelin basic protein demonstrated immunocytochemically in oligodendroglia prior to myelin sheath formation. *Proc. Natl. Acad. Sci. USA* **75**: 2521–2524.
34. STERNBERGER, N.H., R.H. QUARLES, Y. ITOYAMA & H.D. WEBSTER. 1979. Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat. *Proc. Natl. Acad. Sci. USA* **76**: 1510–1514.
35. GILSON, J. & W.F. BLAKEMORE. 1993. Failure of remyelination in areas of demyelination produced in the spinal cord of old rats. *Neuropathol. Appl. Neurobiol.* **19**: 173–181.