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REVIEW ARTICLE

Pathomechanisms of Mutant Proteins in Charcot-Marie-Tooth Disease

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Abstract

We review the putative functions and malfunctions of proteins encoded by genes mutated in Charcot-Marie-Tooth disease (CMT; inherited motor and sensory neuropathies) in normal and affected peripheral nerves. Some proteins implicated in demyelinating CMT, peripheral myelin protein 22, protein zero (P0), and connexin32 (Cx32/GJB1) are crucial components of myelin. Periaxin is involved in connecting myelin to the surrounding basal lamina. Early growth response 2 (EGR2) and Sox10 are transcriptional regulators of myelin genes. Mutations in the small integral membrane protein of lysosome/late endosome, the myotubularin-related protein 2 (MTMR2), and MTMR13/set-binding factor 2 are involved in vesicle and membrane transport and the regulation of protein degradation. Pathomechanisms related to alterations of these processes are a widespread phenomenon in demyelinating neuropathies because mutations of myelin components may also affect protein biosynthesis, transport, and/or degradation. Related disease mechanisms are also involved in axonal neuropathies although there is considerably more functional heterogeneity. Some mutations, most notably in P0, GJB1, ganglioside-induced differentiation-associated protein 1 (GDAP1), neurofilament light chain (NF-L), and dynamin 2 (DNM2), can result in demyelinating or axonal neuropathies introducing additional complexity in the pathogenesis. Often, this relates to the intimate connection between Schwann cells and neurons/axons leading to axonal damage even if the mutation-caused defect is Schwann-cell-autonomous. This mechanism is likely for P0 and Cx32 mutations and provides the basis for the unifying hypothesis that also demyelinating neuropathies develop into functional axonopathies. In GDAP1 and DNM2 mutants, both Schwann cells and axons/neurons might be directly affected. NF-L mutants have a primary neuronal defect but also cause demyelination. The major challenge ahead lies in determining the individual contributions by neurons and Schwann cells to the pathology over time and to delineate the detailed molecular functions of the proteins associated with CMT in health and disease.

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Index Entries: Hereditary neuropathies; myelin; axon; Schwann cell.

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Introduction

Myelin in the peripheral nervous system is generated and maintained by myelinating Schwann cells. This highly specialized cell type enwraps segments of axons with multiple layers of its plasma membrane. The resulting myelin sheath, together with the highly specialized nodes of Ranvier that are regularly arrayed along the myelinated fibers, is responsible for efficient and rapid propagation of action potentials along the nerve. Within this exquisitely regulated system, axons (neurons) and Schwann cells are anatomically and functionally closely connected and tightly regulate each other. At early stages in development, Schwann-cell precursors are dependent on axonal signals for survival and proliferation to ensure that the numbers of Schwann cells and axons are precisely matched. Later, axons are the main determinant of the myelinating phenotype when Schwann cells undergo differentiation into myelinating and nonmyelinating populations (Jessen and Mirsky, 1999, 2002; Michailov et al., 2004). Myelinating Schwann cells, in turn, have a profound effect on axonal properties (Arroyo and Scherer, 2000; Jessen and Mirsky, 2002; Poliak and Peles, 2003; Salzer, 2003; Edgar and Garbern, 2004). During myelination in development or remyelination after injury, juxtacrine signals from Schwann cells mediate the sequential assembly of multiprotein complexes of cell adhesion molecules, ion channels, and scaffolding proteins into distinct domains that are localized around the node of Ranvier. In addition, the axon cytoskeleton, organelle content, and rates of axonal transport are tightly regulated by myelinating Schwann cells.

The dialog between Schwann cells and the accompanying axons is continuous. Neurons or Schwann cells provide individually the "initial trigger" in regulatory events but this first stimulus is immediately followed by signal transduction events that mediate reciprocal interactions between the two cell types. Consequently, Schwann cells and axons/neurons need to be viewed as an intensely communicating dynamic community. Breakdown of this elaborate system results usually in peripheral neuropathies. Hereditary neuropathies are genetically heterogeneous and affect neurons and/or Schwann cells. In the Charcot-Marie-Tooth disease (CMT) group, mutations in several different genes cause similar disease phenotypes. Conversely, different mutations affecting the same gene can lead to different disease phenotypes (Fig. 1, reviewed by Suter and Scherer, 2003; for an updated list of genes and mutations found in CMT as well as the literature references to the individual mutations, *see* http://www.molgen.ua.ac.be/CMTMutations/default.cfm. For classification of CMT and the associated phenotypes, *see* http://www.neuro.wustl. edu/neuromuscular/time/hmsn.html). In demyelinating neuropathies, it is generally assumed that the defect starts in Schwann cells causing dys- and/or demyelination, which in turn leads to length-dependent axonal loss. Importantly, alterations or lack of the myelin sheath have pronounced effects on the axonal caliber, phosphorylation, and packing of neurofilaments, axonal transport, and the organization of ion channels in the axonal membranes (Martini, 2001). Defective axonal transport is the most likely hypothesis to explain the observation that axonal degeneration is maximal at distal nerve endings. Axonal transport is so crucial owing to the extreme polarity and size of motor and sensory neurons that, in humans, may extend for a meter or more. Most axonal components, including the cytoskeletal proteins, synaptic vesicle precursors, and organelles like mitochondria must be transported along the axon. This system must function efficiently and is delicately sensitive to deleterious influences of demyelination in demyelinating neuropathies or mutated proteins in axonal neuropathies with a primary neuronal defect. Defects in axonal transport have not only been put forward as a general critical component of the disease mechanisms in hereditary neuropathies (reviewed by Suter and Scherer, 2003), but also in conjunction with other neurodegenerative diseases, including amyotrophic lateral sclerosis and Alzheimer's disease (reviewed by Roy and Rauk, 2005).

Mutated Myelin Components in Demyelinating CMT

Even before the first genes involved in CMT were identified, it was obvious that mutations affecting structural and functional components of the myelin sheath would be interesting candidates for causing demyelinating forms of neuropathies. Indeed, mutations involving four myelin-associated proteins, peripheral myelin protein 22 (PMP22), protein zero (P0/MPZ), connexin32 (Cx32/GJB1), and periaxin

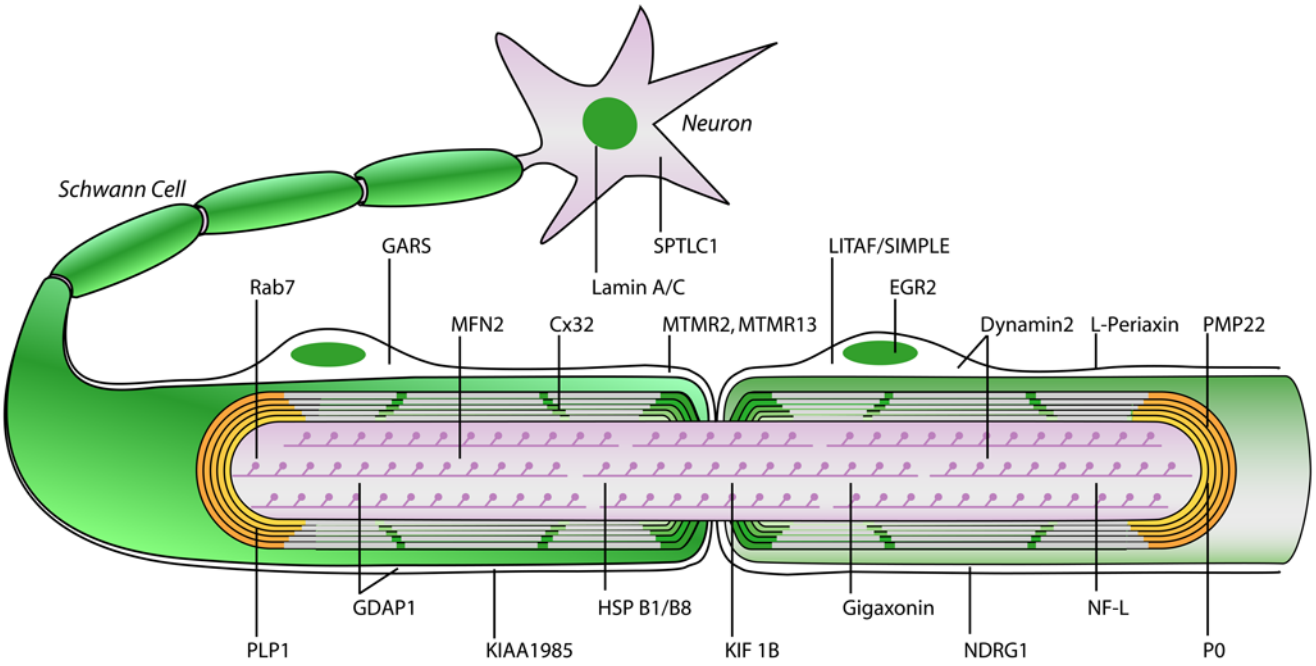

Fig. 1. Schematic overview highlighting proteins that are mutated in CMT. The localization of the normal proteins is depicted. Proteins have been assigned to Schwann cells and/or neurons, respectively, when expression and the observed form of CMT overlap.

(PRX) have been discovered in CMT (Fig. 2, reviewed by Suter and Scherer, 2003).

P0/MPZ and PMP22

P0/MPZ and PMP22 are components of compact myelin and play major structural roles in this highly organized configuration. P0/MPZ is a single-pass transmembrane protein with an immunoglobulin-like domain and accounts for approx 50% of the protein content of peripheral myelin. As predicted by its domain structure, P0/MPZ acts as an adhesion protein connecting adjacent myelin lamellae. These functions have been verified in vitro and in mice with altered P0/MPZ levels (Giese et al., 1992; Martini et al., 1995; Yin et al., 2000). Notably, overexpression as well as haploinsufficiency leads to myelin deficits. Both, the extracellular and the highly positively charged cytoplasmic domains of P0/MPZ are required for the adhesive function (Filbin et al., 1999). Structural analysis of the extracellular domain of P0/MPZ indicates tetrameric homophilic adhesion as the main underlying molecular mechanism (Shapiro et al., 1996).

P0/MPZ mutations cause dominant demyelinating, dominant axonal, and intermediate forms by various mechanisms. A comprehensive analysis revealed that P0/MPZ mutations are clustered in two major groups in relation to the associated phenotypes (Shy, 2004). Severe phenotypes are owing to mutations that are predicted to affect primarily myelin compaction owing to disruption of the structure of P0/MPZ. These mutations appear to affect the myelination process during development and cause early onset neuropathy. Two main mechanisms are involved. Either the mutated P0/MPZ protein is not transported within the cell to the plasma membrane, or the mutated protein is incorporated into myelin but disrupts this structure, presumably by dominant-negative interactions between the mutated and wild-type P0/MPZ protein (Previtali et al., 2000; Shames et al., 2003; Shy, 2004). A second group of P0/MPZ mutations affects predominantly axons and causes a late-onset neuropathy. The corresponding pathomechanisms are less obvious because P0/MPZ expression is confined to Schwann cells. The hypothesis has been put forward that subtle myelin alterations, owing to the

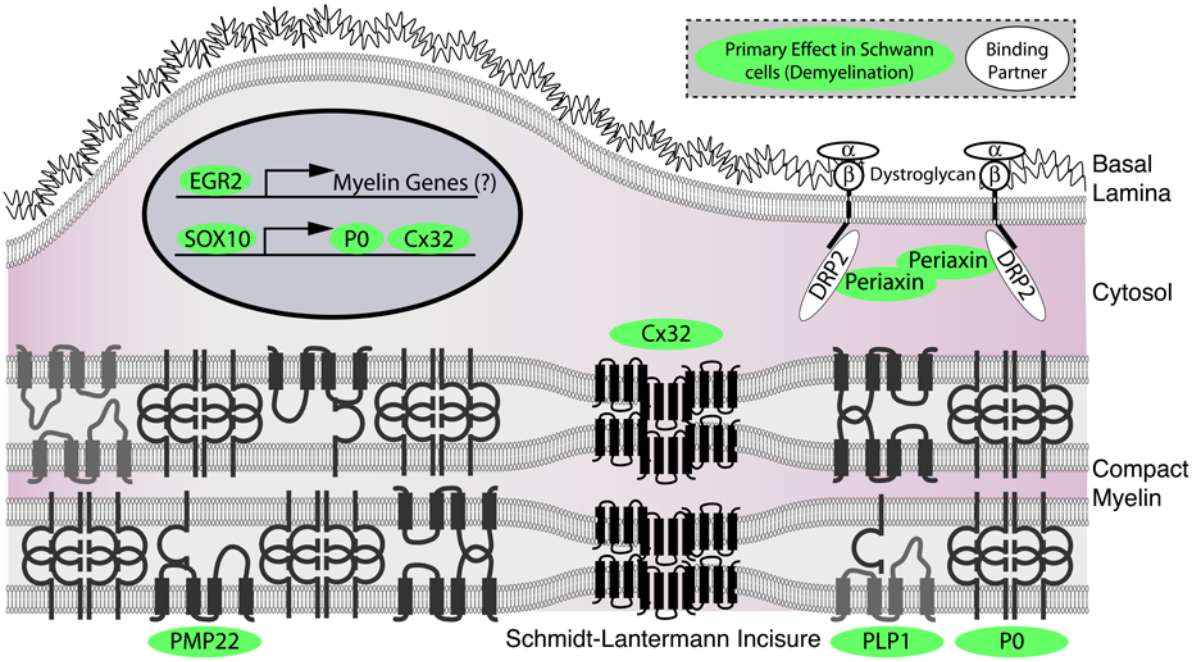

Fig. 2. Proteins involved in the formation of the Schwann-cell myelin sheath.

incorporation of the mutant P0/MPZ protein, affect Schwann cell–neuron interactions leading to axonal damage and loss (Shy, 2004). However, the nature of the exchanged signals is not known at this time. Identifying them is an exciting task for the future.

P0/MPZ has also been suggested to interact with PMP22 to enforce adhesive interactions (Fig. 1; D'Urso et al., 1999; Hasse et al., 2004). This is consistent with the finding that both proteins are spatially and temporally coexpressed during development and in the adult (Hagedorn et al., 1999; Notterpek et al., 1999). PMP22 is a glycosylated membrane component that is commonly referred to as a four-transmembrane protein although alternative membrane topologies remain to be considered as long as direct structural data are not available (Taylor et al., 2000). PMP22 accounts for 2–5% of the myelin protein content and tends to oligomerize (Snipes et al., 1992; Tobler et al., 1999, 2002; Liu et al., 2004). Besides its structural role in myelin, PMP22 regulates cell spreading, cell migration, and apoptosis (Fabbretti et al., 1995; Brancolini et al., 1999, 2000; Sancho, et al., 2001; Roux et al., 2005). PMP22 is also an early constituent of the developing blood–nerve and blood–brain barriers at intercellular junctions (Notterpek et al., 2001; Roux et al., 2004), a finding that is likely to be functionally related to the observation that PMP22 can modulate epithelial morphology and monolayer permeability in cell culture (Roux et al., 2005). In vivo, a functional requirement for PMP22 in junction complexes remains to be established. Mice lacking PMP22 are not obviously affected in epithelia integrity but show a mildly delayed onset of myelination indicating some crucial function of PMP22 in the initial steps of myelination (Carenini et al., 1999). Later, characteristic focal hypermyelinating structures (called tomacula = sausage) are formed, which degenerate with progressing age and remodel the Schwann cell and axonal protein composition by demyelination (Adlkofer et al., 1995; Neuberg et al., 1999). Thus, PMP22 is indispensable for proper myelination and myelin maintenance in peripheral nerves whereas in other tissues, PMP22 function is likely compensated by other members of the PMP22/EMP/MP20 family (Jetten and Suter, 2000). Heterozygous PMP22-null mice revealed haploinsufficiency and mimic genetically, behaviorally, and morphologically the peripheral nerve disorder hereditary neuropathy with liability to pressure palsies (HNPP; Adlkofer et al., 1997). HNPP is a recurrent neuropathy that is

precipitated by minor trauma to peripheral nerves with tomacula as typical morphological feature (Lupski and Chance, 2005). The cellular mechanisms that cause these particular myelin outfoldings have yet to be determined.

Heterozygous intrachromosomal duplication of a segment containing the PMP22 gene on chromosome 17p11 is associated with the by far most common form of inherited form of demyelinating neuropathies (CMT1A; reviewed by Saifi et al., 2003). Transgenic experiments in rodents have definitively established that PMP22 is the disease-causing gene (Huxley et al., 1996; Magyar et al., 1996; Sereda et al., 1996). However, the causative cellular mechanisms of the disease are still largely mysterious. It has been argued that PMP22 may be required in precise stoichiometric amounts in myelin. This hypothesis remains still plausible (Suter and Snipes, 1995). Alternatively, PMP22 overexpression might affect Schwann-cell physiology in more general ways (reviewed by Suter, 2004).

Recently, altered intracellular membrane transport and defects in protein degradation have been implicated in the disease process. Newly synthesized PMP22 has a short half-life within the myelinating Schwann cell (Pareek et al., 1993, 1997) and most of the protein is rapidly degraded via the ubiquitin–proteasome pathway before reaching the Schwann-cell plasma membrane (Notterpek et al., 1999; Ryan et al., 2002). This could be interpreted that the intracellular processing and transport machinery is already operating at the limit of its capabilities with respect to PMP22 biosynthesis. PMP22 overexpression in CMT1A may now overload this fine-tuned system. Proteasomal degradation is one of the possibly associated and damaged processes. This hypothesis is consistent with the finding that overexpression of PMP22 causes intracellular accumulation in vacuolar structures in vitro, but most notably also in PMP22-overpressing rats (Niemann et al., 2000; Chies et al., 2003). Experiments aimed at dissecting the underlying mechanisms revealed that PMP22 overexpression induces the formation of microtubule (MT)-dependent ubiquitinated PMP22 aggregates called aggresomes (Ryan et al., 2002). These structures as well as PMP22 overexpression-induced myelin-like intracellular figures are closely related (Dickson et al., 2002; Chies et al., 2003). Both are likely to be degraded by the process of autophagy (Fortun et al., 2003). Taken together, PMP22 overexpression appears to affect intracellular degradation of membrane components, including PMP22 itself, suggesting a pathomechanism that may also include aberrant turnover of other proteins or even lipids. A second major, seemingly unrelated, pathway of intracellular membrane transport is also affected. On overexpression, PMP22 induces the formation of and accumulates in actin/phosphatidylinositol (4,5)-bisphosphate (PI-4,5-P2)-positive vacuoles (Chies et al., 2003). This results in trapping of membrane proteins by altering ADP-ribosylation factor-6 (Arf6)-regulated endosomal–plasma membrane recycling. Although appealing, the distinct contributions of these altered biosynthetic pathways to the development of demyelinating neuropathies remain largely unclear.

Besides increased PMP22 gene dosage, PMP22 missense mutations are associated with myelination defects. The vast majority of the mutations are dominantly inherited, affect hydrophobic domains of the protein, and lead to more severe phenotypes compared with PMP22 overexpression, often referred to Dejerine-Sottas syndrome (reviewed by Saifi et al., 2003). The mutant proteins fail to reach the cell surface owing to retention in the endoplasmic reticulum (ER) or in the intermediate compartment (Naef et al., 1997; D'Urso et al., 1998; Naef and Suter, 1999; Tobler et al., 1999; Colby et al., 2000; Shames et al., 2003). Because mutant PMP22 and wild-type PMP22 can form homo- and heterodimers and multimers (Tobler et al., 1999, 2002), the retained mutant may block a proportion of wild-type PMP22 from transport to the cell membrane by a dominant-negative mechanism. However, genetic evidence suggests that at least in some mutants, such a mechanism is unlikely to be exclusively responsible for the pathology because some mutated PMP22 proteins exert a deleterious effect in the absence of wild-type PMP22 (Adlkofer et al., 1997). Several potential mechanisms could explain such a "toxic gain-of-function" of mutated PMP22. First, PMP22 interacts with P0 opening the possibility of a *trans*-dominant effect of PMP22 mutants on the trafficking of P0 and vice versa. Interactions between the two proteins during intracellular trafficking, however, are limited, rendering such a pathomechanism unlikely (Shames et al., 2003). Second, increased association of mutated and ER-retained PMP22 with calnexin may lead to a deleterious sequestration of this ER-resident chaperon, causing

a more general defect in protein biosynthesis (Dickson et al., 2002). Third, accumulation of mutant PMP22 could trigger the unfolded protein response in the ER (as suggested for retained proteolipid protein [PLP] mutants [Southwood et al., 2002]) but experimental support for such a signaling is missing. Interestingly, in contrast to the dominantly inherited mutations, the few recessive PMP22 mutations have a low potential for aggregation and are transported to the cell surface, leaving clues toward the associated disease mechanisms largely in the dark (Liu et al., 2004).

The availability of animal models carrying PMP22 missense mutations (Suter et al., 1992a,b; Isaacs et al., 2002) has revealed additional insights into the pathomechanisms. The endosomal–lysosomal pathway (Notterpek et al., 1997) and ubiquitination (Ryan et al., 2002) are upregulated and PMP22-containing aggresomes (Notterpek et al., 1999; Ryan et al., 2002) and myelin-like figures (Dickson et al., 2002), most likely representing autophagic vacuoles (Dunn, 1990) are present in mutant Schwann cells. Because aggresomes are eventually degraded by lysosomes, these observations reflect probably alterations within the same pathway (Fortun et al., 2003). Interestingly, impaired proteasome activity and accumulation of ubiquitinated substrates, including PMP22, has been observed in the mutant PMP22 animals (Fortun et al., 2005), suggesting that aggresome formation is likely to be deleterious in this setting and does not have a beneficial effect in removing potentially toxic misfolded PMP22 (Isaacs et al., 2002).

In summary, remarkable progress has been made in defining the cellular and molecular mechanisms underlying PMP22-based neuropathies suggesting a variety of general and Schwann-cell-specific potential cellular disease mechanisms, ranging from abnormal biosynthesis to abnormal protein processing and degradation, abnormal targeting to myelin, induction of apoptosis, and abnormal function in myelin (Muller, 2000). The challenge remains to determine how these effects are related and connected as well as to assign and determine their individual contributions to the disease. A particularly pressing issue concerns the interrelationship between the disease mechanisms of PMP22 overexpression compared with PMP22 point mutations. Is there a general disease mechanism, or are specific elements involved depending on the nature of the mutation? Recent evidence from transcriptional profiling suggests that both common and specific factors play a role in the pathomechanisms associated with altered PMP22 expression or PMP22 mutations (Giambonini-Brugnoli et al., 2005). Indirect support for potentially diverging disease mechanisms is also provided by earlier studies demonstrating that the effects of PMP22 overexpression on cell spreading and apoptosis require cell surface expression of PMP22 (Brancolini et al., 2000). Because disease-associated dominant PMP22 missense mutants are not transported to the plasma membrane they cannot influence these processes. In addition, a CMT1A-associated PMP22 mutant was unable to trigger the formation of PI-4,5-P2-positive vacuoles (Chies et al., 2003). Further elucidation of these issues, in particular, determining which of the described effects contribute indeed to the CMT pathology caused by a particular PMP22 alteration (Shames et al., 2003; Suter, 2004), will be of major importance for understanding the basic mechanisms underlying the most common form of hereditary neuropathies.

Connexin32

Mutations in the gap junction protein Cx32/GJB1 cause the X-chromosome linked CMT1X. Connexins are tetraspan proteins that oligomerize to form hemichannels in cell membranes. Two hemichannels on apposing membranes form a gap junction. The resulting, highly regulated pore permits the transport of small molecules (usually smaller than 1 kDa) across membranes. In myelin of peripheral nerves, Cx32/GJB1 is present in Schmidt-Lantermann incisures, paranodal loops, and at the internodal zone of partial myelin compaction (Meier et al., 2004). It is generally assumed that the formed gap junctions provide a critical radial pathway directly through the myelin sheath. Disruption of this radial pathway by Cx32/GJB1 mutations may then cause demyelination. Alternatively or concomitantly, alterations of the Cx32/GJB1-based gap junctions between compact and uncompact myelin may contribute to the disease. In any case, the nature of the critical molecules that are exchanged through Cx32/GJB1-specific channels is unclear because

general dye transfer is still possible through gap junctions in incisures of Cx32/GJB1-deficient nerves (Balice-Gordon et al., 1998).

Cx32/GJB1 mutations are spread over the entire protein and cause rather homogeneous disease characteristics. Affected males have a demyelinating phenotype with more pronounced axonal loss than in CMT1A. Experiments in transgenic mice indicate that the initial disease-causing defect occurs in myelinating Schwann cells and axonal loss is secondary (Scherer et al., 2005). Affected women show a variable phenotype, probably proportional to the number of myelinating Schwann cells that inactivate the normal Cx32/GJB1 allele by X-chromosome inactivation. The uniformity of the phenotype in CMT1X peripheral nerves suggests that most Cx32/GJB1 mutants inactivate the protein consistent with null alleles. However, the current mechanistic knowledge is fragmentary. Trafficking of Cx32/GJB1 mutants in mammalian cells is often, but not in all cases, disrupted. Many mutants form nonfunctional channels whereas others form functional channels with altered biophysical characteristics. Some disease-associated mutants form even fully functional channels (Scherer and Paul, 2004). How these mutants cause demyelination remains to be clarified.

Periaxin

PRX mutations are recessively inherited and cause a wide spectrum of demyelinating neuropathies with particularly prominent sensory involvement (reviewed in Takashima et al., 2002). PRX is a membrane-associated protein with a PDZ domain (named after the three proteins in which it was first described: postsynaptic density protein-95, drosophila discs large tumor suppressor gene (dlg) and the tight junction-associated protein ZO-1) that is involved in mediating protein–protein interactions. PRX is mainly expressed by myelinating Schwann cells (Dytrych et al., 1998) although it is also found prominently in junctional complexes in the eye lens (Straub et al., 2003). During myelination, PRX is located close to the adaxonal membrane (near to the axon) but in mature myelin sheaths, it has changed its localization to the abaxonal membrane (near to the basal lamina). In the adult, PRX interacts with the dystroglycan complex through dystrophin-related protein-2 (DRP2) and links the basal lamina to the Schwann-cell cytoskeleton (Sherman et al., 2001). PRX-null mice develop grossly normal myelin fibers (Gillespie et al., 2000). However, closer inspection revealed that cytoplasmic bands (Cajal bands) are disrupted and Schwann-cell elongation during nerve growth is impaired (Court et al., 2004). The resulting decreased internodal distances cause decreased nerve conduction velocities and affected motor function. The altered myelin structure in PRX-null mice appears to be unstable because myelin outfoldings and extensive demyelination are common features in peripheral nerves of older animals (Gillespie et al., 2000). How these findings are related to the broad range of phenotypes associated with PRX mutations is not yet clear, in particular with regard to the pronounced sensory defects. PRX mutations emphasize the general fact, however, that protein complexes involved in extracellular matrix signaling are often affected in diverse neuropathies. This includes merosin-deficient congenital muscular dystrophy, neurofibromatosis, and leprosy (reviewed by Feltri and Wrabetz, 2005).

Proteolipid Protein

Mutations in the PLP1/DM20 gene cause the X-linked leukodystrophyl Pelizaeus-Merzbacher disease (reviewed by Cailloux et al., 2000). In some mutants, this is accompanied by a demyelinating peripheral neuropathy (Garbern et al., 1997; Shy et al., 2003; Vaurs-Barriere et al., 2003). PLP1/DM20, a membrane protein with a putative tetraspan topology, accounts for the majority of CNS myelin protein. It is also expressed by myelinating Schwann cells and occurs in two different splice forms, PLP1 and DM20. Both proteins are identical except that PLP1 carries a 35-amino-acid-long insertion in a putative intracellular domain (corresponding to the major dense line in compact myelin). PLP1-null mutations and PLP1 truncation mutations that affect exclusively the PLP1 isoform, but not DM20, are associated with peripheral neuropathies (Garbern et al., 1997; Shy et al., 2003), suggesting a requirement for the PLP1 isoform for the proper function of myelinated peripheral nerves. The underlying cellular disease mechanisms in this particular

form of neuropathy have yet to be determined (Shy et al., 2003).

Transcription Factors Involved in Myelin Gene Regulation: Early Growth Response 2 and Sry-Related High-Mobility Group Box-Containing Gene 10

The exquisite gene-dosage sensitivity of P0/MPZ and PMP22 indicates that the expression of myelin components is tightly regulated. Thus, transcription factors regulating genes that encode myelin proteins are potential candidates to be mutated in demyelinating neuropathies. In line with this hypothesis, mutations affecting two transcription factors, EGR2/Krox20 and Sox10, have been found in CMT.

EGR2/Krox20 is a zinc finger-containing protein and null mutant mice for this protein do not develop peripheral nerve myelin (Topilko et al., 1994). This is consistent with several recent studies suggesting a broad function of EGR2/Krox20 in the regulation of Schwann-cell myelination by controlling myelin protein gene expression and cholesterol/lipid biosynthesis via the sterol regulatory element binding protein (SREBP) pathway (Nagarajan et al., 2001; Parkinson et al., 2004; Le et al., 2005a; Leblanc et al., 2005). EGR2/Krox20 mutations in human are associated with demyelinating or dysmyelinated forms of CMT (CMT1D, CMT4E, and congenital hypomyelination). Mutations in the zinc finger domain lead to the dominantly inherited form CMT1D. They affect DNA binding negatively and the reduction of DNA binding correlates with the severity of the resulting disease (Warner et al., 1998). A particular mutation, located in the R1 domain of EGR2/Krox20, may affect the interaction between EGR2/Krox20 and its coregulators, the NGFI-A-binding proteins (NABs;Warner et al., 1998; Warner et al., 1999). This mutation is recessively inherited and is classified as CMT4E. The underlying pathogenic mechanisms remain unclear because disturbed coactivation as well as corepression of EGR2/Krox20 by NAB proteins is conceivable (Le et al., 2005b). Mutations in the two NAB-encoding genes themselves have not been found in hereditary neuropathies, most likely because the two NAB proteins have strikingly redundant, but together, indispensable, functions in peripheral nervous system myelination (Le et al., 2005b).

SOX10 encodes a high-mobility group domain-containing transcription factor (reviewed by Mollaaghababa and Pavan, 2003) and some mutations in SOX10 are associated with peripheral neuropathy, demyelinating leukodystrophy, and Waardenburg-Hirschsprung disease (reviewed by Inoue et al., 2002). With regard to the peripheral nervous system, SOX10 is expressed by neural crest stem cells, the progenitors of Schwann cells, and plays a crucial role in progenitor cell maintenance as well as in the control of peripheral gliogenesis (Britsch et al., 2001; Paratore et al., 2001; Kim et al., 2003). SOX10 is a direct transcriptional regulator of P0/MPZ and Cx32/GJB1 providing a mechanistic link to the pathogenesis of other demyelinating peripheral neuropathies (Peirano and Wegner, 2000; Peirano et al., 2000; Bondurand et al., 2001). Strikingly, two CMT1X-causing mutations impair SOX10 function on the mutated P2 promoter of Cx32/GJB1 (Bondurand et al., 2001; Houlden et al., 2004). Conversely, SOX10 mutants identified in patients with peripheral myelin defects fail to transactivate the Cx32/GJB1 promoter.

CMT Owing to Altered Protein Synthesis, Sorting, and/or Degradation

The correct composition and maintenance of membranous compartments in myelinating Schwann cells and in neurons depend on the equilibrium between the synthesis of structural and signaling components and their degradation. Membrane proteins are synthesized in the ER, modified during their transport through the ER and Golgi, and then either directly degraded or inserted into the plasma membrane. Proteins are removed from the plasma membrane by endocytosis. In a first step, proteins are transported to early endosomes where they are sorted according to their fate. Either they are sent in vesicles along microtubles via late endosomes to lysosomes for degradation, or they recycle back to the plasma membrane (Fig. 3). Several proteins that are directly involved in the regulation of these processes are mutated in various forms of CMT.

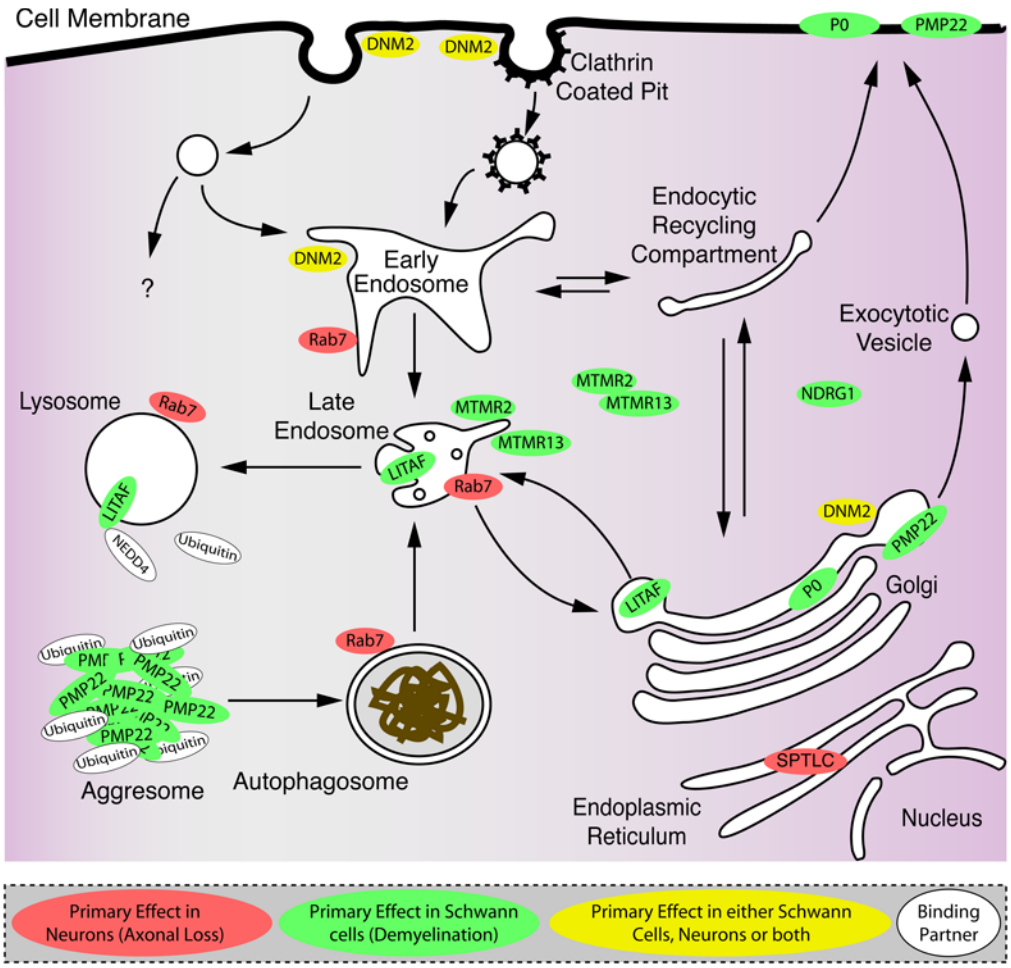

Fig. 3. Proteins related to CMT that are involved in synthesis, sorting, and degradation processes are indicated at their subcellular localization in a schematic drawing.

Endocytosis and Sorting: Myotubularin-Related Proteins 2 and 13, Dynamin 2, Ras-Associated Protein Rab7, and N-myc Downstream-Regulated Gene 1

Myotubularins form a large family of conserved dual-specificity phosphatases with active and inactive members that are involved in the regulation of membrane dynamics (Wishart and Dixon, 2002). Mutations in three genes of this family are associated with human diseases. Mutations in myotubularin cause X-linked myotubular myopathy and mutations in myotubularin-related protein-2 (MTMR2) or MTMR13/SBF2 lead to CMT4B1 or CMT4B2, respectively (Laporte et al., 1996; Bolino et al., 2000; Azzedine et al., 2003; Senderek et al., 2003b). CMT4B1 is classified as a recessive demyelinating neuropathy with prominent myelin outfoldings as particular pathological feature. Consistent with this phenotype, Schwann-cell-specific ablation of MTMR2 is sufficient for the development of myelin outfolding in transgenic mice (Bolino et al., 2004; Bolis et al., 2005; Bonneick et al., 2005). Appreciable levels of MTMR2 are also found in the axon (Berger et al., 2002) and MTMR2 has been reported to interact with the neurofilament light chain (NF-L; Previtali et al., 2003). Active myotubularins dephosphorylate PI-3-P and Pl-3,5-P2 and the disease-associated forms of MTMR2 show drastically reduced phosphatase activity toward these substrates. Thus, loss of phosphatase activity is likely to be involved in the pathogenic mechanism (Walker et al., 2001; Berger et al., 2002).

The substrate specificity of myotubularins points to a function in the regulation of endocytosis, vesicular protein sorting, and protein degradation. This hypothesis is supported by findings that myotubularin mutations block endocytosis in the coelomocytes of *Caenorhabditis elegans* (Dang et al., 2004) and that PI-3-P and PI-3,5-P2 are used as membrane adaptors for proteins involved in early and late phases of the endocytic process. PI-3-P is localized on early endosomes and acts as an adaptor for FYVE domain-containing proteins (Gaullier et al., 1998). The localization and function of PI-3,5-P2 is not as well established. PIKfyve, the kinase which phosphorylates PI-3-P to PI-3,5-P2 is localized on late endosomes indicating that PI-3,5-P2 is also localized in this compartment (Shisheva et al., 2001). MTMR2 binds to PI-3,5-P2 via its GRAM–pleckstrin homology domain but also to other phosphoinositides. Membrane association is achieved as a dimer and tightly regulated. It is only observed on vacuoles after an external activating signal whereas MTMR2 localizes to the cytosol under steady-state conditions (Berger et al., 2003). MTMR2 interacts directly with MTMR13/sbf2, providing a compelling explanation for why mutations in MTMR2 or MTMR13/sbf2 lead to the same pathology (Berger et al., 2006; Robinson and Dixon, 2005). In addition, MTMR2 binds to Dlg1/SAP97 in myelinated nerve fibers but with unknown functional significance (Bolino et al., 2004).

Dynamin2 (DNM2) mutations are associated with the dominant-intermediate CMT type B (DICMTB) (Züchner et al., 2005). Owing to the intermediate electrophysiology and pathology of this phenotype and the ubiquitous expression of DNM2, the disease-relevant cell type(s), neurons, and/or Schwann cells, are not yet clear. DNM2 belongs to the family of large GTPases. Members of this group regulate membrane trafficking, actin-based cytoskeletal dynamics, and membrane fission and fusion of vesicles, mitochondria, and peroxisomes (Praefcke and McMahon, 2004; McNiven, 2005). Dynamins hydrolyze GTP to act as mechanochemical proteins to constrict and deform membranes, including pinching of newly formed clathrin-coated vesicles, indicating a function in early phases of endocytosis (Rappoport and Simon, 2003; Praefcke and McMahon, 2004). All disease-associated mutations identified so far are located in the β3/β4 loop of the DNM2 pleckstrin-homology domain and result in reduced binding to vesicles, reduction of receptor-mediated endocytosis, and a striking reorganization of the microtubular cytoskeleton (Züchner et al., 2005). The relevance of the binding of dynamins to MTs for cytoskeletal dynamics is controversial but is supported by the finding that DNM2 binds to γ-tubulin at the centrosome (Thompson et al., 2004). However, DNM2 is definitively a key regulator of actin dynamics (Buccione et al., 2004; McNiven et al., 2004). DNM2 interacts with actin-binding proteins including profilin, cortactin, and Abp1 (reviewed in Orth and McNiven, 2003). This regulates the formation of membrane tubulation, the formation of podosomes (formation of dynamic membrane portusions in contact to the ECM, similar to focal adhesions), invadopodia (specialized podosomes which can modulate the ECM), and circular dorsal ruffles/waves (cytoplasmatic remodeling to allow motility in response to a stimulus; reviewed in Buccione et al., 2004). How and to what extent these diverse processes of endocytosis, MT organization, and actin-mediated membrane dynamics are altered by the disease-causing mutation in neurons and/or Schwann cells remains an open question. On a speculative note, this mechanism may include synaptic vesicle depletion, possibly secondary to an endocytosis defect at the neuromuscular junction (as described in a dynamin Drosophila mutant; reviewed by McNiven, 2005). Deficient axonal transport or altered polarity and organization of myelinating Schwann cells are also conceivable pathological consequences of DNM2 mutations (reviewed by Scherer and Arroyo, 2002; Salzer, 2003). Elucidation of the mechanistic basis of the dominant effect of the identified DNM2 mutations remains another pressing issue.

Rab7 mutations are associated with the dominant axonal CMT2B. This favors a neuronal disease mechanism (Verhoeven et al., 2003), despite the fact that Rab7 is ubiquitously expressed and is likely to play important roles in neurons and Schwann cells. Rab7 belongs to the large superfamily of small GTPases that are key regulators of many aspects of membrane transport. Rab7 binds to subdomains of early endosomes and escorts proteins via late endosomes to lysosomes (Vonderheit and Helenius, 2005). Rab7 is also required for the endocytosis of glycosphingolipids from the plasma membrane and the transport to the Golgi compartment. In Niemann-Pick

type-C fibroblasts, high levels of glycosphingolipids accumulate in the endocytic compartment and this defect can be corrected by overexpression of Rab7 (Choudhury et al., 2002). This finding provides a tantalizing link to hereditary sensory neuropathy type 1 (HSN1) because affected patients show a similar phenotype compared with patients with Rab7 mutations (strong involvement of sensory components, skin ulcers). HSN1 is caused by mutations in the gene coding for subunit 1 of serine palmitoyltransferase, an enzyme that catalyzes a key step of sphingolipid synthesis (Dawkins et al., 2001). Thus, the pathogenic mechanism in CMT2B and HSN1 might be interrelated. The disease mechanisms of Rab7 may also be linked to axonal transport because Rab7 is involved in lysosomal transport through the effector protein RILP that induces the recruitment of dynein–dynactin motors and dynein mutations can result in axonal degeneration (*see* below).

Although myelin deficiencies are rare in CMT2B, Rab7 may contribute to the phenotype by affecting also the processing and degradation of myelin proteins. Rab7 is involved in the regulation of the autophagic degradation pathway that has been suggested to play a central role in neuropathies owing to PMP22 missense mutations or PMP22 overexpression (Gutierrez et al., 2004).

N-myc downstream-regulated gene 1 (NDRG1) is mutated in the recessive CMT4D (LOM). NDRG1 expression is Schwann-cell-specific in peripheral nerves (Berger et al., 2004), consistent with the demyelinating disease caused by NDRG1 mutations in humans and null mutations in mice (reviewed by Hunter et al., 2003; Okuda et al., 2004). The cellular function of NDRG1 in Schwann cells, however, is not established, although a variety of proposals have been made (reviewed in Berger et al., 2004). The apolipoproteins A-I (APOA1) and A-II (APOA2) are putative NDRG1-interacting proteins (Hunter et al., 2005). These proteins are major components of high-density lipoproteins that regulated lipid distribution within the body. Because myelinating Schwann cells have a high demand on lipids, it is conceivable that NDRG1 mutations affect the interactions with APOA1 or APOA2 causing demyelination. Several additional putative NDRG1 interaction partners with various roles in intracellular vesicle and membrane trafficking have been described, providing a potential functional link to other CMT-related proteins that play a role in such processes (Sugiki et al., 2004; Hunter et al., 2005). Aspartoacyclase was also found as a putative NDRG1-binding partner (Sugiki et al., 2004). This is intriguing because mutations in aspartoacyclase lead to Canavan disease, a leukodystrophy that affects the CNS myelin sheath (Kaul et al., 1993).

Sorting and Degradation: Small Integral Membrane Protein of Lysosome/Late Endosome

Mutations in the small integral membrane protein of lysosome/late endosome (SIMPLE/LITAF) cause the dominant demyelinating neuropathy CMT1C but can also be associated with axonal forms (reviewed by Saifi et al., 2005). LITAF is a putative E3 ubiquitin ligase of the RING finger motif-containing E3 subfamily with several proline-rich sequences (PY motifs) and SH2-binding motifs. Interestingly, such elements also exist in Krox20/EGR2 and Sox10 (Saifi et al., 2005). The first PY motif interacts with NEDD4, a member of the HECT domain-containing E3 ubiquitin ligase subfamily and with WW domain oxidoreductase, a potential tumor-supressor gene (Jolliffe et al., 2000; Ludes-Meyers et al., 2004; Saifi et al., 2005). It has been speculated that NEDD4 ubiquitinates SIMPLE/LITAF, thereby enabling binding to TSG101 (a putative E2 ubiquitin-conjugating enzyme; Pornillos et al., 2002) and regulating endosomal protein sorting (Saifi et al., 2005). However, the disease-relevance of the interactions with the PY motif(s) requires further experimental confirmations because proline-rich recognitions motifs are not always specific. SIMPLE/LITAF partially colocalizes with WW domain oxidoreductase in the Golgi and with LAMP-1 in lysosomes, consistent with a functional role of LITAF in protein degradation (Moriwaki et al., 2001; Ludes-Meyers et al., 2004). Bennett et al. (2004) suggested that SIMPLE/LITAF mutations might affect proper PMP22 degradation in lysosomes and have a related mechanistic effect to PMP22 overexpression in leading to demyelinating neuropathy. Saifi et al. (2005) postulated PMP22 as one of the substrates for a LITAF-mediated ubiquitin-dependent pathway of protein degradation, indirectly supported by the finding that overexpression and point mutations affecting PMP22 cause aggresome formation of

ubiquitinated PMP22 aggregates (Ryan et al., 2002). Consistent with these notions, a case of early-onset CMT disease was recently described in a patient with a duplication of the PMP22 gene in combination with a disease-causing mutation in the SIMPLE/LITAF gene (Meggouh et al., 2005). A misfunction of SIMPLE/LITAF in endosomal sorting or lysosomal functionality may accentuate the effects of PMP22 accumulation resulting in severe demyelination. Taken together, the disease mechanism associated with SIMPLE/LITAF mutations may affect the putative SIMPLE/LITAF ubiquitin ligase activity, alterations of interactions with partners such as NEDD4 and/or TSG101, and/or impaired endosomal protein trafficking and degradation.

Glycyl-tRNA Synthetase in CMT

Mutations in the glycyl-tRNA synthetase (GARS) gene lead to the dominant axonal CMT2D or type-V distal muscular atrophy. This classification is based on the presence or absence of sensory changes. Patients with GARS mutations, however, present a clinical continuum of predominantly motor distal neuronopathy/axonopathy with mild-to-moderate sensory involvement that varies between the families and between members of the same family (Sivakumar et al., 2005). GARS carries out a ubiquitous function in cells by charging the amino acid glycine to its cognate tRNA. It is intriguing that mutations affecting a gene that is involved in such a general physiological function result in a peripheral nerve-specific phenotype. Whether loss-of-function (e.g., haploinsufficiency), gain-of-function, or dominant-negative effects are at work remains to be determined. Because GARS forms oligomers (Woese et al., 2000), dominant-negative mechanisms are likely. However, humans have only one known protein, GARS, which acts as glycyl tRNA synthetase and thus, reduction of its activity in the heterozygous state may specifically affect motor and sensory neurons in the disease owing to their specific metabolic requirements. Alternatively, although less likely based on the pathology (Sivakumar et al., 2005), Schwann cells may be particularly vulnerable to GARS function owing to the high metabolic demands during myelination. In this scenario, the observed axonal loss might be secondary to altered Schwann-cell–axon interactions.

Peripheral Neuropathies Owing to Altered Transport Processes and/or Alterations of the Cytoskeleton

Peripheral nerve fibers contain two specialized cell types, neurons and Schwann cells, which both show astonishing cellular morphologies. The cell bodies of sensory and motor neurons are located in the dorsal root ganglia and in the ventral horn of the spinal cord, respectively. The sensory receptors and the effector muscles lie in the periphery of the body. This distance, which in case of the lower limbs can easily be over 1 m, must be bridged by a continuous extension of a neuron, the axon. Crucial cargo has to be transported along the length of the axon forward and backward, including proteins contained in vesicles and organelles. This requires a highly efficient process, a fact that is underscored by various mutations in cytoskeletal and transport proteins found in CMT. Likewise, mutations in proteins that affect the correct association of organelles and vesicles with the transport machinery are likely causes for CMT (Fig. 4).

Neurofilaments

Neurofilaments are formed by a trimeric protein complex that consists of the NF heavy chain (NF-H; 200 kDa), the medium chain (NF-M; 168 kDa), and the light chain (NF-L; 62 kDa). Mutations in NF-L are associated with dominant axonal and dominant demylinating peripheral neuropathies. NF-L is an abundant cytoskeletal protein in neurons (Lariviere and Julien, 2004). Thus, the observed demyelination is likely be the consequence of altered neuron–Schwann-cell signaling.

NF biosynthesis and transport within the axon is tightly controlled. During development, increases in NF expression, local accumulation, and establishment of proper spacing of NF control the radial growth of the axon (Lariviere and Julien, 2004). Specifically, the tail domain of NF-M, with seven phosphorylated KSP motifs, is an essential target for a myelination-dependent outside-in signaling cascade that determines axonal caliber and conduction velocity (Garcia et al., 2003). This is conceptually important because in demyelinating neuropathies, this signaling is presumably disturbed, causing NF hypophosphorylation, reduced NF

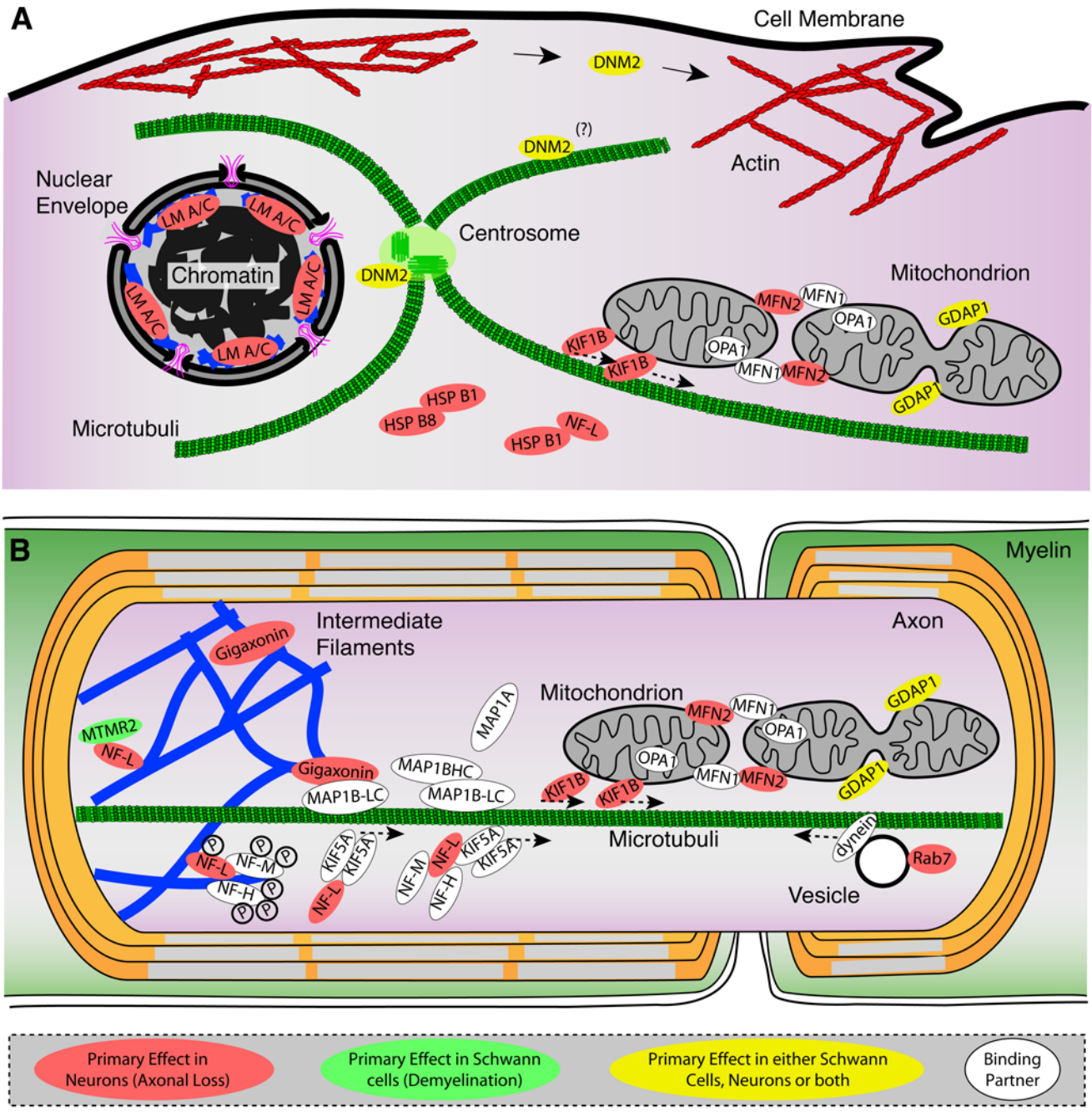

Fig. 4. Proteins related to CMT involved in the organization of the cytoskeleton, transport and mitochondrial dynamics are depicted in a hypothetical cell body **(A)** and in an axon **(B)**.

spacing, reduction in axonal transport, axonal atrophy, and as secondary effects, loss of axons and muscle atrophy (Suter and Scherer, 2003). Thus, phosphorylation of NF appears to play a critical and broad role in the pathogenesis of CMT that can be initiated by neurons or Schwann-cell (myelin) defects.

Newly synthesized NF subunits are transported from the perikaryon along MTs by motor proteins

(Xia et al., 2003). In addition, NFs are transported also in retrograde fashion via cytoplasmatic dynein (LaMonte et al., 2002; Wagner et al., 2004). Whether single or associated NF subunits are transported is debated (Lariviere and Julien, 2004). Transport and spacing are mediated by NF-M and NF-H, whereas NF-L serves as scaffold to assemble the NF complex (Ching and Liem, 1993; Lee et al., 1993). In mouse models, overexpression or loss of one of the three NF subunits result in disturbance of axonal maturation (reviewed in Lariviere and Julien, 2004). Simultaneous overexpression of all three subunits increases the axonal diameter (Xu et al., 1996), demonstrating that functional NF complexes require correct stoichiometry. CMT-linked NF-L mutations affect various aspects of NF biology, including axonal transport of mutant NF, transport of mitochondria, anterograde, and retrograde axonal transport. In addition, some mutants exert a dominant-negative effect on the transport of wild-type NF and cause fragmentation of the Golgi apparatus (Brownlees et al., 2002; Perez-Olle et al., 2002, 2004, 2005). Thus, a generalized axonal transport defect appears to be responsible for the observed neuropathy. The nature of the generated signal(s) causing the prominent myelination defects that are associated with some NF mutations remains an interesting subject for future studies.

Heat-Shock Proteins: 27-kDa Protein 1 and 22-kDa Protein 8

Mutations in the small heat-shock proteins B1 (HSPB1/HSP27) cause dominant axonal CMT2F and distal hereditary motor neuropathy (Evgrafov et al., 2004) whereas mutations in B8 (HSPB8/HSP22) have been described in hereditary motor neuropathy type 2 (Irobi et al., 2004). These two HSPs interact with each other suggesting some common disease mechanisms (Benndorf et al., 2001; Sun et al., 2004). Two additional members of the sHSP family are associated with diseases in humans, αA-crystallin mutations (HSPB4) with cataracts, and αB-crystallin mutations with desmin-related myopathy (Sun and MacRae, 2005). sHSPs contain a conserved C-terminal α-crystallin domain that stabilizes target proteins in a nonaggregated folding-competent state (Lee et al., 1997). In contrast to HSPs with an ATPase domain, like HSP70, sHSPs are unable to unfold misfolded proteins. These features are assumed to form the mechanistic basis why mutations in sHSP lead to the formation of aggregates that probably interfere with cellular functions. Interestingly, coexpression of mutated HSPB1/HSP27 with NF-L in cells with no intermediate filaments resulted in aggregation of NF-L, whereas no such effect was observed with wild-type HSPB1 (Evgrafov et al., 2004). These experiments are intriguing and suggest that mutations in HSPB1 may cause alterations in the NF network providing a potential mechanistic link to NF-L mutations. However, the hypothesis that HSPB1/HSP27 (and possibly also HSPB8/HSP22) are controlling the folding and assembly of NFs in vivo and the causative relationship of this mechanism for the resulting disease remains to be substantiated.

Gigaxonin

The functions of actin fibers, MTs, and intermediate filaments in the cytoskeleton are tightly coupled. This is illustrated by homozygous mutations found in gigaxonin leading to giant axonal neuropathy (GAN) (Bomont et al., 2000; Kuhlenbaumer et al., 2002). Gigaxonin belongs to a protein family that is characterized by an N-terminal BTB (broad-complex, Tramtrack, and Bric a brac) domain and six kelch repeats. BTB/kelch proteins are organizers of the cytoskeletal network and closely linked to the ubiquitin degradation pathway (Stogios and Prive, 2004). Patients with GAN have segmental axonal swellings characterized by NF accumulations in the cerebellum, the cerebral white matter, and peripheral nerves (Bomont et al., 2000). Intermediate filaments, including vimentin, the glial fibrallary acidic protein, and sometimes keratin, are disordered in diseased tissues (Mahadevan et al., 2000). Gigaxonin binds, via its kelch domain, to the MT-associated protein-1B light-chain (MAP1B-LC) enhancing MT stability, and disease-associated gigaxonin mutations lead to loss of gigaxonin–MAP1B-LC interaction probably affecting MT-dependent axonal transport (Ding et al., 2002). Gigaxonin also interacts with the E3-ubiquitin ligase cullin3 like many other BTB-family members (Pintard et al., 2004). Although speculative at this point, gigaxonin may bridge the ubiquitin ligase cullin3 to the potential substrate MAP1B-LC (Pintard et al., 2004).

Kinesin Family Member 1B

MTs serve as tracks that guide directed transport of vesicles, protein complexes, organelles, and chromosomes as they have a directed polarity with the growing plus-end in the periphery and the minus-end

at the centrosome (Howard and Hyman, 2003). Motor proteins, including dyneins and kinesins (KIFs), drive these transport processes by hydrolyzing ATP and converting the energy into mechanical work. Many KIFs move toward the plus end of MTs whereas the majority of the dyneins mediate transport to the minus end. Dynein–dynactin complexes carry out the fast retrograde transport in motor axons. Mutations in dynactin, probably affecting its binding to MTs, cause dominantly inherited motor neuron disease (Puls et al., 2003), highlighting the importance of this transport pathway in motor neuron axons. KIF-mediated, plus-end-directed, or anterograde transport in axons delivers cargo from the neuronal cell body into the periphery (Hirokawa and Takemura, 2005). A mutation in KIF1B was described in a Japanese family affected by dominant axonal CMT2A (Saito et al., 1997; Zhao et al., 2001). The KIF1B gene encodes two different isoforms that are generated by alternative splicing and differ in their C-terminal cargo-binding domain (Nangaku et al., 1994; Zhao et al., 2001). The mutation in CMT2A patients is located in the middle of the ATP-binding site (Zhao et al., 2001) and disrupts the function of both splice variants. KIF1B-α binds to mitochondria and mediates mitochondrial plus-end-directed transport (Nangaku et al., 1994) whereas the cargo-binding domain of the second splice variant KIF1B-β associates with synaptic vesicles precursors. Heterozygous KIF1B-deficient mice display a defect in transporting synaptic vesicle precursors and suffer from progressive muscle weakness (Zhao et al., 2001). These findings, in combination with data demonstrating that the CMT2A mutation in KIF1B-β is associated with decreased ATPase activity and motility, lead to the suggestion that haploinsufficiency of this motor protein is the cause of CMT2A neuropathy, likely owing to impaired transport of synaptic vesicle precursors or affecting neuronal survival (Zhao et al., 2001). Unfortunately mutations in KIF1B were only identified in a single family. Yet, the role of KIF1B in axonal transport was supported by the phenotype of the heterozygous KIF1B mice (Zhao et al., 2001).

Altered Mitochondrial Dynamics in CMT

Fusion of Mitochondria: Mitofusin2

Mitofusin2 (MFN2) is the second gene mutated in dominant axonal CMT2A (Züchner, et al., 2004). MFN2 belongs to the class of large GTPases such as DNM2. MFN2 is a component of the outer mitochondrial membrane and a key regulator of mitochondrial fusion (Santel and Fuller, 2001; Rojo et al., 2002). Together with MFN1, MFN2 forms the tethering complex that brings the outer membranes of two mitochondria into close proximity to allow fusion (Koshiba et al., 2004). Although MFN1 and MFN2 show a high degree of functional redundancy in cell culture experiments, mice lacking either MFN2 or MFN1 die at midgestation owing to placental defects (Chen et al., 2003). Mouse embryonic fibroblasts deficient in MFN1 or MFN2 display an increase of fragmented mitochondria, consistent with central roles of these proteins in mitochondrial fusion. The fragmented mitochondria lose their membrane potential and show impaired mitochondrial transport (Chen et al., 2003). Thus, the underlying disease mechanisms of disease-causing dominant MFN2 mutations might be alterations of the physiology of mitochondria. Alternatively, or in addition, impaired mitochondrial transport may cause a deleterious lack of energy supply in distal parts of axons in CMT2A patients (Shy, 2004; Züchner et al., 2004). It is tempting to speculate that the latter mechanism could explain why mutations in MFN2 and in KIF1B lead to identical phenotypes, yet no obviously altered mitochondrial distribution was observed in KIF1B-deficient mice (Zhao et al., 2001). However, mitochondrial dynamics are a complex system and other contributing factors to the disease phenotype can be envisaged. Blocking mitochondrial fusion reduces respiratory capabilities, interferes with mitochondrial calcium homeostasis, and renders cells more vulnerable to apoptotic stimuli (Chen et al., 2005; Neuspiel et al., 2005; Perfettini et al., 2005; Pich et al., 2005). Interestingly, mutations in another large mitochondrial GTPase, OPA1, are associated with dominant optic atrophy (Alexander et al., 2000). Loss of OPA1 disrupts mitochondrial integrity leading to cytochrome-*c* release (Olichon et al., 2003) and intriguingly, OPA1 functionally cooperates specifically with MFN1 to mediate the fusion of the inner mitochondrial membrane (Chen et al., 2005; Cipolat et al., 2004).

Fragmentation/Fission of Mitochondria: Ganglioside-Induced Differentiation Associated Protein 1

Mutations in ganglioside-induced differentiation-associated protein (GDAP)-1 lead to early-onset

CMT and are a frequent cause of recessive demyelinating, intermediate, and/or axonal forms of CMT (Baxter et al., 2002; Cuesta et al., 2002; Nelis et al., 2002). Neurons and Schwann cells express GDAP1, suggesting that both cell types may contribute to the mixed features of the disease (Niemann et al., 2005). GDAP1 is localized in the outer mitochondrial membrane (Niemann et al., 2005; Pedrola et al., 2005) and regulates the mitochondrial network (Niemann et al., 2005). In contrast to MFN2, GDAP1 induces mitochondrial fragmentation. However, this effect can be counteracted by active MFN2. CMT-associated GDAP1-truncations are not targeted to mitochondria and have lost mitochondrial fragmentation activity. The latter activity is also strongly reduced for disease-associated GDAP1 point mutations (Niemann et al., 2005). The exact role of GDAP1 in the regulation of mitochondrial dynamics remains to be elucidated, particularly how GDAP1 interacts with the rest of the mitochondrial fusion/fission machinery. Nevertheless, the CMT-causing mutation in MFN2 and GDAP1 demonstrate conclusively that proper regulation of mitochondrial dynamics is a critical factor for the proper function of myelinated peripheral nerves. This notion extends the already earlier recognized fact that mitochondrial disturbances are a frequent basis of neurological defects (Bossy-Wetzel et al., 2003; Chen and Chan, 2004).

Lamin A/C in CMT

Mutations in lamin A/C (LMNA) cause autosomal-recessive (AR) CMT2A (De Sandre-Giovannoli et al., 2002; Tazir et al., 2004). Lamins are ubiquitously expressed intermediate filament proteins that form the nuclear lamina. The nuclear lamina is linked to the inner nuclear membrane via integral membrane proteins. Together with the nuclear envelop, the nuclear lamina determines the nuclear shape and organizes the nuclear pore complexes that allow transport in and out of the nucleus (Mounkes and Stewart, 2004). Whereas the chromatin itself organizes independently, the nuclear lamina can provide a scaffold to form distinct subcompartments of the nucleus (Chubb et al., 2002). In this way, the nuclear lamina influences diverse processes such as DNA replication, gene expression, nuclear transport, apoptosis, and intracellular signaling pathways (reviewed in Mounkes and Stewart, 2004). Given this diversity of nuclear lamina functions, it is not surprising that mutations in lamins give rise to a variety of different diseases including muscular dystrophies, cardiomyopathies, lipodistrophy, dysplasia, premature aging (progeria), and AR-CMT2A. Lamin A/C-null mice are affected by axonal loss, axonal enlargement, and nonmyelinated large-caliber axons, somewhat resembling the phenotype of AR-CMT2A (De Sandre-Giovannoli et al., 2002). Recently, an intriguing function for lamins A and C in the protection of cells from physical damage and in the maintenance of the function of transcription factors required for the differentiation of adult stem cells has been proposed (reviewed by Hutchison and Worman, 2004). Particularly, negatively altered protection of neurons from physical damage owing to mechanical influences might be related to the disease mechanism in AR-CMT2A but conclusive experimental evidence to support this speculation is missing.

KIAA1985 in CMT

The gene encoding the KIAA1985 transcript is mutated in recessive demyelinating CMT4C (Senderek et al., 2003a). This transcript encodes a protein of unknown function containing Src homology-3 and tetratricopeptide-repeat motifs. Src homology-3 domains and tetratricopeptide-repeat motifs mediate assembly of protein complexes but interaction partners of KIAA1985 have yet to be described. Elucidation of the function of KIAA1985 and the potential disease mechanisms in CMT4C remains a challenge for the future.

Concluding Remarks

Inherited neuropathies were first described by Charcot, Marie, Tooth and Herringham in 1880s, before Mendelian inheritance was appreciated. As the genetics and molecular causes have been unraveled over the past 20 yr, the complexity of these diseases became apparent. We are just starting to understand the complex relationships between genotypes and phenotypes and how disease mechanisms may converge. As an added benefit, investigating how the different disease-causing genes

cause neuropathy provides a direct path to a better understanding of how axon–Schwann-cell interactions control the development and maintenance of myelinated nerves. Studying the genetics and biology of CMT has provided us with numerous important clues toward our current understanding of the peripheral nervous system. Several key proteins have been identified, or functionally confirmed in this way, and many are still to come, given the numerous CMT-associated loci where the genes are currently being identified. Rather than making the situation more confusing, however, the identification of novel players has provided us with the first examples of how these proteins are interconnected in common pathways and networks. This includes most notably:

1. the regulation of the development and maintenance of the myelin structure,
2. protein biosynthesis, sorting, and degradation,
3. endocytosis, membrane, and vesicle dynamics, including mitochondria, and
4. the crucial role of the cytoskeleton. Many of these processes are dynamically connected within cells and should not be viewed in isolation.

In summary, research on CMT has not only lead to significant progress in our understanding of the disease mechanisms but also allowed exciting studies in the cell and molecular biology of peripheral nerves. Last but not least, such studies have also provided the basis for first rational treatment approaches of CMT1A (Sereda et al., 2003; Passage et al., 2004).

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