The Peripheral Myelin Protein 22 and Epithelial Membrane Protein Family

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The peripheral myelin protein 22 (PMP22) and the epithelial membrane proteins (EMP-1, -2, and -3) comprise a subfamily of small hydrophobic membrane proteins. The putative four-transmembrane domain structure as well as the genomic structure are highly conserved among family members. PMP22 and EMPs are expressed in many tissues, and functions in cell growth, differentiation, and apoptosis have been reported. EMP-1 is highly up-regulated during squamous differentiation and in certain tumors, and a role in tumorigenesis has been proposed. PMP22 is most highly expressed in peripheral nerves, where it is localized in the compact portion of myelin. It plays a crucial role in normal physiological and pathological processes in the peripheral nervous system. Progress in molecular genetics has revealed that genetic alterations in the PMP22 gene, including duplications, deletions, and point mutations, are responsible for several forms of hereditary peripheral neuropathies, including Charcot–Marie–Tooth disease type 1A (CMT1A), Dejerine–Sottas syndrome (DDS), and hereditary neuropathy with liability to pressure palsies (HNPP). The natural mouse mutants Trembler and Trembler-J contain a missense mutation in different hydrophobic domains of PMP22, resulting in demyelination and Schwann cell proliferation. Transgenic mice carrying many copies of the PMP22 gene and PMP22-null mice display a variety of defects in the initial steps of myelination and/or maintenance of myelination, whereas no pathological alterations are detected in other tissues normally expressing PMP22. Further characterization of the interactions of PMP22 and EMPs with other proteins as well as their regulation will provide additional insight into their normal physiological function and their roles in disease and possibly will result in the development of therapeutic tools.

I. Introduction

A growing number of hydrophobic proteins containing four transmembrane domains have been described; on the basis of their sequence and structural similarities these proteins have been divided into different families (*1–3*). One subfamily of transmembrane proteins consists of the peripheral myelin protein 22 (PMP22), the epithelial membrane proteins (EMP-1, -2 and -3), and the more distantly related lens fiber cell protein MP20 (*4–10*). The putative four-transmembrane domain structure as well as the genomic structure of PMP22 and the EMPs are highly conserved, suggesting that the genes encoding these proteins are derived from duplication of a common ancestral gene. Of these proteins, PMP22 is the best characterized and can be regarded as the prototypic member of this family (*11*). Although the precise functions of these proteins have not yet been established, evidence is emerging indicating that they have important roles in the regulation of growth, differentiation, and apoptosis (*4, 6, 12–14*). In addition, PMP22 has been demonstrated to have a crucial function in peripheral nerves, where it plays a role as a component of peripheral nerve myelin and in the regulation of pro-

liferation and differentiation of Schwann cells (*5, 11, 15–19*). The structure and composition of myelin have been extensively studied, in part because of the role of myelin in the pathology of multiple sclerosis and various neuropathies (*20–31*). Genetic alterations in the PMP22 gene, which has been mapped to chromosome 17p11.2–12, have been linked to Charcot–Marie–Tooth type 1A (CMT1A) disease, the most common inherited disorder of the peripheral nervous system (PNS), as well as to several other neuropathies, including Dejerine–Sottas syndrome (DSS) and hereditary neuropathy with liability to pressure palsies (HNPP). Naturally occurring point mutations in the PMP22 gene are responsible for the severe deficiency in myelination in the neurological mouse mutants Trembler (*Tr*) and Trember-J (*Tr-J*) (*32–35*). Genetic models have corroborated the causative role of PMP22 in these neuropathies (*36–39*). Animals overexpressing PMP22 as well as PMP22-null mice show neuropathy with severe demyelination. The fact that duplications as well as deletions in PMP22 can result in various neuropathies indicates that the level of PMP22 expression is a crucial factor in the determination of disease and suggests that PMP22 expression must be under strict control. Studies have provided evidence indicating that PMP22 mutations can result in impaired intracellular processing and trafficking of PMP22 and may be responsible for the changes in Schwann cell physiology and loss of myelin (*40–43*).

Although great insight has been obtained in the biological role of PMP22 in myelination, its precise function at the molecular level has yet to be elucidated. In addition, relatively little is known about the biological functions of PMP22 in nonneuronal tissues and the functions of the recently discovered EMPs. The differential expression of EMPs during various pathways of differentiation and various growth arrest states suggests that they are involved in cellular differentiation and control of proliferation, whereas the increased expression of EMPs in certain carcinoma cells may indicate a possible role in the progression of tumorigenesis (*6, 8, 12, 13, 44, 45*). Thus, EMPs are likely to have important functions in normal physiology and in pathological processes, as has already been demonstrated for PMP22.

In this article, we analyze and compare the structure, regulation, and biological function of this exciting group of transmembrane proteins and review the role of PMP22 in myelination and disease.

II. Cloning and Expression of PMP22 and EMPs

A. PMP22

PMP22 (also known as gas-3, PASII, and SR13) has been cloned from mouse, human, and rat cells (*4, 5, 15, 34, 46–50*). PMP22 was originally iden-

tified together with protein zero (P0) as one of two periodate and Schiff (PASII and PASI) reagent-staining peripheral myelin-specific glycoproteins (*50–52*). PMP22 was first cloned by Schneider and colleagues as the growth arrest-specific (*gas3*) gene as part of a strategy to identify genes induced during growth arrest of murine NIH 3T3 fibroblasts after serum starvation (*4, 53, 54*). It was subsequently cloned from rat and human cells by differential screening of cDNA libraries generated from injured versus noninjured sciatic nerves (*5, 15, 46*) and from *Tr* mice as the gene responsible for a Schwann-cell defect that is characterized by severe hypomyelination and continuing Schwann cell proliferation (*34, 35, 55*).

PMP22 mRNA is widely expressed during embryonic development and in the adult (*5, 7, 15, 16, 18, 56, 57*). PMP22 mRNA is most highly expressed in sciatic nerve, where it is confined to Schwann cells. Immunohistochemistry localized PMP22 to the plasma membrane of Schwann cells and to the compact portion of myelin (*58–60*). This highly ordered membranous sheath facilitates the electrical conduction velocity of myelinated axons. Following injury, PMP22 expression rapidly declines in nerve segments distal to the site of injury and is dramatically induced during nerve regeneration (*5, 16, 18, 56*). Several studies have provided evidence demonstrating that axons provide signals that are required for the induction of PMP22 expression (*5, 15, 58, 61, 62*). PMP22 is also expressed in the central nervous system but at much lower levels than in peripheral nerves. *In situ* hybridization showed strong PMP22 signals in motoneurons of the cranial nerve motor nuclei and spinal cord, whereas the PMP22 signal was very weak in the nuclei of the oculomotor and trochlear nerves and absent in the nucleus of the abducens nerve (*18, 63, 64*). During development, PMP22 is present in potential transverse segments and longitudinal columns in the embryonic mouse brain.

Expression of PMP22 is about 10-fold higher in sciatic nerve compared to that in lung and intestinal tract and about 50- to 100-fold higher than in brain (*5, 7, 15, 57, 65*). In the intestinal tract, the highest levels of PMP22 mRNA expression occur in the colon and cecum, with weaker expression in ileum and jejunum; in the stomach the highest expression is found in the fundus and corpus gastricum. In muscle cells, PMP22 is confined to the innervating myelinated fibers. No PMP22 expression is found in preimplantation embryos (*66*); however, widespread expression occurs in a variety of ectodermal, endodermal, and mesodermal tissues during mouse embryonic development (*56*). Finally, PMP22 expression is induced during nerve growth factor-induced differentiation in pheochromocytoma PC12 cells (*18*).

B. EMP-1

Several laboratories have identified and cloned a number of genes encoding proteins that, based on sequence homology and protein structure, are

closely related to PMP22. The first of these genes, named officially by the Human Gene Nomenclature Committee, encoded epithelial membrane protein-1 (EMP-1), also referred to as CL-20, TMP, B4B, and PAP (*6–8, 44, 45, 67*); the gene has been cloned from a number of different cDNA libraries. It was isolated from a tracheobronchial epithelial cell cDNA library as a gene that is differentially expressed during squamous cell differentiation (*6, 45*). The gene for EMP-1 was also identified by differential display, using RNA from different fractions of bone marrow cells, in a strategy to identify transcripts that are highly expressed in precursor cells of the immune system (*67*). This study showed that EMP-1 expression is restricted to a particular subset of B cell progenitor cells that do not express cytoplasmic mu chain but are positive for CD19 and negative for CD20.

The gene for EMP-1 has also been cloned from a subtracted cDNA library enriched for transcripts highly expressed in a mouse c-myc-induced brain tumor (*8*). Although the EMP-1 gene was identified as being highly expressed in brain tumors compared to normal brain, little difference was observed in the levels of PMP22 expression. EMP-1 is also highly expressed in embryonic stem (ES) cells land its expression is down-regulated after differentiation of ES cells into embryoid bodies. The latter is accompanied by a decrease in the expression of c-myc mRNA. The correlation between EMP-1 and c-myc expression in ES and brain tumor cells may indicate that EMP-1 serves as a direct or indirect target for c-myc regulation (*8*). In addition, these observations demonstrate an association between increased expression of EMP-1 and certain malignancies, and may suggest a role for EMP-1 in the progression of tumorigenesis. The latter is supported by a study that identified EMP-1 as the progression-associated protein (PAP) by the differential display technique using RNA isolated from mammary carcinoma MCF-7 cells and NCI/ADR-RES (formerly MCF-7/ADR) cells, which, in contrast to MCF-7 cells, are estrogen receptor negative, adriamycin resistant, vimentin positive, and invasive *in vitro* and *in vivo* (*44*). EMP-1 was found to be highly expressed in NCI/ADR-RES cells as well as in a G361 melanoma and in SW480 colorectal adenocarcinoma cells. However, EMP-1 is not generally highly expressed in carcinoma cell lines because EMP-1 expression was not detected in a series of leukemia and lymphoma cell lines. The expression of EMP-1 may depend on the cell type or a specific characteristic, such as the activation of a specific oncogene. Investigation of several human mammary carcinoma cell lines with different metastatic characteristics revealed a correlation between expression of EMP-1 and their invasive and metastatic properties (*13*). These findings further strengthen a role for EMPs in tumorigenesis.

EMP-1 mRNA is found in many tissues, where it is mostly expressed as a 2.8-kb transcript; however, another transcript of about 1.8 kb has been found

in some tissues (*6, 7, 44, 45, 57, 67*). This shorter transcript may be derived from the use of an alternative polyadenylation signal. The 2.8-kb EMP-1 transcript has a 3′ untranslated region (UTR) of about 2 kb that contains several AUUUA instability motifs. These motifs may play a role in the posttranscriptional regulation of EMP-1. EMP-1 is highly expressed in the intestinal tract, stomach, lung, skin, placenta, and heart, but little expression is found in brain, peripheral blood mononuclear cells, and spleen (*7, 44, 45, 57, 67*). In the stomach, EMP-1 mRNA is abundant in the fundic region whereas expression is much lower in the corpus and pylorus. In the intestinal tract, the cecum, colon, and rectum contain the highest level of EMP-1 mRNA. EMP-1 is also highly expressed in squamous tissues, including skin and esophagus, in which increased EMP-1 mRNA expression is associated with induction of squamous differentiation (*6, 45*). Although EMP-1 is coexpressed with PMP22 in peripheral nerve, EMP-1 expression increases following distal nerve injury, in sharp contrast to the expression pattern of PMP22 (*7*). These results indicate that the expression of these two genes is under distinct controls.

C. EMP-2 and EMP-3

EMP-2 (also referred to as XMP) and EMP-3 (also named YMP or HNMP-1) were first discovered as expressed sequence tags (ESTs) that exhibited homology to PMP22 and EMP-1 (*8, 9*). EMP-3 has also been cloned from a mouse and human peripheral blood monocyte cDNA library (*68*). Both EMP-2 and -3 mRNAs are expressed in many tissues as multiple transcripts (*9, 68*). These different mRNAs may be generated by either alternative splicing or by the usage of alternative polyadenylation signals. EMP-2 is most prominently expressed as 5.0- and 5.5-kb transcripts in ovary, heart, intestine, and lung. An additional 1.0-kb mRNA was detectable in several tissues and in liver it was the major transcript. Spleen, brain, thymus, and peripheral blood leukocytes express very low levels of EMP-2 mRNA. EMP-3 is expressed in most tissues as a 1.0- and 1.4-kb mRNA (*9, 68*). It is most abundant in peripheral blood leukocytes, heart, lung, ovary, colon, spleen, and intestine, and is found at low levels in brain, liver, and kidney. EMP-3 is highly expressed in monocytes, macrophages, and microglia. It is expressed in several hematopoietic-derived cells but in particular in cells of monocytic lineage and in the monocytic cell line U-937. Several studies have provided evidence for a developmental regulation of EMP-2 and -3. For example, both are highly expressed in fetal kidney and at much lower levels in adult kidney. EMP-2 expression is much higher in adult liver compared to fetal liver, whereas the reverse is true for EMP-3. EMP-3 mRNA is expressed at low levels in adult sciatic nerve and is rapidly induced in the nerve distal to the injury (*68*). Immunohistochemistry showed that in the adult peripheral nerve EMP-3 protein was detectable in the axon core but not in the myelin sheath,

whereas after injury immunoreactivity is associated with regions of proliferating Schwann cells.

Comparison of the expression patterns of PMP22 and the various EMPs shows that these genes are coexpressed in many but not all tissues. Whether there is functional overlap between family members awaits further characterization of their functions. Although functional redundancy could explain the lack of phenotype outside the nervous system in PMP22-null mutants, the inverse correlation with growth arrest in some cell types and the cell type-specific expression of PMP22 and EMPs suggest that these proteins have very specific and different functions.

III. Structure of PMP22 and EMPs

The genes of the PMP22/EMP family encode hydrophobic integral membrane proteins of about 18 kDa in size that contain four conserved transmembrane domains (TMDs) (*4–6, 8, 9, 15, 34, 44–46, 48–50, 67, 68*). Figure 1 shows a comparison between the amino acid sequence of members of the PMP22/EMP protein family. The amino acid sequence, and in particular the hydrophobic domains, of each member is highly conserved between species. For example, human PMP22 shares 87% identity with mouse PMP22, and the amino acid sequence of EMP-1 is about 76% conserved between humans, rabbits, and rats. The amino acid sequence between members of this family is only moderately conserved. Human PMP22 shares 35, 39, and 41% identities with human EMP-1, -2, and -3, respectively. In contrast to the amino acid sequence, the TMD structure between the PMP22/EMP proteins is highly conserved. Computer predictions suggest that each protein contains four TMDs and two extracellular domains, the lengths of which are very similar in PMP22 and EMPs. Theoretical models based on computer modeling further illustrate the close structural relationship between these proteins (*7, 8, 19, 21, 22, 25*). Figure 2 shows a comparison of topographical models that depict the four-TMD structures of human PMP22 and EMP-1.

The hydrophobic amino terminus of these proteins functions as a signal peptide to guide expression of the protein to the membrane. This signal peptide is not cleaved, indicating integral membrane proteins (*4, 5, 7*). Analysis of the expression of an octapeptide-tagged PMP22 in HeLa cells showed that PMP22 is synthesized in the endoplasmic reticulum of transfected cells land after passing through the Golgi becomes localized in the plasma membrane (*19*). These studies also suggested that the N terminus is facing the cytoplasmic compartment. Recent experiments have revealed that only a small fraction of PMP22 synthesized in Schwann cells becomes complex glycosylated and that a large portion of PMP22 is rapidly degraded (*69*). As will be dis-

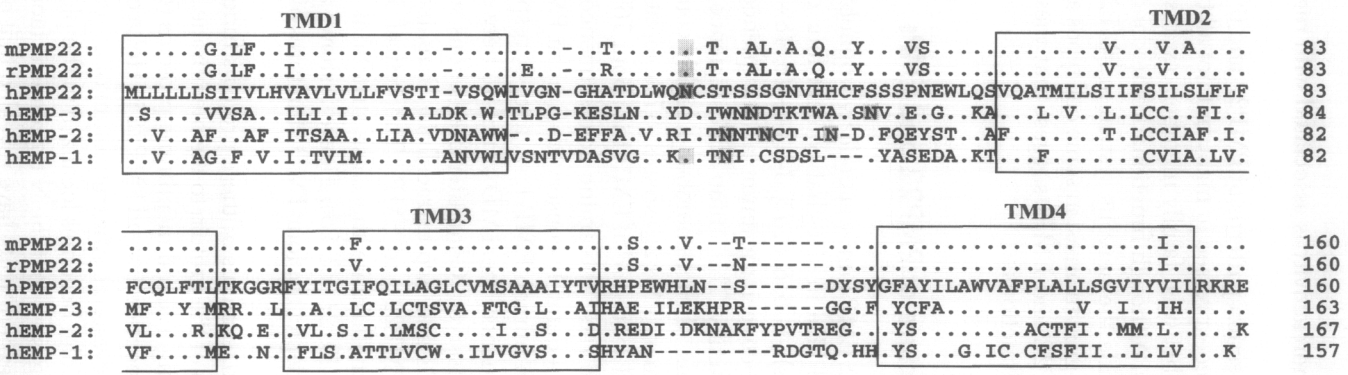

FIG. 1. Amino acid sequence comparison of human (h), mouse (m), and rat (r) PMP22 and EMP-1, -2, and -3 (*4–6, 8, 9, 15, 34, 44–46, 48–50, 67, 68*). A dot indicates an amino acid identical to that in human PMP22; dashes indicate gaps. The four transmembrane domains (TMDs) are indicated. Putative N-linked glycosylation sites are shaded.

cussed below, defects in intracellular sorting of PMP22 appear to be a major mechanism involved in various neuropathies (*40, 42*).

In particular, the second TMD is highly conserved between PMP22 and EMPs. This finding is in agreement with the concept that this particular region constitutes a functionally important domain. The importance of this region, as well the other TMDs, is supported further by studies demonstrating that most PMP22 mutations associated with hereditary and sensory neuropathies are mapped to these TMDs (*21, 23–25, 29, 30*). It is tempting to speculate that mutations in the TMDs of EMPs could be involved in disease as well.

Because of the small size of the intracellular loops, it appears unlikely that they are involved in specific interactions with intracellular proteins and, therefore, in intracellular signaling. In contrast, the extracellular loops would be able to interact with other molecules and could have functional significance. The first hydrophilic region of PMP22/EMPs, between the first and second hydrophobic domains, forms an extracellular loop that contains one or more consensus sequences for N-linked glycosylation (Fig. 1). Several studies have demonstrated that PMP22 and EMP-1 are glycosylated (*4, 5, 12, 70*). The presence of the N-glycosylation inhibitor tunicamycin of treatment with *N*-glycosidase F causes deglycosylation and a shift in the migration of these proteins on polyacrylamide gel electrophoresis. In addition, human and cat PMP22 and rabbit EMP-1 have been reported to react with an antibody that recognizes the HNK-1 antibody (*6, 19, 71, 72*). This antibody identifies carbohydrate structures found on a number of cell surface glycoproteins, including P0, neural adhesion protein *N*-CAM, and myelin-associated protein MAG and members of the tenescin family, as well as some glycolipids and proteoglycans containing specific sulfated glycoconjugates (*71, 73–75*). Many of these proteins have been reported to function in cell adhesion, cell to cell, and cell to extracellular matrix interactions. On this basis, it has been suggested that PMP22/EMPs may be implicated in adhesive processes.

Peripheral myelin proteins, including PMP22 and P0, are regulated similarly during development and peripheral nerve injury. X-Ray crystallography has revealed that the extracellular domain of P0 forms a tetramer on the plane of the membrane, with four molecules arranged around a hole (*76*). It has been suggested that other proteins, and possibly PMP22, may be associated with this tetrameric complex (*19*). The colocalization in compact myelin, the similar patterns of expression during development (*16*), and the fact that mutations affecting PMP22 or P0 are both associated with certain neuropathies could support such a hypothesis. In addition, the ordered structure and specific function of myelin imply a requirement for specific interactions between various myelin components (*21, 27, 28*). Mutations or changes in the stoi-

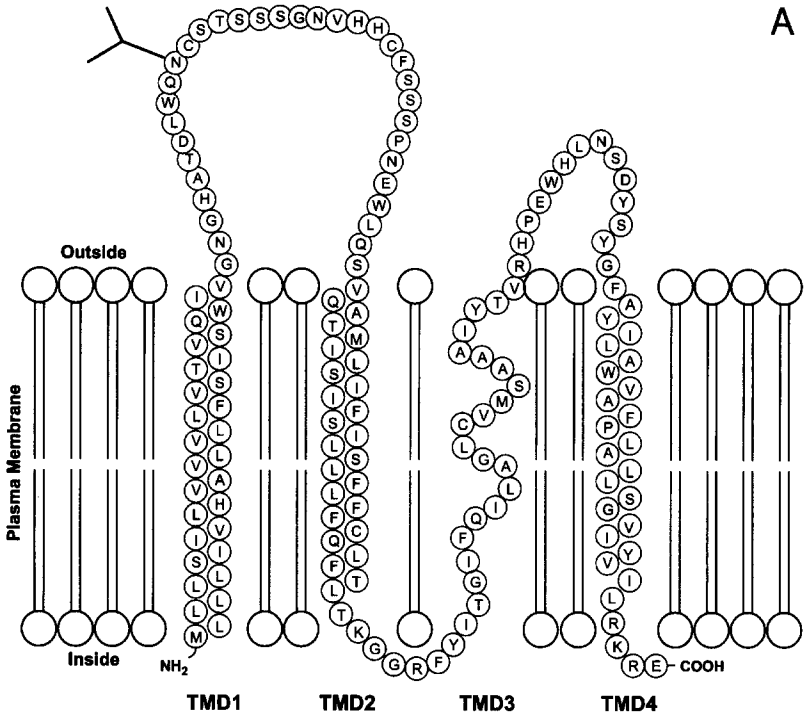

FIG. 2. Schematic view of the predicted structure of human PMP22 (A) and EMP-1 (B) in a lipid bilayer (*6–8, 19, 21, 22, 25, 45*). The Y-shape symbol indicates a putative N-linked carbohydrate chain. The four transmembrane domains (TMDs) and the intracellular and extracellular sides of the membrane are indicated.

chiometry of these proteins may be detrimental to the structure and function of these protein complexes. Such a hypothesis may also explain the importance of PMP22 dosage in the cause of neuropathies.

A number of proteins have been identified that are more distantly related to PMP22 and EMPs, including MP20, a major component of the eye lens (also named MP19 and LIM2) (*10, 77, 78*) and oligodendrocyte-specific protein (OSP) (*79, 80*). Although structurally similar, MP20 exhibits a smaller degree of homology (20–25%) with PMP22 and EMPs. MP20 has been reported to colocalize with connexin 46 at junctional plaques in the fiber cells of the lens (*81, 82*), suggesting a role in cell adhesion or intercellular communication. OSP was cloned from a spinal cord cDNA library. OSP exhibits a 48% similarity and 21% identity with PMP22 (*79*). OSP mRNA is expressed in oligodendrocytes and in CNS myelin (*80*). A role for OSP in the control of oligodendrocyte proliferation has been suggested.

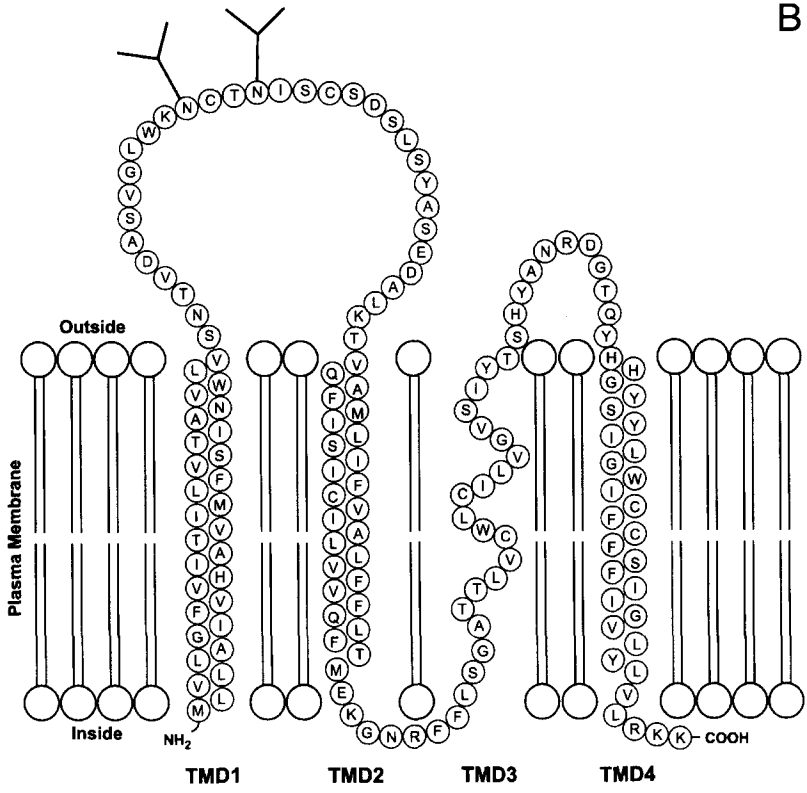

FIG. 2. (*continued*).

IV. Genomic Structure and Regulation

A. Genomic Structure and Chromosomal Localization

The genomic structures of PMP22, EMP-1, and EMP-3 have been determined (*45, 57, 65, 68*). The human PMP22 gene spans about 40 kb whereas the human EMP-1, mouse EMP-1, and mouse EMP-3 genes encompass about 21, 6, and 4.8 kb, respectively. The gene for PMP22 consists of six exons that are evolutionarily conserved in humans and rats. Exons 1A and 1B are alternatively transcribed, yielding two different mRNAs (*65*). These RNAs are identical in their coding sequence but differ in the sequence of their 5′ untranslated region. These findings imply that the expression of these two mRNAs is regulated by two different promoters, P1 and P2. Exon 2 encodes the N terminus consisting of the first TMD, and exon 3 encodes the first extracellular loop of PMP22, which contains the glycosylation site.

Exon 4 encodes the second and half of the third TMD and exon 5 covers the remaining of the third TMD, the second extracellular loop, the fourth TMD, and the 3′ UTR. Comparison of the genomic structures shows that the exon–intron structure of the PMP22 gene is highly conserved with those of EMP-1 and -3 (Fig. 3). As with the PMP22 gene, the coding region of these genes is encoded by four exons (exons 2–5). In addition, the positions of introns are completely conserved between genes for PMP22, EMP-1, and EMP-3 (*45, 57, 68*), corroborating that these genes belong to the same family and are likely derived from duplications of a common ancestral gene. However, the genomic structure of the MP20 gene is not as highly conserved as that of PMP22 and EMP, in agreement with the notion that it is a more distantly related member of this family (*10*).

The PMP22 gene has been mapped to human chromosome 17p11.2–p12, and genetic changes in this gene have been linked to various neuropathies, as will be discussed more extensively below (*48, 83*). Mouse and rat PMP22 genes were mapped to chromosome 11 and 10q22, respectively (*34, 84*). The EMP-1 gene has been mapped to human chromosome 12p12 (*6, 45*), and mouse EMP-1, -2, and -3 genes were mapped to chromosomes 6, 16, and 7, respectively (*85, 86*).

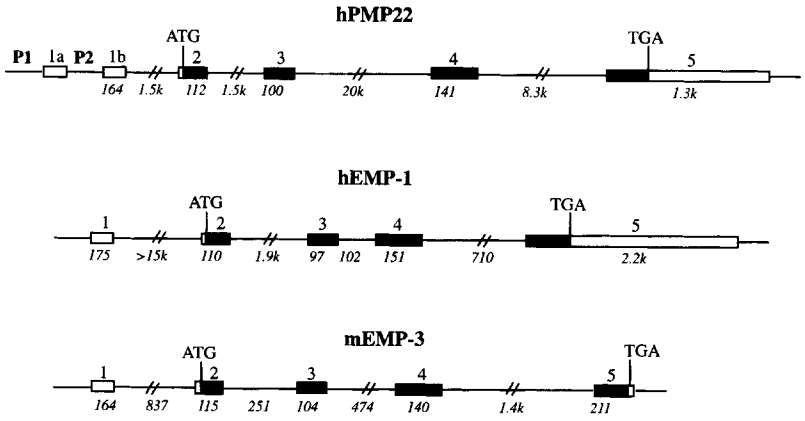

FIG. 3. Schematic view of the genomic structure of human (h) PMP22 and EMP-1 and mouse (m) EMP-3 (*45, 57, 65, 68*). The open boxes mark the exons and the black boxes denote the coding regions. Start and stop codons are indicated. The numbers above the genes refer to exons. The italic numbers below the genes indicate the sizes of the introns and exons. The PMP22 gene generates two transcripts through alternative splicing of exons 1a and 1b. These two transcripts are regulated by two alternative PMP22 promoters, P1 and P2. The exon–intron junctions are conserved between PMP22, EMP-1, and EMP-3.

B. Regulation of PMP22 and EMPs

Although relatively little is known about the regulation of EMPs, a number of studies have provided insight into the control of PMP22 expression. These studies have demonstrated that PMP22 is under a complex control that involves transcriptional as well as posttranscriptional mechanisms. In addition, PMP22 protein can be regulated posttranslationally at the level of protein stability and trafficking (*40–42*). As stated above, the PMP22 gene generates by alternative splicing two different transcripts that are regulated by two different promoters, P1 and P2 (Fig. 3) (*65*). Characterization of these promoter regions showed that the P1 promoter contains a TATA-box-like sequence 30 bp upstream of the cap site of the corresponding mRNA, whereas no TATA-box-like sequence could be found in the P2 promoter. The first 0.35 kb of the upstream sequence of the P2 promoter contains a high GC content and has many characteristics of a housekeeping promoter. Although several putative enhancer elements can be identified, including two NF1 sites in the P1 promoter and overlapping AP-2 and Sp1 sites in the P2 promoter, further deletion and point mutation analyses are required in order to understand the importance of these sites. Because several other myelin proteins, such as P0 and MBP, are regulated in peripheral nerves in a manner similar to that of PMP22, their regulation may involve some common transcriptional factors and enhancer sites. Several transcription factors have been shown to play critical roles in regulating the phenotype of Schwann cells, including Oct-6 (tst-1, SCIP) and Erg-2 (Krox-20), both of which are required for normal development of the myelinating phenotype (*28, 87*). The importance of Erg-2 in Schwann cell differentiation was supported by a study showing a link between dominant missense mutations in Erg-2 and hereditary myelinopathies (*88*). Whether these transcription factors regulate PMP22 transcription directly or indirectly by controlling early stages in Schwann cell development awaits further study (*28, 87*).

The P1 and P2 promoters are used in different cell types and under different conditions. Although both transcripts are detected in all tissues examined, transcripts containing exon 1a are predominant in peripheral nerve whereas transcripts containing exon 1b are preferentially found in all other human and rat tissues (*65*). These results suggest that expression of PMP22 in different tissues is preferentially regulated by one of the two promoters.

As mentioned previously, PMP22 expression in the sciatic nerve is tightly regulated during development and after injury (*5, 7, 15, 16, 18, 56–58*). Myelin formation and PMP22 are dramatically induced during early postnatal development, and following peripheral nerve injury; PMP22 expression is suppressed and subsequently up-regulated during nerve regeneration. Although both transcripts show similar changes in expression, regulation of the

exon 1a-containing transcript correlates more tightly with the induction of PMP22 during development and with the changes in PMP22 expression after injury (*65*). Forskolin, an activator of adenylate cyclase, elevates PMP22 expression in cultured Schwann cells through increased cAMP levels (*65, 70*). Treatment of Schwann cells with forskolin induces both PMP22 transcripts to the same extent whereas exon 1b transcripts remain the dominant mRNA species. Whether the cyclic AMP response elements (CREs) present in the PMP22 promoter regulatory region are involved in this induction has yet to be elucidated. The up-regulation of PMP22 expression during growth arrest in rat embryo fibroblasts is also related to an increase in both transcripts. No change in the ratio between the two transcripts was observed and exon 1b-containing transcripts remain the most abundant mRNA species (*65*). In these cells, PMP22 may be regulated by a posttranscriptional mechanism involving changes in the stability of PMP22 transcripts (*4*).

Several studies have reported a regulatory role for progesterone in the regulation of myelination. Progesterone has been shown to promote myelination in the peripheral nervous system and a role for progesterone in nerve repair has been proposed (*89*). This stimulation in myelination may be due to increased expression of myelin protein genes in Schwann cells. The latter is in agreement with reports showing that Schwann cells express progesterone receptors and contain high levels of progesterone. This concept is further supported by a recent study demonstrating that in cultured rat Schwann cells progesterone increased transcriptional activation of a reporter gene through the P1 but not the P2 promoter of PMP22 (*90*). In contrast, progesterone was unable to induce P1-dependent transactivation in the mammary carcinoma cell line T47D, which also expresses progesterone receptors, demonstrating a cell type-specific activation of this promoter. The progesterone antagonist RU486 was unable to inhibit the effect of progesterone and rather acted as a weak agonist. Elevated levels of cAMP have been reported to change the action of RU486 from an antagonist to an agonist. The fact that Schwann cells were treated with forskolin during RU486 treatment probably explains its action as a weak agonist in the activation of the P1 promoter.

Genetic changes in the promoter regulatory region of PMP22 could lead to alterations in its regulation and result in disease. Analysis of the P1 promoter of PMP22 in a set of patients with CMT1A and HNPP, in which no duplication/deletion nor a mutation in the coding region of the CMT1A/HNPP genes was detected, identified in one autosomal dominant CMT1A patient a base change in the noncoding exon 1a of PMP22 (*91*). This change, however, did not cosegregate with the disease in the family. This study appears to suggest that mutations in the P1 promoter and 5′ untranslated exon will not be a common genetic cause of CMT1A and HNPP.

V. Putative Functions of PMP22 and EMPs in Cell Growth and Differentiation

A. Roles in Growth Control

Despite the progress in the molecular genetics of PMP22, relatively little is known beyond its function in the PNS. PMP22-null mice develop normally except for the PNS despite extensive PMP22 expression in nonneuronal tissues (*36*). Several studies have provided evidence indicating that PMP22 may play a role in growth control and apoptosis. PMP22 is induced during growth arrest of NIH 3T3 cells and embryonic fibroblasts after serum starvation or at confluence, whereas its expression becomes down-regulated after serum addition (*4, 54, 65*). PMP22 is also highly expressed in serum-starved adipoblasts, contact-inhibited adipoblasts, and postmitotic adipocytes (*92*), and PMP22 mRNA expression in rat Schwann cells follows the growth-arrest-specific pattern observed in NIH 3T3 cells (*5, 14*). PMP22 is also induced in rat pheochromocytoma PC12 cells during neuronal differentiation after nerve growth factor (NGF) treatment (*18*). Although these studies demonstrate a positive correlation between growth arrest and expression of PMP22, other studies do not, or show a negative correlation. In C6 glial cells, NGF-induced growth arrest has no effect on PMP22 expression (*18*). Moreover, in rat L6 myoblasts, PMP22 mRNA increases only slightly when cells are grown to confluence and become quiescent, and is dramatically down-regulated when cells are induced to differentiate terminally into myotubules (*93*). These observations suggest that expression of PMP22 does not correlate generally with growth arrest and may depend on the cell type, the class of growth arrest signal, or a particular pathway of terminal differentiation.

Overexpression of PMP22 in NIH 3T3 cells significantly inhibits cell growth and induces apoptosis as judged by several morphological criteria, such as cell surface blebbing and typical changes in nuclear architecture (formation of apoptotic bodies) (*12, 94*). In addition, nucleosomal DNA fragmentation was detected by a terminal deoxynucleotidyl transferase-mediated dUTP–biotin nick end-labeling (TUNEL) assay. The extent of cell death appears to correlate with the level of PMP22 expression. *N*-Acetylcysteine and ascorbic acid are able to prevent this induction, suggesting that generation of reactive oxygen intermediates may be part of the mechanism that leads to this type of apoptosis (*12*). Mutant PMP22 containing point mutations as found in CMT1A, showed a significant reduced ability to induce apoptosis in NIH 3T3 cells (*12*), whereas mutant PMP22^{Tr}, although inhibiting cell growth, did not promote cell death (*94*). Overexpression of PMP22 in cultured Schwann cells by retroviral transfection also markedly reduced the rate of proliferation but did not cause apoptosis (*14, 95*). Con-

versely, expression of PMP22 in the antisense orientation reduced PMP22 protein levels and enhanced proliferation. However, proliferation of cultured Schwann cells that carry the CMT1A duplication appears to be decreased (*95*). Cell cycle analysis demonstrated that overexpression of PMP22 in Schwann cells increases the number of cells in G_0/G_1 and reduces the number of cells in S and G_2/M phases of the cell cycle. This suggests that PMP22 delays the transition of cells from G_0/G_1 to the S phase, or growth arrest cells in the G/G_1 phase. The significance of these *in vitro* studies in relation to the observations *in vivo* is still difficult to assess because both overexpression and reduced PMP22 expression lead to Schwann cell hypertrophy (*36–38*). It is also interesting to note in this context that a loss of Schwann cells in biopsies from CMT1A and HNPP patients has been reported to be due to apoptosis (*96*). However, another study concluded that CMT1A does not involve induction of apoptosis in Schwann cells but rather alterations in Schwann cell differentiation (*97*).

Little is known about the function of EMPs in growth control. Expression of EMP-1 has been correlated with both growth arrest and growth stimulation (*6–8, 45, 67*). Overexpression of EMP-1 in COS-7 cells has been reported to cause growth inhibition by a mechanism distinct from apoptosis (*67*). In tracheobronchial epithelial cells and epidermal keratinocytes, EMP-1 expression correlates with irreversible growth arrest during terminal differentiation (*6, 45*). However, in NIH 3T3 cells, expression of EMP-1 mRNA is high in proliferating cells and low in growth-arrested cells, suggesting the inverse pattern of expression as observed for PMP22 (*7, 8*). Moreover, EMP-1 is highly expressed in several tumor cell lines (*8, 13, 44*).

The mechanism by which PMP22 and EMPs regulate cell cycle progression and apoptosis has yet to be established. Several investigators have proposed a role for PMP22 and EMPs in cell-to-cell interaction and cell adhesion. It is conceivable that the effects of PMP22 and EMPs on cell growth are mediated through changes in these cellular processes because many studies have demonstrated the importance of cell-to-cell and cell–extracellular matrix interactions in cell cycle control.

B. Functions in Differentiation

In a number of cell systems expression of PMP22 and EMPs appear to correlate with differentiation. As discussed previously, maturation of Schwann cells and induction of differentiation in PC12 cells by NGF is associated with increased PMP22 expression, whereas the reverse has been shown for differentiation of L6 cells into myotubules (*5, 18, 65, 93*). PMP22 and EMPs are relatively highly expressed in certain regions of the stomach and intestinal tract (*7*). In the gastric gland, stem cells in the isthmus/neck region rapidly proliferate, migrate, and differentiate. Intense EMP-1 im-

munoreactivity was detected in these regions as well as in the epithelium of the gastric pit containing the mature differentiated cells, whereas cells toward the base were negative. EMP-1 protein was associated with the plasma membrane and equally distributed between the apical, basic, or lateral membrane. These studies appear to indicate a role for EMP-1 in this process of differentiation.

EMP-1 mRNA expression is highly induced during squamous cell differentiation in rabbit and human tracheobronchial epithelial cells in culture, as well as in human epidermal keratinocytes (*6, 45*). Squamous differentiation occurs in many different tissues either as a normal process or as part of a pathological process. Under vitamin A deficiency or toxic or mechanical injury, the normal mucociliary tracheobronchial epithelium transforms into a squamous epithelium. Squamous differentiation is a multistep process in which cells undergo irreversible growth arrest followed by expression of specific squamous marker genes, such as cornifin and transglutaminase (*98, 99*). Induction of squamous differentiation by a number of different signals, including treatment with the phorbol ester phorbol myristate acetate (PMA) and growing cells to confluence, results in increased expression of EMP-1. Retinoids, analogs of vitamin A, inhibit the expression of squamous differentiated markers and suppress the expression of EMP-1 mRNA (*6, 45*). EMP-1 mRNA is highly expressed in several squamous epithelia, including those of the skin, tongue, and esophagus. *In situ* hybridization analysis in several tissues has shown that EMP-1 mRNA is localized in the suprabasal differentiated layers of squamous epithelia. These results suggest that the expression of EMP-1 closely correlates with the induction of squamous differentiation. Although cells undergo irreversible growth arrest during squamous differentiation, it appears that EMP-1 expression is related to expression of the squamous phenotype rather than to growth arrest.

VI. PMP22 in Myelination and Disease

A. Introduction

Much of our current knowledge concerning the function of PMP22 has been derived from its involvement in hereditary motor and sensory neuropathies in human and rodents (*100*). The first hints of an important function of PMP22 in the nervous system were revealed in the early 1990s by differential hybridization screening approaches; these were aimed at the identification of regulated genes discovered after sciatic nerve lesions identified high levels of PMP22 transcripts in peripheral nerves (*5, 15, 61, 101–103*). The crucial role of PMP22 in the development and maintenance of the

PNS became apparent soon thereafter through the discovery that the spontaneous mouse mutants *Tr* and *Tr-J* carry spontaneous point mutations affecting the PMP22 protein (*21, 34, 104*). These mouse mutants had been suggested previously as potential animal models for severe forms of congenital hereditary motor and sensory neuropathies (HMSNs) called Dejerine-Sottas syndrome (*105–107*). This hypothesis proved to be correct in that families with severe HMSNs have been shown to carry mutations identical to those found in *Tr-J* (*108*) and *Tr* (*109*). In the following sections, we review what is known about the role of PMP22 from studies in human genetics, then proceed to the role of PMP22 in the nervous system, and discuss the lessons that we have learned from different mouse and rat PMP22 mutants as well as human pathology.

B. PMP22 in Human Genetics

Fine mapping of the mouse PMP22 gene on chromosome 11 revealed a colocalization with the known *Tr* locus, which had been determined previously by classical breeding experiments (*34, 104*). Most interestingly, this locus lies on a chromosomal segment potentially syngenic to parts of the short arm of human chromosome 17 at 17p11.2. This region had been linked to the most common form of HMSN (*110, 111*) [70% of all cases (*112, 113*)], Charcot–Marie–Tooth disease type 1A, named in honor of its discoverers, the two French neurologists Jean Martin Charcot and Pierre Marie (*114*) and the English physician Howard Henry Tooth (*115*). CMT1A belongs to a heterogeneous group of neurological disorders (*29, 116*) and is characterized by autosomal dominant inheritance. Usual onset of CMT is in the second decade of life, manifested by distally pronounced progressive muscle weakness of the legs and hands associated with variable degrees of sensory loss (*117, 118*). The clinical phenotypes vary considerably even within the same family and in identical twins (*119*), indicating the involvement of epigenetic factors. Histologically, sural nerve biopsies from CMT1A patients show demyelination and remyelination accompanied by Schwann cell proliferation, leading to the characteristic formation of so-called onion bulb structures, which consist of supernumerary Schwann cells and Schwann cell processes concentrically arranged around the thinly myelinated axon (*120*). As a consequence of the observed myelin deficiencies, reduced nerve conduction velocities (NCVs) can be used reliably for the diagnosis of CMT1A (*117, 118*). Fine mapping of the CMT1A locus revealed that the disorder is associated in the vast majority of cases with an approximately 1.5-megabase (Mb) intrachromosomal duplication (*121, 122*). Subsequent studies showing that patients with large segmental trisomies, including the CMT1A-linked duplication, were also affected by the clinical hallmarks of CMT1A suggested that the CMT1A phenotype can result from a gene dosage effect (*123, 124*). Based on the findings

in *Tr* and *Tr-J*, the PMP22 gene was proposed as the responsible dosage-sensitive gene within the CMT1A duplication (*34*). Cloning of the human PMP22 cDNA and gene (*46, 65*) and mapping within the CMT1A duplication were consistent with this hypothesis (*20, 46, 83, 125*). Furthermore, the subsequent finding of more than a dozen different missense point mutations in the PMP22 gene of *de novo* and familial HMSN of different severities provided additional support (*100*). Correlative studies on phenotype–genotype relationships indicated that the clinical phenotypes and the associated pathologies tend to be more severe in CMT1A patients affected by PMP22 point mutations if compared to cases with increased PMP22 gene dosage (*120, 126*). Closer inspection revealed that the CMT1A duplication consists of a tandem repeat that arises from an unequal crossing-over due to misalignment of repetitive sequences (termed CMT1A-REPs) during meiosis (*127*). These CMT1A-REPs flank the CMT1A monomer unit on the normal chromosome 17 and are present in an additional copy on the CMT1A-duplicated chromosome (*23*). Sequence analysis of CMT1A-REP revealed an insect-derived, functionally defective, mariner transposon-like element (MITE) near a recombination hot spot potentially mediating strand exchanges via cleavage by a transposase at or near the 3′ end of the element (*128*). Such a facilitating mechanism may explain the high frequency of *de novo* recombination events observed in isolated CMT1A (*129*).

The proposed mechanism for the generation of the CMT1A duplication suggests that the reciprocal deletion should also occur rather frequently. Indeed, such intrachromosomal deletions have been found in the comparatively mild motor and sensory neuropathy, hereditary neuropathy with liability to pressure palsies (*130, 131*). Two distinct sex-dependent mechanisms appear to be involved in the chromosomal rearrangements leading to the CMT1A duplication and HNPP deletion. Data support the hypothesis that rearrangements of paternal origin, essentially duplications (*132*), are indeed generated by unequal meiotic crossing-over between the two chromosome 17 homologs. Duplications and deletions of maternal origin, however, seem to result from an intrachromosomal process, either unequal sister chromatid exchange or, in the case of deletion, excision of an intrachromatidal loop (*133, 134*). Clinically, HNPP is characterized as an autosomal dominant recurrent neuropathy that is precipitated by minor trauma to peripheral nerves and may become a chronic disease resembling demyelinating CMT with age (*135, 136*). Key histological features of HNPP are focal hypermyelination with a sausagelike appearance (called tomacula) on teased nerve fiber preparations (*137*). Decreased PMP22 gene dosage is very likely to be the major (if not the sole) contributing factor to HNPP, because heterozygous frameshift mutations, which probably yield PMP22-null alleles, have been found to be associated with HNPP (*138–140*).

The combined data from human genetics indicate that the normal function of PMP22 is sensitive to specific missense mutations, which are almost exclusively localized in putative membrane-associated domains (*4, 42, 100*), and to altered gene dosage either increased to 1.5-fold in CMT1A, or decreased to half the normal level in HNPP. Complementary PMP22 expression studies on biopsies from CMT1A duplication and HNPP deletion patients revealed that the altered gene dosage is also reflected at the PMP22 mRNA and protein level (*141–147*). The definitive proof, however, that altered PMP22 gene dosage is sufficient to cause HMSN was provided by the generation of appropriate transgenic animals (see below). On the basis of the work described above, the analysis of alterations affecting the PMP22 gene has now become a routinely used and highly reliable genetic tool for the effective diagnosis of PMP22-based HMSN (*148, 149*).

C. PMP22 in Myelination

As discussed above, PMP22 expression is by far the highest in peripheral nerves (*5, 15*), where the protein is localized in the compact portion of myelin (*16, 58*). Low expression can also be found in the plasma membranes of nonmyelinating Schwann cells (*60*). A thorough understanding of the molecular and cellular mechanisms of gene regulation, biosynthesis, targeting, insertion, stability, and turnover of myelin components in normal and disease states is required for the elucidation of disease mechanisms in dysmyelinating and demyelinating disorders (*28, 29, 100, 116, 150, 151*). In the case of PMP22, transcriptional and posttranscriptional regulatory mechanisms have been well described (*4, 53, 54, 90, 92, 152–155*), but there is also an emerging picture on the relevance of posttranslational regulation. As expected for a membrane glycoprotein that is targeted to the Schwann cell plasma membrane and incorporated into compact myelin, PMP22 is synthesized in the rough endoplasmic reticulum and passes through the Golgi apparatus to the cell surface (*19, 40*). Extended studies revealed that most of the newly synthesized PMP22 in Schwann cells is rapidly degraded in the endoplasmic reticulum (*69*) and that only a small proportion of PMP22 acquires complex glycosylation and accumulates in the Golgi compartment. The protein appears to be translocated to the Schwann cell membrane in detectable amounts only in the presence of axonal contact and myelination. Nevertheless, the rapid turnover of PMP22 in Schwann cells remains unchanged by the myelination process. This is of particular interest in the light of findings that defective intracellular transport appears to be a common disease mechanism associated with PMP22 mutations (*40–42*). These results suggest that impaired trafficking of mutated PMP22 protein affects Schwann cell physiology, which in turn leads to myelin instability and loss. Additional data, however, suggest that there are likely to be subtle molecular differences in how

different PMP22 mutations may lead to disease. Specifically, *Tr-J* mutant PMP22 protein, which is largely accumulated in a pre-Golgi transport intermediate compartment (ERGIC) in transfected cells, forms heterodimers with wild-type (wt) PMP22, and this interaction causes some of the wt protein also to be retained in the ERGIC (*43*). This mechanism may result in decreased trafficking of wt PMP22 to the plasma membrane and lead, in combination with a toxic gain of function through the intracellular accumulation of *TrJ* PMP22, to the observed dysmyelinating neuropathy (*108, 156*).

D. Natural Mouse Mutants Carrying PMP22 Mutations

The natural mouse mutant *Tr*, found as a spontaneous mouse mutation in a regular breeding colony almost 50 years ago (*157*), was followed by the discovery of a second mouse strain, called *Tr-J*, with similar behavior (*35, 158*). Clinically, these mouse strains are similarly affected by an unsteady gait and weakness of the hind limbs and represent excellent animal models for PMP22 missense mutation-based neuropathies in humans (*108, 109*). The name "Trembler" was given to these mice because they exhibit a conspicuous axial tremor and show stress-induced "convulsions." Studies have demonstrated that these observations are not due to epileptic phenomena, consistent with the histological findings that the central nervous system appears normal in *Tr* and that there are no epileptic cortical neuronal discharges (*159*). The peculiar behavior is instead caused by neuromyotonia, an increased muscle stiffness due to hyperactivity of motor units (*160, 161*). These findings may also explain the high incidence of tremor associated with CMT (*162*) and the often-observed muscle cramps in these patients (*163*).

The *Tr* mutation replaces the small and neutral amino acid residue glycine at position 150, located in the last hydrophobic domain of the PMP22 protein, by the bulky and charged amino acid residue aspartate, leading to a protein trafficking defect (*34, 40, 41*). As a consequence, these mutants are affected by severe hypomyelination of peripheral nerves (*32, 35, 105, 164–167*), continuous Schwann cell proliferation (*168*), and reduced NCV (*33*). Furthermore, a delay in the onset of myelination (*35*) and some structural abnormalities in the myelin remnants of heterozygous *Tr* mice have been described (*169*). Homozygous *Tr* mice are more affected than heterozygous siblings, lacking virtually all myelinated fibers (*55*).

Consistent with the high expression of PMP22 by myelinating Schwann cells, elegant grafting experiments revealed that the defect in *Tr* is largely Schwann cell autonomous (*170*). In agreement with the observed hypomyelination, myelin components, including PMP22, a strongly down-regulated (*171–173*) and the lipid metabolism is severely altered, potentially con-

tributing as a downstream effect to the observed pathology (*174–177*). Later studies demonstrated that *Tr* Schwann cells have also a profound influence on the associated axons. This is manifested by decreased axonal caliber, increased neurofilament density, slow axonal transport, alterations of a kinase-phosphatase system acting on neurofilaments, and changes in the composition and phosphorylation of the microtubule cytoskeleton and its associated proteins (*178–181*). Thus, in addition to being an animal model for HMSN, the *Tr* mouse can be regarded as an excellent model system to study Schwann cell–axon interactions, including the role of myelinating Schwann cells in the morphogenesis of the node of Ranvier (*62, 150, 182–186*). Interestingly, results similar to those observed in *Tr* grafting experiments have been obtained by studying xenografts of biopsies from an HMSN patient carrying a PMP22 point mutation into nude mice (*31, 187*). Nevertheless, although these experiments suggest a minor role of neuronally expressed PMP22 in the HMSN disease process (*64*), some contributions cannot be formally excluded.

Tr-J mice carry a missense mutation that alters a leucine residue to a proline residue at position 16 in the first hydrophobic domain of PMP22. Similar to *Tr*, this replacement affects the intracellular trafficking of PMP22 (*41, 43*). The resulting pathology is overall less pronounced than in *Tr* but is qualitatively similar (*35*). Thinly myelinated axons associated with Schwann cell onion bulbs due to aberrant Schwann cell proliferation, signs of demyelination and remyelination, abnormalities in myelin compaction, and indications of altered axon–glia interactions are present (*156, 188, 189*). Myelin instability leads to an increased turnover of myelin components, which are degraded by the lysosomal pathway (*156*). Furthermore, the steady-state levels of protein components of compact myelin, including PMP22, protein zero, and myelin basic protein, are decreased. In contrast, myelin-associated glycoprotein, which is excluded from the compact portion of myelin, is affected only by altered glycosylation (*156, 190, 191*).

A third described (*192*) spontaneous PMP22 mouse mutant named *Tr-Ncnp* is associated with an in-frame deletion of exon IV (*65*) and thus is lacking the second and part of the third putative membrane associated domains. Although the behavioral and overall pathological abnormalities are comparable to *Tr* and *Tr-J*, *Tr-Ncnp* is unique in that giant vacuolar formation in the sciatic nerve of homozygous animals is observed. These structures vary in size and show features of abnormally swollen endoplasmatic reticulum of nonmyelinating Schwann cells. In addition, significant cell death was found in the nerves of these animals. Given the previously discussed findings of altered intracellular transport in *Tr* and *Tr-J* and the clearly divergent phenotype of *Tr-Ncnp* compared to mice carrying a PMP22-null allele (see below), the *Tr-Ncnp* mutation may lead to a specific or even more general disruption of intracellular transport processes, ultimately resulting in cell death.

E. Artificially Generated PMP22 Mutants

1. Rodents with Increased PMP22 Gene Dosage

The understanding of molecular mechanisms of a given disease greatly benefits from appropriate experimental models that closely reflect the biology of the disorder. The discussed examples of *Tr* and *Tr-J*, which have already provided numerous crucial clues to our current understanding of PMP22 missense mutation-based HMSN, are excellent examples to support this claim. Thus, several different approaches have been used to generate animal models for CMT1A associated with increased PMP22 gene dosage. Transgenic mice and rats were established carrying different copy numbers of an approximately 40-kb cosmid insert containing exclusively the mouse PMP22 gene (*37, 38*). These animals developed dysmyelinating hereditary neuropathies, proving that increased PMP22 gene dosage along is sufficient to cause PNS myelin deficiencies. Mice carrying approximately 16 or 30 copies of the PMP22 gene displayed a severe congenital hypomyelinating neuropathy with an almost complete lack of myelinated large-caliber axons (*100*) in combination with marked slowing of nerve conduction (*38*). Affected nerves contained an increased number of nonmyelinating Schwann cells that aligned in association with axons without forming extensive cellular onion bulbs. However, empty basal laminae, most probably remnants of degenerated supernumerary Schwann cells and their processes, were a common feature. Interestingly, the mutant Schwann cells were characterized by a promyelination-like state as indicated by the expression of embryonic Schwann cell markers. These results have been interpreted in that Schwann cells associated with large-caliber axons are impaired in their differentiation into the myelinating phenotype, leading to a disorder comparable to severe cases of hereditary motor and sensory neuropathies.

In parallel, the same *PMP22* transgene was used to establish a line of rats with increased *PMP22* gene dosage (*37*). These animals carry three transgenic *PMP22* copies and resemble the CMT1A phenotype rather closely, as evidenced by gait abnormalities caused by a peripheral hypomyelination, Schwann cell hypertrophy, and abundant onion bulb formation as well as muscle weakness. Electrophysiological recordings indicated reduced NCV, closely resembling the findings in CMT1A patients. However, similar to CMT1A patients, a high variability in the visible, electrophysiological, and pathological phenotypes was observed. Interestingly, the severity of myelin abnormalities was more pronounced in ventral roots (consisting of motor fibers) compared to dorsal roots (sensory fibers). Similar tendencies suggesting that motor nerves are more affected than sensory nerves have also been noted in other animal models for CMT and are not specific for alterations affecting PMP22 (*193*). When bred to homozygosity, the transgenic animals

completely failed to elaborate myelin. Molecular analysis in heterozygous and homozygous rats revealed overexpression of transgenic PMP22 mRNA with some variability but correlated with the severity of the corresponding clinical phenotypes. These data are consistent with the results obtained from transgenic mice that carry different copy numbers of a yeast artificial chromosome (YAC) containing the human PMP22 gene (*194*). In these mice, a correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in NCV has been observed (*195*).

2. Mice with Decreased PMP22 Gene Dosage

In order to analyze the molecular function of PMP22 and to generate an animal model for HNPP that is due to a heterozygous deletion including the PMP22 gene (*124*), mice carrying an inactivated PMP22 gene were generated using homologous recombination in embryonic stem cells (*36*). Mice that completely lack PMP22 are viable but develop walking difficulties due to progressive weakness of the hind limbs first visible at the age of approximately 2 weeks. Morphological examinations reveal a mildly delayed onset of myelination in young animals, indicating a crucial role of PMP22 in the initial steps of myelination followed by the characteristic formation of mainly paranodal but also internodal tomacula. These hypermyelination structures appear to be unstable and degenerate with age. In older animals, classical signs of demyelination and remyelination, including thinly myelinated axons and Schwann cell onion bulbs associated with very slow NCV, become predominant, although some tomacula can still be found (*36, 100*). As a consequence of the neural phenotype, a muscular atrophy develops, characterized by extensive type grouping of muscle fibers and ultraterminal axonal sprouting. Interestingly, no obvious pathological alterations have been detected in other tissues that normally express PMP22. Whether this is due to more subtle effects yet to be discovered or possible compensatory effects by other members of the PMP22/EMP/MP20 family remains to be determined.

Heterozygous "knock-out" PMP22 (+/0) mice have retained only one PMP22 gene copy and genetically mimic HNPP. Indeed, these animals display morphological and electrophysiological features similar to those observed in the human disease (*196*). Tomacula, as the pathological hallmarks of HNPP, develop progressively in young PMP22 (+/0) mice. In older mice, subtle electrophysiological abnormalities are detected accompanied by a significant number of abnormally swollen and degenerating tomacula. Furthermore, thinly myelinated axons and Schwann cell onion bulbs indicate ongoing demyelination and remyelination. Similar findings have also been described in an alternative mouse model for HNPP that has been generated using the Schwann cell-specific P0 promoter to drive rat antisense PMP22 RNA expression (*39*). The observations are reminiscent of the disease pro-

gression in HNPP and may explain why some aging HNPP patients develop a chronic form of HMSN with similarities to CMT1 (*135*). Furthermore, the intrinsic instability of tomaculous myelin might also be involved in the transient symptoms experienced by HNPP patients after nerve trauma.

The availability of various PMP22 alleles in mice was also beneficial to gain important additional information on disease mechanisms. In particular, heterozygous *Tr* mutants (*Tr*/+) display severe hypomyelination of peripheral nerve fibers whereas PMP22 (+/0) mice are characterized by focal hypermyelination, suggesting that the *Tr* mutation does not generate a PMP22-null allele. The comparison of various combinations of PMP22 alleles revealed that the *Tr* allele can act as a true gain-of-function mutation on a null background (*Tr*/0) as well as in homozygous *Tr* animals (*Tr*/*Tr*) (*197*).

F. Lessons from PMP22-Based Neuropathies and Open Questions

The analysis of animal models for PMP22-related neuropathies and detailed neurological and pathological examinations of CMT1A patients have contributed considerably to our current knowledge of the function of PMP22. One of the most tantalizing remaining questions refers to the pathogenesis of HMSN due to altered PMP22 gene dosage. Because there is good evidence that altered PMP22 expression is causative in these diseases, a detailed analysis of PMP22 gene regulation appears warranted. The findings of dominant and recessive missense mutations in conserved functional domains of Erg-2 in patients diagnosed with congenital hypomyelination and CMT1 (*88*) point toward transcription factors that may regulate myelin protein genes as promising candidates for starting such a molecular analysis (*28*). However, other questions concerning the molecular functions and interactions of PMP22 may require novel approaches, such as regulated alterations of gene dosage *in vivo* (*198, 199*). Recombinant PMP22 expression *in vitro* suggested that PMP22 may be involved in the regulation of proliferation of Schwann cells and NIH 3T3 fibroblasts (*14, 94*). Furthermore, PMP22 might regulate apoptosis in NIH 3T3 fibroblasts but not in cultured Schwann cells (*12, 200*). Data from human nerve biopsies (*96*) and from mouse models (*192*) indicate, however, that increased Schwann cell apoptosis can be present *in vivo* if PMP22 is altered. Based on the available *in vitro* data and immunohistochemical and morphological analysis of CMT1A biopsies (*95, 97, 201*). CMT1A was proposed to be the result of sequential events. This process may start with the genetic lesion of a PMP22 gene duplication, followed by altered levels of PMP22 expression, progressing to the modulation of Schwann cell proliferation and abnormal differentiation, and culminating in defective myelination and altered myelin stability (*30, 200*). This interpretation is sup-

ported by the finding that marker proteins for nonmyelinating Schwann cells are abundantly expressed in nerves of PMP22-overexpressing mice (*38*). However, cultured Schwann cells derived from transgenic rats with increased PMP22 gene dosage showed no obvious proliferation defects (*37*), in contrast to observations using Schwann cells derived from CMT1A patients (*95*). Furthermore, postnatally modified PMP22-overexpressing Schwann cells applied in an *in vitro* myelination system revealed no effect on initial myelin spiraling and myelin compaction (*202*), although animal models suggest that PMP22 overexpression can alter or even prevent normal myelination. Thus, expression of PMP22 in embryonic Schwann cells may be critical (*87*). Finally, approaches directed toward the elucidation of disease-causing function of PMP22 have to include and distinguish between early effects and late secondary effects, including the influence of immune system-related responses that are potentially involved in the pathogenesis (*160, 203–205*).

VII. Summary

Based on the crucial role that PMP22 plays in normal physiological and pathological processes in the PNS, PMP22 and EMPs form an important family of proteins. The future challenge is to extend our knowledge of the biological functions of EMPs and PMP22 in neuronal as well as nonneuronal tissues and to determine what (additional) roles these proteins may have in disease. Characterization of specific PMP22/EMP interactions with other proteins may be required to determine the precise molecular function of these proteins, and increased understanding of their regulation will not only provide insight into their function but may also provide therapeutic tools.

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