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ABSTRACT: The Trembler mouse suffers from a dominantly inherited autosomal mutation that results in an abnormal myelination of the peripheral nervous system. Biochemical studies have shown that dysmyelination is the primary event, demyelination being a late-occurring process. The expression of myelin protein genes has been studied. The steady-state levels for *PMP22* mRNA represent 10 and 5% of normal values in the nerves of heterozygous and homozygous Trembler, respectively. This is due to a reduced expression of the specific transcript driven by the promoter 1 of the *PMP22* gene. Collective results indicate that Trembler dysmyelination is not necessarily the consequence of a large accumulation of the mutated PMP22 protein. Moreover, it appears that the situation in the Trembler is different from that encountered in most CMT1A patients, where an increased *PMP22* gene dosage is responsible for the disease. Therefore, the Trembler mutant is perhaps not an ideal model for this human neuropathy.

INTRODUCTION

The Trembler mutation arose spontaneously in 1946 in a colony maintained by Dr. C. Auerbach at the Institute of Animal Genetics, Edinburgh. None of the ancestors of these mice had been treated with mutagenic agents. The first description of the mutant[1] showed an autosomal dominant transmission of the mutation. Clinical symptoms, spastic paralysis and a generalized tremor that ceases when the animal is at rest, become manifest after 10 to 14 days of age. Young animals exhibit transient seizures when stimulated, and these convulsions seem to disappear with age.[1] The mutants do not show abnormal electrocorticograms.[2] Histologic examination of the nervous system shows a peripheral "onion bulb" neuropathy.[3-6] Hypomyelination is the main abnormality, although demyelination and onion bulb formation have been described in older animals, suggesting ongoing remyelination. At all ages, many fibers are found in the "promyelin"state, in which differentiating Schwann cells have attained a one-to-one relationship with axons but have not yet formed myelin. It is clear from the myelinated fibers observed that compact myelin formation is incomplete; the ratio of myelin thickness to axon diameter is reduced, and the myelin

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undergoes rapid degeneration. The Schwann cells become reactive to the presence of myelin debris and undergo proliferation. These processes lead to early onion bulb formation.[3–6] In these mutants, nerve conduction velocities are abnormally low, less than 10 m/sec (normal, nearly 50 m/sec), and fibers share other physiological properties of demyelinated nerves, such as an increased refractory period and an inability to transmit trains of impulses.[7] Elegant experiments using nerve transplantation techniques have demonstrated that the neuropathy in Trembler is due to a disorder of the Schwann cells and not of the axons.[8,9] Hypomyelination may be reproduced in regenerated grafted nerves when Trembler Schwann cells are transplanted into nerves of normal mice. In reciprocal experiments, axons in Trembler nerves were correctly myelinated by normal Schwann cells.[8,9] Although the Schwann cells in unmyelinated fibers of Trembler mice are phenotypically normal, the characteristic hypomyelination and increased Schwann cell proliferation of Trembler nerves are replicated after transplantation of the Trembler cervical sympathetic trunk, an unmyelinated nerve, into the richly myelinated sural nerves of normal mice.[10] This finding indicates that the Trembler phenotype is expressed only by those Trembler Schwann cells challenged to form myelin.

Numerous biochemical studies of the Trembler peripheral nervous system (PNS) were performed before 1985. They have been reviewed by Bourre and collaborators[11] and Hogan and Greenfield.[12] Our purpose here is to summarize the data obtained on the Trembler mutant over the last 15 years and to examine if this mutant can be considered as a model of Charcot-Marie-Tooth type 1A disease (CMT1A).

THE TREMBLER MOUSE IS A DYSMYELINATING MUTANT

Numerous studies indicate that the synthesis and accumulation of several lipid species and proteins considered to be characteristic of, or specific to, myelin are abnormal in the peripheral nerves of the Trembler mouse.

We have carried out, both *in vitro* and *in vivo*, detailed comparative analyses of lipid accumulation and metabolism in the peripheral nerves of normal and Trembler mice. We examined the quantitative evolution of 10 polar lipids in the sciatic nerves of normal and Trembler mice between the ages of 3 days and 60 days.[13] In normal nerves, the polar lipids accumulate slowly until the age of 9 days. Then, a period of rapid accumulation takes place until 18 days of age, after which the phospholipid content plateaus, while the glycolipid content continues to increase but at a slower rate. In Trembler nerves, the accumulation of all polar lipids studied, except phosphatidylcholine and the hydroxysulfatides, is abnormal from the earliest stages of postnatal development, the cerebrosides being the lipids the most severely affected at all ages. The accumulation of neutral lipids has also been studied.[14] Cholesterol and triacylglycerols accumulate in normal nerves throughout the myelination period, while cholesteryl esters, whose presence is frequently interpreted as an indication of ongoing membrane degradation processes, are never observed. In Trembler nerves, the cholesterol level is low from the earliest stages of development. Triacylglycerol levels are normal in Trembler nerves during the first three weeks, but thereafter their quantity diminishes significantly. On the other hand, cholesteryl esters were detected in the mutant nerves, but not until the age of 18 days, suggesting that myelin degradation is relatively late setting in. The saturated very-long-chain fatty acyl groups (VLCFA: 20-24 carbon atoms), which are mainly, but not exclusively, associated with the galactosylsphingolipids and are highly enriched in normal myelinated nerves, are deficient in the Trembler nerves at all ages studied, the deficiency increasing from about 2.5-fold at 5 days to 26-fold at 60 days.[15] A significant but much lesser decrease has also been observed for the more ubiquitous shorter acyl chains (16–18 carbon atoms).[15] The selective deficiency of the saturated VLCFA accumulation was not due to abnormal palmitate synthase or stearic acid synthetase activities.[15,16] *In vitro* and *in vivo* studies of fatty acid metabolism in

the sciatic nerves of normal and Trembler mice[17–19] have demonstrated that the synthesis of lipids the most characteristic of myelin, VLCFA, and galactosyl-cerebrosides containing VLCFA is severely diminished in the mutant's nerves, and that the cerebrosides that are synthesized have an abnormal fatty acyl content. *In vitro* studies[18,19] indicated that the most important factor intervening in the Trembler cerebroside deficit was the lack of ceramide synthesis. An *in vivo* kinetic study revealed that the defective "myelin-specific" sphingolipid synthesis was due to the constitution of a qualitatively and quantitatively abnormal fatty acid substrate pool destined to be incorporated into sphingolipids via the ceramide pathway,[20] whereas the synthesis of the more ubiquitous glycerolipids via the Kennedy pathway, although reduced, occurred normally.[21] Taken together, these studies of lipid accumulation and biosynthesis indicate that the severe myelin sphingolipid anomalies in the Trembler peripheral nerves stem from a general regulatory dysfunction of the processes involved in myelin formation. In other words, as a consequence of the mutation, the Trembler mutant appears to be incapable of initiating metabolic processes required for the synthesis of the lipids characteristic of normal myelin sheaths.

When we started our work, the protein composition of the adult Trembler PNS was known,[12,22,23] but few data were available concerning the accumulation of the myelin proteins during postnatal development. First, we measured the total amount of proteins accumulating in the developing sciatic nerve. As seen in FIGURE 1, there is no significant difference between normal and Trembler mice. This result indicates that this biochemical parameter is not a good index of the myelination status of the sciatic nerve.

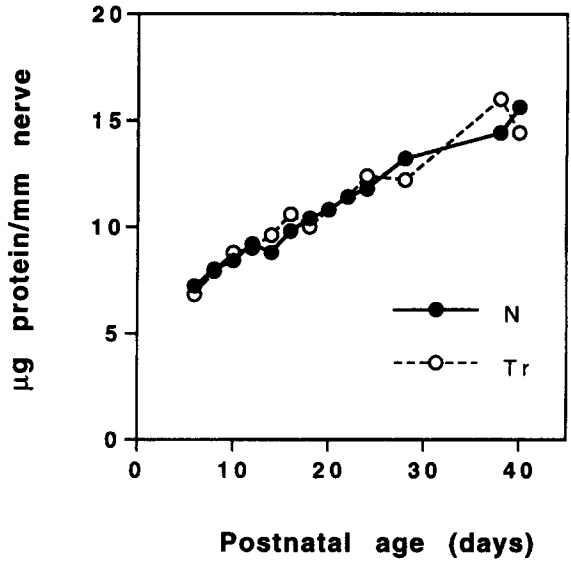

FIGURE 1. Evolution of protein quantity during sciatic nerve development. Both sciatic nerves from individual mice were dissected out, their lengths were measured, and they were delipidated in a $CHCl_3$/MeOH (2:1, vol/vol) solution. The proteins were then extracted with 100–200 µl of 1% SDS for 2–3 hours at room temperature. We checked that further extraction did not yield any more proteins. The protein concentrations of the extracts were determined by using a modified Lowry procedure.[63,64] Results are expressed as micrograms protein/mm of sciatic nerve. Each point represents the mean value from 5–8 independent determinations.

Concerning the myelin proteins, it was already known that the levels of glycoprotein P_0 and myelin basic proteins (MBP) are strongly decreased in adult Trembler nerves.[22,23] By using the Western blot technique with specific P_0 and MBP antibodies raised in our laboratory, we measured the accumulation of these proteins in the sciatic nerves of normal and Trembler mice during postnatal development.[24] As early as 4 days postnatal, the quantity of P_0 detected in the Trembler nerve is half that detected in the control. Thereafter, the amount of P_0 protein increases in the normal sciatic nerve, whereas the quantities in Trembler reach a plateau. Finally, after one month, the P_0 level in the nerves appears to remain constant and there is 5–6 times more P_0 protein in normal adult sciatic nerves than in those of Trembler. Concerning the MBP, the situation is similar to that of P_0, except that the deficit in MBP species measured in the mutant is more important than that observed for the P_0 protein. Indeed, in 12-day-old Trembler mice, MBP amounts represent only 4% of the control, and they are not detectable in adult mutant nerves. Nevertheless, the distribution of the different MBP species is identical in Trembler and normal PNS, indicating that the regulation of the alternative splicing of the *MBP* gene is not affected by the mutation.[24] The accumulation of the myelin-associated glycoprotein (MAG), which is expressed during, and is believed to be involved in, the early stages of myelination, was also monitored in the Trembler PNS; MAG quantities appeared to be similar in control and mutant nerves.[23] Nevertheless, MAG has a higher than normal apparent molecular mass in the sciatic nerves of Trembler due to an increased content of α-sialic acid and galactose.[25]

We also examined the accumulation of histone proteins, the amount of which is indicative of the number of cells in a given tissue. FIGURE 2 shows the data obtained for normal

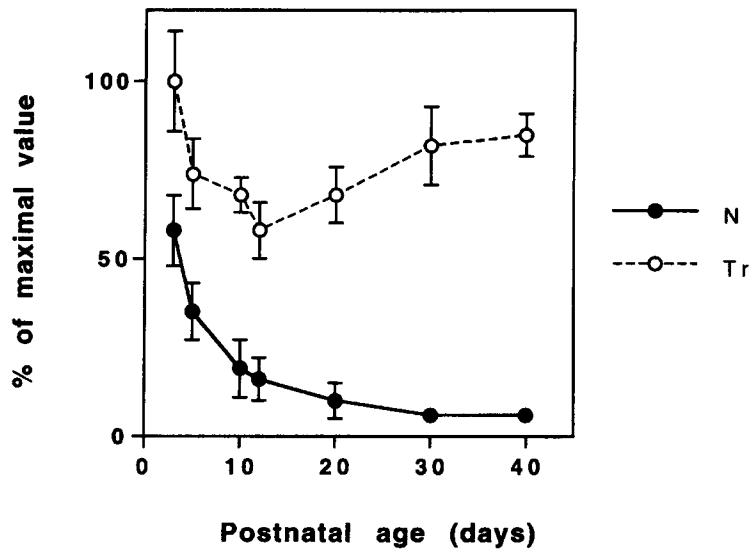

FIGURE 2. Histone H4 content during the postnatal development of mouse sciatic nerve. For each sample, 30 μg of sciatic nerve proteins were separated by (12.5%) SDS-PAGE and transferred onto nitrocellulose sheets. Immunodetection was performed using antisera raised against purified histones from calf thymus and radiolabeled protein A. The corresponding autoradiograms were analyzed using a densitometer. The quantity of histone H4 per millimeter of sciatic nerve was calculated, and the results are expressed as percent of the maximal value. Data are given as the mean ± SEM (bars) of three separate experiments.

and Trembler mice. During the period studied, the amount of histones was always higher in Trembler than in control nerves. However, two phases can be distinguished. Up to the age of 12 days, the cell number decreases similarly in Trembler and normal nerves. Later on, an abnormal cell proliferation becomes apparent in Trembler nerves, whereas the cell density continues to decrease and reaches a stable value around postnatal day 30 in control nerves. Similar results were obtained by quantitation of the sciatic nerve DNA content.[24]

These developmental and metabolic studies not only illustrate the general inability of the Trembler mutant to initiate myelinogenesis-related metabolisms correctly, but they also show that the primary disorder in the Trembler PNS is one of dysmyelination, which can be detected biochemically as early as 4 days after birth. The observed demyelination process is a secondary event that becomes manifest at the end of the second postnatal week, as seen by the rise in histone levels[24] and the appearance of cholesteryl esters.[14]

THE HOMOZYGOUS TREMBLER MICE

Until 1987, all morphological and biochemical studies were performed on heterozygous Trembler mice. This was mainly due to the dominant character of the mutation which made it impossible to distinguish the Tr/Tr from their Tr/+ littermates on the basis of the severity of clinical symptoms. Nevertheless, genetic tests of Trembler offspring of Tr/+ × Tr/+ intercrosses revealed the presence of homozygotes that were viable and fertile.[1] Therefore, we decided to study the biochemical composition of such Tr/Tr sciatic nerves. Using the ELISA technique, we showed that the sciatic nerve P_0 protein content of heterozygous Trembler mice is, respectively, 54% and 37% of normal values at 8 and 20 days. In homozygous Trembler mice, the P_0 level represents, respectively, 15% and 8% of normal values at 8 and 20 days.[26,27] Therefore, the dysmyelination observed in the Trembler homozygotes is more pronounced than that reported in the heterozygotes, indicating that the Trembler mutation is not fully penetrant. The same year, Henry and Sidman published a morphological study showing that myelination in Tr/Tr and Tr/+ represents respectively 1% and 30% of myelination in normal littermates.[28] Therefore, a negligible amount of myelin is synthesized in the PNS of homozygous Trembler mice, and the low level of myelin detected in the heterozygous mutant is due to the activity of the normal allele.

EXPRESSION OF MYELIN PROTEIN GENES

A new myelin protein, named PMP22 for peripheral myelin protein of 22 kDa, was identified and cloned by two independent groups[29–31] in 1991. The corresponding gene became an immediate candidate for mutation in Trembler, and Suter *et al.* identified a point mutation leading to the replacement of a glycine by an aspartic acid residue at position 150 of the protein.[32] This nonconservative mutation introduces a charged amino acid into a putative transmembrane domain of PMP22. A similar point mutation has also been identified in the allelic Tr-J mouse.[33]

These observations led us to investigate the expression of the *PMP22* gene in the Trembler PNS. Using the Northern blot technique, we measured the steady-state *PMP22* mRNA levels in the sciatic nerve of normal and Trembler mice, and compared these results with the steady-state levels of mRNAs encoding the major peripheral nervous system myelin proteins.[34] A summary of the data obtained in 8-day-old animals is shown in FIGURE 3. The mRNA quantities for the proteolipid protein (PLP), 2′, 3′-cyclic nucleotide phosphodiesterase (CNP), and MAG are moderately lowered in the mutant nerve when compared to the control. This may reflect the fact that the expression of the CNP and PLP genes is not under strict axonal control in the PNS. Concerning MAG, our result is in

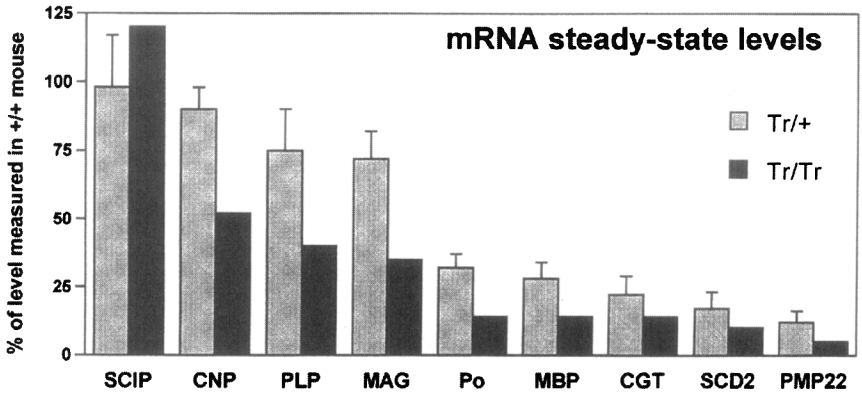

FIGURE 3. Steady-state levels of myelin protein mRNAs in 8-day-old Tr/+ and Tr/Tr sciatic nerves. Mouse sciatic nerve total RNA was separated by 1.5% agarose/3% formaldehyde gel electrophoresis, blotted onto Hybond-N nylon membrane, and each blot was hybridized successively with ^{32}P-labeled cDNA probes specific for either SCIP, CNP, PLP, MAG, P_0, MBP, CGT, SCD2, or PMP22. Quantitations were performed by densitometric scanning of the autoradiograms after correction for RNA levels between samples using the GAPDH results. For each mRNA, results are presented as the percentage of the level measured in the normal mouse. For the Tr/+ mice, the data are given as the mean ± SEM (bars) of 3–5 different experiments. Data for Tr/Tr mouse are mean values from two separate experiments.

agreement with those of morphological studies showing that Trembler Schwann cells are able to initiate the very early steps of myelination, when Schwann cells have singled out and ensheathed the individual axons. This was confirmed by data obtained with the transcription factor SCIP, which is also believed to be involved in the early stages of Schwann cell differentiation towards a myelinating phenotype.[35] SCIP mRNA levels are normal or slightly elevated in the Tr/+ and Tr/Tr nerves. The situation is different for genes involved in the accumulation of myelin membrane around the axons and the subsequent compaction. For example, P_0 and MBP mRNA levels are severely affected by the mutation.[36] At postnatal days 12 and 40, the P_0 mRNA level in Trembler sciatic nerves represents only 40% and 7%, respectively, of the normal content. In the Trembler PNS, MBP mRNAs, barely detectable at days 8 or 40, reach a maximum at day 12 that is only 25% of the normal. This is also the case for genes encoding enzymes involved in the synthesis of myelin lipids, such as ceramide galactosyl transferase[37] and stearoyl-CoA desaturase,[38] whose expression levels are very low in the Trembler sciatic nerve. Nevertheless, the main result is that *PMP22* gene expression is the most severely affected in the mutant nerve. The steady-state levels measured in the Tr/N and Tr/Tr represent 10% and 5% of normal values, respectively. Therefore, the consequences of Trembler mutation are the synthesis of an abnormal PMP22 protein and a lack of upregulation of the corresponding gene after the onset of myelination. Further studies from Bosse *et al.*[39] and Suter *et al.*[40] showed that *PMP22* expression is regulated by two alternatively used promoters, which are located immediately upstream of two alternative 5′-non-coding exons (exons 1A and 1B). It has been shown that the expression of exon 1A-containing *PMP22* transcript correlates tightly with the formation of myelin, whereas the exon 1B-containing transcript is induced to a lesser extent during the same period.[40] Using the RT-PCR technique, we monitored the level of expression of these two different *PMP22* mRNAs in the Trembler PNS. We showed that the steady-state levels of the exon 1A-containing transcript were greatly

reduced in heterozygous and homozygous Trembler mice when compared to normal animals.[41] Such a difference was not observed for the exon 1B-containing transcript. Indeed, the exon 1A/exon 1B ratio in normal sciatic nerve evolved from 0.8 in 5-day-old animals to 1.4 and 2 in 15-day-old and 50-day-old animals, respectively. The ratio was 0.4 in 15-day-old and 50-day-old heterozygous Trembler mice. A similar ratio, around 0.4, was measured in tissues expressing low amounts of PMP22 protein, such as adult mouse brain and liver (Garbay, unpublished results). Moreover, this ratio is only 0.01 in the sciatic nerves of 15-day-old Tr/Tr mice (FIG. 4). These results show that the qualitative expression of the *PMP22* gene is altered in the Trembler mutant mouse, the transcript originating from promoter 1 being present at very low levels in the Tr/+ PNS and almost undetectable in the Tr/Tr PNS.

IS THE CENTRAL NERVOUS SYSTEM AFFECTED IN THE TREMBLER MUTANT?

Young heterozygous and homozygous Trembler mice exhibit transient seizures when stimulated, and these convulsions seem to disappear in animals older than one month.[1] During the last 15 years, we have been working with two different colonies of Trembler mice. One is maintained in the B6CBA background, the second in the C57Black background. We have never noticed any biochemical or morphological difference between the animals of the two colonies. However, seizures have been much more frequent, intense,

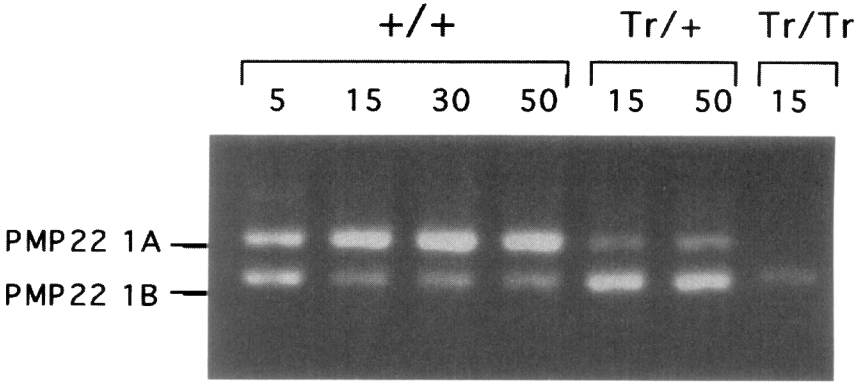

FIGURE 4. Co-amplification of exon 1A- and exon 1B-containing mRNA. After identification of the mouse genotype, the sciatic nerves were pooled, and total cytoplasmic RNA from 5-, 15-, 30-, and 50-day-old +/+ mice; 15- and 50-day-old Tr/+mice; and 15-day-old Tr/Tr mice was isolated using the guanidinium isothiocyanate procedure, followed by cesium chloride centrifugation. The amount of RNA was determined by spectrophotometry at 260 nm. One microgram of the purified RNA was reverse-transcribed using random hexanucleotides with a first-strand synthesis kit (Amersham). A 1-µl aliquot from the 20-µl, single-stranded cDNA mixture was diluted in the 50-µl PCR reaction mix, and 25 amplification cycles (30 sec at 94°C, 45 sec at 60°C, and 90 sec at 72°C) were performed using a trio-thermoblock apparatus (Biometra), AmpliTAQ DNA polymerase (Appligene) and exon 1A-specific, exon 1B-specific, and reverse PMP22 primer/exon 5 in the same reaction. Thereafter, 5 µl of each PCR reaction were analyzed on a 2% agarose gel poured and run in TAE buffer. Quantitation of the DNA bands was performed using a Videocopy/Densylab image analysis system (Bioprobe). The primers used were as follows: exon 1A-specific: 5′-AGC TCC ACC AGA GAA CAT CT-3′; exon 1B-specific: 5′-AGC ACA GCT GTC TTT GGG GA-3′; reverse PMP22 primer/exon 5: 5′ GCT GCA CTC ATC ACG CAC AG-3′. Using these sets of primers, the exon 1A- and the exon 1B-specific cDNA fragments were 530 bp and 482 bp long, respectively.

and lethal in animals maintained in the C57Black background. As stated by I.R. Griffiths, the occurrence of seizures is not expected in such mutations affecting the PNS, so CNS lesions should be sought.[42] Few studies have analyzed the Trembler brain. The electrocorticograms are reported to be normal,[2] and no major morphological abnormalities have been found. However, some histologic signs of hypermyelination have been reported.[43]

Using the Northern blot technique, we investigated the steady-state levels of mRNA encoding myelin protein genes in 8-day-old normal and Trembler brains. We measured a two- to fourfold increase in MBP and PLP mRNAs in Trembler samples.[44] However, it should be noted that the level of expression of the *PMP22* gene is similar in +/+, Tr/+ and Tr/Tr mouse brains.[34] Nevertheless, because the expression of *PMP22* is detected in certain populations of motor neurons in the brain stem and spinal cord of normal mice,[45] it may be worth having a closer look at the Trembler CNS.

TREMBLER STUDIES AND PMP22 FUNCTION

As a component of compact myelin, PMP22 plays a structural role. This glycoprotein carries an N-linked glycosylation site in its first putative extracellular loop that may be involved in mediating adhesive processes.[46] Hence, its function in myelin may be similar to that of the P_0 glycoprotein, although PMP22 represents only 2–5% of PNS myelin proteins against 50–60% for P_0. More recently, it has been suggested that myelin proteins have other functions besides maintaining the integrity of the myelin membrane. Several hypotheses have been raised concerning the biological role of the wild-type and mutated PMP22 proteins, and it is interesting to compare them with observations made in the Trembler mutant.

The first hypothesis is that PMP22 is involved in the regulation of cell growth; when NIH3T3 cells are shifted from a proliferation state to a resting state, a concomitant elevation of the *PMP22* mRNA steady-state level is observed.[47] Moreover, recent *in vitro* experiments showed that an overexpression of the Trembler PMP22 protein alters the cell growth of NIH3T3 fibroblasts.[48,49] The hypothesis is also sustained by the *in vivo* observations that a downregulation of *PMP22* expression occurs in proliferating Schwann cells during development or after nerve injury.[50] In fact, there is a correlation between the Schwann cell proliferation that occurs in the Trembler nerve and an abnormally low level of *PMP22* gene expression. Taken together, these data support the view that *PMP22* may play a role in cell growth regulation. Nevertheless, there are several arguments against this hypothesis. First, continuous Schwann cell proliferation is not observed in the Trembler sciatic nerve. Morphological studies have shown that the onset of myelination is normal in Trembler nerves[3,4,6] until the differentiating Schwann cells have attained a one-to-one relationship with the axons. This would not be possible if the Schwann cells were not able to withdraw from the proliferation cycle. Our data concerning the histone and DNA quantities in the mutant PNS indicated a decrease in Schwann cell numbers during the first postnatal week, when the first signs of dysmyelination were already clearly visible. Later on, the demyelination process begins and Schwann cell proliferation takes place.[24] Finally, abnormal cell proliferation occurs neither in the unmyelinated nerves of the Trembler PNS, nor in other tissues expressing the *PMP22* gene. Therefore, we do not believe that the putative *PMP22* role in cell growth regulation is supported by the observations made in Trembler.

A more recent hypothesis is that an abnormal accumulation of the mutated PMP22 protein in the Schwann cell cytoplasm is triggered by the Trembler defect. This is sustained by data from the groups of Professor Müller[51] and Professor Suter.[52] They have demonstrated that the transformation of cultured Schwann, HeLa, and COS-7 cells with plasmids directing the expression of the mutated PMP22 protein triggers an abnormal intracellular accumulation of PMP22. The mutant proteins are not inserted into the plasma membrane but accumulate in the endoplasmic reticulum and Golgi compartments.

These authors proposed that the impaired trafficking of mutated PMP22 affects Schwann cell physiology, leading to myelin instability and loss. Such an aberrant PMP22 intracellular traffic has been proved to be responsible for the neuropathy observed in the Tr-J mutant.[53] In these mice, double-immunolabeling experiments showed an accumulation of PMP22 in endosomal/lysosomal structures. A similar approach has been employed by Naef and collaborators in the Tr/+ mutant. In 8-month-old mice, although PMP22 immunoreactivity was found in compact myelin, it was also abundant in the Schwann cell cytoplasm. However, it is important to note that such an abnormal PMP22 accumulation was not detected in 18-day-old animals.[52] Although Trembler dysmyelination is manifest during the first postnatal week, the absence of an abnormal PMP22 accumulation in the Schwann cells of 18-day-old animals indicates that an abnormal trafficking of the mutated PMP22 is not the primary defect leading to the Trembler neuropathy. Such an abnormal accumulation may be a secondary event. Moreover, we have demonstrated that the expression of the *PMP22* gene in the Trembler PNS is very low. The *PMP22* mRNA steady-state levels measured in 8-day-old Tr/+ and Tr/Tr represent 10% and 5% of normal values, respectively.[34] Such a low level of *PMP22* gene expression does not seem to be compatible with an overload of the intracellular compartments.

At the moment, we are unable to offer any alternative hypothesis concerning the biological role of PMP22 that would be compatible with the data obtained with the Trembler mouse and in transfection experiments. Moreover, PLP mutations have been known for more than 15 years, but no definitive function has yet been assigned to this myelin protein. The road to the elucidation of the PMP22 function may be a difficult one.

CONCLUSION

Is the Trembler mutant a good model for CMT1A disease? In both cases, the mutation affects the *PMP22* gene, there is a specific alteration of the PNS myelin, and demyelination and Schwann cell proliferation are observed. However, in most CMT1A patients, a *PMP22* gene duplication is responsible for the disease, three copies of the gene being present in the genome instead of two.[54] Thus, CMT1A neuropathy is likely to be triggered by an overexpression of the normal PMP22 protein. The case is very different for the Trembler mouse. Although we have demonstrated that the level of *PMP22* gene expression in the mutant PNS is very low, the absence of normal PMP22 protein amounts in Trembler cannot be the cause of the observed dysmyelination. Indeed, PMP22 knock-out mice display a totally different neuropathy from Trembler mice; hypermyelination and demyelinating neuropathy being their main characteristics.[55] Taken together, these data tend to indicate rather that the Trembler mutation leads to the gain of a new, abnormal PMP22 function.[56]

Another difference between Trembler and CMT1A is that dysmyelination is the primary defect in Trembler, demyelination and Schwann cell proliferation being secondary events. This is demonstrated by the fact that myelin synthesis aborts very early during the development of the PNS of homozygous Trembler mice.[26–28] In CMT1A, the primary defect is demyelination, indicating that some myelination occurs during PNS development. Thus, we believe that the recently developed transgenic models, with extra copies of the normal *PMP22* gene inserted into the genome,[57,58] are better animal models of the CMT1A pathology than the Trembler mouse. Nevertheless, Trembler and Trembler-J mutants are excellent animal models for those rare cases of CMT1A where point mutations of the PMP22 protein are responsible for the disease.[59–62] Moreover, the Trembler mutant is a good tool for studying both the biosynthetic pathways necessary for PNS myelination and the regulation of the genes involved in the biosynthesis of myelin constituents.

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