Peripheral myelin protein 22 (PMP22) is expressed in many tissues but mainly by Schwann cells as a component of compact myelin of the peripheral nervous system (PNS). Mutations affecting PMP22 are associated with hereditary motor and sensory neuropathies. Although these phenotypes are restricted to the PNS, PMP22 is thought to play a dual role in myelin formation and in cell proliferation.

We describe the cloning and characterization of epithelial membrane protein-1 (EMP-1), a putative four-transmembrane protein of 160 amino acids with 40% amino acid identity to PMP22. EMP-1 and PMP22 are co-expressed in most tissues but with differences in relative expression levels. EMP-1 is most prominently found in the gastrointestinal tract, skin, lung, and brain but not in liver. In the corpus gastricum, EMP-1 protein can be detected in epithelial cells of the gastric pit and isthmus, while gastric gland in a pattern consistent with plasma membrane association. EMP-1 and PMP22 mRNA levels are inversely regulated in the degenerating rat sciatic nerve after injury and by growth arrest in NIH 3T3 fibroblasts.

The discovery of EMP-1 as the second member of a novel gene family led to the identification of the lens-specific membrane protein 20 (MP20) as a third but distant relative. The proteins of this family are likely to serve similar functions possibly related to cell proliferation and differentiation in a variety of cell types.

The 22-kDa peripheral myelin protein (PMP22) is a hydrophobic protein of 160 amino acids with four predicted transmembrane domains (1, 2). Point mutations in PMP22 and aberrant expression of the PMP22 gene are associated with various hereditary peripheral motor and sensory neuropathies (3). In particular, the spontaneous mouse mutants Trembler and Trembler-J carry point mutations in the pmp22 gene (4, 5). In humans, the majority of patients suffering from the autosomal dominant demyelinating neuropathy Charcot-Marie-Tooth disease type 1A (6) bear 1.5-megabase intrachromosomal duplication of chromosome 17p11.2–12 that includes the PMP22 gene (7–10). Rare point mutations in the PMP22 gene have also been found in non-duplication Charcot-Marie-Tooth disease type 1A patients (11–13) and in the severe congenital peripheral neuropathy Dejerine-Sottas syndrome (14). Furthermore, the relatively mild, recurrent peripheral neuropathy with liability to pressure palsies is associated with the reciprocal deletion to the Charcot-Marie-Tooth disease type 1A duplication (15, 16).

As anticipated from the phenotype of PMP22 mutant organisms, PMP22 is mainly expressed by myelinating Schwann cells in the peripheral nervous system (PNS) where it is incorporated into compact myelin (1, 17–19). PMP22 mRNA and protein have also been found in motorneurons, and transcripts have been identified in various adult tissues, including the brain, intestine, lung, and heart (8, 20–22). Furthermore, PMP22 mRNA expression is widespread during mouse embryonic development (23). Tissue-specific expression and regulation of PMP22 is controlled by a complex genetic mechanism involving two alternative promoter sequences (24, 25). While the distal promoter is specifically activated in myelinating Schwann cells, the more proximal promoter was found to be active in all known PMP22-expressing tissues (24).

Tissue culture experiments using NIH 3T3 cells and primary dermal fibroblasts revealed that PMP22 is up-regulated under growth arrest conditions, e.g. serum deprivation or density growth arrest, suggesting a potential role for PMP22 in cell proliferation (20, 27). In support of this hypothesis, recent studies employing retrovirus-mediated gene transfer of PMP22 into cultured Schwann cells suggest a pronounced influence of PMP22 expression on the length of the G1 phase (28).

Based on these findings, it was proposed that PMP22 serves a general role in cell physiology and an additional specialized function in PNS myelin (29). However, all known mutations affecting the PMP22 are associated with a phenotype restricted to the PNS, and no consistent abnormalities in non-neural tissues have been detected, even in genetically engineered mice that are completely devoid of PMP22 (30). The most likely explanation for these apparently contradictory results is to postulate specific mechanisms that can compensate for the lack of PMP22 in non-neural tissues. Such processes may involve molecules that are structurally and/or functionally related to PMP22. However, although the putative membrane topology of PMP22 is similar to the gap junction-forming connexin protein family (31) or the tight junction component occludin (32), PMP22 does not display convincing amino acid sequence identity with any other known protein.

In this study, we report the identification of a PMP22-related transcript and the characterization of its encoded protein,
which have designated epithelial membrane protein-1 (EMP-1) based on its tissue expression pattern. EMP-1 and PMP22 are significantly related in their overall structure and primary amino acid sequences and define a novel gene family that also includes a more distant relative, the lens-specific membrane protein MP20 (33).

EXPERIMENTAL PROCEDURES

Animals and Surgery—Male SIV rats (8 weeks old; University of Zurich, Switzerland) were anesthetized by intraperitoneal injection of a mixture of ketamine and choral hydrate (17). Both sciatic nerves were exposed and the left nerve was cut out approximately 2 cm above the hip joint (17). Animals were sacrificed 4 days after injury in a CO2 atmosphere. For isolation of RNA and protein, tissues were excised and either lysed directly into 5 M GT buffer (5 M guanidinium isothiocyanate in 25 mM NaOH, pH 7.2, 1 M KCl, 0.5 M NaCl, 10 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS) or snap-frozen in liquid nitrogen.

Cloning and Sequencing of EMP-1 cDNA—Recombinant clones were isolated from a fetal rat intestine cDNA library. Plasmid inserts were analyzed by automated sequencing in both orientations. The resulting nucleotide sequences were compared with the GenBank™ database to identify known and related sequences. EMP-1 cDNA was identified as being significantly related to rat PMP22, and the 98-base pair cDNA clone was subcloned into both strands for confirmation. The longest open reading frames by translation in all frames. The longest open reading frame was compared with protein data bases and again showed significant similarity to PMP22. Computer-assisted sequence analysis was performed using the GCG software package to predict hydrophobicity profiles, identify signal peptide cleavage sites, and predict surface probability. The EMP-1 and PMP22 polypeptide sequences were compared using the Pileup program and depicted graphically by Prettypplot to show amino acid residues conserved between EMP-1 and the PMP22 species homologues.

In Vitro Transcription and Translation—Both the EMP-1 and mouse PMP22 cDNAs were cloned into the pCDNA-3 expression vector downstream of the T7 promoter. The plasmid inserts were linearized and transcribed/translated in vitro using the SP6 (glycosylation) and prolactin (signal peptide cleavage) according to the manufacturer’s instructions. The synthesis and translation product was electrophoresed on a 10% SDS-PAGE gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Schleicher & Schuell) using a semi-dry blotter (Bio-Rad). The proteins were electrotransferred to nitrocellulose (Schleicher & Schuell) membrane (Hybond N, Amersham Corp.) by capillarity with buffer A (10 mM Tris pH 7.2, 150 mM NaCl, 0.05% Tween 20) for 2 h at 4°C. The membrane was blocked by incubation in 5% dry milk powder followed by a 1-h incubation with 1:500 anti-EMP-1 antibodies diluted in buffer A and washing with buffer A three times before exposure to x-ray film (RX, Fuji) overnight at −70°C.

Isolation of Total RNA and Northern Blot Analysis—Total RNA was extracted from rat tissues using a modified acid guanidinium thiocyanate-phenol-chloroform (AG, Technopark Zürich, Switzerland). Preimmune serum (1:500) was used as a negative control. Antibodies—Anti-peptide antibodies were raised to synthetic peptides representing the first and second putative extracellular loops of EMP-1. Employing a random hexamer priming (Oligo-Glenalting kit, Pharmacia Biotech Inc.) protocol, 20 µg of total RNA was loaded onto a 1% agarose gel, run in 0.5x TBE (100 mM Tris-HCl, pH 8.3, 0.5 mM EDTA) buffer and transferred to nylon membranes (Hybond N, Amersham Corp.) by capillarity with buffer A for 2 h at 4°C. The membrane was blocked by incubation in 5% dry milk powder followed by a 1-h incubation with 1:500 anti-EMP-1 antibodies diluted in buffer A and washing with buffer A three times before exposure to x-ray film (RX, Fuji).
dilution of primary antisera in phosphate-buffered saline containing 0.2% Tween 20 followed by 2 h with a 1:200 dilution of Texas Red-labeled donkey anti-rabbit immunoglobulin (Jackson Immunoresearch Laboratories). After extensive washing in phosphate-buffered saline containing 0.2% Tween 20, sections were coated with AF1 (Citifluor), and coverslips were mounted. Immune reactivity was visualized by high resolution confocal microscopy. Preimmune serum (1:500) as primary antiserum was used as a negative control.

RESULTS

Cloning of an EMP-1 cDNA and Predicted Structure of the EMP-1 Protein—During a fetal rat intestine cDNA sequencing project, a 981-base pair cDNA containing an open reading frame of 480 nucleotides was identified (Fig. 1). The predicted EMP-1 polypeptide of 160 amino acid residues has a calculated molecular weight of approximately 18 kDa, and computer-assisted analysis reveals that amino acid residues 1–28, 64–89, 95–117, and 134–157 represent four hydrophobic, potentially membrane-spanning domains (Fig. 2 B). The amino-terminal 16 amino acid residues have the characteristics of a signal peptide including a signal peptidase cleavage site after alanine 16 (Fig. 1). Furthermore, a single motif for putative N-linked glycosylation is present between the first and second hydrophobic domains at asparagine 43 (underlined). The sequence has been submitted to the EMBL GenBank™ under the accession number Z54212.

EMP-1 Is a Member of a Gene Family—Comparison of the EMP-1 cDNA sequence to the GenBank™ database identified PMP22 as the closest known relative with a nucleotide identity of 58% over the open reading frame (data not shown). Both the predicted EMP-1 protein and PMP22 are polypeptides of 160 amino acids that show 40% amino acid identity (Fig. 2A). The putative four membrane-spanning regions of EMP-1 and PMP22 are particularly well conserved. The first and second of these hydrophobic domains exhibit the highest degree of amino acid identity at 54 and 67%, respectively, while the third and fourth are only 30 and 37% identical.

Fig. 2B depicts a theoretical model of the EMP-1 protein structure based on the hydrophobicity profile and the suggested structure of PMP22. Filled circles represent identical amino acid residues that are shared by rat EMP-1 and rat PMP22, while divergent residues are shown as open circles. The positions of the amino acids in PMP22 known to cause hereditary motor and sensory neuropathies (21) when mutated are highlighted in the EMP-1 sequence as diamonds (Fig. 2B). Interestingly, all of these mutations lie within the putative membrane-spanning domains, and five of the six residues are conserved in rat EMP-1. The conservation of these amino acid residues suggests that they may be of functional significance.

Additional data base searches with the EMP-1 and PMP22 sequences revealed that both display 30% amino acid identity to the lens fiber cell protein MP20 (33). MP20 is a 173-amino acid protein with similar structural features to EMP-1 and PMP22 (Fig. 2C). If MP20 is compared with PMP22 and EMP-1 simultaneously, the amino acid identity increases to 36% including strongly conserved motifs in the putative transmembrane domains (Fig. 2C).

EMP-1 Is Glycosylated in Vitro but Its Signal Sequence Is Not Cleaved—The EMP-1 cDNA shown in Fig. 1 was transcribed and translated in vitro (Fig. 3). The translation product in the absence of CMMs has an apparent molecular mass of approximately 18 kDa, which is in agreement with the calculated molecular mass of the EMP-1 protein. Translation in the presence of CMM results in a 4–6-kDa increase in molecular mass consistent with the presence of the single putative N-linked glycosylation site in the EMP-1 amino acid sequence (Fig. 3). This migrational shift can be reversed by deglycosylating the EMP-1 cDNA and amino acid sequences of rat EMP-1. The 981 base pairs contain an open reading frame of 480 base pairs starting with an ATG codon at nucleotide 1 and terminating with a TAA stop codon at position 481. A potential cleavable signal peptide is highlighted in boldface letters (residues 1–16). A single motif for putative N-linked glycosylation is present between the first and second hydrophobic domains at asparagine 43 (underlined). The sequence has been submitted to the EMBL GenBank™ under the accession number Z54212.
Fig. 2. Predicted EMP-1 structure and amino acid sequence comparison of PMP22/EMP-1 family members. A, amino acid sequence comparison between rat EMP-1 and rat, mouse, and human PMP22. Identical residues are boxed. B, hypothetical topology of EMP-1 in a lipid bilayer based on computer-assisted hydrophobicity plots and secondary structure predictions. Residues identical to rat PMP22 are filled. Most of the point mutations in PMP22 known to result in hereditary peripheral neuropathies (diamonds) are conserved at the corresponding positions in EMP-1. The Y-shaped symbol indicates a potential N-linked carbohydrate chain. C, amino acid comparison of the three known PMP22/EMP-1/MP20 family members. The residues conserved between MP20 and PMP22 or EMP-1 are shown in white on black.
Characterization of EMP-1

EMP-1 mRNA and PMP22 mRNA Are Differently Regulated After Sciatic Nerve Injury—The highest levels of PMP22 are found in myelinating Schwann cells of the PNS, and expression is down-regulated in the distal portion of the rat sciatic nerve following crush or cut injury (1, 2, 17, 38). We have examined EMP-1 mRNA levels in the sciatic nerve under the same conditions. The sciatic nerves of 2-month-old rats were bilaterally exposed and cut unilaterally. Four days after injury, the degenerating distal portion of the traumatized nerve and, as a control, the undamaged contralateral nerve were removed, and total RNA was isolated. Northern blot analysis reveals that EMP-1 transcripts are present at considerably lower levels in the adult sciatic nerve than PMP22 transcripts; Fig. 5 represents exposures of 1 h for PMP22 and approximately 36 h for EMP-1. Furthermore, EMP-1 expression increases in the distal nerve following injury, in sharp contrast to PMP22 (Fig. 5). These findings demonstrate that, although EMP-1 and PMP22 are coexpressed in PNS nerves, they appear to be differently regulated.

EMP-1 and PMP22 mRNA Expression in Schwann Cells Is Inversely Regulated In Vitro—In order to substantiate the hy-

FIG. 3. In vitro transcription and translation of EMP-1 and PMP22 cDNAs. The [35S]methionine metabolically labeled proteins were separated by reducing 15% SDS-PAGE. pcDNA-1 was used as a control, and no nonspecific proteins can be detected. The EMP-1 cDNA generated an 18-kDa protein that shows a tendency to aggregate (EMP-1). Transcription and translation of the EMP-1 cDNA in the presence of CMM results in a reduced rate of migration of the protein (EMP-1 + CMM). This reduced migration is reversed by deglycosylation with N-glycosidase F (EMP-1 deglycosylated). The PMP22 cDNA generates an 18-kDa protein whose apparent molecular weight increases by 4–6 kDa when the reaction is performed in the presence of CMM (PMP22 + CMM). Treatment with N-glycosidase F reduced the molecular mass back to 18 kDa (PMP22 deglycosylated). Neither EMP-1 nor PMP22 are substrates for signal peptidase; approximately 50% of the protein is processed when translated in the presence of CMM (c) (Promega technical manual). α-factor was used as a control for N-linked glycosylation and was completely modified in the reactions (b) (Promega technical manual).

Translation product with N-glycosidase F (Fig. 3). The deglycosylated protein migrates identically to the unglycosylated EMP-1 protein, suggesting that the putative N-terminal signal peptide is not removed during EMP-1 protein biosynthesis. A similar modification of PMP22 was seen with CMM, confirming previous reports of endogenous PMP22 carrying an uncleaved signal peptide (37).

Expression Patterns of EMP-1 mRNA and PMP22 mRNA Are Qualitatively Similar but Quantitatively Different—To elucidate the distribution of EMP-1 mRNA in the rat, we used the EMP-1 cDNA (Fig. 1) to probe Northern blots of total RNA extracted from various tissues. EMP-1 transcripts can be found in all organs examined with the exception of the liver (Fig. 4, a and b). The most prominent EMP-1 mRNA expression is observed in tail-derived skin and in the gastrointestinal tract. To examine a potential specific regional expression pattern, RNA was extracted from different regions of the gastrointestinal tract. Interestingly, we found that EMP-1 mRNA is not uniformly expressed throughout the stomach of the rat. The fundic region exhibits high levels of EMP-1 mRNA, while expression in the corpus and pylorus is much lower. In the intestinal tract, the cecum and large intestine (colon and rectum) contain the highest levels of EMP-1 mRNA. EMP-1 transcripts are also detectable throughout the small intestine but at far lower levels than in the fundus of the stomach, the cecum, and large intestine. Considerable amounts of EMP-1 mRNA, similar to the expression in the duodenum, are also found in the brain and lung. Low level EMP-1 expression is detectable in the heart, kidney, spleen, thymus, and skeletal muscle.

All tissues expressing EMP-1 mRNA contain 2.8-kb EMP-1 transcripts. In some regions of the gastrointestinal tract, however, including the fundus, ileum, cecum, and colon, additional transcripts of approximately 1.7 kb hybridize with the EMP-1 cDNA (Fig. 4a). Prolonged washing of the blots at high stringency did not result in the preferential loss of one of the signals relative to the other (data not shown), hence, we favor the
null
of the small intestine is considerably lower than in the other regions of the intestinal tract, in accordance with the reduced mRNA levels found in these tissues (Figs. 4 and 6A). Very low levels of the 25-kDa EMP-1 protein can also be detected in the lung (Fig. 6A), spleen, and thymus (data not shown).

In addition to the 25-kDa protein, both EMP-1 antisera detect a similar array of larger proteins in the intestine (Fig. 6B and data not shown). The presence of these additional immunoreactive species varies from experiment to experiment and between tissues (data not shown). In general, the additional bands are most prominent in lysates containing higher amounts of EMP-1 protein. Since the two antisera are directed against independent regions of EMP-1 protein, these larger immunoreactive species are likely to represent aggregated molecules, a phenomenon frequently seen with highly hydrophobic proteins.

Although the level of EMP-1 protein observed in some tissues does not strictly correlate with EMP-1 mRNA expression, EMP-1 protein can only be found in tissues where EMP-1 mRNA expression is seen. No immunoreactive proteins are detected by either antiserum in lysates of the EMP-1 mRNA-negative liver (Fig. 6A).

Detection of Recombinant EMP-1 Protein by Immunofluorescence in Transiently Transfected COS Cells—To test the suitability of the polyclonal antisera to detect EMP-1 protein on frozen sections of rat tissue, we first assessed the specificity of the anti-loop 1 and anti-loop 2 antisera in COS cells transiently expressing EMP-1. COS cells were transfected by electroporation with a construct containing the EMP-1 cDNA under the transcriptional control of the cytomegalovirus promoter. Forty-eight hours later, cells were fixed, and antisera were used to detect EMP-1 expressing cells. After transfection, both antisera identified approximately 25% of the cells with strong immunoreactivity (Fig. 7). Neither antisera nor preimmune sera recognized control COS cells transfected with the parental expression vector without EMP-1 insert (data not shown). Since detection with anti-loop 2 antisera was more efficient than with the anti-loop 1 antisera, the former antibody was predominantly used in subsequent studies.

EMP-1 Is Expressed in Epithelial Cells of Rat Intestine—Fig. 8a shows a schematic representation of the topology of the rat gastric mucosa. The epithelial cells of the gastric pit are produced from stem cells in the isthmus/neck region of the gastric gland. These cells differentiate during their migration toward the gastric pit from which they are extruded (exfoliated) from the tip of the villus (reviewed by Gordon and Hermiston (44)). Transverse sections across the gastric pit show epithelial cells that are organized in circles around the intestinal lumen (Fig. 8a).

Ten-μm frozen sections of corpus gastricum were stained with anti-loop 2 antiserum. Strong immunoreactivity was detected in the outer epithelial cells of the gastric mucosa from the tip of the villus down toward the isthmus and neck of the gastric gland (Fig. 8, b and c). In transverse section, the EMP-1 immunoreactivity appears to be associated with the plasma membrane of epithelial cells in the gastric pits (Fig. 8b). The epithelial cells deeper in the gastric gland show little or no immunoreactivity, and specific labeling was not detectable in the base of the gastric gland or in the submucosal muscle layer (Fig. 8b).

**DISCUSSION**

We report the cloning and characterization of the epithelial membrane protein EMP-1, a hydrophobic polypeptide of 160 amino acid residues. Computer-aided analysis revealed that EMP-1 shows 40% amino acid identity to PMP22, a PNS myelin protein that is responsible for inherited peripheral neuropathies. EMP-1 and PMP22 display similar hydrophobicity profiles, suggesting that both proteins contain four membrane-associated, potentially membrane-spanning domains. Thus, we propose that EMP-1 and PMP22 are two members of a gene family that also includes the lens fiber cell protein MP20, one of the major protein components of the mammalian eye lens (33, 40, 41), as a distantly related third family member.

The high degree of identity at the amino acid level suggests that EMP-1 and PMP22 may serve similar functions. Close examination of the amino acid sequences of these proteins reveals that the hydrophobic regions, in particular the first two transmembrane domains, are highly conserved, suggesting that they are of particular functional importance. This hypothesis is further supported by the finding that the hydrophobic domains are the most strongly conserved regions between PMP22 species homologues. Interestingly, the amino acid residues in PMP22 that are sites of mutation in hereditary peripheral neuropathies are located within putative transmembrane domains, and the majority of these mutated amino acid residues are also conserved at the corresponding positions of EMP-1 and MP20.

The N-terminal signal peptides of both EMP-1 and PMP22 contain consensus sequences for signal peptidase cleavage. However, the signal sequence of PMP22 is not cleaved efficiently in myelinating Schwann cells as demonstrated by N-terminal sequencing of purified PMP22 protein (37). Since the N terminus of EMP-1 is also not cleaved when synthesized in the presence of CMM, we hypothesize that the EMP-1 signal peptide is not removed during biosynthesis in vivo. This situation is reminiscent of the structurally related connexin family of gap junction proteins, where a specific mechanism has been postulated to prevent aberrant N-terminal processing (31). Furthermore, MP20 does not contain a signal peptide cleavage consensus sequence, and N-terminal sequencing has shown it to be unmodified at its N terminus in vivo (42).

The most interesting conservation within the putative extracellular domains of EMP-1 and PMP22 concerns the consensus sequence for an N-linked glycosylation. This glycosylation site in PMP22 carries a modified carbohydrate chain containing the L2/HNK-1 epitope, a structure which has been implicated in cell-cell recognition and adhesion processes (for recent review see Schachner and Martini (1995)) (43). Although the presence and nature of carbohydrate moieties linked to EMP-1 remains to be determined, an N-linked glycosylation in the identical
position of EMP-1 may be involved in cell recognition processes in the epithelium of the intestine.

EMP-1 and PMP22 are co-expressed in a wide range of tissues, and particularly high levels of transcripts for both proteins are found in the intestinal tract. The gastrointestinal tract is characterized by a continual and rapid renewal of its epithelial surface that continues throughout the animal’s life. Pluripotent stem cells anchored in the isthmus/neck regions of the gastric gland give rise to progeny displaying increased proliferation and reduced potentiality, which progress to terminally differentiated mature cells (44). During this differentiation process, the cells are highly migratory, with proliferation, migration, and differentiation all being tightly coupled. EMP-1 is found mainly in the proliferation and differentiation zones of the outer gastric gland as well as in the mature epithelial cells of the gastric pit region. In these cells, EMP-1 appears to be associated with the plasma membrane, with no clear distinction between the basal, apical, and lateral aspects.

PMP22 has been suggested to play a role in the control of cell proliferation. In support of this hypothesis, evidence has been presented that modulation of PMP22 levels in cultured Schwann cells has a pronounced influence on the cell cycle (28). In these experiments, overexpression of PMP22 increased the length of the G1 phase, while reduced expression resulted in a

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**Fig. 8. Immunofluorescent localization of EMP-1 protein expression in the corpus gastricum.**

- **a**, schematic view of the gastric mucosa showing the proliferative zone in the neck/isthmus region of the gastric gland and the migration of the differentiating epithelial cells toward the gastric pit.
- **b**, low magnification view of the gastric mucosa labeled with the polyclonal anti-EMP-1 loop 2 antiserum and detected with a Texas Red-labeled donkey anti-rabbit antibody. Intense immunoreactivity can be detected in the epithelial cells of the outer mucosa. No immunoreactive cells can be found toward the base of the gastric pit or in the submucosal muscle layer (sm). The intense labeling of the isolated cells at the base of the gastric mucosa is not specific, as it is also present in control sections incubated with preimmune serum (not shown).
- **c**, higher magnification of the labeled epithelial cells in the pit region. The migrating, differentiating epithelial cells in the isthmus express high levels of EMP-1 protein.
- **d**, transmitted light view of the region shown in panel c. e, cross-section through the gastric pit shows intense plasma membrane-associated labeling of the epithelial cells but no labeling of the mesenchyme. No staining is seen in transverse sections across the base of the gastric gland (not shown).
- **e**, transmitted light view of the section shown in panel c. The scale bars shown are 100 μm for b and 60 μm for panels c-f.
These findings are reminiscent of the crystallin protein family (reviewed in Ref. 26). Crystallins are expressed in many tissues as enzymes or stress proteins. Through various evolutionary processes, they have subsequently been recruited as structural components of the lens. In some cases, high expression in the lens has been achieved using tissue-specific promoters or enhancers. Whether such mechanisms also apply to other known or yet to be found PMP22/EMP-1/MP20 family members remains to be seen.

In conclusion, PMP22 has been widely regarded as mainly a structural component of PNS myelin. Our description of EMP-1 and the concomitant identification of the PMP22/EMP-1/MP20 gene family, the expression pattern of these proteins in mainly epithelial tissues, and the observed differences in regulation of PMP22 and EMP-1 during the cell cycle support the concept that these proteins play multiple roles in cell biology. These functions may be related to both the switch from proliferation to differentiation as well as the maintenance of critical functions in the differentiated state.

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