Brain Pathology 8: 771-793(1998)

## SYMPOSIUM: Transgenic Models of Neurodegeneration

# Mouse Models of Myelin Diseases

**H. Werner[1], M. Jung[1], M. Klugmann[1], M. Sereda[1], I.R. Griffiths[2], K.-A. Nave[1]**

[1] Zentrum für Molekulare Biologie (ZMBH), Universität Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany
[2] Applied Neurobiology Group, Dept. of Veterinary Clinical Studies, University of Glasgow, Glasgow G61 1QH, Scotland

### 1. Overview

In the nervous system of higher vertebrates, myelin is a unique cellular specialization that provides the physical basis for rapid saltatory impulse conduction: by electrically insulating axonal segments, myelin sheaths restrict action potentials to the nodes of Ranvier. Consequently, myelinated axons transmit signals up to 100 times faster than non-myelinated fibers of an equal caliber. At the same time, myelin sheaths reduce the ionic currents across axonal membranes and stabilize the extracellular milieu within rapidly-firing axon bundles. In the central nervous system (CNS), myelin is assembled by oligodendrocytes which originate from the subventricular zone. In the peripheral nervous system (PNS), the same function is provided by Schwann cells which derive from the neural crest. Despite these embryological and major morphological differences, the mature myelin sheath in the CNS and PNS is remarkably similar in ultrastructure, which results from the spiral wrapping of an axonal segment by the extended glial cell membrane. The tight association ("compaction") of the intracellular and extracellular membrane surfaces yields unique adhesion zones within myelin, referred to as *major dense line* and *intraperiod line*, respectively (Figure 1). As we will discuss in some detail, myelin-specific genes expressed in oligodendrocytes and Schwann cells encode structural proteins many of which are assembled into the compact sheath and required for the normal myelin architecture. One can consider the myelin sheath as an "extracellular" glial organelle that remains metabolically coupled to the glial cell body throughout life.

Not surprisingly, diseases of myelination, whether acquired or inherited, are severe disorders of nervous system function. White matter diseases (leukodystrophies) comprise a heterogeneous group of genetic disorders that affect synthesis, composition, or turnover of CNS myelin components. Clinically, they are characterized by motor disturbances, especially pyramidal and cerebellar symptoms, sensory losses, mental deterioration and susceptibility to seizures. Many leukodystrophies are lethal. Also peripheral neuropathies which are the result of a myelin defect originating in Schwann cells can have a wide range of genetic causes. From a pathophysiological point of view, *dysmyelinating* diseases are developmental defects, whereas *demyelinating* diseases are degenerative in nature. Dysmyelinations may be due to a genetic defect in a structural myelin component, but as we will discuss, some mutations that affect axon-glia communication or the stability of myelin (at an early age) may have features of both, dys- and demyelination. Understanding these pathomechanisms has been a major challenge when human myelin diseases were first defined at the DNA level, and continues to be an unresolved issue for many myelin disorders.

Basic research in cellular and developmental neurobiology as well as the analysis of mutations in mice, which serve as models for corresponding human diseases, have made substantial progress in recent years. Today most of the abundant structural proteins in myelin are molecularly defined. Mutations in their genes have been identified either in naturally occuring mouse mutants or were generated by gene targeting techniques

Corresponding author:
Dr. Klaus-Armin Nave, Zentrum für Molekulare Biologie (ZMBH) Universität Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Fed. Rep. Germany; Tel.: (+49) 6221-546898; Fax:(+49) 6221-545894;
E-mail: nave@sun0.urz.uni-heidelberg.de

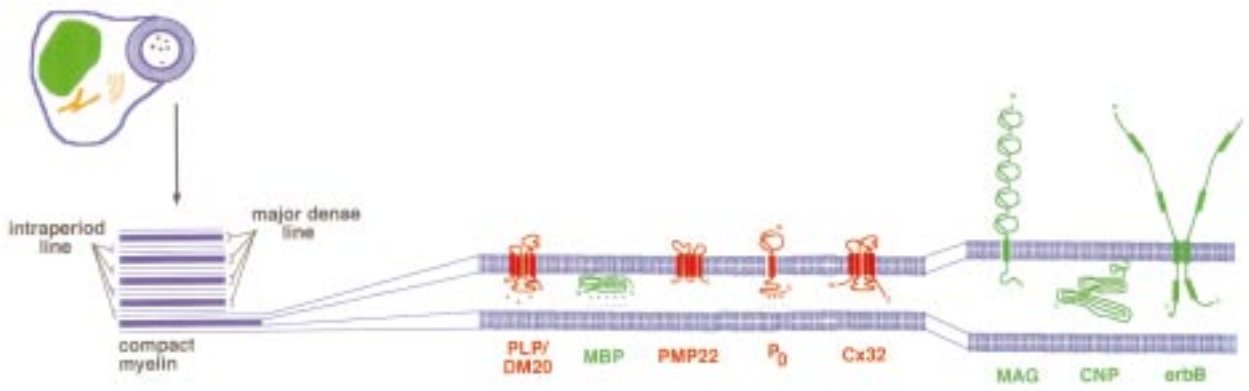


**Figure 1.** Schematic depiction of a myelin-forming glial cell, the ultrastructure of compacted myelin, and structural proteins discussed in this review. Membrane proteins which are associated with a human myelin disease are shown in red. Other glial proteins for which such an involvement has not been demonstrated in humans (or only in mouse mutants) are depicted in green. Structural proteins of compacted myelin are lined up in the middle, and proteins located in uncompacted regions or facing the axon are shown to the right. Abbreviations: PLP/DM20, proteolipid protein; MBP, myelin basic protein; PMP22, peripheral myelin protein of 22kDa; P0, protein zero; Cx32, connexin of 32 kDa; MAG, myelin-associated glycoprotein; CNP, cyclic nucleotide phosphodiesterase. Some membrane proteins are involved in axon-glia communication prior to myelination, e.g. receptor tyrosine kinases (ErbB).

(or both), and several have been associated with human myelin diseases. More recently, also the function of myelin-specific lipids was approached by generating mice lacking enzymes of the myelin-associated lipid metabolism. Finally, the function of some regulatory molecules, i.e. glial transcription factors and cell signalling molecules, which are required prior to myelin assembly, has been studied, and some of their genes could be analyzed in mouse mutants. Already the number of myelin genes analyzed in mutant mice exceeds the number of myelin diseases in humans, and will further grow as new transgenic tools are being developed. In this review, we look at what mouse mutants have revealed about the normal biology of myelin-forming glial cells and, where applicable, what they have revealed about the corresponding human myelin diseases. Rather than providing a detailled summary of these studies (for in depth reviews see 25, 120, 161, 164, 107), we will emphasize some of the emerging concepts in “myelin genetics.”

## 2. Early development

Developmental processes are frequently regulated at the level of gene transcription. The analysis of myogenic and neuronal differentiation has demonstrated that some transcription factors can induce developmental programs at the end of which cell type-specific proteins are expressed. Although little progress has been made in identifying the factors which control myelin genes as their *bona fide* target genes (71), some genetic defects of glial cell development could be attributed to abnormal gene regulation. One well-studied example in the Schwann cell lineage is the “POU domain” transcription factor SCIP (for *suppressed cAMP inducible POU*; also termed Tst-1 or Oct-6). SCIP is expressed transiently in differentiating myelin-forming glial cells in both the CNS and PNS (114, 194) although the protein is certainly not glial cell-specific. In SCIP-overexpressing Schwann cells, the activity of genes that encode myelin-specific structural proteins is actually inhibited, suggesting that this factor serves as a repressor of myelin genes, whereas in heterologous cells, SCIP binding can transactivate reporter genes (some of these discrepancies may be solved with the identification of a SCIP coactivator). Later, a model was put forward in which SCIP stabilizes committed Schwann cells in the “promyelin” stage, a transitory stage between proliferation and myelination (194). Would the absence of SCIP therefore result in premature myelination? Initially, the phenotype of transgenic mice, expressing a dominant-negative form of SCIP supported this idea (179), however two independently generated lines of SCIP/Oct6/Tst-1-deficient mice revealed a more complex function (12, 75). Homozygous SCIP-mutants which survive the early perinatal period (there is a high lethality due to breathing problems) display a dysmyelination defect: Schwann cells segregate one-to-one with the axons that they engulf, but they express only the early myelin markers (such as the myelin-associated glycoprotein, MAG) and appear to be arrested at this promyelin stage. There is little axonal wrapping and expression of the later myelin genes, such as P0 and MBP, is decreased.

Surprisingly however, after prolonged survival, many Schwann cells continue their terminal differentiation and assemble myelin with a significant temporal delay (75). Thus, in the absence of this transcription factor, myelination arrests transiently as if SCIP only facilitates a myelination program in glia that can be executed also in its absence. No SCIP-associated human disease has yet been recognized.

The zinc-finger protein Krox-20, independently termed EGR-2, was first identified as an "immediate early gene" in differentiating neurons, but appears to be also involved in the activation of myelin genes in Schwann cells (23, 194). Known as a marker of rhombomers 3 and 5 in the developing mouse embryo, it was a serendipidous finding that the targeted disruption of Krox-20 resulted in both altered hindbrain segmentation (149, 166) and a complete block of Schwann cell differentiation at the promyelin stage (170). This myelination arrest is remarkably similar to that of SCIP mutant mice. However, the Krox-20 function is genetically "downstream", because SCIP expression is unaffected in Krox-20 deficient mice, whereas SCIP mutants also lack Krox-20 (D. Meijer, personal communication). The identification of Krox-20 as a myelin-associated gene has led to a search for human mutations among unclassified peripheral myelin diseases. Recently, several Krox-20 gene mutations were identified in patients with peripheral neuropathies (175). In one family, a missense mutation (the substitution I268→N) underlies even a severe congenital hypomyelination (CHN), most likely the direct phenocopy of the loss-of-function phenotype in mice. In two other families with autosomal-dominant Charcot-Marie-Tooth disease (CMT-1) and one with CHN, the primary defects were identified as amino acid substitutions R409→W, S382→R, and D383→Y, respectively. As they are located within the highly conserved zinc-finger domain of Krox-20, its ability to bind to DNA may have been lost. The autosomal-dominant pattern of inheritance could either indicate a dominant-negative effect at the protein level or a haploinsuffiency in humans (175). Heterozygous Krox-20 mutant mice are apparently normal - but, as we will see later, mouse and man can differ in the timecourse and phenotypic expression of a myelin disease.

These two examples have shown that transcription factors which function in various cell-types can be associated with distinct myelination defects. The lack of other transcription factors, expressed earlier in the glial cell lineage, may cause the complete absence of myelin-forming Schwann cells or may be tolerated with respect to myelination. Examples are the "paired box" protein Pax-3, detectable in the mouse neural crest from embryonic day 8.5 (E8.5), i.e. before Schwann cell precursors migrate into the PNS, and declining in abundance as myelination begins (83). Natural Pax-3 mutant mice (*splotch* and *splotch delayed*) die at different embryonic ages without limb muscles and no (or almost no) Schwann cells. The *splotch* mutant mouse is considered a model for the Waardenburg syndrome. Although point mutations of the human Pax-3 gene have been identified in this heterogeneous disorder (which is characterized by congenital abnormalities of various neural crest-derived tissues) dysmyelination is not a feature of the human disease (167). Another group of DNA binding proteins, Sox ("SRY box") proteins, define a group of transcription factors homologous to the sex-determining factor SRY. The protein Sox-10, recently cloned from primary rat Schwann cells, is expressed in both the Schwann cell and oligodendrocyte lineage (92). Although its genuine target genes are not known, some synergistic effects with SCIP were observed in promoter transactivation assays (2-3 fold activity when compared to SCIP alone) in which Sox-10 by itself failed to activate the reporter gene. Mutations in the Sox-10 gene were identified in patients with the Waardenburg-Shah syndrome (WS4) (128) suffering from deafness, pigmentary disturbances and aganglionic megacolon, but myelination is again not disturbed. Also the corresponding mouse mutant *Dom* ("dominant megacolon"; 159) appears normally myelinated. Thus, although expression data would have suggested an important role for these transcription factors in the entire neural crest-derived cell lineage, myelin-forming Schwann cells appear not to be affected. We will come back to the concept that loss-of-funcion mutations are frequently "masked" by compensatory mechanisms.

### 3. Gain-of-function mutations

For several decades, naturally occurring mouse mutants with myelination defects have been considered animal models for inherited myelin diseases in humans, based on the obvious homology in the mode of disease transmission and in brain pathology, but without knowledge of the underlying primary defect (154). Only within the last 15 years, molecular cloning of the myelin-specific proteins has led to the identification of underlying mutations in both the mouse mutants and human diseases. At first, it seemed likely that mutations cause the loss of a functional gene product, and that this loss of function underlies any given myelin defect. It soon became clear, however, that defects in quite different genes can underlie the same myelin pathology, whereas

allelic mutations within the same gene may cause diseases which are, by clinical criteria, distinct entities. This wealth of molecular information has been used since to reclassify several myelin diseases according to molecular-genetic criteria (102, 150). In the following, we will summarize evidence that one reason for this complexity is the fact that point mutations frequently alter the structure of a protein. In autosomal-dominant diseases, point mutations may lead to products with abnormal properties ("aberrant gain of function") that disrupt, in a dominant-negative fashion, the function of the normal protein (for example within an oligomeric membrane protein assembly) or are simply "toxic" to the cell, or both. As we will see later, dysmyelination following a gene duplication may be considered a special case in this group of diseases. An important tool to distinguish possible loss-of-function and gain-of-function effects is the creation of a *null* allele by gene targeting.

Well-studied examples which illustrate the detrimental effect of a mutant gene product are the X-linked *jimpy* (jp) mutation in the mouse, and its alleles *jp-msd*, *jp-4J*, *rumpshaker*, and *md* (in the rat), which are all caused by point mutations in the gene for myelin proteolipid protein (PLP). These mutants provide accurate mouse models for the connatal form of Pelizaeus-Merzbacher disease (PMD) in humans and its milder form Spastic Paraplegia type-2 (120). The disease-associated PLP is the most abundant integral membrane protein of CNS myelin (94, 111, 178) and thought to stabilize membrane adhesion along the intraperiod line. By its hypothetical structure, PLP belongs into a heterogeneous group of "four-helix-bundle" membrane proteins (130), which also includes other myelin proteins, such as peripheral myelin protein-22kDa (PMP22) and connexin-32 (Cx32), with their N- and C-termini oriented towards the major dense line (Figure 1). PLP and its smaller isoform DM20 (derived by alternative mRNA splicing) are highly conserved in evolution (145) which suggests that they engage in protein-protein interactions. In *jimpy* mice, the mutation of a RNA splice site in PLP intron 4 causes the aberrant loss of exon 5 and a frameshift of translation that deletes the fourth transmembrane domain (122, 121). Most other point mutations in the PLP gene cause single residue substitutions, such as the conservative exchange A242→V (in the fourth transmembrane domain) of the *jp-msd* allele (55). Nevertheless, the cellular consequences appear remarkably similar in *jp* and *jp-msd* mice: the entire CNS remains largely unmyelinated, and whereas oligodendrocyte precursor cells are increased in number, all newly differentiated oligodendrocytes die with typical features of apoptosis (157, 60, 98). PLP mutant mice show severe tremors and seizures, and most animals die at the end of the third postnatal week (for a comparison of clinical features in dysmyelinated mice and human PMD see ref. 120).

It is noteworthy that the jp defect differs from that of the PLP missense mutation (I186→T) in *rumpshaker* mice (jp-rsh) (146). Here, the hypomyelination is very moderate and affected hemizygous mice have a normal life span (62), largely due to improved CNS myelination and normal numbers of oligodendrocytes. The identical substitution was rediscovered in a patient with X-linked spastic paraplegia type 2 (SPG-2) (87), a myelin disease which also involves the PLP gene but with a milder course and clinically distinct from PMD (143). Why would conservative substitions in jp-msd be more detrimental to oligodendrocytes than non-conservative changes in mouse models of SPG-2? We believe that residues of the transmembrane regions which are conserved within the family of DM20-related proteins are critical for alignment of the four alpha helices, protein folding, and possibly oligomerisation.

Transgenic experiments, in which a wildtype PLP-transgene was expressed in hemizygous *jimpy* mice yielded the first direct evidence that a functional complementation is not possible in these mutants (118, 147). Since there is indirect evidence that PLP engages in homophilic interactions, and possibly oligomerization (see below), a lack of transgenic complementation could be due to dominant-negative effects. That mutant PLP indeed exerts an aberrant gain-of-function effect (independant of the presence of wildtype PLP) was revealed by gene targeting experiments (13, 14) and the complete inactivation of this gene (86). PLP/DM20-deficient oligodendrocytes which lack even trace amounts of abnormal PLP polypeptides have no obvious differentiation defect and are able to myelinate CNS axons of all calibers (86). The PLP/DM20 loss-of-function phenotype was instead unexpectedly mild, at least at early ages: only the intraperiod line of compacted myelin was abnormally condensed, similar to what has been documented in the few myelinated areas of the CNS in *jp* mice and *md* mutant rats (37, 38). Although PLP knockout mice have thus provided a formal proof that mutant forms of PLP are not tolerated by oligodendrocytes, the molecular mechanisms of this "toxicity" remain to be defined. As discussed later, studying protein misfolding and intracellular retention in transfected cells may provide an answer to this problem.

A second example in which myelin pathology is not

adaequately explained by the myelin protein's loss of function are the point mutations in the gene for PMP22, a glycosylated membrane protein of myelin-forming Schwann cells (158). PMP22, which also belongs into the diverse group of "four-helix bundle" proteins, is localized in compacted myelin (Figure 1) but its normal function is obscure. PMP22 expression is not restricted to Schwann cells and the gene was discovered independently as a growth arrest-specific transcript in cultures of NIH3T3 cells (termed *gas3* ; 148). It has therefore been suggested that PMP22 and two highly related membrane proteins of epithelial cells (168, 24) are involved in the regulation of cell proliferation and apoptosis. Point mutations of the PMP22 gene as well as gene duplications (see below) have been associated with the most frequent peripheral neuropathy in humans, Charcot-Marie-Tooth disease type1A. One major argument for the initial assignment of PMP22 as the responsible disease gene was the identification of point mutations in the murine PMP22 gene of two dysmyelinated mutants, *Trembler* (*Tr*; mouse chromosome 11) and *Trembler -J* (162, 163). In *Trembler* , a missense mutation causes the non-conservative substitution G150→D within the predicted fourth transmembrane domain. The dysmyelination is PNS-specific and formally dominant, as heterozygous mice exhibit progressive impairment of the hindlimbs and show occasional convulsions. At the cellular level, peripheral nerves display aberrant Schwann cell growth, onion bulb formation and thinly myelinated axons. Homozygous mutants show an even more pronounced disease phenotype, but it has been noted that the animals' life span is not reduced (67). The identical mutation has been found in a human patient with the Dejerine-Sottas-Syndrome (DSS) (74), a neuropathy clinically more severe than CMT1A.

Again, direct evidence that the disease phenotype in mice constitutes more than the complete loss of PMP22 function came from mice with a targeted disruption of the gene (1). Although peripheral myelin was clearly abnormal in the complete absence of PMP22 (a focal hypermyelination frequently proceded to myelin breakdown), the myelin pathology was very different from that of *Trembler* mutants which were congenitally dysmyelinated. Also mice with one copy of the *Trembler* allele and one copy of the *null* allele (Tr/-) completely lack peripheral myelin (2). Thus, PMP22 point mutations are likely to cause dysmyelination, at least in part, through an aberrant gain-of- function effect.

The disease phenotype of *Trembler-J* mice is more severe than that of *Trembler* (even though slightly more myelin is made in the *J*-allele). The primary *Trembler-J* defect, a substitution L16→P in the first transmembrane domain of PMP22 (163) results in early lethality of homozygotes. Interestingly, the same substitution was rediscovered in a human patient diagnosed with CMT-1 rather than DSS (172). Thus, the clinical severities associated with PMP22 point mutations do not necessarily correspond in mouse and man, which suggests that modifier genes determine the clinical picture. The analysis of nerve biopsy material from one patient with the *Trembler-J* type mutation revealed little immunoreactivity of PMP22 in myelin due to the retention of the mutant protein in Schwann cells (32). Similar observations have been made in the analysis of Tr nerves, suggesting that the mutant gene products do not reach their normal destination and interfere with myelination (119). Taken together, the PMP22 disease phenotype is also the combination of both loss- and aberrant gain-of-function effects.

The lessons learned from mutations in the PLP and PMP22 genes are also relevant for a third myelin disease, the X-linked form of CMT which is associated with mutations of the connexin-32 (Cx32) gene. Cx32 belongs to a family of gap junction-forming membrane proteins (they too share the four-helix-span topology) and is expressed in Schwann cells and oligodendrocytes, as well as in many non-glial cell types. Within PNS myelin, Cx32 is concentrated in non-compacted regions (Schmidt-Lanterman incisures and paranodal loops) where the protein is postualted to form 'reflexive' gap junctions between adjacent myelin membrane leaflets. These Cx-32 junctions may enable the diffusion of metabolites and signalling molecules through the myelin sheath (15). More than 150 different point mutations have been identified in human patients with CMT-X (11, 144). As seen in families with X-linked PMD/SPG-2, the obligate carrier females may be clinically normal or partly affected (144). Again, the random X-inactivation in females does not allow to distinguish easily whether the Schwann cell pathology follows a Cx32 loss-of-function or an aberrant gain-of-function effect, or both. When mutant and wildtype Cx32 proteins were expressed in "paired" Xenopus oocytes in order to study connexin function through electric coupling (18), most of the CMT-X mutant isoforms appeared to be non-functional. In addition, when coexpressed with wildtype Cx32, there were dominant-negative effects, but the relevance of these data for the *in vivo* situation is difficult to interprete. When Cx32-deficient mice were generated by homologous recombination they revealed no obvious behavioural phenotype (reviewed in 107). However, at older ages they devel-

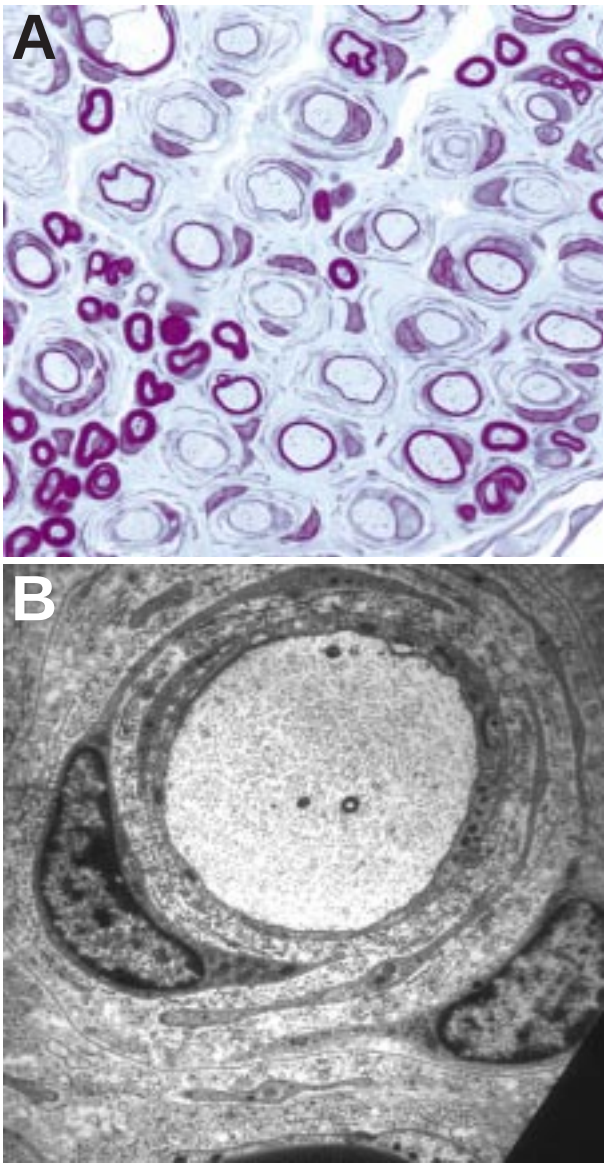


**Figure 2.** Hypomyelination of the peripheral nervous system in the CMT rat (151), a transgenic model of Charcot-Marie-Tooth disease type 1A, in which 1.5-fold overexpression of PMP22 turns the normal gene into disease gene. **A.** Section of the ventral root stained with Toluidine blue. Small-caliber axons are associated with myelin of normal thickness, whereas most large-caliber axons are thinly myelinated and show 'onion bulbs'. **B.** Electronmicrograph of a single 'onion bulb' with redundant basal laminas and collagen deposits. Reproduced with permission from Sereda et al. (151).

oped a progressive peripheral demyelination with typical onion bulb formation, whereas central myelin remained unaffected (6). It is possible that the complete loss of Cx32 causes a rather mild course of disease. Alternatively, all mutations of the Cx32 gene (in mouse and man) are functional *nulls*, but the species differ widely in disease expression. The answer to this question may require the engineering of point mutations into the mouse Cx32 gene.

Taken together, the examples have demonstrated (i) that it is surprisingly difficult to predict the cellular effects of a mutation merely from structural considerations of the affected protein, and (ii) that there may be natural limitations using short-lived mice to fully model human myelin diseases. Although the natural mutants have provided important information about disease mechanisms, it is more difficult than expected to derive the normal cellular function of these myelin proteins from the mutant phenotype.

#### 4. Gene dosage problems

An unexpected finding in human myelin diseases, that was also reproduced in transgenic animals, was the sensitivity of glial cells to abnormal myelin gene dosage, and myelin gene overexpression as a cause of myelinopathies. Although inherited neuropathies have been known for over a century (reviewed in 40), insight into their molecular pathology has been gained only within the last years. A variety of pathogenic mechanisms have been revealed by which mutations in myelin-associated genes can cause a myelinopathy, but the gene dosage model has been most intriguing as it states that the increased (or decreased) expression level of a normal gene is sufficient to turn it into a disease gene. Most carefully studied in this respect are Charcot-Marie-Tooth disease (CMT) and 'hereditary neuropathy with liability to pressure palsies' (HNPP), linked to the PMP22 gene on human chromosome 17, and its corresponding rodent models (165).

The most common inherited peripheral neuropathy, CMT (the prevalence is 1 in 2500), is characterized by a dominant mode of inheritance and, clinically, the progressive atrophy of distal muscles which begins usually within the first two decades of life (40). Nerve conduction velocities (NCV) are mildly to moderately reduced and peripheral nerve biopsies show segmental demyelination and remyelination. This, and Schwann cell proliferation result in hypertrophic changes, known as 'onion bulb' formation (Figure 2). HNPP is also a dominantly inherited demyelinating neuropathy, but with clinical features distinct from CMT. HNPP patients show recurrent episodes of a focal neuropathy at vulnerable sites of nerve compression and mildly reduced NCVs. Hallmark is the appearance of distinctive focal foldings of the myelin sheath, termed 'tomacula'. Dejerine-Sottas-Syndrome (DSS), a neuropathy more severe

than CMT, has an early onset in infancy and is associated with progressive dysmyelination and very slow NCV. Clinically, it may be difficult to differentiate DSS from congenital hypomyelination (CH). All these dys- and demyelinating neuropathies constitute a continuum of disease expression rather than defined disease entities. Mutations affecting either one of three myelin-associated genes account for the majority of cases: the gene for peripheral myelin protein-22 (PMP22; in CMT1A and HNPP); myelin protein zero (MPZ or PO; in CMT1B), and connexin-32 (Cx-32; in CMT-X) (164, 165, 125, 174, 144).

In patients with CMT1A, an interstitial duplication in chromosome 17 accounts for nine out of ten sporadic cases (69). The duplication usually originates from an unequal crossover event between two copies of a repeat sequence, which are spaced in tandem some 1.5 Mb apart from each other. This 'hotspot' of recombination is associated with a transposon-like sequence related to Mariner elements in Drosophila (180, 137). A corresponding loss of the 1.5 Mb region underlies HNPP. In CMT1A, increased steady state levels of PMP22 mRNA have been reported, at least at early stages of the disease, consistent with a 1.5-fold increase in PMP22 gene dosage (189, 65, 81). Biochemically, the amount of PMP22 in compact peripheral myelin is increased in CMT1A and decreased in HNPP (173, 58). However, the increase of PMP22 mRNA and protein is only measurable prior to the onset of disease; later the steady state levels appear normal or even decreased, probably secondary to the demyelination itself. This and other evidence suggests that having only one copy of PMP22 leads to HNPP, two copies are the normal gene dosage, three copies cause CMT1A, and four copies cause a severe DSS-like phenotype, indicating a direct correlation of PMP22 gene dosage with disease severity.

The overexpression (and underexpression) of PMP22 in transgenic rodents causes a phenotype similar to CMT and HNPP, providing formal proof that PMP22 itself is the critical dosage-sensitive gene in the duplicated 1.5 Mb region (1, 72, 151, 2, 103). Indeed, different lines of transgenic mice carrying increasing numbers of the human PMP22 gene (contained in a yeast artificial chromosome) revealed a correlation between the number of transgene copies, the level of PMP22 expression and the clinical phenotype (73). Interestingly, this study revealed a threshold of approximately 2-fold of the normal expression level (i.e. the ratio of mouse:human PMP22 mRNA being 1.0), above which demyelination occured. These results indicate that overexpression of PMP22 mRNA in humans is close to such a threshold level and that the clinical variation in patients could be due to subtle interindividual variations in the expression level.

A line of PMP22-transgenic rats with hypomyelination, onion bulb formation (Figure 2), slowed NCVs, and motor impairment provides a good model for CMT1A caused by a PMP22 gene duplication (151). On closer inspection young transgenic rats also show unmyelinated sciatic nerve axons in the absence of myelin breakdown, which is not a typical feature in humans (but note that human biopsies are usually taken from the sensory suralis nerve only). The rat pathology suggests that the principle disease mechanisms operate, at least in part, prior to myelin assembly. Other fibres, which are initially myelinated, show demyelination with typical signs of myelin breakdown as the disease progresses. Thus, also the CMT rat phenotype constitutes a complex overlay of dys- and demyelination.

When bred to homozygosity, 4-week old transgenic rats completely fail to elaborate myelin, presumably following a further increase in PMP22 expression (a similar dysmyelination has been observed in one line of PMP22-transgenic mice; 103). Interestingly, Schwann cells are arrested abnormally at the 'promyelin' stage, when they are segregated with axonal segments at a 1:1 ratio (151). This pathology resembles congenital amyelination and indicates a very early block in the myelin assembly process. In contrast to the pathology of SCIP-deficient mice (see above), the PMP22-dependent myelination arrest appears not to interfere with the 'molecular' differentiation of the affected Schwann cells, as visualized by the high-level induction of the major myelin genes (S. Niemann, M. Sereda., U. Suter, I. Griffiths, K.-A. Nave, in preparation).

PMP22 loss- and gain-of-function effects are clearly different. Mice homozygous for a PMP22 null allele show a delayed onset of myelination and focal hypermyelination ('tomacula') at young age, followed by a CMT-1 like disease with demyelination, onion bulbs, axonal loss and a strong clinical phenotype (1, 2). Heterozygous PMP22 knock-out mice (which are models for HNPP) demonstrate the expected haploinsufficiency with similar symptoms as found in the human disease. Clearly, the overall defect of PMP22-deficiency is quite different from that of a point mutation in the PMP22 gene of *Trembler* (Tr) mice, as discussed earlier (162). This strongly suggests that the protein structure in this mutant exerts an aberrant dominant-negative effect rather than a simple loss-of-function.

Taken together, Schwann cells are extremely sensitive to PMP22 gene dosage, but it remains elusive what

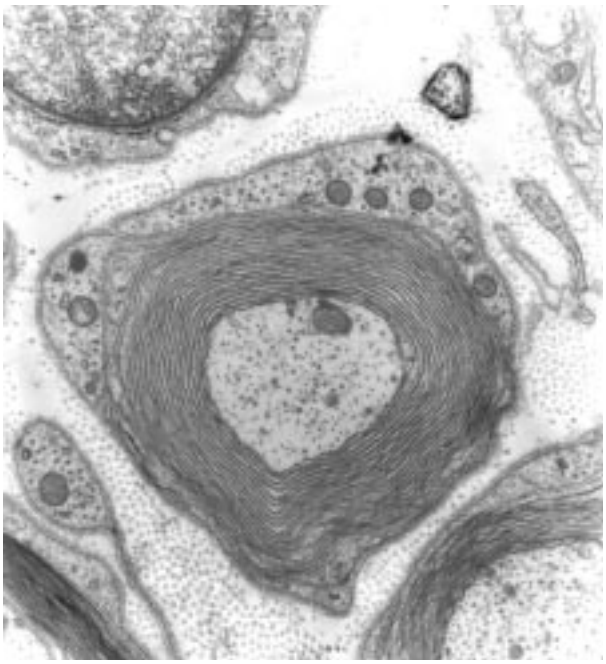

**Figure 3.** Myelin protein P0 is required for the normal compaction of myelin in the peripheral nervous system. Electronmicrograph of a cross-sectioned axon from the sciatic nerve of an adult mouse that is homozygous for the targeted inactivation of the P0 gene (56). The axon is enwrapped by multiple layers of membrane but the periodicity and the spacing of lamellae is altered. Mutations of the human P0 gene underlie Charcot-Marie-Tooth disease type 1B (kindly provided by R. Martini, S. Carenini, and M. Schachner).

mechanisms cause demyelination. It is tempting to speculate that the stoicheometry between PMP22 and other cellular proteins in Schwann cells must be carefully balanced to assemble and maintain the myelin sheath.

Gene dosage effects have also been observed in heterozygous mutant mice with reduced expression of other myelin genes. P0, for instance, is a highly conserved cell adhesion molecule and exclusively expressed by myelinating Schwann cells. It constitutes the major protein component (50%) of peripheral myelin (95). Cristallographic data suggest that myelin membrane adhesion is mediated by the formation of P0 homotetramers within the myelin membrane, which interact in trans with other tetramers of the apposed membrane (152). Not surprisingly, mice with a homozygous null mutation of the P0 gene are severely hypomyelinated, and myelin fails to compact (Figure 3). This and the secondary degeneration of many axons result in a severe peripheral neuropathy beginning at about 3 weeks of age (56). It was interesting to find that also heterozygous mice undergo a mild demyelination, first detectable at about 4 months of age. At that time mice lacking one P0 allele are behaviourally normal but develop a slowly progressive demyelination with onion bulb formation and remyelination, the typical signs of a peripheral neuropathy (106). Thus, P0 haploinsufficiency in mice causes a myelin disease within several months. The question arises whether the autosomal-dominant forms of human CMT1B, when caused by point mutations in the human P0 gene, are likewise caused by the loss of one functional allele, or alternatively by the aberrant gain-of-function of one mutant allele, or both. An interesting case is CMT1B with a P0 "near" null allele (resulting from a severely truncated protein). Patients heterozygous for this mutation show a rather mild neuropathy, wheras their homozygous children (essentially representing a complete null allele) present with a DSS phenotype (174). The authors conclude that the mild forms of CMT1B are associated with the loss of one functional allele and that aberrant gain-of-function effects underlie severe forms of CMT1B, and DSS or CH. Thus, half the normal P0 gene dosage may be sufficient to initiate myelination, but is not enough to maintain myelin in the adult, and there are some obvious differences in the time course of disease between mouse and man (see below). In general, the late-onset demyelination is most obvious in large caliber motor fibres, and less pronounced in the (cutaneous) sensory nerves and dorsal roots, irrespective of the primary genetic defect. Unpublished data strongly suggests that also overexpression of the normal P0 gene is not tolerated (183), a transgenic experiment not known to correspond to a human neuropathy.

In the CNS, oligodendrocytes are sensitive to the dosage of the PLP/DM20 gene. This was first noticed, unexpectedly, when these proteolipids were overexpressed in transgenic mice in which they cause a lethal dysmyelination when homozygous (80, 136). Mice heterozygous for either one of two transgenes described by Readhead et al. appeared normal for several months of age (136) but started to demyelinate at older ages (>1 year). Interestingly, this late-onset disease includes vacuolar spongiform degeneration of the white matter and clear axonal involvement (5). Another line of transgenic mice transmitting 70 copies of a human cDNA for the DM-20 proteolipid was reported to develop a late onset demyelination with features of an inflammatory disease (76, 108) While inflammation was not a feature in the mice expressing genomic transgenes (5), the molecular pathology remains a poorly understood consequence of a very modest proteolipid overexpression. All these findings in mice are in good agreement with a report

showing that a small intrachromosomal duplication of the human Xq21 region (including the PLP locus) causes a complex congenital syndrom including Pelizaeus-Merzbacher disease (28). That duplications of the normal PLP gene underlie other cases of PMD was originally viewed as rare (42), but turned out to be the most common cause of this disease, affecting about 50% of all familial cases (68, 120).

Recently, the gene for 2', 3''-cyclic nucleotide 3'-phosphodiesterase (CNP) was found to be dosage sensitive, at least in the mouse. CNP which is enriched in myelin-forming cells has the enzymatic activity of hydrolysing cyclic nucleotides, but its *in vivo* function is unclear (160). Transgenic overexpression of CNP leads to a lack of myelin compaction in mice, 'redundant' myelin membrane wraps, and intramyelin vacuoles, however in the absence of obvious behavioral abnormalities (61). This novel pathology may reflect aberrant glial differentiation at very early stages of development (188): CNP-transgenic oligodendrocytes seemed to mature earlier than in wildtype cells, at least when monitored by the temporal profile of MBP and PLP gene expression.

Finally, the gene for myelin basic protein (MBP), encoding a major membrane-associated protein of myelin, may also be dosage sensitive. *Shiverer* mice lack MBP due to a deletion of the MBP gene (see below), and they are dysmyelinated throughout the CNS. Whereas heterozygous *shiverer* mutants appear normal with half their MBP gene dosage, humans with a similar condition appear to be less tolerant: patients with a small deletion at the tip of the long arm of chromosome 18 (termed '18q- syndrome'), including the MBP locus, show incomplete myelination in the CNS as visualized by MRI (54). Again, the specific sensitivity to abnormal myelin gene dosage (here: haploinsufficiency) only at more advanced stages of development remains to be explained.

Myelin diseases may have been among the first to reveal the consequences of abnormal gene dosage in a genetically defined system. It is anticipated, however, that some of the basic pathomechanisms, specifically when associated with gene overexpression, may not be specific to glial cells and applicable to other hereditary diseases and aneuploidy syndromes.

### 5. Compensatory mechanisms

As mentioned before, the myelin mutant *shiverer* defines a natural *null* allele of the MBP gene, because none of the six protein isoforms (ranging in size from 14 - 21.5 kd) can be synthesized by oligodendrocytes and Schwann cells (139, 140, 112). In homozygous mutants, the CNS is severely hypomyelinated and the few myelin wraps detectable are generally uncompacted and devoid of their major dense line (141). The introduction of a genomic wild-type MBP-transgene into *shiverer* mice has led to a partial correction of the mutant phenotype with a restoration of the major dense line in myelin (135). This landmark study has been considered direct proof for the presumed role of MBP in the myelin architecture. It also demonstrated that the degree of myelin assembly correlates well with the amount of MBP synthesis (129, 153). It is less well known that areas with compacted CNS myelin of normal thickness and periodicity have also been observed in *shiverer* mice (141) even before the underlying molecular defect was known, raising the question in which way these "exceptional" areas differ from the majority of hypomyelinated CNS regions. It is possible but unproven that other proteins, such as the recently identified 'myelin-oligodendrocyte basic protein' (MOBP), are responsible for myelin heterogenity in *shiverer*-mice. MOBP is a basic soluble protein found in the compacted regions of normal CNS myelin (185). That some proteins of myelin would share a function with MBP was first suggested when the PNS of *shiverer* mice was analyzed but was found to be morphologically unaffected (132, 84). A good candidate to compensate for peripheral MBP function in *shiverer* mice was P0, the major cell adhesion protein of PNS myelin, because the intracellular basic domain of P0 is localized at the major dense line (95). Indeed, mouse mutants double-deficient in both P0 and MBP expression (P0-/- * shi/shi) synthezise a peripheral myelin sheath that lacks the major dense line, whereas P0 -/- mice lack more specifically the 'extracellular' adhesion at the intraperiod line of myelin, as illustrated in Figure 3 (105, 56). Thus, P0 and MBP show a partial overlap in function, and P0 is sufficient to suppress the peripheral myelin pathology in *shiverer* mice. It is interesting that this compensation works in the absence of obvious sequence relationship in the primary structure of both proteins.

Redundancy in function is also the most likely explanation of the rather subtle phenotype of mice lacking the myelin-associated glycoprotein (MAG). This 100 kD membrane protein belongs to the immunoglobulin superfamily of proteins and was strongly implicated in cell-adhesion and axon-glial recognition, due to its very restricted localization at the adaxonal surface of the myelin sheath (104). However, MAG-deficient mice assemble myelin without gross abnormalities (113, 97, 96, 8). It is only at the electron microscopic level that

subtle defects have been observed. These included multiply myelinated axons, redundant myelin loops, and uncompacted regions at axon-glia interfaces ('cytoplasmic collars') (97, 113). In aged MAG mutants (>9 month), some oligodendroglial pathology was noted, consisting of enlarged processes with myelin-like vesicles and amorphous granular inclusions reminiscent of a dying-back oligodendropathy (93). With minor exceptions (see below) also peripheral myelin is assembled normally in the absence of MAG.

In the search for compensatory mechanisms, the neural cell-adhesion molecule N-CAM has been an attractive candidate to substitute for MAG function, at least in the developing PNS. The primary hint was the observation that N-CAM was upregulated in Schwann cells of MAG-deficient mice, and most prominent in myelinated regions where MAG is localized normally (113). However, the morphological analysis of the respective (MAG * N-CAM) double mutant mouse could not confirm this hypothesis: peripheral myelination appeared uncompromised, even though axonal degeneration was noted months earlier than in simple MAG-deficient mice (20). Whether other neural cell adhesion molecules, such as L1, provide the underlying compensatory MAG function remains to be defined. It is also possible that the major function of MAG relates to glia-axonal signalling, rather than to cellular adhesion, and is compensated for by different molecules in glia. Future double mutant mice should help to clarifiy this issue.

### 6. Abnormal myelin lipids

Lipids are by far the most abundant molecules in myelin but experimental genetics has only recently be used to address lipid function. Galactocerebroside (GalC) and its sulfatide are the major myelin lipid components and thought to participate in oligodendrocyte development and protein trafficking. Targeted disruption of the gene encoding the enzyme UDP-galactose:ceramide galactosyltransferase (CGT), which is required for GalC synthesis, creates mice devoid of GalC, sulfatide, and their hydroxylated forms (25, 16). CGT-deficient mice exhibit tremors, mild ataxia, and their life span is reduced to a few months. Ultrastructurally, normal myelin is assembled but a progressive demyelination causes significant degeneration and vacuolization of white matter areas in mutant CNS (25). Moreover, GalC-deficient myelin is instable and tends to split along the intraperiod line during fixation, similar to CNS myelin in PLP-deficient mice. Nerve conduction velocities are already decreased in younger mutants (in the presence of myelin) and decline further as these mice age. The molecular mechanisms causing the virtual loss of rapid impulse propagation are not fully understood and there is no simple correlation with the loss of myelin. A more detailed analysis revealed paranodal abnormalities, reflected by numerous heminodes and the abscence of the electron dense 'transverse bands' that may represent axo-glial junctions (39). The disruption of axonal-glial communication in the paranodal regions may explain some of the electophysiological deficits. A more severe dysmyelinated phenotype may have been prevented by compensatory mechanisms, including the presence of the alternate glycoconjugate glucosyl-ceramide (GlcC instead of GalC). Abscence of GalC may be tolerated better in the PNS than in the CNS, although there are conflicting reports as to the physiological consequences (17). The CGT-deficient mouse must be considered an orphan model, because at present no human disease is known to be linked to this gene.

### 7. Glial - axonal communication

Myelin assembly is preceded by a bi-directional communication between axons and glial cells (36, 27, 177), and some interactions are maintained throughout life. In peripheral neuropathies, axonal involvement is ultimately responsible for most of the impairment of motor functions, independently of whether the disease is primarily glial in origin (as in CMT-1) or axonal (CMT-2). The targeted inactivation of myelin-specific genes in mice has also revealed surprising impairments of axonal structure and function, suggesting that the extent and significance of normal glia-to-axon communication has been underestimated.

In development, the first known interaction between axons and their associated glial cells is mediated by axonal neuregulins and their glial receptors. The latter are a family of tyrosine kinases (erbB2, -3 and -4), distantly related to the EGF receptor, which provide a signalling network also in non-neural tissues. On the ligand side, three genes are known to encode neuregulins and give rise to several alternatively spliced transcripts (126, 22, 21, 190). Neuregulin-1 (also called ARIA, GGF, heregulin or NDF) has three major splice isoforms (termed neuregulin-1-I, -II, and -III) which are all potent mitogens and differentiation factors for epithelial, muscle, and neural cells *in vitro*. This view was corroborated by the analysis of mice with an inactivated neuregulin-1 gene (109). Although these mutants die already *in utero* (with a lethal failure of heart development), a role of neuregulin-1 in PNS development was indicated

because the number of Schwann cell precursors in sciatic nerves was severely reduced when compared to wild-type controls. It remains to be determined whether fewer stem cells have been destined to become Schwann cells or whether the proliferation rate of the committed Schwann cells is abnormally low. Recently, the Schwann cell promoting activity was assigned to the neuregulin-1-III isoform, as transgenic mice that only express this variant (thereby lacking type I and II) develop a normal number of Schwann cell precursors (110).

The biological effects of neuregulins depend on the receptor(s) expressed by the target cell. Which are the relevant receptors for myelin-formimg glial cells? Based on the expression pattern, it was assumed that erbB3 receives the neuregulin-1 signal on Schwann cells, whereas erbB4 performs a similar role on oligodendroglial cells. Both erbB3 and erbB4 are active only after heterodimerization with erbB2, a tyrosine kinase lacking an own receptor domain (reviewed in ref. 4). Mice lacking expression of erbB3 are deficient for Schwann cell precursors and Schwann cells (Figure 4). Moreover, as a secondary effect (most likely not mediated by neuregulins), initially established peripheral axonal connections (motor and sensory) degenerate, confirming bi-directional communication between neuronal and glial cells in the developing PNS (138). The molecular nature of this axonal 'trophic' support by cells of the Schwann cell lineage awaits to be identified.

To understand the role of neuregulins and their receptors for oligodendrocytes and their precursor cells, mice lacking erbB4 were generated (53). These mutants showed early neuronal defects in the CNS but died too early to show defects in neuron-to-oligodendrocyte communication. Because all mice with inactivated neuregulin receptors die *in utero*, the further analysis of glial cell development requires the reinvestigation in 'conditional' knock out mice bypassing the heart failure.

To enable vertebrate axons for rapid saltatory conduction of nerve impulses, voltage dependent ion channels must be clustered. Initially spread uniformly along axons, clustering gives rise to the regularly spaced, electrically active nodes of Ranvier. As this molecular rearrangement of channel proteins is closely interrelated with myelination itself, it has been speculated to be also a function of glial cells. For many years, MAG has been a good candidate to drive axonal channel proteins away from the myelinated internodal region. However, when the PNS of transgenic mice lacking MAG was analyzed, the membrane distribution of sodium channels appeared unaltered (171). In contrast, CNS axons of *shiverer* mice (lacking MBP expression) showed sodium channels

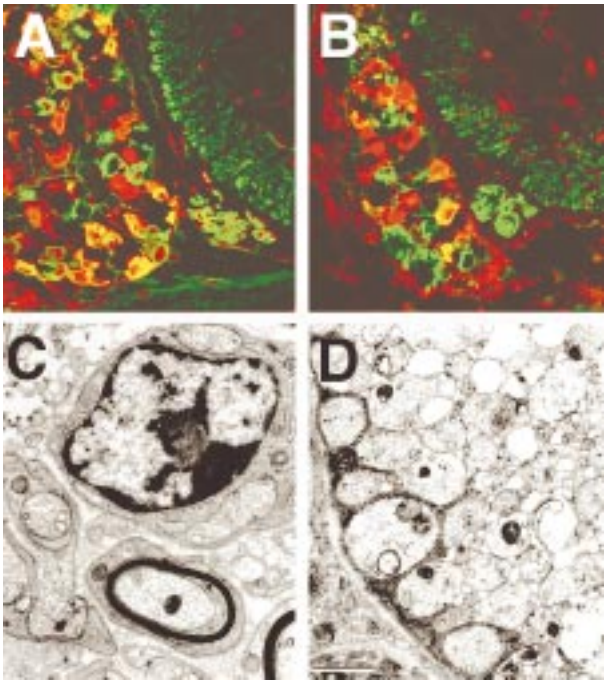


**Figure 4.** The glial erbB3 receptor and its axonal ligand neuregulin-1 comprise an essential signalling system in the developing PNS. Spinal cord and nerve roots from mouse embryos with a mutation of the erbB3 gene lack Schwann cell precursors in the developing motor and sensory fibre tracts (138). **A**, **B.** Embryonic DRG neurons (left half in **A** and **B**) are shown in close proximity to the spinal cord (right half) and ventral roots (lower right) of normal mice (in **A**) and mutant mice lacking erbB3 (in **B**). The staining of neurofilaments (in green) and the Schwann-cell marker S100 (in red) reveals that CNS and DRG appear normal, but that nerves of the ventral root are not associated with Schwann cells. **C**, **D.** Electron micrographs of the ventral roots of E18.5 mice. Heterozygous mice (in **C**) show Schwann cells associated with axons and elaborating compacted myelin. Mice homozygous for the erbB3 mutation (in **D**) have tightly packed axons without contact to Schwann cells. Reproduced with permission from Riethmacher et al. (1997)

unclustered on dysmyelinated axons, indicating that oligodendrocytes are involved in axonal sodium channel distribution (123). Another line of evidence came from a cell culture system, when Kaplan et al. found that retinal ganglion cells develop axonal clusters of sodium channels (including interacting cytoskeletal protein ankyrin-G) only when cultured in the presence of oligodendrocytes or oligodendrocyte-conditioned medium (82). Once sodium channel clusters were formed, their maintenance was independent of oligodendroglial support, at least for two weeks *in vitro*, presumably because underlying cytoskeletal elements kept channel proteins from redistributing. Whether a similar mechanism operates in the PNS is unknown, and the diffusible glia-derived factor remains to be defined at the molecuar

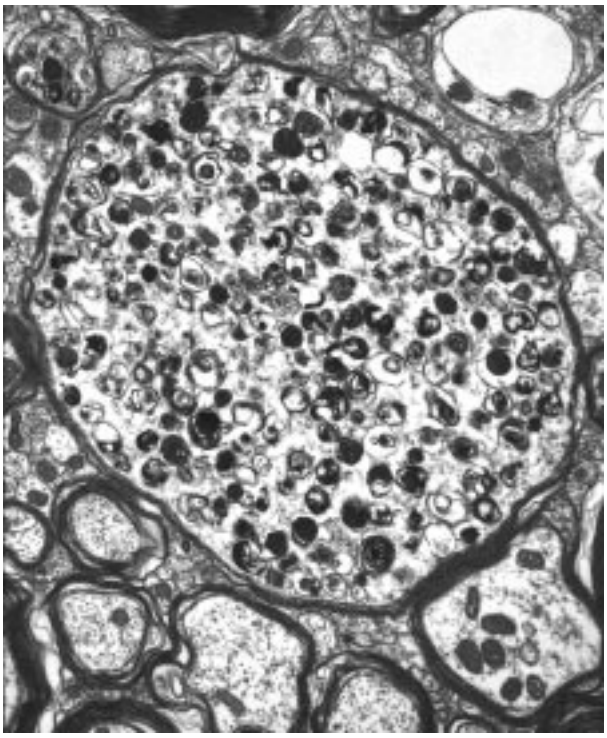

**Figure 5.** Lack of PLP/DM20 from CNS myelin has profound effects on the integrity of myelinated axons in older mice (63). Depicted in this electron micrograph is a cross-section of the optic nerve, showing a large axonal swelling filled with accumulated membranous organelles. Such swellings are most likely caused by the local break-down of axonal transport and result, some time later, in the degeneration of the affected axons.

level.

A structural role of MAG in axonal-glial adhesion was less obvious than expected (97, 113, 20), but long-term observations of MAG-mutant mice identified onion-bulb formations in the PNS, paranodal myelin tomaculi, and degenerating axons, suggestive of a mild peripheral neuropathy (49. 64, 107). The re-investigation of mice lacking MAG also led to the idea that MAG is involved in glia-to-axon communication thereby regulating stuctural features of the myelinated axons, at least in the PNS (187). A principal observation was the reduced phosphorylation of neurofilaments (NF-M and NF-H), resulting in an increased neurofilament density, and consequently a reduced diameter of myelinated PNS axons.The expression level of NF-L and NF-M was also decreased (by Western blot analysis of sciatic nerve homogenates). Although it is likely that MAG itself mediates the underlying glia-to-axon communication (the axonal 'MAG receptor' is still undefined), it can not be excluded that some of the downstream effects are indirect and involve other glial cell surface molecules.

Transgenic experiments have also revealed an unexpected role of proteolipid protein in the communication between oligodendrocyte and axon. From what was known about the natural PLP mutations in mouse and man (discussed above), it was quite unexpected that oligodendrocytes with a targeted disruption of the PLP gene assemble CNS myelin as an apparently normal multilayered structure. PLP-deficient oligodendrocytes enwrap axons of all calibers and only high-power magnification has revealed that the compacted extracellular surfaces of myelin appear more condensed when compared to wildtype (86). Moreover, a reduced physical stability of myelin leads to an artefactual delamination (13), but this is far away from the fatal consequences of dysmyelination that results when misfolded proteolipids are expressed. Instead, PLP and DM20 may play a role in maintaining axonal integrity. After myelination has been completed in PLP-deficient mice, axons throughout the CNS show abnormal swellings filled with membranous dense bodies, mitochondria, and phosphorylated neurofilaments (63), suggesting an impaired axonal transport (Figure 5). As these accumulations occur predominantly (but not exclusively) in the distal paranode, the underlying defect may be an impairment of retrograde axonal transport. These swellings and the subsequent degeneration of axons are not a feature of unmyelinated fibers, indicating that axons become 'dependent' on oligodendrocytes some time after myelin ensheathment (63). Speculations on the mechanisms are guided by two observations. First, the axonal support appears to be locally restricted. This is indicated by female mice heterozygous for the PLP *null* allele which is randomly X-inactivated in oligodendrocytes. In these chimeras, wildtype oligodendrocytes of the optic nerve are not able to support axons segments ensheathed by mutant oligodendrocytes, i.e. along their entire length. Second, biophysical data suggest that proteolipids form ionic pores in a lipid bilayer (33, 34, 85). This suggests a novel role of PLP in direct axon-glial signalling or in maintaining the microenvironment of myelinated CNS axons.

The cytoskeletal organization of myelinated axons depends, among other factors, on the controlled expression of neurofilaments and their pattern of phosphorylation (reviewed in ref. 184). The latter is fine-tuned by axonal protein kinases and phosphatases, which in turn are regulated by various signalling cascades. A growing number of membrane proteins has been implied in the communication between axons and their associated glial cells. In addition to the examples already discussed,

these include 'classical' cell adhesion molecules, such as L1 (70, 79, ), growth factors such as IGF-1 (9, 186), basic FGF (66) or Reg-2 (99), the glial receptor-type protein-tyrosin phosphatase RPTP-β (99, 19), the mammalian neurexin-IV-homologue Caspr (for contactin-associated transmembrane receptor) (41), and contactin/F3 itself (133, 89). It is anticipated that transgenic techniques will help to clarify the function of these proteins in axon-glia interaction and within the concert of others. The research focus of how myelin-forming cells and target axons interact *in vivo* has slightly shifted from myelination to general aspects of cellular communication. These questions are clearly more difficult to answer, but we speculate that some of the unclassified neuropathies relate to proteins that contribute to long-term glial-axonal communication.

### 8. *In vitro* studies

The function of myelin genes should be tested, whenever possibe, in the living organism, but often times *in vitro* experiments (referring to myelin genes experimentally expressed in non-glial cells) allow to ask specific questions that are very difficult to study *in vivo*, usually at the protein level. Here, we focus on experiments in which "transgenic" cells were used to investigate some principal properties and structure-function relationship of myelin proteins and their mutant isoforms.

A good example for studying myelin protein function *in vitro* is protein zero (P0), the major integral membrane protein of PNS myelin. When cDNAs encodig P0 were transfected into fibroblasts, the immunoreactive protein accumulated at contact sites of neighboring cells, allowing the formation of junctional zones reminiscent of the intraperiod line in myelin (29). At the same time, the self-adhesive properties of P0 were demonstrated in CHO cells which aggregated in suspension when overexpressing P0 at their cell surface (44). Using this aggregation assay, the expression of various mutant isoforms was compared and it could be shown that the integrity of both the extracellular and cytoplasmic domains of P0, as well as its glycosylation sites is required for efficient homophilic binding (45, 181, 191). These data, when combined with X-ray crystallography of P0 (152) and the analysis of mutant mice (56), give rise to the current model of P0 as a tetrameric membrane protein that binds *in trans* to another P0 complex on the adjacent myelin membrane. The basic cytoplasmic domain of P0 binds to a phospholipid bilayer *in vitro* (35), supporting the concept that P0 also contributes to the major dense line *in vivo* (132, 84). What can fibroblasts reveal about CMT1B and autosomal-dominant mutations of the human P0 gene? Coexpression of wild-type P0 and C-terminally truncated isoforms interfered with cell aggregation, even though the wild-type protein was delivered to the cell surface (182). This suggests that mutations of P0 can act in a dominant-negative fashion also within myelin, at least in the severe forms of CMT1B and DSS. As discussed before, milder courses of disease may be due to the mere loss of one functional P0 allele (haploinsufficiency).

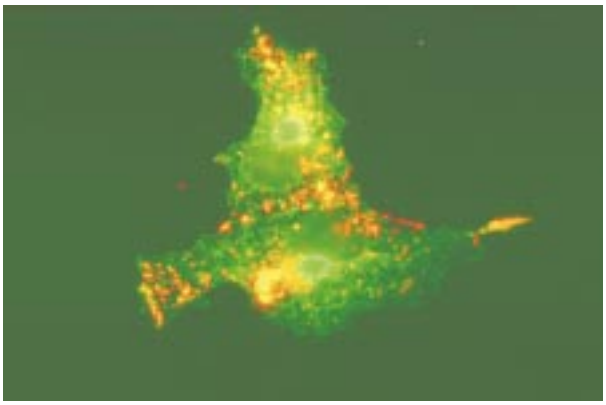

**Figure 6.** A conformation-sensitive epitope of PLP, termed O10, has been used to reveal protein misfolding by amino acid substitutions which cause dysmyelination in mouse and man (78). Shown in this two-color overlay is the distribution of wild-type PLP in transfected COS-7 cells, first localised by the monoclonal antibody O10 that recognizes the properly folded PLP (in red), and double-stained with a polyclonal antibody against the carboxyl-terminus of PLP (in green). Note that O10 immunoreactivity appears to be restricted to the trans-Golgi network, whereas full-length PLP polypeptides are also abundant in the endoplasmic reticulum. Superimposition of images yields an orange-yellow color. Most likely, emergence of the O10 epitope is associated with a post-translational step of protein maturation, possibly the oligomerisation of PLP. Mutant forms of the protein which cause dysmyelination *in vivo* lack the O10 epitope.

Proteolipid proteins were studied following ectopic expression in heterologous cells, in order to better understand the subcellular disease mechanism of the natural mutants. Gow et al. first described that PLP and DM20, when cloned from mutant mice or patients with PMD, is largely retained in the endoplasmic reticulum of transiently transfected COS-7 cells (57). This transportation-arrest is caused by protein misfolding (78, 58), as shown directly by the loss of the conformation-sensitive O-10 epitope of PLP and DM20 (Figure 6). Moreover, DM-20 carrying single residue substitutions associated with a mild phenotype *(rumpshaker*; spastic paraplegia type-2) has a greater probability to reach the cell surface Thus, the degree of ER-retainment in transfected cells correlates with the severity of a clinical pheno-

type *in vivo* (59, 169). Depending on the molar ratio of two cDNA expression vectors, when cotransfected into COS-7 cells, misfolded proteolipids also effect the intracellular distribution of the wild-type isoforms (77, 59), demonstrating homophilic interactions of proteolipid proteins, possibly by oligomerization. Different from P0 (and in agreement with myelination in knock-out mutants), ectopically overexpressed PLP is not a strongly self-adhesive molecule (155).

Clearly, COS-7 cells and other cell lines tolerate overexpression of PLP and the accumulation of misfolded proteolipids at a level that causes oligodendrocyte death. Since the intracellular accumulation of PLP has been shown for mutant oligodendrocytes *in vivo* (142, 60), an "ER-overload response" could be the apoptotic trigger. Alternatively, as a quite unspecific side effect, the PLP accumulation in the secretory pathway may reduce the normal transport of other proteins to the cell surface, such as growth factor receptors, and contribute indirectly to increased cell death. Such an effect would be expected stronger for primary cells than for transformed cells that resist apoptosis.

PLP shows many parallels with the peripheral myelin protein PMP22 which has also been studied *in vitro*, in order to understand the molecular mechanisms preceding demyelination in CMT disease. PMP22 is a glycoprotein and, like PLP/DM20, a tetraspan membrane protein. The latter was by itself supported in *in vitro* studies, employing epitope-tagged versions of PMP22 overexpressed in transfected fibroblasts (30). In transfected fibroblasts, mutant isoforms of PMP22 (e.g. those cloned from *Trembler* mice) are efficiently retained in the ER, suggesting that mutations either interfere with normal intracellular trafficking (119, 32), or that a normal retention of the unfolded PMP22 is not overcome. In fact, most of the defective protein enters an endosomal-lysosomal pathway (124), at least in Schwann cells. Similar to the behaviour of proteolipids, it was observed that mutant PMP22 also arrests the normal trafficking of wild-type PMP22 out of the ER of COS-7 cells (119). However, in transfected Schwann cells neither PMP22 from *Trembler* (G150D) nor from *Trembler-J* mice (L16P) was able to block the transport of the wildtype protein completely (32).

Gene dosage effects are more difficult to model *in vitro*. Schwann cells obtained from newborn PMP22-overexpressing transgenic rats (151) have rather normal growth characteristics *in vitro*. However, Schwann cells from newborn rats forced to overexpress cloned PMP22 cDNAs following retroviral infection show a significant reduction of cell growth (193). High-level overexpression of wildtype (but not mutant) PMP22 in 3T3 fibroblasts induces apoptosis (43). Whether or not these observations are directly relevant for the disease mechanism operating in human Schwann cells (following 1.5-fold PMP22 overexpression) remains to be determined.

Taken together, ectopic expression of myelin membrane proteins is easily achieved in heterologous cells and allows the mapping of functional domains of a protein (i.e. for adhesion) or to visualize protein misfolding by the impairment of its intracellular transport. However, there are clear limitations to the use of hetrologous cells, and cell surface expression of a myelin protein in fibroblasts is not equal to the same protein entering the myelin compartment (and vice versa). These simplified systems will continue to be useful for monitoring protein behaviour in the context of mutations, and specific protein interactions. However, they are likely to be insufficient models for "myelin-specific" events, such as directed protein trafficking within a polarized cell or vesicle fusion with the growing myelin membrane.

### 9. Limitations of mouse models

The amino acid sequence of PLP is 100% conserved between humans and mice, but the effects of a PLP null mutation appear surprisingly different when analyzed in these species. Whereas mice have a normal motor development until adulthood in the complete absence of PLP expression (86), it is clear that some human patients diagnosed clinically with PMD and abnormal motor development carry a mutation that is equivalent to a *null* allele (134, 156, 51). These patients which are more mildly affected than classical PMD cases (similar to SPG-2) also develop a peripheral neuropathy resembling CMT1, whereas there is no peripheral myelin involvement obvious in the PLP-deficient mouse (14, 86, 63). Are there fundamental differences between rodent and human myelin? The biochemical composition is nearly identical and it is unlikely that PLP, being so highly conserved in evolution, serves different functions in mammalian species. More likely, PLP is essential for a long-term stability of the myelin architecture that relates to age. Mice assemble myelin within only 1-2 weeks and are "aged" animals within 1-2 years. Myelination in humans continues for several years, and myelin architecture must be preserved for decades. We hypothesise that in the absence of proteolipids, the myelin sheath is at a higher risk of premature breakdown and will degenerate within a certain time. Such a disease model assumes that a fixed rate of progression has features of a developmental disorder in children (PMD/SPG-2) as it interferes with normal motor devel-

opment, whereas mice are too short -lived to even show this pathology (instead the CNS axonal dystrophy is most prominent; Figure 5). In contrast, the "developmental" PLP defects in the connatal form of human PMD are adequately modeled in *jimpy* mice: the childhood disease which progresses over 2-3 years is "squeezed" into the first postnatal weeks of the mouse.

A similar model may explain why it has been difficult to generate a genuine mouse model for adrenoleukodystrophy, a lipid storage disorder that affects CNS myelin. The X chromosome-linked ALD gene (X-ALD) was identified by positional cloning and found to encode a peroxisomal membrane protein (116, 117). By structural homology, the adrenoleukodystrophy-protein (termed ALDP) is a member of the superfamily of ATP-binding cassette (ABC) transporters, and expressed in a variety of cell types (100) including oligodendrocytes (47). Biochemically, the peroxisomal beta-oxidation of very long-chain fatty acids (VLCFA) is defective in ALD patients. This causes the accumulation of VLCFAs, and frequently adrenal insufficieny and progressive CNS demyelination (for review see 115, 131), but the relationship of the lipid storage disorder and myelin degeneration is not fully understood. Possibly, VLCFA deposition alters the biophysical properties of membranes and myelin stability (7), but experimental data are lacking. To establish a mouse model and address this question directly, several groups have generated ALDP-deficient mice (46, 88, 101). The biochemical analyses of these mutants, which represent *null* alleles, demonstrated the expected impairment of peroxisomal beta-oxidation. However, none of the animals developed neurological symptoms (within 15 months of age; Forss-Petter et al. unpublished observation), nor were any lesions in CNS myelin apparent, the hallmark of human ALD. Thus, the much needed animal model for the test of experimental treatments is still lacking (although ALDP-knockout mice could be used for the experimental treatment of the VLCFA storage disorder). It is possible that 'timing' is again a critical parameter, and that the onset of myelin degeneration (in humans at the age of 4-5 years) is not any earlier in mice. One alternative explanation is that the functional compensation of ALDP differs in mouse and man, because the spatio-temporal expression of ALDP and two related peroxisomal transporters is not the same. Another alternative to be considered is the action of modifier genes.

#### 10. Modifier genes and epigenetic factors

Some disease genes, such as PLP and ALDP, are associated with monogenic leukodystrophies in humans, but the affected patients show a remarkable degree of clinical variability. In fact, each disease has such a spectrum of clinical expression that it was classified by different names. For the PLP gene, these are Pelizaeus-Merzbacher disease (PMD) and X-linked spastic paraplegia type 2 (SPG-2) (reviewed in ref. 120). For the ALDP gene, these are the severe cerebral adrenoleukodystrophy in childhood and the non-lethal, adult-onset adrenomyeloneuropathy (AMN) (7). Point mutations affecting the same nucleotide have been associated with very different degrees of neurological involvement, often times within an affected family, suggesting that the genotype-phenotype correlation is more complex (120, 115).

Some of these phenomena have been also observed in mice. The spontaneous mouse mutant *rumpshaker* carries a point mutation in exon 4 of the PLP gene, but when compared to other natural mutations of this gene, the phenotype is very mild and the lifespan is normal (146). Surprisingly, upon crossing this mutation from the original genetic background (a C3H x 101 hybrid) into the strain C57BL/6, the behavioural phenotype became more dramatic and included seizures and premature death as early as postnatal day 40 (M. Klugmann, A. Schneider, I. Griffiths, K.-A.Nave, unpublished observation). Most likely, it is the presence or absence of a specific allele of one or more other genes, which are not linked to the disease gene but which influence disease progression as "modifier genes." These genes have not been identified, but good candidate genes are those associated with the transcriptional activation of the PLP gene or the seizure susceptibility of mice.

Also for X-ALD, there is no simple correlation between the type of a mutation and the severity of the neurological phenotype. Specific point mutations in the ALDP gene may give rise to demyelinating ALD, AMN, or the absence of any neurological symptoms (10, 111). In general, the endocrinological abnormalities of X-ALD are independent of the development of leukodystrophy. In one study of 55 patients, no correlation was found between the mutation type and the reduction of cortisol synthesis (91). The inability to reproduce a cerebral demyelination phenotype in ALDP knock-out mice (a 129/Sv x C57BL6 hybrid) could result form the "wrong" set of modifier genes. This may be a principal problem of the mouse species or one that can be solved by systematic cross-breeding of the mutant allele into different mouse strains (see also ref. 48)

In addition to the effect of modifier genes, myelin diseases have also revealed clear "epigenetic effects," and the clinical variability is sometimes not attributable to genetic differences. In X-ALD, this phenomenon is supported by a case report of monozygotic twins (90).

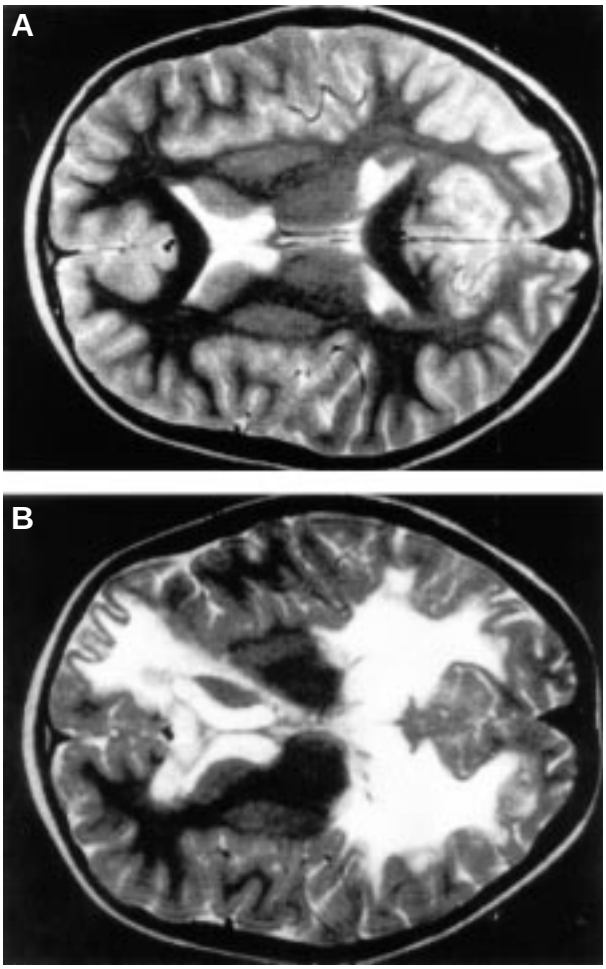


**Figure 7.** 'Epigenetic factors' are molecularly still undefined, but influence disease expression in X-linked adrenoleukodystrophy and other myelin disorders. Shown are the MRI images from two monozygotic twins with a mutation in the X-ALD gene. Although both brothers share the mutation and all possible modifier genes, a progressive demyelination is found only in one boy (90). However, in both brothers the VLCFA levels were elevated to approximately the same degree. **A.** T2-weighted axial MRI of the 11 year-old boy without clinical signs and normal myelination. **B.** Similar MRI of his twin brother, revealing a severe demyelination in the frontal and parieto-occipital white matter (reproduced with permission from Korenke et al. (90)). Such 'epigenetic effects' may be relevant for future disease prevention and therapy, and are, hopefully, amenable to a systematic analysis in mouse models.

One of two boys showed a severe CNS demyelination and behavioural abnormalities at the age of 11 years when his brother was unaffected, even when analyzed by MRI (Figure 7). A similar situation has been reported in a family with CMT1A, in which one of two identical twins developed a peripheral neuropathy whereas the other was unaffected (52). The molecular basis of

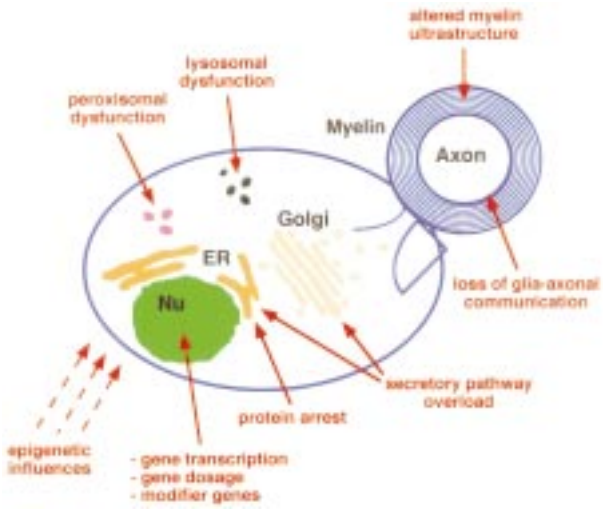


**Figure 8.** Schematic view of a myelin-forming glial cell and its organelles to 'localize' various pathomechanisms identified in inherited myelin diseases. It appears that even minor impairments of basic cellular functions can affect the delicate balance of myelin synthesis and degradation in mouse and man, and can disrupt the long-term stability of the compacted myelin sheath. Not indicated are secondary effects on the integrity of the myelinated axon. For details see text.

these clinical differences is not known, but we anticipate that transgenic mouse models will allow a systematic investigation of some of these epigenetic effects. If successful, these findings may hold a key for human disease prevention and therapy.

### 11. Summary

Dys- and demyelination are the common endpoints of several inherited diseases of glial cells, which elaborate myelin and which maintain the myelin sheath very much like an "external" cellular organelle. Whereas some of the genes that are affected by mutations appear to be glial-specific, other genes are expressed in many cell types but their defect is restricted to oligodendrocytes or Schwann cells. Many of the disease genes and their encoded proteins have been studied with the help of mouse models, and a number of different molecular pathomechanisms have emerged which have been summarized in Figure 8. Some of the new concepts in the field, which have been addressed in this review, have only emerged because similar pathomechanisms were discovered for different myelin proteins. Mouse models have clearly helped to address both, the molecular pathology of myelin diseases and the normal function of myelin genes, but as discussed in this review, these questions turned out to be very different. Despite the progress in understanding the role of the abundant

myelin proteins, there also remain a number of open questions that concern, among other things, the initial axon-glia recognition, the assembly process of the myelin sheath, and the long-term interaction of axons with their myelinating glia. Finally, animal models of human neurological diseases should not be restricted to the study of pathology, but they should also contribute to the development of experimental treatments. It is encouraging that a few attempts have been made.

**Acknowledgement**

We thank J. Rami for excellent secretarial help. Work in the authors' lab was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 317) and the EC Biomed-2 programme.

**References**

1. Adlkofer K, Martini R, Aguzzi A, Zielasek J, Toyka KV, Suter U (1995) Hypermyelination and demyelinating peripheral neuropathy in PMP-22-deficient Mice. *Nature Genet* 11:274-280
2. Adlkofer K, Frei R, Neuberg DH, Zielasek J, Toyka KV, Suter U (1997) Heterozygous peripheral myelin protein 22-deficient mice are affected by a progressive demyelinating tomaculous neuropathy. *J Neurosci* 17:4662-4671
3. Adlkofer K, Naef R, Suter U (1997) Analysis of compound heterozygous mice reveals that the Trembler mutation can behave as a gain-of-function allele. *J Neurosci* Res 49:671-680
4. Alroy I, Yarden Y (1997) The ErbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions. *FEBS Lett* 410:83-86
5. Anderson TJ, Schneider A, Barrie JA, Klugmann M, McCulloch MC, Kirkham D, Kyriakides E, Nave K-A, Griffiths IR (1998) Increased dosage of the proteolipid protein gene causes late-onset neurodegeneration. *J. Comp. Neurol.* 394: 506-519
6. Anzini P, Neuberg DH, Schachner M, Nelles E, Willecke K, Zielasek J, Toyka KV, Suter U, Martini R (1997) Structural abnormalities and deficient maintenance of peripheral nerve myelin in mice lacking the gap junction protein connexin 32. *J Neurosci* 17:4545-4551
7. Aubourg P (1996) X-linked adrenoleukodystrophy. In Moser HW (ed): *Handbook of Clinical Neurology Volume 22: Neurodystrophies and Neurolipidoses.* Amsterdam: Elsevier, pp 447-483
8. Bartsch S, Montag D, Schachner M, Bartsch U (1997) Increased number of unmyelinated axons in optic nerves of adult mice deficient in the myelin-associated glycoprotein (MAG). *Brain Res* 762:231-234
9. Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F (1995) Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. *Neuron* 14:717-730
10. Berger J, Molzer B, Fae I, Bernheimer H (1994) X-linked adrenoleukodystrophy (ALD): a novel mutation of the ALD gene in 6 members of a family presenting with 5 different phenotypes. *Biochem Biophys Res Commun* 205:1638-1643
11. Bergoffen J, Scherer SS., Wang S, Oronzi SM, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. *Science* 262: 2039-2041
12. Bermingham JR Jr, Scherer SS, O'Connell S, Arroyo E, Kalla KA, Powell FL, Rosenfeld MG (1996) Tst-1/Oct-6/SCIP regulates a unique step in peripheral myelination and is required for normal respiration. *Genes Dev* 10:1751-1762
13. Boison D, Stoffel W (1994) Disruption of the compacted myelin sheath of axons of the central nervous system in proteolipid protein-deficient mice. *Proc Natl Acad Sci (USA)* 91: 11709-11713
14. Boison D, Bussow H, D'Urso D, Muller HW, Stoffel W (1995) Adhesive properties of proteolipid protein are responsible for the compaction of CNS myelin sheaths. *J Neurosci* 5:5502-5513
15. Bone LJ, Deschenes SM, Balice-Gordon RJ, Fischbeck KH, Scherer SS (1997) Connexin32 and X-linked Charcot-Marie-Tooth disease. *Neurobiol Dis* 4:221-230.
16. Bosio A, Binczek E, Stoffel W (1996) Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. *Proc Natl Acad Sci (USA)* 93: 13280-13285
17. Bosio A, Bussow H, Adam J, Stoffel W (1998) Galactosphingolipids and axono-glial interaction in myelin of the central nervous system. *Cell Tissue Res* 292:199-210
18. Bruzzone R, White TW, Scherer SS, Fischbeck KH, Paul DL (1994) Null mutations of connexin32 in patients with X-linked Charcot-Marie-Tooth disease. *Neuron* 13:1253-1260
19. Canoll PD, Petanceska S, Schlessinger J, Musacchio JM (1996) Three forms of RPTP-beta are differentially expressed during gliogenesis in the developing rat brain and during glial cell differentiation in culture. *J Neurosci* Res 44:199-215
20. Carenini S, Montag D, Cremer H, Schachner M, Martini R (1996) Absence of the myelin-associated glycoprotein (MAG) and the neural cell adhesion molecule (N-CAM) interferes with the maintenance, but not with the formation of peripheral myelin. *Cell Tissue Res* 287:3-9
21. Carraway KL, Weber JL, Unger MJ, Ledesma J, Yu N, Gassmann M, Lai C (1997) Neuregulin-2, a new ligand of ErbB3/ErbB4-receptor tyrosine kinases. *Nature* 387:512-516
22. Chang H, Riese DJ 2nd, Gilbert W, Stern DF, McMahan UJ (1997) Ligands for ErbB-family receptors encoded by a neuregulin-like gene. *Nature* 387:509-512
23. Chavrier P, Zerial M, Lemaire P, Almendral J, Bravo R, Charnay P (1988) A gene encoding a protein with zinc fingers is activated during G0/G1 in cultured cells. *EMBO J* 7:29-35

24. Chen Y, Medvedev A, Ruzanov P, Marvin KW, Jetten AM (1997) cDNA cloning, genomic structure, and chromosome mapping of the human epithelial membrane protein CL-20 gene (EMP1), a member of the PMP22 family. *Genomics* 41:40-48
25. Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, Suzuki K, Popko B (1996) Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. *Cell* 86: 209-219
26. Coetzee T, Suzuki K, Popko B (1998) New perspectives on the function of myelin galactolipids. *Trends Neurosci* 21:126-130
27. Colello RJ, Pott U (1997) Signals that initiate myelination in the developing mammalian nervous system. *Mol Neurobiol* 15:83-100
28. Cremers FP, Pfeiffer RA, van de Pol TJ, Hofker MH, Kruse TA, Wieringa B, Ropers HH (1987) An interstitial duplication of the X chromosome in a male allows physical fine mapping of probes from the Xq13-q22 region. *Hum Genet* 77:23-27
29. D'Urso D, Brophy PJ, Staugaitis SM, Gillespie CS, Frey AB, Stempak JG, Colman DR (1990) Protein zero of peripheral nerve myelin: biosynthesis, membrane insertion, and evidence for homotypic interaction. *Neuron* 4:449-460
30. D'Urso D, Müller HW (1997) Ins and outs of peripheral myelin protein-22: mapping transmembrane topology and intracellular sorting. *J Neurosci* Res 49:551-562
31. D'Urso D, Schmalenbach C, Zoidl G, Prior R, Muller HW (1997) Studies on the effects of altered PMP22 expression during myelination in vitro. *J Neurosci* Res 48:31-42
32. D'Urso D, Prior R, Greiner-Petter R, Gabreels-Festen AA, Muller HW (1998) Overloaded endoplasmic reticulum-Golgi compartments, a possible pathomechanism of peripheral neuropathies caused by mutations of the peripheral myelin protein PMP22. *J Neurosci* 18:731-740
33. de Cozar M, Lucas M, Monreal J (1987) Ionophoric properties of the proteolipid apoprotein from bovine brain myelin. *Biochem Int* 14:833-841
34. Diaz RS, Monreal J, Lucas M (1990) Calcium movements mediated by proteolipid protein and nucleotides in liposomes prepared with the endogenous lipids from brain white matter. *J Neurochem* 55:1304-1309
35. Ding Y, Brunden KR (1994) The cytoplasmic domain of myelin glycoprotein P0 interacts with negatively charged phospholipid bilayers. *J Biol Chem* 269:10764-10770
36. Doyle JP, Colman DR (1993) Glial-neuron interactions and the regulation of myelin formation. *Curr Opin Cell Biol* 5:779-785
37. Duncan ID, Hammang JP, Trapp BD (1987) Abnormal compact myelin in the myelin-deficient rat: absence of proteolipid protein correlates with a defect in the intraperiod line. *Proc Natl Acad Sci U S A* 84:6287-6291
38. Duncan ID, Hammang JP, Goda S, Quarles RH (1989) Myelination in the jimpy mouse in the absence of proteolipid protein. Glia 2:148-154
39. Dupree JL, Coetzee T, Blight A, Suzuki K, Popko B (1998) Myelin galactolipids are essential for proper node of Ranvier formation in the CNS. *J Neurosci* 18: 1642-1649
40. Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (1993) *Peripheral neuropathy Vol 2.* W.B. Saunders Company, Philadelphia
41. Einheber S, Zanazzi G, Ching W, Scherer S, Milner TA, Peles E, Salzer JL (1997) The axonal membrane protein Caspr, a homologue of neurexin IV, is a component of the septate-like paranodal junctions that assemble during myelination. *J Cell Biol* 139:1495-1506
42. Ellis D, Malcolm S (1994) Proteolipid protein gene dosage effect in Pelizaeus-Merzbacher disease . *Nature Genet* 6: 333-334
43. Fabbretti E, Edomi P, Brancolini C, Schneider C (1995) Apoptotic phenotype induced by overexpression of wild-type gas3/PMP22: its relation to the demyelinating peripheral neuropathy CMT1A. *Genes Dev* 9:1846-1856
44. Filbin MT, Walsh FS, Trapp BD, Pizzey JA, Tennekoon GI (1990) Role of myelin P0 protein as a homophilic adhesion molecule. *Nature* 344:871-872
45. Filbin MT, Tennekoon GI (1993) Homophilic adhesion of the myelin P0 protein requires glycosylation of both molecules in the homophilic pair. *J Cell Biol* 122:451-459
46. Forss-Petter S, Werner H, Berger J, Lassmann H, Molzer B, Schwab MH, Bernheimer H, Zimmermann F, Nave KA (1997) Targeted inactivation of the X-linked adrenoleukodystrophy gene in mice. *J Neurosci Res* 50:829-843
47. Fouquet F, Zhou JM, Ralston E, Murray K, Troalen F, Magal E, Robain O, Dubois-Dalcq M, Aubourg P (1997) Expression of the adrenoleukodystrophy protein in the human and mouse central nervous system. *Neurobiol Dis* 3:271-285
48. Frankel WN (1998) Mouse strain backgrounds: more than black and white. *Neuron* 20:183
49. Fruttiger M, Montag D, Schachner M, Martini R (1995) Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity. *Eur J Neurosci* 7:511-515
50. Gabriel JM, Erne B, Pareyson D, Sghirlanzoni A, Taroni F, Steck AJ (1997) Gene dosage effects in hereditary peripheral neuropathy. Expression of peripheral myelin protein 22 inCharcot-Marie-Tooth disease type 1A and hereditaryneuropathy with liability to pressure palsies nerve biopsies. *Neurology* 49:1635-1640
51. Garbern JY, Cambi F, Tang XM, Sima AA, Vallat JM, Bosch EP, Lewis R, Shy M, Sohi J, Kraft G, Chen KL, Joshi I, Leonard DG, Johnson W, Raskind W, Dlouhy SR, Pratt V, Hodes ME, Bird T, Kamholz J (1997) Proteolipid protein is necessary in peripheral as well as central myelin. *Neuron* 19:205-218
52. Garcia CA, Malamut RE, England JD, Parry GS, Liu P, Lupski JR (1995) Clinical variability in two pairs of identical twins with the Charcot-Marie-Tooth disease type 1A duplication. *Neurology* 45:2090-2093
53. Gassmann M, Lemke G (1997) Neuregulins and neuregulin receptors in neural development. *Curr Opin Neurobiol* 7:87-92

54. Gay CT, Hardies LJ, Rauch RA, Lancaster JL, Plaetke R, DuPont BR, Cody JD, Cornell JE, Herndon RC, Ghidoni PD, Schiff JM, Kaye CI, Leach RJ, Fox PT (1997) Magnetic resonance imaging demonstrates incomplete myelination in 18q-syndrome: evidence for myelin basic protein haploinsufficiency. *Am J Med Genet* 74:422-431

55. Gencic S, Hudson LD (1990) Conservative amino acid substitution in the myelin proteolipid protein of jimpymsd mice. *J Neurosci* 10:117-124

56. Giese KP, Martini R, Lemke G, Soriano P, Schachner M (1992) Disruption of the PO gene in mice leads to abnormal expression of recognition molecules, and degeneration of myelin and axons. *Cell* 71:565-576

57. Gow A, Friedrich VL, Lazzarini RA (1994) Many naturally occurring mutations of myelin proteolipid protein impair its intracellular transport. *J Neurosci* Res 37:574-583

58. Gow A, Gragerov A, Gard A, Colman DR, Lazzarini RA (1997) Conservation of topology, but not conformation, of the proteolipid proteins of the myelin sheath. *J Neurosci* 17:181-189

59. Gow A, Lazzarini RA (1996) A cellular mechanism governing the severity of Pelizaeus-Merzbacher disease. *Nat Genet* 13:422-428

60. Gow A, Southwood CM, Lazzarini RA (1998) Disrupted proteolipid protein trafficking results in oligodendrocyte apoptosis in an animal model of Pelizaeus-Merzbacher disease. *J Cell Biol* 140:925-934

61. Gravel M, Peterson J, Yong VW, Kottis V, Trapp B, Braun PE (1996) Overexpression of 2',3'-cyclic nucleotide 3'-phosphodiesterasein transgenic mice alters oligodendrocyte development and produces aberrant myelination. *Mol Cell Neurosci* 7:453-466

62. Griffiths IR, Scott I, McCulloch MC, Barrie JA, McPhilemy K, Cattanach BM (1990) Rumpshaker mouse: a new X-linked mutation affecting myelination: evidence for a defect in PLP expression. *J Neurocytol* 19:273-283

63. Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 280, 1610-1613.

64. Guenard V, Montag D, Schachner M, Martini R (1996) Onion bulb cells in mice deficient for myelin genes share molecular properties with immature, differentiated non-myelinating, and denervated Schwann cells. *Glia* 18:27-38

65. Hanemann CO, Stoll G, D'Urso D, Fricke W, Martin JJ, Van-Broeckhoven C, Mancardi GL, Bartke I, Müller HW (1994) Peripheral myelin protein-22 expression in Charcot-Marie-Tooth disease type 1a sural nerve biopsies. *J Neurosci Res* 37:654-659

66. Harari D, Finkelstein D, Bernard O (1997) FGF plays a subtle role in oligodendrocyte maintenance in vivo. *J Neurosci Res* 49:404-415

67. Henry EW, Sidman RL (1988) Long lives for homozygous trembler mutant mice despite virtual absence of peripheral nerve myelin. *Science* 241:344-346

68. Hodes ME, Dlouhy SR (1996) The proteolipid protein gene: double, double, ... and trouble. *Am J Hum Genet* 59: 12-15

69. Hoogendijk JE, Hensels GW, Gabreels-Festen AA, Gabreels FJ, Janssen EA, de Jonghe P, Martin JJ, van Broeckhoven C, Valentijn LJ, Baas F, et al. (1992) De-novo mutation in hereditary motor and sensory neuropathy type I. *Lancet* 339:1081-1082

70. Hortsch M (1996) The L1 family of neural cell adhesion molecules: old proteins performing new tricks. *Neuron* 17:587-593

71. Hudson LD, Ko N, Kim JG (1996) Control of myelin gene expression. In: Jessen KR, Richardson WD (eds): *Glial cell development: basic principles and clinical relevance* (Oxford: Bios Scientific Publishers) pp. 101-121

72 Huxley C, Passage E, Manson A, Putzu G, Figarella-Branger D, Pellissier JF, Fontes M (1996) Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum Mol Genet* 5:563-569

73. Huxley C, Passage E, Robertson AM, Youl B, Huston S, Manson A, Saberan-Djoniedi D, Figarella-Branger D, Pellissier JF, Thomas PK, Fontes M (1998) Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. *Hum Mol Genet* 7:449-458

74. Ionasescu VV, Searby CC, Ionasescu R, Chatkupt S, Patel N, Koenigsberger R (1997) Dejerine-Sottas neuropathy in mother and son with same point mutation of PMP22 gene. *Muscle Nerve* 20:97-99

75. Jaegle M, Mandemakers W, Broos L, Zwart R, Karis A, Visser P, Grosveld F, Meijer D (1996) The POU factor Oct-6 and Schwann cell differentiation. *Science* 273:507-510

76. Johnson RS, Roder JC, Riordan JR (1995) Over-expression of the DM-20 myelin proteolipid causes central nervous system demyelination in transgenic mice. *J Neurochem* 64:967-976

77. Jung M, Schneider A, Nave KA. (1995) Dominant-negative action of mutations in the PLP/DM-20 gene and direct interaction of PLP polypeptides in vivo. *J Neurochem* 64: S101.

78. Jung M, Sommer I, Schachner M, Nave KA (1996) Monoclonal antibody O10 defines a conformationally sensitive cell- surface epitope of proteolipid protein (PLP): evidence that PLP misfolding underlies dysmyelination in mutant mice. *J Neurosci* 16:7920-7929

79. Kadmon G, Altevogt P (1997) The cell adhesion molecule L1: species- and cell-type-dependent multiple binding mechanisms. *Differentiation* 61:143-150

80. Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, Aruga J, Okano H, Mikoshiba K (1994) Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. *Neuron* 13: 427-442

81. Kamholz J, Shy M, Scherer S (1994) Elevated expression of messenger RNA for peripheral myelin protein 22 in biopsied peripheral nerves of patients with Charcot-Marie-Tooth disease type 1A. *Ann Neurol* 36:451-452

82. Kaplan MR, Meyer-Franke A, Lambert S, Bennett V, Duncan ID, Levinson SR, Barres BA (1997) Induction of sodium channel clustering by oligodendrocytes. *Nature* 386:724-728

83. Kioussi C, Gross MK, Gruss P (1995) Pax3: a paired domain gene as a regulator in PNS myelination. *Neuron* 15:553-562

84. Kirschner DA, Ganser, AL (1980) Compact myelin exists in the absence of basic protein in the shiverer mutant mouse. *Nature* 283: 207-210

85. Kitagawa K, Sinoway MP, Yang C, Gould RM, Colman DR (1993) A proteolipid protein gene family: expression in sharks and rays and possible evolution from an ancestral gene encoding a pore-forming polypeptide. *Neuron* 11:433-448

86. Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA (1997) Assembly of CNS myelin in the absence of proteolipid protein. *Neuron* 18:59-70

87. Kobayashi H, Hoffman EP, Marks HG (1994) The rumpshaker mutation in spastic paraplegia. *Nat Genet* 7:351-352

88. Kobayashi T, Shinnoh N, Kondo A, Yamada T (1997) Adrenoleukodystrophy protein-deficient mice represent abnormality of very long chain fatty acid metabolism. *Biochem Biophys Res Commun* 232:631-636

89. Koch T, Brugger T, Bach A, Gennarini G, Trotter J (1997) Expression of the immunoglobulin superfamily cell adhesion molecule F3 by oligodendrocyte-lineage cells. *Glia* 19: 199-212

90. Korenke GC, Fuchs S, Krasemann E, Doerr HG, Wilichowski E, Hunneman DH, Hanefeld F (1996) Cerebral adrenoleukodystrophy (ALD) in only one of monozygotic twins with an identical ALD genotype. *Ann Neurol* 40:254-257

91 Korenke GC, Roth C, Krasemann E, Hufner M, Hunneman DH, Hanefeld F (1997) Variability of endocrinological dysfunction in 55 patients with X-linked adrenoleucodystrophy: clinical, laboratory and genetic findings. *Eur J Endocrinol* 137:40-47

92. Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M (1998) Sox10, a novel transcriptional modulator in glial cells. *J Neurosci* 18:237-250

93. Lassmann H, Bartsch U, Montag D, Schachner M (1997) Dying-back oligodendrogliopathy: a late sequel of myelin-associated glycoprotein deficiency. *Glia* 19:104-110

94. Lees MB, Brostoff SW (1984) Proteins of myelin. In: *Myelin* (Ed. Morrell P) 197-224, Plenum Press, New York

95. Lemke G, Axel R (1985) Isolation and sequence of a cDNA encoding the major structural protein of peripheral myelin. *Cell* 40:501-508

96. Li C, Trapp B, Ludwin S, Peterson A, Roder J (1998) Myelin associated glycoprotein modulates glia-axon contact in vivo. *J Neurosci* Res 51:210-217

97. Li C, Tropak MB, Gerlai R, Clapoff S, Abramow-Newerly W, Trapp B, Peterson A, Roder J (1994) Myelination in the absence of myelin-associated glycoprotein. *Nature* 369:747-750

98. Lipsitz D, Goetz BD, Duncan ID (1998) Apoptotic glial cell death and kinetics in the spinal cord of the myelin-deficient rat. *J Neurosci* Res 51:497-507

99. Livesey FJ, O'Brien JA, Li M, Smith AG, Murphy LJ, Hunt SP (1997) A Schwann cell mitogen accompanying regeneration of motor neurons. *Nature* 390:614-618

100. Lombard-Platet G, Savary S, Sarde CO, Mandel JL, Chimini G (1996) A close relative of the adrenoleukodystrophy (ALD) gene codes for a peroxisomal protein with a specific expression pattern. *Proc Natl Acad Sci U S A* 93:1 265-1269

101. Lu JF, Lawler AM, Watkins PA, Powers JM, Moser AB, Moser HW, Smith KD (1997) A mouse model for X-linked adrenoleukodystrophy. *Proc Natl Acad Sci (USA)* 94:9366-9371

102. Lupski JR (1998) Charcot-Marie-Tooth disease: lessons in genetic mechanisms. *Mol Med* 4: 3-11.

103. Magyar JP, Martini R, Ruelicke T, Aguzzi A, Adlkofer K, Dembic Z, Zielasek J, Toyka (1997) Impaired differentiation of Schwann cells in transgenic mice with increased PMP22 gene dosage. *J Neurosci* 16:5351-5360

104. Martini R, Schachner M (1986) Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. *J Cell Biol* 103:2439-2448

105. Martini R, Mohajeri MH, Kasper S, Giese KP, Schachner M (1995a) Mice doubly deficient in the genes for P0 and myelin basic protein show that both proteins contribute to the formation of the major dense line in peripheral nerve myelin. *J Neurosci* 15:4488-4495

106. Martini R, Zielasek J, Toyka KV, Giese KP, Schachner M (1995b) Protein zero (P0)-deficient mice show myelin degeneration in peripheral nerves characteristic of inherited human neuropathies. *Nature Genet* 11:281-286

107. Martini R, Schachner M (1997) Molecular bases of myelin formation as revealed by investigations on mice deficient in glial cell surface molecules. *Glia* 19:298-310

108. Mastronardi FG, Ackerley CA, Arsenault L, Roots BI, Moscarello MA (1993) Demyelination in a transgenic mouse: a model for multiple sclerosis. *J Neurosci Res* 36:315-324

109. Meyer D, Birchmeier C (1995) Multiple essential functions of neuregulin in development. *Nature* 378:386-390

110. Meyer D, Yamaai T, Garratt A, Riethmacher-Sonnenberg E, Kane D, Theill LE, Birchmeier C (1997) Isoform-specific expression and function of neuregulin. *Development* 124:3575-3586

111. Milner RJ, Lai C, Nave KA, Lenoir D, Ogata J, Sutcliffe JG (1985) Nucleotide sequences of two mRNAs for rat brain myelin proteolipid protein. *Cell* 42:931-939

112. Molineaux SM, Engh H, de Ferra F, Hudson L, Lazzarini RA (1986) Recombination within the myelin basic protein gene created the dysmyelinating shiverer mouse mutation. *Proc Natl Acad Sci U S A* 83:7542-7546

113. Montag D, Giese KP, Bartsch U, Martini R, Lang Y, Bluthmann H, Karthigasan J, Kirschner DA, Wintergerst ES, Nave KA, et al (1994) Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. *Neuron* 13:229-246

114. Monuki ES, Kuhn R, Weinmaster G, Trapp BD, Lemke G (1990) Expression and activity of the POU transcription factor SCIP. *Science* 249:1300-1303

115. Moser HW, Powers JM, Smith KD (1995) Adrenoleukodystrophy: molecular genetics, pathology, and Lorenzo's oil. *Brain Pathol* 5:259-266

116. Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, Poustka AM, Mandel JL, Aubourg P (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature* 361:726-730

117. Mosser J, Lutz Y, Stoeckel ME, Sarde CO, Kretz C, Douar AM, Lopez J, Aubourg P, Mandel JL (1994) The gene responsible for adrenoleukodystrophy encodes a peroxisomal membrane protein. *Hum Mol Genet* 3:265-271

118. Nadon NL, Arnheiter H, Hudson LD (1994) A combination of PLP and DM20 transgenes promotes partial myelination in the jimpy mouse. *J Neurochem* 63:822-833

119. Naef R, Adlkofer K, Lescher B, Suter U (1997) Aberrant protein trafficking in Trembler suggests a disease mechanism for hereditary human peripheral neuropathies. *Mol Cell Neurosci* 9:13-25

120. Nave KA, Boespflug-Tanguy O (1996) X-linked developmental defects of myelination: from mouse mutants to human genetic diseases. *Neuroscientist* 2:33-43

121. Nave KA, Bloom FE, Milner RJ (1987) A single nucleotide difference in the gene for myelin proteolipid protein defines the jimpy mutation in mouse. *Neurochem* 49:1873-1877

122. Nave KA, Lai C, Bloom FE, Milner RJ (1986) Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing. *Proc Natl Acad Sci U S A* 83:9264-9268

123. Noebels JL, Marcom PK, Jalilian-Tehrani MH (1991) Sodium channel density in hypomyelinated brain increased by myelin basic protein gene deletion. *Nature* 352:431-434

124. Notterpek L, Shooter EM, Snipes GJ (1997) Upregulation of the endosomal-lysosomal pathway in the trembler-J neuropathy. *J Neurosci* 17: 4190-4200

125. Patel PI, Lupski JR (1994) Charcot-Marie-Tooth disease: a new paradigm for the mechanism of inherited disease. *Trends Genet* 10:128-133

126. Peles E, Ben-Levy R, Tzahar E, Liu N, Wen D, Yarden Y (1993) Cell-type specific interaction of Neu differentiation factor (NDF/heregulin) with Neu/HER-2 suggests complex ligand-receptor relationships. *EMBO J* 12:961-971

127. Peles E, Nativ M, Campbell PL, Sakurai T, Martinez R, Lev S, Clary DO, Schilling J, Barnea G, Plowman GD, et al (1995) The carbonic anhydrase domain of receptor tyrosine phosphatase beta is a functional ligand for the axonal cell recognition molecule contactin. *Cell* 82:251-260

128. Pingault V, Bondurand N, Kuhlbrodt K, Goerich DE, Prehu MO, Puliti A, Herbarth B, Hermans-Borgmeyer I, Legius E, Matthijs G, Amiel J, Lyonnet S, Ceccherini I, Romeo G, Smith JC, Read AP, Wegner M, Goossens M (1998) SOX10 mutations in patients with Waardenburg-Hirschsprung disease. *Nat Genet* 18:171-173

129. Popko B, Puckett C, Lai E, Shine HD, Readhead C, Takahashi N, Hunt SW, Sidman RL, Hood L (1987) Myelin deficient mice: expression of myelin basic protein and generation of mice with varying levels of myelin. *Cell* 48:713-721.

130. Popot JL, Pham Dinh D, Dautigny A (1991) Major myelin proteolipid: the 4-alpha-helix topology *J Membr Biol* 120:233-246

131. Powers JM, Moser HW (1998) Peroxisomal disorders: genotype, phenotype, major neuropathologic lesions, and pathogenesis. *Brain Pathol* 8:101-120

132. Privat A, Jaque C, Bourre JM, Dupouye P, Baumann N (1979) Absence of the major dense line in myelin of the mutant mouse 'shiverer'. *Neurosci Lett* 12:107-112

133. Ranscht B (1988) Sequence of contactin, a 130-kD glycoprotein concentrated in areas of interneuronal contact, defines a new member of the immunoglobulin supergene family in the nervous system. *J Cell Biol* 107:1561-1573

134. Raskind WH, Williams CA, Hudson LD, Bird TD (1991) Complete deletion of the proteolipid protein gene (PLP) in a family with X-linked Pelizaeus-Merzbacher disease. *Am J Hum Genet* 49:1355-1360

135. Readhead C, Popko B, Takahashi N, Shine HD, Saavedra RA, Sidman RL, Hood L (1987) Expression of a myelin basic protein gene in transgenic shiverer mice: correction of the dysmyelinating phenotype. *Cell* 48:703-712

136. Readhead C, Schneider A, Griffiths I, Nave KA (1994) Premature arrest of myelin formation in transgenic mice with increased dosage of the proteolipid protein gene. *Neuron* 12:583-595

137. Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny DM, Gibbs RA, Lupski JR (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nature Genet* 12:288-297

138. Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C (1997) Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature* 389:725-730

139. Roach A, Boylan K, Horvath S, Prusiner SB, Hood LE (1983) Characterization of cloned cDNA representing rat myelin basic protein: absence of expression in brain of shiverer mutant mice. *Cell* 34:799-806

140. Roach A, Takahashi N, Pravtcheva D, Ruddle F, Hood L (1985) Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. *Cell* 42:149-155

141. Rosenbluth J (1980) Central myelin in the mouse mutant shiverer. J Comp Neurol 194: 639-648

142. Roussel G, Neskovic NM, Trifilieff E, Artault JC, Nussbaum JL (1987) Arrest of proteolipid transport through the Golgi apparatus in Jimpy brain. *J Neurocytol* 16:195-204

143. Saugier-Veber P, Munnich A, Bonneau D, Rozet JM, Le Merrer M, Gil R, Boespflug-Tanguy O (1994) X-linked spastic paraplegia and Pelizaeus-Merzbacher disease are allelic disorders at the proteolipid protein locus. *Nat Genet* 6:257-262

144. Scherer SS, Bone LJ, Deschenes SM, Fischbeck K, Balice-Gordon RJ (1997) The role of gap junction protein connexin32 in the myelin sheath. in: *Cell biology and pathology of myelin: evolving biological concepts and therapeutic approaches* (Eds. R.M. Devon, R. Doucette, B.H.J. Juurlink, A.J. Nazarali, D.J. Schreyer, and V.M.K. Verge). Plenum Press, New York, pp. 83-102.

145. Schliess F, Stoffel W (1991) Evolution of the myelin integral membrane proteins of the central nervous system. *Biol Chem Hoppe Seyler* 372:865-874

146. Schneider A, Montague P, Griffiths I, Fanarraga M, Kennedy P, Brophy P, Nave KA (1992) Uncoupling of hypomyelination and glial cell death by a mutation in the proteolipid protein gene. *Nature* 358:758-761

147. Schneider AM, Griffiths IR, Readhead C, Nave KA (1995) Dominant-negative action of the jimpy mutation in mice complemented with an autosomal transgene for myelin proteolipid protein. *Proc Natl Acad Sci U S A* 92:4447-4451

148. Schneider C, King RM, Philipson L (1988) Genes specifically expressed at growth arrest of mammalian cells. *Cell* 54:787-793

149. Schneider-Maunoury S, Topilko P, Seitandou T, Levi G, Cohen-Tannoudji M, Pournin S, Babinet C, Charnay P (1993) Disruption of Krox-20 results in alteration of rhombomeres 3 and 5 in the developing hindbrain. *Cell* 75:1199-1214

150. Seitelberger F (1995) Neuropathology and genetics of Pelizaeus-Merzbacher disease. *Brain Pathol* 5: 267-273

151. Sereda M, Griffiths I, Puhlhofer A, Stewart H, Rossner MJ, Zimmerman F, Magyar JP, Schneider A, Hund E, Meinck HM, Suter U, Nave KA (1996) A transgenic rat model of Charcot-Marie-Tooth disease. *Neuron* 16:1049-1060

152. Shapiro L, Doyle JP, Hensley P, Colman DR, Hendrikson WA (1996) Crystal structure of the extracellular domain from P0, the major structural protein of the peripheral nerve myelin. *Neuron* 17:435-449

153. Shine HD, Readhead C, Popko B, Hood L, Sidman RL (1992) Morphometric analysis of normal, mutant, and transgenic CNS: correlation of myelin basic protein expression to myelinogenesis. *J Neurochem.* 58:342-349

154. Sidman RL, Dickie MM, Apple SH (1964) Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. *Science* 144:309-311

155. Sinoway MP, Kitagawa K, Timsit S, Hashim GA, Colman DR (1994) Proteolipid protein interactions in transfectants: implications for myelin assembly. *J Neurosci* Res 37:551-562

156. Sistermans EA, de Wijs IJ, de Coo RF, Smit LM, Menko FH, van Oost BA (1996) A (G-to-A) mutation in the initiation codon of the proteolipid protein gene causing a relatively mild form of Pelizaeus-Merzbacher disease in a Dutch family. *Hum Genet* 97:337-339

157. Skoff RP (1982) Increased proliferation of oligodendrocytes in the hypomyelinated mouse mutant-jimpy. *Brain Res* 248:19-31

158. Snipes GJ, Suter U, Welcher AA, Shooter EM (1992) Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). *J Cell Biol* 117:225-238

159. Southard-Smith EM, Kos L, Pavan WJ (1998) Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. *Nat Genet* 18:60-64

160. Sprinkle TJ (1989) 2',3'-cyclic nucleotide 3'-phosphodiesterase, an oligodendrocyte-Schwann cell and myelin-associated enzyme of the nervous system. *Crit Rev Neurobiol* 4:235-301

161. Stoffel W, Bosio A (1997) Myelin glycolipids and their functions. *Curr Opin Neurobiol* 7: 654-661

162. Suter U, Welcher AA, Ozcelik T, Snipes GJ, Kosaras B, Francke U, Billings-Gagliardi S, Sidman RL, Shooter EM (1992a) Trembler mouse carries a point mutation in a myelin gene. *Nature* 356:241-244

163. Suter U, Moskow JJ, Welcher AA, Snipes GJ, Kosaras B, Sidman RL, Buchberg AM, Shooter EM (1992b) A leucine-to-proline mutation in the putative first transmembrane domain of the 22-kDa peripheral myelin protein in the trembler-J mouse. *Proc Natl Acad Sci U S A* 89:4382-4386

164. Suter U, Snipes GJ (1995a) Peripheral myelin protein 22: facts and hypotheses. *J Neurosci* Res 40:145-151

165. Suter U, Snipes GJ (1995b) Biology and genetics of hereditary motor and sensory neuropathies. *Annu Rev Neurosci* 18:45-75

166. Swiatek PJ, Gridley T (1993) Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20. *Genes Dev* 11:2071-2084

167. Tassabehji M, Newton VE, Liu XZ, Brady A, Donnai D, Krajewska-Walasek M, Murday V, Norman A, Obersztyn E, Reardon W, et al (1995) The mutational spectrum in Waardenburg syndrome. *Hum Mol Genet* 11:2131-2137

168. Taylor V, Suter U (1996) Epithelial membrane protein-2 and epithelial membrane protein-3: two novel members of the peripheral myelin protein 22 gene family. *Gene* 175:115-120

169. Thomson CE, Montague P, Jung M, Nave KA, Griffiths IR (1997) Phenotypic severity of murine Plp mutants reflects in vivo and in vitro variations in transport of PLP isoproteins. *Glia* 20:322-332

170. Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P (1994) Krox-20 controls myelination in the peripheral nervous system. *Nature* 371:796-799

171. Vabnick I, Messing A, Chiu SY, Levinson SR, Schachner M, Roder J, Li C, Novakovic S, Shrager P (1997) Sodium channel distribution in axons of hypomyelinated and MAG null mutant mice. *J Neurosci* Res 50:321-336

172. Valentijn LJ, Baas F, Wolterman RA, Hoogendijk JE, van den Bosch NH, Zorn I, Gabreels-Festen AW, de Visser M, Bolhuis PA (1992) Identical point mutations of PMP-22 in Trembler-J mouse and Charcot-Marie-Tooth disease type 1A. *Nat Genet* 2:288-291

173. Vallat JM, Sindou P, Preux PM, Tabaraud F, Milor AM, Couratier P, LeGuern E, Brice A (1996) Ultrastructural PMP22 expression in inherited demyelinating neuropathies. *Ann Neurol.* 39:813-817

174. Warner LE, Hilz MJ, Appel SH, Killian JM, Kolodry EH, Karpati G, Carpenter S, WattersGV, Wheeler C, Witt D, Bodell A, Nelis E, Van Broeckhoven C, Lupski JR (1996) Clinical phenotypes of different MPZ (P0) mutations may include Charcot-Marie-Tooth type 1B, Dejerine-Sottas, and congenital hypomyelination. *Neuron* :451-460

175. Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR (1998) Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. *Nat Genet* 4:382-384

176. Warner LE, Reiter LT, Murakami T, Lupski JR (1996) Molecular mechanisms for Charcot-Marie-Tooth disease and related demyelinating peripheral neuropathies. *Cold Spring Harb Symp Quant Biol* 61:659-671

177. Waxman SG (1997) Axon-glia interactions: building a smart nerve fiber. *Curr Biol* 7:R406-R410

178. Weimbs T, Stoffel W (1992) Proteolipid protein (PLP) of CNS myelin: positions of free, disulfide- bonded, and fatty acid thioester-linked cysteine residues and implications for the membrane topology of PLP. *Biochemistry* 31:12289-12296

179. Weinstein DE, Burrola PG, Lemke G (1995) Premature Schwann cell differentiation and hypermyelination in mice expressing a targeted antagonist of the POU transcription factor SCIP. *Mol Cell Neurosci* 6:212-229.

180. Wise CA, Garcia CA, Davis SN, Heju Z, Pentao L, Patel PI, Lupski JR (1993) Molecular analyses of unrelated Charcot-Marie-Tooth (CMT) disease patients suggest a high frequency of the CMTIA duplication. *Am. J. Hum. Genet.* 53:853-63

181. Wong MH, Filbin MT (1994) The cytoplasmic domain of the myelin P0 protein influences the adhesive interactions of its extracellular domain. *J Cell Biol* 126:1089-1097

182. Wong MH, Filbin MT (1996) Dominant-negative effect on adhesion by myelin Po protein truncated in its cytoplasmic domain. *J Cell Biol* 134:1531-1541

183. Wrabetz L, Feltri M, Quattrini A, Arona M, Trapp B, Messing A (1996) Increased P0 glycoprotein gene dosage causes a dysmyelinating peripheral neuropathy in transgenic mice. *Soc Neurosci Abstr* 22: 1980

184. Xu Z, Dong DL, Cleveland DW (1994) Neuronal intermediate filaments: new progress on an old subject. *Curr Opin Neurobiol* 4:655-661

185. Yamamoto Y, Mizuno R, Nishimura T, Ogawa Y, Yoshikawa H, Fujimura H, Adachi E, Kishimoto T, Yanagihara T, Sakoda S (1994) Cloning and expression of myelin-associated oligodendrocytic basic protein. A novel basic protein constituting the central nervous system myelin. *J Biol Chem* 269:31725-31730

186. Ye P, Carson J, D'Ercole AJ (1995) In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. *J Neurosci* 15:7344-7356

187. Yin X, Crawford TO, Griffin JW, Tu Ph, Lee VM, Li C, Roder J, Trapp BD (1998) Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. *J Neurosci* 18:1953-1962

188. Yin X, Peterson J, Gravel M, Braun PE, Trapp BD (1997) CNP Overexpression induces aberrant oligodendrocyte membranes and inhibits MBP accumulation and myelin compaction. *J. Neurosc. Res*.50:238-47

189. Yoshikawa H, Nishimura T, Nakatsuji Y, Fujimura H, Himoro M, Hayasaka K, Sakoda S, Yanagihara T (1994) Elevated expression of messenger RNA for peripheral myelin protein 22 in biopsied peripheral nerves of patients with Charcot-Marie-Tooth disease type 1A. *Ann. Neurol.* 35:445-450

190. Zhang D, Sliwkowski MX, Mark M, Frantz G, Akita R, Sun Y, Hillan K, Crowley C, Brush J, Godowski PJ (1997) Neuregulin-3 (NRG3): a novel neural tissue-enriched protein that binds and activates ErbB4. *Proc Natl Acad Sci U S A* 94:9562-9567

191. Zhang K, Filbin MT (1994) Formation of a disulfide bond in the immunoglobulin domain of the myelin P0 protein is essential for its adhesion. *J Neurochem* 63:367-370

192. Zhang K, Merazga Y, Filbin MT (1996) Mapping the adhesive domains of the myelin Po protein. *J Neurosci* Res 45:525-533

193. Zoidl G, Blass-Kampmann S, D'Urso D, Schmalenbach C, Müller HW (1995) Retroviral-mediated gene transfer of the peripheral myelin protein PMP22 in Schwann cells: modulation of cell growth. *EMBO J* 14:1122-1128

194. Zorick TS, Syroid DE, Arroyo E, Scherer SS, Lemke G (1996) The transcription factors SCIP and Krox-20 mark distinct stages and cell fates in Schwann cell differentiation. *Mol Cell Neurosci* 8:129-145