Axon-Glial Signaling and the Glial Support of Axon Function

Klaus-Armin Nave[1] and Bruce D. Trapp[2]

[1]Department of Neurogenetics, Max Planck Institute of Experimental Medicine, D-37075 Göttingen, Germany; email: nave@em.mpg.de

[2]Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195; email: trappb@ccf.org

Annu. Rev. Neurosci. 2008. 31:535–61

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

This article's doi: 10.1146/annurev.neuro.30.051606.094309

0147-006X/08/0721-0535$20.00

Key Words

oligodendrocytes, Schwann cells, myelination, axonal transport, growth factors, energy metabolism, neurodegenerative diseases

Abstract

Oligodendrocytes and Schwann cells are highly specialized glial cells that wrap axons with a multilayered myelin membrane for rapid impulse conduction. Investigators have recently identified axonal signals that recruit myelin-forming Schwann cells from an alternate fate of simple axonal engulfment. This is the evolutionary oldest form of axon-glia interaction, and its function is unknown. Recent observations suggest that oligodendrocytes and Schwann cells not only myelinate axons but also maintain their long-term functional integrity. Mutations in the mouse reveal that axonal support by oligodendrocytes is independent of myelin assembly. The underlying mechanisms are still poorly understood; we do know that to maintain axonal integrity, mammalian myelin-forming cells require the expression of some glia-specific proteins, including CNP, PLP, and MAG, as well as intact peroxisomes, none of which is necessary for myelin assembly. Loss of glial support causes progressive axon degeneration and possibly local inflammation, both of which are likely to contribute to a variety of neuronal diseases in the central and peripheral nervous systems.

Contents

INTRODUCTION

In all complex nervous systems (except for those of the coelenterates) neuronal cells coexist with glial cells. This finding suggests that neuron-glia interactions are principal features of neural function. For example, in the *Drosophila* nervous system, glial cells form diffusion barriers, cover neuronal cell bodies, and align with axons in the peripheral nervous system (PNS). Indeed, the glial cells that wrap anterior and posterior commissural fibers in *Drosophila* (**Figure 1**) resemble the nonmyelin-forming Schwann cells of vertebrates because they associate with multiple axons without myelinating them (Klämbt et al. 2001). Although the numerical glia-to-neuron ratio is low in the ganglia and fiber tracts of invertebrates, this ratio has increased as the vertebrate nervous system has grown larger. Although glial cell numbers may have been overestimated in the past (Herculano-Houzel & Lent 2005), glia clearly outnumber neurons in the primate brain (Sherwood et al. 2006).

PNS: peripheral nervous system

CNS: central nervous system

Researchers generally assume that glial cells, which lack electrical excitability, support and modulate neuronal function, but what exactly is meant by the term support is still poorly understood. Moreover, experimental in vivo evidence for the known functions of glia, except for myelination (see below), is remarkably scarce. Research has identified few clearly microglia- or astrocyte-specific human diseases. Astrocytes have been implicated in forming the blood-brain barrier (Hawkins & Davis 2005), neurotransmitter reuptake (Hertz & Zielke 2004), metabolic coupling of synaptic activity (Magistretti 2006), and injury response. Whereas astrocytes are the predominant glial cell type in the mammalian cortex, the largest proportion of all central nervous system (CNS) glia is oligodendrocytes, which are most abundant in (but not restricted to) white-matter tracts.

In this review, we first compare the development and morphology of oligodendrocytes with Schwann cells in the peripheral nervous system (PNS). This will lead to a discussion of the first functional aspect of axon-glia interactions, the relevant axonal signals that initiate and regulate glial cell differentiation and myelination, processes with clear differences between the PNS and CNS. Here, key signaling molecules have recently been identified, including neuregulins and neurotrophins. Axonal electrical activity is also a likely regulator of glial ensheathment. In the second part of this review of axon-glia interactions, we review unexpected findings revealing that long-term axonal function and survival depend on the association of axons with ensheathing glial cells, not necessarily on the myelin sheath. Although it is mechanistically least understood, the view emerges that glial cell ability to support axons in the brain white matter could have a major impact on the course of human neurological and psychiatric diseases.

MYELINATING GLIAL CELLS

Oligodendrocytes are known for their role in axon myelination, which enables rapid saltatory impulse propagation. In fact, the

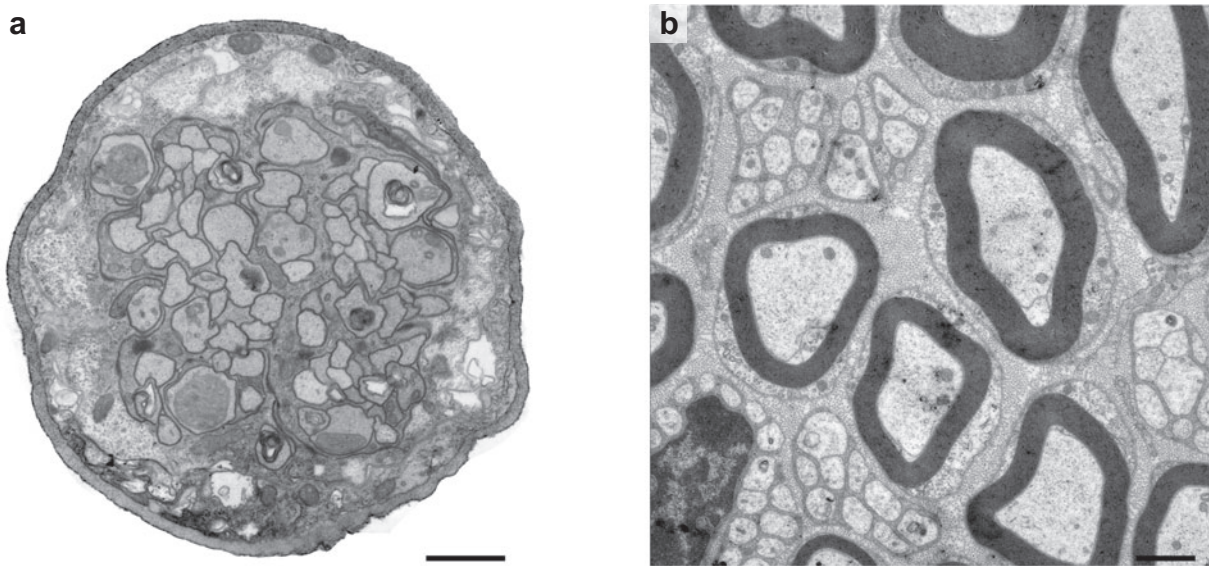

Figure 1

Electron micrograph of a peripheral nerve in *Drosophila* (panel *a* provided by C. Klämbt), showing glial cell processes that interdigitate and engulf single axons. The relationship is strikingly similar to that of nonmyelin-forming Schwann cells that engulf small-caliber C-fiber axons in the mouse sciatic nerve (*b*). Scale bar, 1 μm.

electrophysiology of myelinated nerves is one of the best understood concepts of nervous system function. Moreover, some human neurological diseases are caused primarily by myelinating glial cells, such as leukodystrophies, in the CNS. In the PNS, Schwann cells outnumber the axons that they ensheath because each cell is associated with a short axonal segment. Schwann cell dysfunctions lead to demyelinating neuropathies, which include some frequently inherited neurological disorders. One emerging concept indicates that primary myelin diseases can affect in many ways the functional integrity and survival of the axons ensheathed by defective glial cells. As visualized in different myelin mutant mice, glia's failure to support axon function and survival is surprisingly not proportional to the more obvious structural defects of the ensheathment.

Myelin extends from the glial plasma membrane, which spirally enwraps an axonal segment and is condensed into a multilamellar-compacted sheath, typically depicted by electron microscopy in cross-sections (**Figure 1*b***). Although cell biologists have understood for many years the ultrastructure of mature myelin and the relationship between axons and Schwann cells or oligodendrocytes, knowledge about the spatiotemporal development of myelin as an extracellular organelle is still elusive. Although axon-glial contact, axonal engulfment, and the first rounds of spiral ensheathment can be documented, all later stages of myelination are difficult to capture by light or electron microscopy, including the dynamics of myelin membrane deposition and membrane compaction, as well as the formation of nodal specializations and paranodal junctions.

Whereas glial engulfment of axons is, in evolutionary terms, an early feature of complex nervous systems, myelination is a late invention. In fact, spiral membrane ensheathment of axonal segments with ion channel clustering at node-like structures has been independently developed in vertebrates and several invertebrate clades (Davis et al. 1999, Schweigreiter et al. 2006, Hartline & Colman 2007). This finding suggests that glials have been repeatedly

recruited from a more ancestral, poorly defined function within axon bundles into a new role of providing a multilayered membrane ensheathment for saltatory impulse propagation.

Although the morphological design and ultrastructure of multilayered glial ensheathments vary between different (invertebrate and vertebrate) species, some basic features are conserved. For example, large axons are the first to recruit glial wrappings, and these receive more layers than do smaller axons. In vertebrates, this well-preserved ratio between axonal diameter and myelinated fiber diameter was recognized many years ago (Donaldson & Hoke 1905, Friede 1972) and is now frequently used as a measure (termed the g-ratio) to quantify myelination in development and disease states. These comparisons across species strongly suggest that axon-derived signals recruit glial cells to differentiate into myelinating glia and that this axonal signal is quantitatively related to axon size. Such a recruitment must have occurred independently several times during evolution.

In vertebrates, all myelinating glial cells share key subcellular and ultrastructural features, including the spiral ensheathment of axons, nodes, and paranodal specializations, and tight membrane compaction, suggesting a single developmental origin of myelin in the vertebrate lineage. Only the most ancestral surviving species of fish (e.g., cyclostomes, such as lamprey or hagfish) have unmyelinated nerves (Bullock et al. 1984), whereas beginning with cartilaginous fish, all present-day vertebrates exhibit myelinated central and peripheral axons. In the CNS, multipolar oligodendrocytes generally interact with multiple axons. In white-matter tracts, only larger axons are myelinated, whereas in the optic nerve or within the cortex, for example, oligodendrocytes can also ensheath very small axons. In the PNS, myelination begins when Schwann cells sort out single axons from the bundle of multiple axons that they are engulfing (Jessen & Mirsky 2005). Typically, only axons larger than 1 μm in diameter are sorted, and myelination itself increases axonal diameter (de Waegh et al. 1992). Smaller axons remain engulfed by nonmyelinating Schwann cells and stay grouped together in Remak bundles, with thin glial processes interdigitating each axon, a process remarkably similar to the glial engulfment of axons in invertebrates such as *Drosophila* (**Figure 1*a***).

Vertebrate myelin evolved ~600 million years ago and has allowed fish to develop fast escape reflexes, leading to the genetic selection of both myelinated prey and myelinated predators. Thus, myelination must have been an important driving force in early vertebrate evolution and has become essential for all freely moving terrestrial vertebrates. Its vital function becomes obvious in animals that fail to make myelin and in myelin disease in humans. Natural mouse mutants with central or peripheral dysmyelination suffer from severe motor defects, ataxia, and seizures and die prematurely, often just days or weeks after birth. In humans, the absence of myelination can be observed in leukodystrophies and neuropathies, two heterogenous groups of neurological disorders. In the primate nervous system, the integrity of myelin is subject to clear degenerative changes with aging (Sandell & Peters 2003).

Novel mouse mutants have recently been generated that harbor oligodendrocyte-specific gene defects and are fully myelinated but that later develop progressive axonal loss and die prematurely. These models provide in vivo proof that myelinating glial cells preserve axon function and survival, independent of myelination. Moreover, human patients with peripheral neuropathies have recently been identified who suffer, by genetic criteria, from a primary Schwann cell disease, but exhibit mostly clinical features of axonal degeneration in apparently well-myelinated nerves. As detailed below, novel disease models may help better define the supportive function of axon-ensheathing glia that is independent of myelination. Nonmyelinating (Remak) Schwann cells may represent an ancestral type of glia that has been preserved in the mammalian PNS. Exploring Remak cell function will lead to a better understanding of fundamental axon-glia interactions. The evolutionary steps leading to

myelination are difficult to study. However, a related question is how the two alternate fates of present-day Schwann cells are molecularly controlled (i.e., the fate of a myelinating versus nonmyelinating cell). Recent experiments involving transgenic and mutant mice revealed that the axonal growth factor neuregulin-1 (NRG1) plays an important role not only in this developmental decision, but also in the subsequent regulation of myelin membrane growth.

Through axonal growth factor expression, vertebrate neurons recruit immature glial cells to multiply and to follow and ensheath the growing axonal process to secure long-term glial support. This vital function is still required when the ensheathing cells begin to wrap the axonal segments with a multilayered myelin membrane for rapid impulse propagation.

AXONAL SIGNALS THAT RECRUIT GLIAL CELLS

In the PNS, the developmental program of glial cells is controlled entirely by axonal signals, from the proliferation of the neural crest–derived precursor cells to the differentiation of mature myelin-forming Schwann cells (for a detailed review, see Jessen & Mirsky 2005). The dependency on axonal influences is also a feature of developing oligodendrocytes but is much less pronounced compared with Schwann cells in corresponding in vivo (axonal lesion) and in vitro (neuron-glia coculture) studies. This difference and the distinct response of cultured oligodendrocytes and Schwann cells to neuronal growth factors (see below) suggest that CNS glial cells have acquired additional mechanisms to control myelin. Several observations support the idea that neurons have recruited ensheathing glial cells to obtain glial support and myelination (see Colello & Pott 1997).

That axons control myelination, and thus exert visible control over Schwann cell behavior, was first suggested by nerve-grafting experiments. Classical studies by Aguayo and colleagues showed that Schwann cells that cannot make myelin in unmyelinated nerves can nevertheless do so in a remyelination experiment (i.e., when confronted with regenerating axons from a myelinated nerve as a tissue graft). Thus axonal signals rather than Schwann cell lineage must be responsible for myelination control (Aguayo et al. 1976, Weinberg & Spencer 1976).

NRG1: neuregulin-1

Peripheral axons more than 1 μm in diameter are typically myelinated (Peters et al. 1991), whereas smaller C-fiber axons remain unmyelinated and grouped in Remak bundles, suggesting that myelination is a function of axon size (Murray 1968, Smith et al. 1982). Voyvodic (1989) experimentally showed a direct correlation between axon size and myelination, which strongly suggested a causal relationship. When unmyelinated postganglionic nerves of the salivary gland were surgically hemisected, the surviving axons were left with approximately twice the normal target size and neurotrophic support. This resulted in neurotrophin-induced axon growth, and the increase of axonal diameter was sufficient to recruit resident Remak Schwann cells for myelination. The most likely explanation was that the number of signaling molecules from the enlarged axonal surface increased, reaching a critical threshold level for myelination, but these studies could not identify the responsible signals.

Numerous in vitro studies have demonstrated that cultured Schwann cells respond to axonal signals, such as contact-dependent mitogens (Salzer et al. 1980). Pure glial cultures that myelinate axons in vitro can be experimentally manipulated and have led to the identification of axon-derived regulatory factors (see below) and second messenger pathways (Jessen & Mirsky 2005). Oligodendrocytes also respond to axonal signals (Goto et al. 1990, Kidd et al. 1990, McPhilemy et al. 1991, Scherer et al. 1992), but their differentiation in culture also proceeds in the absence of neuronal signals (Dubois-Dalcq et al. 1986, Ueda et al. 1999). Moreover, myelination by oligodendrocytes is regulated by a balance of promoting and inhibiting factors (Coman et al. 2005, Rosenberg et al. 2006). For example, axonal ensheathment by oligodendrocyte processes is inhibited by the

presence of PSA-NCAM and L1 on the axon (Charles et al. 2000). The extent to which the downregulation of these inhibitors is a physiological switch and rate-limiting step of myelination is not known. Neurons may utilize a battery of signals to control the mitotic division and differentiation of associated glial cells that provide long-term axonal support. Later in development, axonal signals specifiy which axons are myelinated and help match myelin sheath thickness to axon caliber.

Neuregulins

NRG1 is part of a family of neuronal growth factors that stimulate myelinating glial cells in vitro and in vivo. NRG1 is also found outside the nervous system and is essential for normal mammary and cardiac development. With different start sites for transcription and alternative mRNA splicing, the *NRG1* gene codes for at least 15 different proteins. These proteins share an epidermal growth factor–like signaling domain that is necessary and sufficient for the activation of ErbB receptor tyrosine kinases, which are expressed by oligodendrocytes and Schwann cells (for details, see Adlkofer & Lai 2000, Falls 2003, Esper et al. 2006, Nave & Salzer 2006).

NRG1 binds to ErbB3, a membrane protein lacking a kinase domain, or to ErbB4 but not directly to ErbB2 receptor tyrosin kinase (which must heterodimerize with either ErbB3 or ErbB4). Three NRG1 subgroups have been defined on the basis of their amino termini; NRG1 type I (also termed heregulin, neu differentiation factor, or acetylcholine receptor–inducing activity) and NRG1 type II (glial growth factor) were either secreted or shed from the axon following proteolytic processing. These factors have an immunoglobulin-like domain, bind to heparan sulfate proteoglycans in the extracellular matrix, and thus act in a paracrine fashion. In contrast, NRG1 type III (also termed SMDF) is defined by a second transmembrane (cysteine-rich) domain, remains associated with the membrane after proteolytic cleavage, and serves as a juxtacrine axonal signal (Schroering & Carey 1998). NRG1 processing enzymes include the tumor-necrosis factor-alpha-converting enzyme for NRG1 type I shedding and the beta-amyloid converting enzyme, which likely activates NRG1 type III (Horiuchi et al. 2005, Hu et al. 2006, Willem et al. 2006).

Although NRG1 has been implicated in numerous neural functions (such as neuronal migration, synaptogenesis, and glutamatergic neurotransmission), its best understood function is the neuronal and axonal regulation of Schwann cell development (reviewed in Garratt et al. 2000a, Corfas et al. 2004, Britsch 2007). Beginning with the specification of glial precursors in the embryonic neural crest (Shah et al. 1994), the entire Schwann cell lineage is controlled, at least in part, by NRG1. The classical finding that axonal membranes stimulate Schwann cell proliferation in vitro (Salzer et al. 1980) can be explained largely by the activity of membrane-associated NRG1 as a Schwann cell mitogen. Its expression by axons secures the necessary number of glial cells for normal ensheathment (Morrissey et al. 1995, Jessen & Mirsky 2005), provided that Schwann cells express ErbB2 and ErbB3 receptors (Riethmacher et al. 1997, Garratt et al. 2000b). NRG1/ErbB signals are amplified by the PI3 kinase pathway (Maurel & Salzer 2000, Ogata et al. 2004). Repopulation and remyelination of a crush-injured nerve by dedifferentiated Schwann cells do not require ErbB2 expression, as suggested by inducible gene targeting in adult mice (Atanasoski et al. 2006).

Following expansion of the Schwann cell pool in the developing peripheral nerve, axon-bound NRG1 type III is required for the differentiation of the myelinating Schwann cell phenotype (Leimeroth et al. 2002, Taveggia et al. 2005). This function includes the quantitative control of myelin membrane growth because myelin sheath thickness (as determined by g-ratio measurements) is a function of total axonal NRG1 that is presented to the ensheathing Schwann cell. Hence, in mice with reduced *NRG1* gene expression, peripheral myelin sheaths are thinner than in wild type,

and in transgenic mice that overexpress NRG1 type III [in dorsal root ganglia (DRG) and motoneurons], peripheral myelin is thicker than normal (Michailov et al. 2004). The proper axonal presentation of NRG1 is essential for myelination control, possibly in the context of axonal laminin (Colognato et al. 2002), because it cannot be replaced by paracrine NRG1 signaling (Zanazzi et al. 2001). If the level of axonal NRG1 type III stays below the threshold, the associated axons are not sorted and myelinated but remain grouped together as a Remak bundle with a single nonmyelinating Schwann cell. Experimental NRG1 type III axonal overexpression is sufficient, however, to trigger axonal sorting and myelination in vitro (Taveggia et al. 2005). Thus, the expression level of NRG1 in neurons and on the axonal surface is responsible for a lineage decision made by mammalian Schwann cells. A similar genetic switch in early vertebrate evolution may have led to the recruitment of ensheathing glia and the invention of myelin.

Many axons are myelinated first by Schwann cells and then by oligodendrocytes as they enter the spinal cord and vice versa. Moreover, Schwann cells can invade the injured spinal cord and ensheath central axons. These observations suggest that the axonal signals for myelination are conserved in the CNS and PNS (Colello & Pott 1997). Indeed, oligodendrocytes respond to NRG1 in vitro and ex vivo (Canoll et al. 1996; Vartanian et al. 1997, 1999; Fernandez et al. 2000; Flores et al. 2000; Calaora et al. 2001; Sussman et al. 2005), and transgenic expression of a dominant-negative ErbB4 construct in oligodendrocytes leads to hypomyelination (Roy et al. 2007). The analysis of conditional mouse mutants that completely lack NRG1 in cortical projection neurons (as early as E11.5) unexpectedly failed to show any reduction of myelin assembly in the subcortical white matter or the spinal cord (B. Brinkmann et al., under review). Nevertheless, transgenic NRG1 overexpression in cortical neurons induced a significant hypermyelination that was not restricted to the NRG1 type III isoform (B. Brinkmann et al., under review). These observations suggest that central axons regulate oligodendrocytes using distinct mechanisms from Schwann cells, no longer requiring NRG1 as an instructive myelination signal. Perhaps a simple system of axon-glia interactions (represented by NRG1 type III/ErbB signaling to Schwann cells) has been superseded in CNS evolution by a more complex regulation involving other growth factors and signaling systems still to be identified. This hypothesis is consistent with distinct responses from oligodendrocytes and Schwann cells to neurotrophins.

Neurotrophins

Neurotrophins compose a family of target-derived growth factors [nerve growth factor (NGF), BDNF, NT3, and NT4/5] well-known for their effect on neuronal survival. They also play a role in dendritic pruning and neurotransmitter release and have been implicated in neurodegenerative diseases (reviewed in Chao et al. 2006). Growth factors that regulate neuron survival may later stimulate axon myelination. Voyvodic (1989) suggested that small-caliber axons with elevated access to target-derived neurotrophins can grow in diameter and trigger Schwann cell myelination. In vitro, specific candidate factors can be tested by coculturing myelinating glial cells with DRG axons. Chan et al. (2004) demonstrated that NGF stimulates the myelination of such DRG axons by Schwann cells. NGF's effect was restricted to TrkA-expressing neurons, however, which suggests an indirect mechanism mediated by another axonal signal rather than direct glial stimulation (Rosenberg et al. 2006). This factor could be NRG1 because (Schwann cell–derived) NGF causes a rapid release of soluble NRG1 isoforms from the axon (Esper & Loeb 2004). Myelination in similar DRG-oligodendrocyte cocultures was unexpectedly inhibited by NGF, which suggests that oligodendrocytes and Schwann cells respond differently to the same axonal signals (Chan et al. 2004). Unfortunately, it is difficult to exclude the notion that NGF perturbs oligodendrocyte differentiation

MS: multiple sclerosis

CMT: Charcot-Marie-Tooth disease

SPG2: spastic paraplegia type 2

in vitro (Casaccia-Bonnefil et al. 1996), thereby overriding axonal myelination signals (Rosenberg et al. 2006). In Schwann cells (but not in oligodendrocytes), the low-affinity neurotrophin receptor $p75^{NTR}$ is required for efficient myelination (as shown by blocking antibodies) and mediates the stimulatory effects of BDNF. In contrast, the neurotrophin NT3 is a Schwann cell mitogenic signal, acting via TrkC receptors, and with proliferation and differentiation being incompatible, NT3 is an inhibitor of myelination (Cosgaya et al. 2002).

Electrical Activity

Oligodendrocytes proliferate poorly in the postnatal optic nerve of rodents injected with tetrodotoxin to block sodium-dependent action potentials (Barres & Raff 1993). Demerens et al. (1996) showed that tetrodotoxin blocks myelination in vivo and in vitro. Indeed, axon-glial signaling includes axonal spiking activity because ATP release and adenosin generation are monitored by glial cells (for details, see Fields & Burnstock 2006). In the PNS, the axonal release of ATP (Stevens & Fields 2000) inhibits Schwann cell differentiation and myelination via purinergic P2 receptor signaling. In contrast, oligodendrocytes expressing purinergic P1 receptors are stimulated by the axonal release of adenosin (Stevens et al. 2002). Recent studies demonstrated that the activity-dependent release of ATP from axons in the CNS stimulates nearby astrocytes to release the cytokine leukemia inhibiting factor, which in turn stimulates oligodendrocyte myelination (Ishibashi et al. 2006), and defects in this tripartite pathway may cause dysmyelination. For example, in Alexander disease (a rare human leukodystrophy), the primary defect resides in astrocytes that lack the expression of the glial fibrillary acidic protein. Given that myelinated axons remain myelinated as they enter or exit the spinal cord, the identification of the many different mechanisms by which axonal signals regulate oligodendrocytes and Schwann cells was quite unexpected.

RECRUITED GLIAL CELLS PROTECT AXON FUNCTION AND SURVIVAL

Axon-ensheathing cells have been recruited throughout evolution specifically for myelination and rapid impulse propagation, yet genetic evidence suggests the existence of a more ancestral function of these glia in axonal support. This hypothesis is strongly supported by the positive influence of myelinating glial on axon caliber (Windebank et al. 1985, Colello et al. 1994, Kirkpatrick et al. 2001) and progressive axonal degeneration found in human neurological diseases that affect oligodendrocytes, such as multiple sclerosis (MS) (Trapp & Nave 2008) and leukodystrophies (see below). Inherited peripheral neuropathies, when caused by Schwann cell dysfunction [Charcot-Marie-Tooth (CMT) disease type 1], also present with progressive axon loss that marks a clinically relevant final common pathway for all CMT diseases (Nave et al. 2007). For oligodendrocytes, animal models with spontaneous or induced mutations have provided experimental evidence supporting an oligodendrocytic role in endogenous neuroprotection, which must be distinct from myelin's role.

MYELIN PROTEINS IN NEURODEGENERATIVE DISEASE

Developmental defects of oligodendrocytes and CNS myelination cause leukodystrophies. There has been considerable research on Pelizaeus-Merzbacher disease (PMD)/spastic paraplegia type-2 (SPG2), a prototype of leukodystrophy with early onset dysmyelination and demyelination (Pelizaeus 1885, Merzbacher 1909, Johnston & McKusick 1962; reviewed in Nave & Boespflug-Tanguy 1996, Garbern 2007). With the discovery of *Plp1* mutations in corresponding mouse models (Nave et al. 1986, Hudson et al. 1987, Schneider et al. 1992) and human PMD and SPG2 patients (Hudson et al. 1989, Saugier-Veber et al. 1994), PMD/SPG2 is now defined as a genetic defect

of the X-linked proteolipid protein (*PLP1*) gene (for details, see Inoue 2005).

PLP (30 kDa) and its smaller splice isoform (DM20) are abundant tetraspan proteins found in CNS myelin of higher vertebrates. One cellular function of PLP/DM20, which is not essential for myelination itself, is the stabilization of compacted myelin membranes by serving as molecular struts (Klugmann et al. 1997). However, some subtle developmental functions of myelin in PLP-deficient mice are now well understood (Boison et al. 1995, Yool et al. 2001, Rosenbluth et al. 2006). PLP is a cholesterol-binding protein in lipid rafts (Simons et al. 2000), and mice doubly deficient in PLP and the closely related proteolipid M6B (also binding to cholesterol) exhibit a severe CNS dysmyelination with an altered myelin cholesterol content (H.B. Werner & K.-A. Nave, manuscript in preparation). Thus, proteolipids may be required to enrich cholesterol as an essential lipid of myelination (Saher et al. 2005) and to transport other proteins into the myelin compartment efficiently (Werner et al. 2007).

In mice and humans, most *Plp1* point mutations and even duplications of the human *PLP1* gene cause severe dysmyelination, triggered by PLP/DM20 misfolding (Jung et al. 1996), abnormal cysteine-cross-links (Dhaunchak & Nave 2007), endoplasmic reticulum retention (Gow & Lazzarini 1996), the unfolded protein response (Southwood et al. 2002), and finally oligodendrocyte death. The short life span of mutant mice therefore masks the functions of oligodendrocytes in adult mice for long-term axonal function and integrity. For example, *Plp1* mutant *jimpy* mice die at four weeks of age at a time when most of their axons are intact (Meier & Bischoff 1975). Moreover, all axonal abnormalities seen in *jimpy* mice (or *Plp1* mutant *md* rats) were originally thought to result from the absence of myelin (Rosenfeld & Friedrich 1983, Barron et al. 1987).

The first insights into oligodendrocytes supporting axonal survival independently of myelination came with the analysis of genetic null mutants in the *Plp1* gene. These mice develop normally and are long-lived, which suggests that PLP is dispensable for myelination and that oligodendrocyte death in *jimpy* mice follows the expression of truncated PLP (Nave et al. 1986, Klugmann et al. 1997). *Plp1 null* mice, however, develop a late-onset (>12 months) neurodegenerative disease caused by progressive axonal loss throughout the CNS, preferentially small-caliber axons in long spinal tracts, and eventually premature death (Griffiths et al. 1998). Many months before the onset of clinical symptoms, this type of Wallerian degeneration is preceded by axonal swelling. Swellings occur in fully myelinated axons and are either organelle rich or filled with nonphosphorylated neurofilaments. They begin at the paranodal region, which may possibly be a bottleneck of axonal transport. Swellings and transection bulbs can be readily visualized by immunohistochemical staining of the amyloid precursor protein, when locally trapped, or by electron microscopy (**Figure 2**). Abnormal swellings likely reflect the complete breakdown of the fast axonal transport because they are developmentally preceded by a significant reduction of the axonal transport rate (initially the retrograde transport). This has been demonstrated for the optic nerve of 60-day-old *Plp1 null* mice that are clinically and histopathologically still unaffected (Edgar et al. 2004b).

With this late onset of ataxia (but no tremor or seizures), *Plp1 null* mice are genetically and clinically bona fide models for SPG2, a milder allelic form of PMD (Johnston & McKusick 1962, Saugier-Veber et al. 1994). Likewise, human patients with a null mutation of *PLP1* (Raskind et al. 1991) have a milder course of disease, dominated by slowly progressive degeneration of long spinal cord axons (Garbern et al. 2002) rather than dysmyelination at infant age. Reduced N-acetyl aspartate levels in the brains of PMD patients (Bonavita et al. 2001), however, suggest that some axonal involvement is a general feature of PLP-related diseases, provided there is a long enough survival time (see below).

PLP: proteolipid protein

DM20: smaller splice isoform of PLP

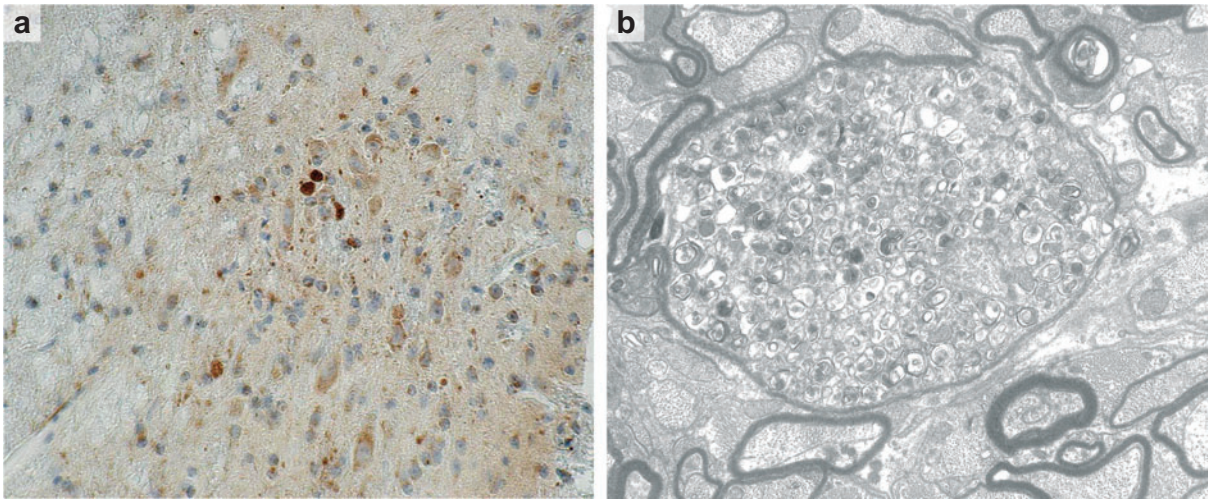

Figure 2

(*a*) Immunostaining and (*b*) electron microscopy of axonal swelling in the white matter of adult mice with a disruption of the oligodendroglial *Cnp1* gene. Note the presence of normally myelinated axons. Figure adapted from Lappe-Siefke et al. (2003).

The exact role of PLP or its alternative spliced isoform DM20 in axonal preservation is still unknown. Mice with the *rumpshaker* mutation in *Plp1* (Schneider et al. 1992) are long-lived and hypomyelinated and have reduced amounts of mutant DM20 and trace amounts of PLP incorporated into myelin. These mice also exhibit late-onset axonal degeneration (Edgar et al. 2004a), suggesting that *rumpshaker* DM20 may be sufficiently folded to reach the myelin compartment but unable to support axonal integrity fully. One study found axonal swellings in myelinated mice that lack selective expression of the PLP isoform (Stecca et al. 2000), although this phenotype was not as early as in the absence of both PLP and DM20 (Spörkel et al. 2002).

A second gene specifically expressed in myelinating glial cells and recently associated with the oligodendroglial support of axons is *Cnp1*. The encoded protein 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP or RIP) is widely used as a marker protein for myelin-forming glial cells (Vogel & Thompson 1988, Watanabe et al. 2006). CNP is specifically associated with noncompacted myelin regions (i.e., inner mesaxon, paranodal loops, and Schmidt-Lantermann incisures) but is absent from the compacted sheath (Braun et al. 1988, Trapp et al. 1988). The enzymatic activity of CNP in vivo is unclear because 2′,3′-cyclic nucleotides are not found in the brain and only known from RNA metabolism. CNP, when experimentally overexpressed, induces process outgrowth in cultured cells (Lee et al. 2005) and premature abnormal myelination in transgenic mice (Gravel et al. 1996, Yin et al. 1997). CNP is expressed outside the nervous system but at lower levels. A CNP-related protein in fish, termed RICH (regeneration induced CNP homolog), is expressed by retinal ganglion cells during axonal outgrowth, which suggests that CNP/RICH performs certain functions during active membrane growth. Thus, similar to other myelin proteins, CNP may have been recruited in evolution by myelin-forming glia from a more general cellular function. The *Cnp1* gene encodes two CNP isoforms; the larger one (CNP2) harbors an amino-terminal mitochondrial targeting sequence (Lee et al. 2006). Both forms are acylated and isoprenylated at their carboxyl terminus (Gravel et al. 1994), which explains their efficient association with cellular membranes. CNP also interacts with the actin

CNP: 2′,3′-cyclic nucleotide 3′-phosphodiesterase

skeleton and microtubules (DeAngelis & Braun 1996, Bifulco et al. 2002, Lee et al. 2005), as well as with mitochondria, in which the longer CNP isoform can be imported, at least in nonglial cells (McFerran & Burgoyne 1997, Lee et al. 2006). Whether CNP functions by associating mitochondria and/or RNA with the oligodendroglial cytoskeleton, as speculated, requires experimental support.

Mice with targeted disruption of the *Cnp1* gene develop on schedule and are fully myelinated, but they develop widespread and progressive axonal swellings (Lappe-Siefke et al. 2003). The phenotype is clinically more severe than that of *Plp1 null* mice, with a much earlier onset and premature death by ~12 months of age. Myelin sheaths are normally compacted in the absence of CNP, which emphasizes that axonal problems are not caused by a thin or physically unstable myelin sheath. Many oligodendroglial paranodes become disorganized before the onset of clinical symptoms (Rasband et al. 2005), which suggests that the absence of CNP alters the normal communication between axons and oligodendrocytes. That the paranodal changes are the major cause of axonal dysfunction and degeneration is unlikely because the first swellings (as early as postnatal day 5 in the optic nerve) and enlargement of the inner tongue precede the corresponding paranodal changes (J.M. Edgar et al., submitted). The axonal swellings also ultimately indicate an energy-related metabolic problem. The exact molecular mechanisms remain to be defined.

PLP and CNP are myelin-associated proteins expressed by oligodendrocytes and Schwann cells, but the axonal degeneration phenotype in null mutant mice is CNS specific. Yin et al. (1998) reported perturbed axonal integrity in the PNS for the myelin-associated glycoprotein (MAG). MAG is a member of the immunoglobulin superfamily (Lai et al. 1987) and has properties of a cell-adhesion protein and signaling molecule localized to the periaxonal membranes of all myelinating glia, as well as to paranodal loops and Schmidt-Lantermann incisures in Schwann cells (Martini & Schachner 1986, Trapp et al. 1989). MAG is not present on axons. MAG's function was expected to involve specific axon-glia recognition, adhesion, and signaling, but the phenotype of MAG-deficient mice (Li et al. 1994, Montag et al. 1994) was not informative, most likely because MAG's function was masked by the presence of other adhesion molecules such as N-CAM and L1 (for details, see Bartsch 2003) or the recently identified Necl4 protein (Maurel et al. 2007, Spiegel et al. 2007). Some minor developmental abnormalities were documented, including multiply ensheathed axons or the delay of optic nerve myelination (Bartsch et al. 1995, 1997). Moreover, oligodendrocytes in aged mice exhibited degenerative changes (dying back oligodendrogliopathy), a pathological feature previously described in some MS lesions (Lassmann et al. 1997, Weiss et al. 2000). These phenotypes, however, fail to identify the normal function of this protein. Instead, MAG's role as one of several myelin-associated and Nogo receptor-dependent inhibitors of axonal regeneration has gained much attention (e.g., Domeniconi et al. 2002). Studies suggest that myelin inhibitors prevent inappropriate axonal sprouting. Resident microglial cells are another likely target of repulsive MAG signaling (F. Orfaniotou & K.-A. Nave, manuscript in preparation).

MAG: myelin-associated glycoprotein

The inhibition of axonal growth cones by MAG demonstrates that this myelin protein possesses signaling domains that communicate with neuronal/axonal receptors. An important finding in this respect is that MAG modulates the physical caliber of the myelinated axons on Schwann cells. Despite the morphologically normal myelination of *Mag* mutant mice, peripheral axons remain significantly smaller in diameter when compared with wild-type mice (Yin et al. 1998), presumably because their neurofilaments are hypophosphorylated and thus more densely packed. The reduction in axonal diameter was strikingly more pronounced in paranodal regions of the myelin internode. Because much of the paranodal atrophy occurred after myelin was

formed, and because myelin collapsed on the shrunken axon, axonal segments were hypermyelinated. The occurrence of hypermyelinated focal segments or tomacula, as found in certain demyelinating neuropathies (e.g., CMT4B), suggests that MAG may be one of the hypothesized glia-to-axon signals that may explain why normal myelination must maintain axonal calibers (de Waegh et al. 1992). This dependence of axon size on glial ensheathment was originally discovered in the myelin-deficient *Trembler* mouse, a natural point mutation of the *Pmp22* gene, and was later confirmed in the CNS of dysmyelinated *shiverer* mice, a natural deletion mutant of the *Mbp* gene (Roach et al. 1985, Brady et al. 1999).

In humans, inherited demyelinating PNS neuropathies (CMT disease) can be caused by a plethora of primary glial defects, comprising mutations in Schwann cell–specific or ubiquitously expressed genes (for details, see Suter & Scherer 2003, Berger et al. 2006, Nave et al. 2007). Most detailed clinical studies and nerve biopsy analyses have been performed using patients with defects of the myelin-associated proteins PMP22 (in CMT1A), MPZ (in CMT1B), and connexin-32 (in CMT1X). Transgenic and mutant animal models of the corresponding *Pmp22*, *Mpz*, and *Gjp1* genes provided formal proof of concept for the genetic cause of each disease and also helped further dissect disease mechanisms. Demyelinating CMT diseases present with progressive muscle weakness and sensory loss, in addition to the strong reduction of nerve conduction velocity (NCV), which demonstrates that a length-dependent loss of motor and sensory axons must be caused by primary Schwann cell dysfunction. In *Mpz* mutant mice, peripheral axon loss is also preceded by focal swellings (Ey et al. 2007). In a transgenic model of demyelinating neuropathy, caused by overexpression of the wild-type *Mpz* gene in Schwann cells (Yin et al. 2004), the authors observed synaptic retraction and loss of the neuromuscular end plate that preceded the visible axonal degeneration of lower motoneurons. Although the molecular details are not understood, presynaptic dysfunctions may constitute an independent mechanism of target denervation that is clinically relevant to myelin disease.

By definition, all demyelinating neuropathies exhibit morphological signs of myelin pathology as the underlying cause of slowed NCV, which suggests that myelin per se could be required to maintain axonal integrity. It was therefore a major advance when specific mutations in the Schwann cell–specific *MPZ* gene were identified that caused CMT type 2 with normal NCV (i.e., the axonal form of CMT disease) rather than CMT1B (Marrosu et al. 1998, Senderek et al. 2000, Boerkoel et al. 2002). Although they are mechanistically not well understood, separate functions of Schwann cells in myelination (preserved in CMT2) and in axonal support (lost in all CMT forms) may have been uncoupled, reminiscent of *Cnp1* and *Plp null* mutations in myelinating oligodendrocytes. Nonmyelinating Schwann cells also support axonal integrity, as shown for L1-mutant mice, in which Remak Schwann cells fail to properly engulf C-fiber axons that degenerate (Haney et al. 1999). Transgenic overexpression of nonfunctional ErbB receptors in these Schwann cells also causes dysfunction and loss of C-fiber axons (Chen et al. 2003), which supports the hypothesis that axonal support is an ancestral glial function that precedes myelination.

Thus, when comparing myelin disorders in the CNS and PNS, all ensheathing glial cells are essential to maintain long-term axonal integrity. However, the consequences of demyelination and axon loss are regionally different. The enormous plasticity of the brain may be able to mask a slowly progressive degeneration of the subcortical axons for a long time, and a substantial axonal loss may remain clinically silent in MS patients (Trapp & Nave 2008). However, length-dependent axon loss in the spinal cord (e.g., in patients with SPG2) is more difficult to mask. Finally, the progressive loss of peripheral axons (e.g., in patients with CMT disease) causes invariable muscle denervation and sensory losses that cannot be hidden by neural plasticity.

POSSIBLE MECHANISMS OF GLIAL SUPPORT

Long-term demyelination is associated with axon loss (Raine & Cross 1989, Trapp et al. 1998). However, in comparing axon loss in different myelin diseases in mice and humans, there is no simple correlation between the degree of demyelination and axonal involvement. This discrepancy suggests that the absence of myelin alone is not the only cause of neurodegeneration. Nevertheless, demyelination is likely to increase dramatically the energy consumption of fast spiking axons in white-matter tracts, which may perturb axon function. One hypothetical model of how reduced axonal energy balance can trigger calcium-dependent proteolysis of a demyelinated axon is discussed elsewhere in this volume (Trapp & Nave 2008). Glial support, as discussed here, is a feature of fully myelinated axons that requires an alternative model.

Myelin-Associated Toxicity

One must critically discuss whether axonal perturbation as observed in myelinated *Plp* and *Cnp1 null* mice results from the loss of support, or alternatively from a possible gain of toxicity of the PLP-deficient and CNP-deficient myelin wraps. Although no toxic mechanisms are presently known, this alternate hypothesis is difficult to disprove. In a natural mosaic situation (e.g., random inactivation of the X chromosome–linked *Plp* gene in heterozygous females), axonal swellings are clearly detectable, although at reduced frequency (Griffiths et al. 1998). Likewise, after transplantation of *Plp* mutant oligodendrocytes into *shiverer* white-matter tracts, axonal swellings can be locally induced (Edgar et al. 2004b). Although they are compatible with some glial toxicity, these observations are equally well explained by a short range of oligodendroglial support. Only myelinated axons are dependent on support (Griffiths et al. 1998). One might thus speculate that myelination per se could be toxic for axons unless the ensheathing glial cells can support the axonal segment that they have myelinated. Indeed, compacted myelin membranes may act as a destructive shield to impair the axon's otherwise ready access to outside nutrients, metabolites, oxygen, and other important molecules. Thus a specific glial transport apparatus might be required to compensate for these restrictions and to prevent toxic myelination effects. These hypothetical functions would collectively qualify as glial support.

Myelin-Independent Mechanisms

The prodromal phenotype of *Plp null* mice includes axonal transport defects reminiscent of mitochondrial disorders in the CNS, and it suggests that in the presence of myelin, an underlying low energy balance leads to axon loss. We suggest that myelin-independent glial support requires axonal engulfment and a molecular apparatus that is part of, but not identical to, the myelin sheath. In addition to the genes mentioned above, other oligodendrocyte defects have been associated with a similar loss of axonal support, for example, in mice lacking the synthesis of GalC and sulfatide, two myelin-specific glycosphingolipids that are essential for paranodes forming axo-glial junctions (Garcia-Fresco et al. 2006, Marcus et al. 2006). However, not all myelin defects are associated with axonal loss. For example, *Mbp null* (i.e., *shiverer*) mice lack the expression of myelin basic protein (MBP), are severely dysmyelinated in the CNS, and die prematurely. However, they exhibit no obvious signs of axonal swelling or degeneration (Rosenbluth 1980, Inoue et al. 1981, Nixon 1982, Griffiths et al. 1998). In *shiverer* mice, electrophysiological signs of conduction block are caused by an abnormal distribution of potassium channels (Sinha et al. 2006) not by Wallerian degeneration. Increased energy needs in *shiverer* cause a twofold-higher density of mitochondria (Andrews et al. 2006). Thus, *Mbp null* mice are dysmyelinated with functional axons, whereas *Plp null* mice are fully myelinated with widespread axonal degeneration. Clearly, PLP must serve a unique function in axonal support that goes beyond its

CNTF: ciliary neurotrophic factor

structural role of stabilizing compacted myelin membranes (Boison et al. 1995, Klugmann et al. 1997). This notion has been independently proven in transgenic knockout mice, in which PLP oligodendroglial expression was swapped with MPZ expression, the major cell adhesion molecule from PNS myelin (Yin et al. 2006). Although MPZ could form an adhesive strut between adjacent myelin membranes (similar to PLP), it did not allow oligodendrocytes to support axonal survival.

PLP is a tetraspan myelin protein and, by itself, is unlikely to support axonal survival. Recently, the biochemical analysis of myelin purified from *Plp null* mice revealed secondary abnormalities of the myelin proteome, which suggested that PLP is required for the transport of other proteins that may contribute to oligodendroglial support into the growing myelin compartment. One protein that is nearly absent from PLP-deficient myelin is Sirt2, an NAD^+-dependent deacetylase expressed most strongly in oligodendrocytes (Werner et al. 2007).

Our understanding of the glial support of axons is still in its infancy, and the mechanisms involved are likely complex. Comparisons at the ultrastructural level of just *Plp* and *Cnp1 null* mice revealed significant differences in the onset and distribution of prodromal axonal changes (J.M. Edgar et al., manuscript in preparation). The observation that *Plp*Cnp1* double mutants exhibit an earlier and much more severe course of disease than does either single mutant indicates additive effects that are compatible with the involvement of different protective mechanisms (H.A. Werner & K.A. Nave, unpublished observations).

Neurotrophic Factors

Myelinating glia may provide trophic support for axons, but the extent to which this support involves neurotrophins and related forms of glia-to-neuron growth factor signaling is not known. The function of neurotrophins has been reviewed in detail (Huang & Reichardt 2001), also with respect to myelinating glial cells (Rosenberg et al. 2006). Target-derived neurotrophins stimulate axon-bearing neurons, which likely alters the expression of axonal cues for myelination (Chan et al. 2004). In addition, ample evidence demonstrates that neurotrophins can stimulate the proliferation and differentiation of myelinating glial cells directly (Barres et al. 1994, Kumar et al. 1998, McTigue et al. 1998, Du et al. 2003), but they may also have detrimental effects (Casaccia-Bonnefil et al. 1996). Although all these functions are important for oligodendrocyte development and remyelination (and thus indirectly for axonal support), there is scarce experimental evidence that myelinating glia provide continuous axonal support by releasing trophic factors. Neurotrophins are expressed by Schwann cells (Chan et al. 2001) and in satellite glia of cultured DRG neurons. Here, the expression of NT3 and NGF can be stimulated by axotomy, causing the release of nitric oxide, and nitric oxide–induced neurotrophins are likely neuroprotective (Thippeswamy et al. 2005). There is obviously great interest in using neurotrophins in the prevention of axon loss in CMT disease. The systemic application of NT3 has even been tested clinically in a small number of patients with demyelinating neuropathy CMT1A with encouraging results (Sahenk et al. 2005).

Two other factors with reported survival functions in the nervous system and some expression in myelinating glia must be considered as candidates for glial trophic support. The ciliary neurotrophic factor (CNTF) is expressed by Schwann cells (Sendtner et al. 1994), but the mechanisms of release are unclear because this cytoplasmic cytokine is not exocytosed like growth factors are. In mice, CNTF is required for motoneuron survival and the long-term integrity of myelinated axons in the PNS (Gatzinsky et al. 2003). Also, in mice with experimental allergic encephalomyelitis, an immunological model of human MS, the absence of CNTF causes a more severe disease (Linker et al. 2002). A small but significant percentage (2.3%) of the Japanese population is homozygous for a null mutation of this gene (Takahashi et al. 1994), and this genetic polymorphism is not obviously linked to a neurological disease

such as amyotrophic lateral sclerosis or CMT (Van Vught et al. 2007). This observation raises the question, at least in humans, of the relevance of CNTF in endogenous neuroprotection by myelinating glial cells.

The glia cell line–derived growth factor (GDNF) is a ligand of the ret receptor tyrosine kinase (Durbec et al. 1996) and signals independently by N-CAM and the fyn serine/threonine kinase (Paratcha et al. 2003). GDNF, along with several related proteins, is expressed inside and outside the nervous system (Suter-Crazzolara & Unsicker 1994), including Schwann cells (Springer et al. 1995, Trupp et al. 1995) in which GDNF is upregulated upon axotomy (Hammaberg et al. 1996). Mouse mutants of this gene model Hirschsprungs disease (Moore et al. 1996, Pichel et al. 1996, Sanchez et al. 1996) with the loss of enteric ganglia, which resembles early glial and neural crest defects (Inoue et al. 1999, Paratore et al. 2001). GDNF has been intensively studied for its survival effect on dopaminergic neurons (Beck et al. 1995), but it is also a survival factor for embryonic motoneurons (Henderson et al. 1994). The beneficial role of GDNF in the treatment of neuropathic pain (Boucher et al. 2000) could relate to neuroprotective effects normally exerted by Schwann cells. However, direct evidence showing that GDNF would be required to maintain axonal integrity in myelinated fibers is lacking. Similar to neurotrophins, promoting the differentiation of myelinating glia could provide a neuroprotective effect (Wilkins et al. 2003). When GDNF was injected into intact peripheral nerves, it caused Schwann cells to proliferate and to sort and myelinate small-caliber C-fiber axons (Hoke et al. 2003). This study also demonstrates that trophic factors can, in principle, overcome developmental thresholds and change the glial phenotype from nonmyelinating Schwann cells to myelin-forming Schwann cells, similar to axonal NRG1.

An endogenous neuroprotective molecule, synthesized by oligodendrocytes, may be prostaglandin D. In the dysmyelinated mouse mutant *twitcher*, upregulated expression of the oligodendroglial prostaglandin D synthase correlated with neuronal survival, and *Pgds *twitcher* double mutants had a more severe phenotype (Taniike et al. 2002). This enzyme is also induced in MS lesions (Kagitani-Shimono et al. 2006), but the mechanisms of action are not known.

GDNF: glial cell line–derived factor

Metabolic Support

Both oligodendrocyte and Schwann cell defects have been associated with a length-dependent axon loss. The most plausible, but least understood, mechanism of axonal preservation by ensheathing glial cells may be metabolic support (Spencer et al. 1979). Rapidly conducting white-matter axons consume a large fraction of the brain's energy supply, and oligodendrocytes are vulnerable to hypoxia and glucose deprivation (Fern et al. 1998). In dysmyelinated and demyelinated axons, the number of mitochondria is increased (Mutsaers & Carrol 1998, Andrews et al. 2006). Most ATP is required for membrane repolarization and for fast axonal transport of vesicles and organelles, occurring at a rate of micrometers per second (Hollenbeck & Saxton 2006). The latter includes axonal mitochondria, which travel with frequent stops and restarts (Misgeld et al. 2007). Mitochondria often pause at the node of Ranvier, which is thought to indicate the site of highest metabolic activity (Fabricius et al. 1993), an observation more obvious in the PNS (I. Griffiths, personal communication). Although the nodal region harbors Na^+/K^+ channels and is the site of most transmembrane ionic flow, most ATP is consumed by membrane repolarization, and Na^+/K^+ ATPases are dispersed along the entire internodal membrane of myelinated PNS and CNS axons (Alberti et al. 2007; E. Young, J.H. Fowler, G.J. Kidd, A. Chang, R. Rudick, E. Fisher & B. Trapp, submitted). We also note that mitochondria, when pausing at the nodal region, are in closest proximity to the glial paranodal loops, which form a highly specialized axon-glial junction. In the PNS of Caspr-deficient mice, which lack the normal paranodal ultrastructure, even more

PEX5: peroxin-5

mitochondria are abnormally retained at the intra-axonal membrane surface beneath the disrupted paranode, and many of these mitochondria have a swollen morphology (Einheber et al. 2006). This finding suggests that axonal mitochondrial transport is regulated, at least in part, by glial contact and that myelinating cells contribute to normal mitochondrial functions within axons.

In healthy axons, ATP is present in micromolar concentrations, but we predict that an impairment of (ATP-dependent) axonal transport ultimately affects the normal turnover rate and integrity of mitochondria themselves. This indicates a potentially vicious cycle for even minor or highly localized metabolic problems. Mitochondrial involvement can lead to local entrapment and axonal swellings as visible signs of system collapse, which leads to Wallerian degeneration. This hypothesis is supported by the axonal pathology of specific myelin mutants with reduced axonal transport and axonal swellings that closely resemble those in mitochondrial disorders (Ferreirinha et al. 2004), constituting the final common pathway of hereditary spastic paraplegia. Moreover, reduced axonal ATP decreases Na^+/K^+ ATPase (thus elevating axoplasmic Na^+) and ATP-dependent Na^+/Ca^{2+} exchanger activity. The latter can operate in reverse and exchange axoplasmic Na^+ for extracellular Ca^{2+}, at least under pathological conditions (low ATP, high Na^+). Elevated axoplasmic Ca^{2+} causes further damage to axonal and mitochondrial proteins, which introduces a second vicious pathological cycle (Stys et al. 1992, Li et al. 2000).

In the CNS, astrocytes provide free lactate to neurons for ATP generation (hypothesized as the "lactate shuttle"; for details, see Magistretti 2006). In white-matter tracts, this action can involve the mobilization of stored glycogen (Brown et al. 2003). Whether myelinating glial cells also provide energy-rich metabolites to the axons they ensheath is not well analyzed in vivo. In explant systems, non-myelin-forming Schwann cells take up glucose, which they transfer to axons, possibly as lactate equivalents via gap junctions (Vega et al. 2003). The generation of PEX5-deficient mutants lacking functional peroxisomes selectively in oligodendrocytes in vivo recently provided a link between the loss of specific peroxisomal pathways in myelinating glia and the progressive degeneration of axonal integrity in adult mice (Kassmann et al. 2007). We anticipate that a systematic genetic dissection of metabolic pathways in oligodendrocytes and Schwann cells of mutant mice will identify those pathways required for axonal support.

Mouse mutants lacking functional peroxisomes in glia myelinate normally, but similar to dysmyelinated mice that overexpress PLP in oligodendrocytes (Readhead et al. 1994), they exhibit axon loss, late-onset demyelination, and neuroinflammation in areas affected by degenerative changes. Unlike other myelin mutants, inflammation includes the infiltration of activated CD8(+) T cells, and, in peroxisomal mutants, perivascular B-cell infiltrates as well (Ip et al. 2006, Kassmann et al. 2007). Such observations demonstrate that specific oligodendrocyte defects contribute to, if not trigger, inflammatory demyelinating diseases, which is relevant to the unknown etiology of human MS (Trapp & Nave 2008).

CONCLUSION

Research on glial cells in the long-term support of axonal function and in endogenous neuroprotection has just begun. One conceptual link between axonal myelination in higher vertebrates and myelin-independent axonal support is that both mechanisms have evolved to meet the energy demands of rapidly conducting long axonal tracts. Loss of these functions can trigger a vicious cycle of pathological changes, including reduced axonal transport and retention of mitochondria, leading to axonal swelling and calcium-dependent Wallerian degeneration. Genetic data have now provided strong in vivo evidence that neurodegenerative diseases of the CNS and PNS can result from primary defects in oligodendrocytes and Schwann cells, respectively, although the affected glial cells

reveal no obvious developmental defects. The view emerges that, except for clearly dysmyelinating and demyelinating disorders, some glial diseases may share features of neurodegenerative disorders. Because axonal degeneration is seen in many neurological diseases, dysfunction of glia and glial cell aging should be considered disease-modifying factors.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors apologize to colleagues whose relevant work could not be cited because of space restrictions. We thank C. Klämbt and G. Saher for providing electron micrographs. We also thank J. Edgar, I. Griffiths, C. Kassmann, G. Saher, and H. Werner for helpful discussion and critical reading of the manuscript. K.-A.N. is supported by grants from the Deutsche Forschungsgemeinschaft, the European Union (FP6), the BMBF (Leukonet), the Hertie Institute of MS Research, the National MS Society, the Del Marmol Foundation, and Olivers Army. B.D.T. is supported by NIH grants NS038186, NS038667, and NS029818.

LITERATURE CITED

Adlkofer K, Lai C. 2000. Role of neuregulins in glial cell development. *Glia* 29:104–11

Aguayo AJ, Charron L, Bray GM. 1976. Potential of Schwann cells from unmyelinated nerves to produce myelin: a quantitative ultrastructural and radiographic study. *J. Neurocytol.* 5:565–73

Alberti S, Gregorio EA, Spadella CT, Cojocel C. 2007. Localization and irregular distribution of Na,K-ATPase in myelin sheath from rat sciatic nerve. *Tissue Cell* 39:195–201

Andrews H, White K, Thomson C, Edgar J, Bates D, et al. 2006. Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse. *J. Neurosci. Res.* 83:1533–39

Atanasoski S, Scherer SS, Sirkowski E, Leone D, Garratt AN, et al. 2006. ErbB2 signaling in Schwann cells is mostly dispensable for maintenance of myelinated peripheral nerves and proliferation of adult Schwann cells after injury. *J. Neurosci.* 26:2124–31

Barres BA, Raff MC. 1993. Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* 361:258–60

Barres BA, Raff MC, Gaese F, Bartke I, Dechant G, Barde YA. 1994. A crucial role for neurotrophin-3 in oligodendrocyte development. *Nature* 367:371–75

Barron KD, Dentinger MP, Csiza CK, Keegan SM, Mankes R. 1987. Abnormalities of central axons in a dysmyelinative rat mutant. *Exp. Mol. Pathol.* 47:125–42

Bartsch U. 2003. Neural CAMS and their role in the development and organization of myelin sheaths. *Front Biosci.* 8:477–90

Bartsch U, Montag D, Bartsch S, Schachner M. 1995. Multiply myelinated axons in the optic nerve of mice deficient for the myelin-associated glycoprotein. *Glia* 14:115–22

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.* 762:231–34

Beck KD, Valverde J, Alexi T, Poulsen K, Moffat B, et al. 1995. Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain. *Nature* 373:339–41

Berger P, Niemann A, Suter U. 2006. Schwann cells and the pathogenesis of inherited motor and sensory neuropathies (Charcot-Marie-Tooth disease). *Glia* 54:243–57

Bifulco M, Laezza C, Stingo S, Wolff J. 2002. 2′,3′-cyclic nucleotide 3′-phosphodiesterase: a membrane-bound, microtubule-associated protein and membrane anchor for tubulin. *Proc. Natl. Acad. Sci. USA* 99:1807–12

Boerkoel CF, Takashima H, Garcia CA, Olney RK, Johnson J, et al. 2002. Charcot-Marie-Tooth disease and related neuropathies: mutation distribution and genotype-phenotype correlation. *Ann. Neurol.* 51:190–201

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.* 15:5502–13

Bonavita S, Schiffmann R, Moore DF, Frei K, Choi B, et al. 2001. Evidence for neuroaxonal injury in patients with proteolipid protein gene mutations. *Neurology* 56:785–88

Boucher TJ, Okuse K, Bennett DLH, Munson JB, Wood JN, McMahon SB. 2000. Potent analgesic effects of GDNF in neuropathic pain states. *Science* 290:124–27

Brady ST, Witt AS, Kirkpatrick LL, de Waegh SM, Readhead C, et al. 1999. Formation of compact myelin is required for maturation of the axonal cytoskeleton. *J. Neurosci.* 19:7278–88

Braun PE, Sandillon F, Edwards A, Matthieu JM, Privat A. 1988. Immunocytochemical localization by electron microscopy of 2′3′-cyclic nucleotide 3′-phosphodiesterase in developing oligodendrocytes of normal and mutant brain. *J. Neurosci.* 8:3057–66

Britsch S. 2007. The neuregulin-I/ErbB signaling system in development and disease. *Adv. Anat. Embryol. Cell Biol.* 190:1–65

Brown AM, Tekkök SB, Ransom BR. 2003. Glycogen regulation and functional role in mouse white matter. *J. Physiol.* 549:501–12

Bullock TH, Moore JK, Fields RD. 1984. Evolution of myelin sheaths: both lamprey and hagfish lack myelin. *Neurosci. Lett.* 48:145–48

Calaora V, Rogister B, Bismuth K, Murray K, Brandt H, et al. 2001. Neuregulin signaling regulates neural precursor growth and the generation of oligodendrocytes in vitro. *J. Neurosci.* 21:4740–51

Canoll PD, Musacchio JM, Hardy R, Reynolds R, Marchionni MA, Salzer JL. 1996. GGF/neuregulin is a neuronal signal that promotes the proliferation and survival and inhibits the differentiation of oligodendrocyte progenitors. *Neuron* 17:229–43

Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV. 1996. Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature* 383:716–19

Chan JR, Cosgaya JM, Wu YJ, Shooter EM. 2001. Neurotrophins are key mediators of the myelination program in the peripheral nervous system. *Proc. Natl. Acad. Sci. USA* 98:14661–68

Chan JR, Watkins TA, Cosgaya JM, Zhang C, Chen L, et al. 2004. NGF controls axonal receptivity to myelination by Schwann Cells or oligodendrocytes. *Neuron* 43:183–91

Chao MV, Rajagopal R, Lee FS. 2006. Neurotrophin signalling in health and disease. *Clin. Sci.* 110:167–73

Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, et al. 2000. Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. *Proc. Natl. Acad. Sci. USA* 97:7585–90

Chen S, Rio C, Ji RR, Dikkes P, Coggeshall RE, et al. 2003. Disruption of ErbB receptor signaling in adult nonmyelinating Schwann cells causes progressive sensory loss. *Nat. Neurosci.* 6:1186–93

Colello RJ, Pott U. 1997. Signals that initiate myelination in the developing mammalian nervous system. *Mol. Neurobiol.* 15:83–100

Colello RJ, Pott U, Schwab ME. 1994. The role of oligodendrocytes and myelin on axon maturation in the developing rat retinofugal pathway. *J. Neurosci.* 14:2594–605

Colognato H, Baron W, Avellana-Adalid V, Relvas JB, Baron-Van Evercooren A, et al. 2002. CNS integrins switch growth factor signalling to promote target-dependent survival. *Nat. Cell Biol.* 4:833–41

Coman I, Barbin G, Charles P, Zalc B, Lubetzki C. 2005. Axonal signals in central nervous system myelination, demyelination and remyelination. *J. Neurol. Sci.* 233:67–71

Corfas G, Velardez MO, Ko CP, Ratner N, Peles E. 2004. Mechanisms and roles of axon-Schwann cell interactions. *J. Neurosci.* 24:9250–60

Cosgaya JM, Chan JR, Shooter EM. 2002. The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science* 298:1245–48

Davis AD, Weatherby TM, Hartline DK, Lenz PH. 1999. Myelin-like sheaths in copepod axons. *Nature* 398:571

De Angelis DA, Braun PE. 1996. 2′,3′-cyclic nucleotide 3′-phosphodiesterase binds to actin-based cytoskeletal elements in an isoprenylation-dependent manner. *J. Neurochem.* 67:943–51

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, et al. 1996. Induction of myelination in the central nervous system by electrical activity. *Proc. Natl. Acad. Sci. USA* 93:9887–92

de Waegh SM, Lee VM, Brady ST. 1992. Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. *Cell* 68:451–63

Dhaunchak A, Nave KA. 2007. A common mechanism of PLP/DM20 misfolding leading to cysteine-mediated ER retention in oligodendrocytes and Pelizaeus-Merzbacher disease. *Proc. Natl. Acad. Sci. USA* 104:17815–18

Domeniconi M, Cao Z, Spencer T, Sivasankaran R, Wang KC, et al. 2002. Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron* 35:283–90

Donaldson HH, Hoke GW. 1905. The areas of the axis cylinder and medullary sheath as seen in cross sections of the spinal nerves of vertebrates. *J. Comp. Neurol.* 15:1–16

Du Y, Fischer TZ, Lee LN, Lercher LD, Dreyfus CF. 2003. Regionally specific effects of BDNF on oligodendrocytes. *Dev. Neurosci.* 25:116–26

Dubois-Dalcq M, Behar T, Hudson L, Lazzarini RA. 1986. Emergence of three myelin proteins in oligodendrocytes cultured without neurons. *J. Cell Biol.* 102:384–92

Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, et al. 1996. GDNF signalling through the Ret receptor tyrosine kinase. *Nature* 381:789–93

Edgar JM, McLaughlin M, Barrie JA, McCulloch MC, Garbern J, Griffiths IR. 2004a. Age-related axonal and myelin changes in the rumpshaker mutation of the Plp gene. *Acta Neuropathol. (Berl.)* 107:331–35

Edgar JM, McLaughlin M, Yool D, Zhang SC, Fowler JH, et al. 2004b. Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic paraplegia. *J. Cell Biol.* 166:121–31

Einheber S, Bhat MA, Salzer JL. 2006. Disrupted axo-glial junctions result in accumulation of abnormal mitochondria at nodes of Ranvier. *Neuron Glia Biol.* 2:165–74

Esper RM, Loeb JA. 2004. Rapid axoglial signaling mediated by neuregulin and neurotrophic factors. *J. Neurosci.* 24:6218–27

Esper RM, Pankonin MS, Loeb JA. 2006. Neuregulins: versatile growth and differentiation factors in nervous system development and human disease. *Brain Res. Rev.* 51:161–75

Ey B, Kobsar I, Blazyca H, Kroner A, Martini R. 2007. Visualization of degenerating axons in a dysmyelinating mouse mutant with axonal loss. *Mol. Cell Neurosci.* 35:153–60

Fabricius C, Berthold CH, Rydmark M. 2003. Axoplasmic organelles at nodes of Ranvier. II. Occurrence and distribution in large myelinated spinal cord axons of the adult cat. *J. Neurocytol.* 22:941–54

Falls DL. 2003. Neuregulins: functions, forms, and signaling strategies. *Exp. Cell Res.* 284:14–30

Fern R, Davis P, Waxman SG, Ransom BR. 1998. Axon conduction and survival in CNS white matter during energy deprivation: a developmental study. *J. Neurophysiol.* 79:95–105

Fernandez PA, Tang DG, Cheng L, Prochiantz A, Mudge AW, Raff MC. 2000. Evidence that axon-derived neuregulin promotes oligodendrocyte survival in the developing rat optic nerve. *Neuron* 28:81–90

Ferreirinha F, Quattrini A, Pirozzi M, Valsecchi V, Dina G, et al. 2004. Axonal degeneration in paraplegin-deficient mice is associated with abnormal mitochondria and impairment of axonal transport. *J. Clin. Invest.* 113:231–42

Fields RD, Burnstock G. 2006. Purinergic signalling in neuron-glia interactions. *Nat. Rev. Neurosci.* 7:423–36

Flores AI, Mallon BS, Matsui T, Ogawa W, Rosenzweig A, et al. 2000. Akt-mediated survival of oligodendrocytes induced by neuregulins. *J. Neurosci.* 20:7622–30

Friede RL. 1972. Control of myelin formation by axon caliber (with a model of the control mechanism). *J. Comp. Neurol.* 144:233–52

Garbern JY. 2007. Pelizaeus-Merzbacher disease: genetic and cellular pathogenesis. *Cell. Mol. Life Sci.* 64:50–65

Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, et al. 2002. Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. *Brain* 125:551–61

Garcia-Fresco GP, Sousa AD, Pillai AM, Moy SS, Crawley JN, et al. 2006. Disruption of axo-glial junctions causes cytoskeletal disorganization and degeneration of Purkinje neuron axons. *Proc. Natl. Acad. Sci.USA* 103:5137–42

Garratt AN, Britsch S, Birchmeier C. 2000a. Neuregulin, a factor with many functions in the life of a Schwann cell. *Bioessays* 22:987–96

Garratt AN, Voiculescu O, Topilko P, Charnay P, Birchmeier C. 2000b. A dual role of erbB2 in myelination and in expansion of the Schwann cell precursor pool. *J. Cell Biol.* 148:1035–46

Gatzinsky KP, Holtmann B, Daraie B, Berthold CH, Sendtner M. 2003. Early onset of degenerative changes at nodes of Ranvier in alpha-motor axons of Cntf null (−/−) mutant mice. *Glia* 42:340–49

Goto K, Kurihara T, Takahashi Y, Kondo H. 1990. Expression of genes for the myelin-specific proteins in oligodendrocytes in vivo demands the presence of axons. *Neurosci. Lett.* 117:269–74

Gow A, Lazzarini RA. 1996. A cellular mechanism governing the severity of Pelizaeus-Merzbacher disease. *Nat. Genet.* 13:422–28

Gravel M, DeAngelis D, Braun PE. 1994. Molecular cloning and characterization of rat brain 2′,3′-cyclic nucleotide 3′-phosphodiesterase isoform 2. *J. Neurosci. Res.* 38:243–47

Gravel M, Peterson J, Yong VW, Kottis V, Trapp B, Braun PE. 1996. Overexpression of 2′,3′-cyclic nucleotide 3′-phosphodiesterase in transgenic mice alters oligodendrocyte development and produces aberrant myelination. *Mol. Cell Neurosci.* 7:453–66

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, et al. 1998. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 280:1610–13

Haney CA, Sahenk Z, Li C, Lemmon VP, Roder J, Trapp BD. 1999. Heterophilic binding of L1 on unmyelinated sensory axons mediates Schwann cell adhesion and is required for axonal survival. *J. Cell Biol.* 146:1173–84

Hartline DK, Colman DR. 2007. Rapid conduction and the evolution of giant axons and myelinated fibers. *Curr. Biol.* 17:R29–35

Hawkins BT, Davis TP. 2005. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol. Rev.* 57:173–85

Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C, et al. 1994. GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. *Science* 266:1062–64

Herculano-Houzel S, Lent R. 2005. Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. *J. Neurosci.* 25:2518–21

Hertz L, Zielke HR. 2004. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci.* 27:735–43

Hoke A, Ho T, Crawford TO, LeBel C, Hilt D, Griffin JW. 2003. Glial cell line derived neurotrophic factor alters axon Schwann cell units and promotes myelination in unmyelinated nerve fibers. *J. Neurosci.* 23:561–67

Hollenbeck PJ, Saxton WM. 2005. The axonal transport of mitochondria. *J. Cell Sci.* 118:5411–19

Horiuchi K, Zhou HM, Kelly K, Manova K, Blobel CP. 2005. Evaluation of the contributions of ADAMs 9, 12, 15, 17, and 19 to heart development and ectodomain shedding of neuregulins beta1 and beta2. *Dev. Biol.* 283:459–71

Hu X, Hicks CW, He W, Wong P, Macklin WB, et al. 2006. Bace1 modulates myelination in the central and peripheral nervous system. *Nat. Neurosci.* 9:1520–25

Huang EJ, Reichardt LF. 2001. Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* 24:677–736

Hudson LD, Berndt JA, Puckett C, Kozak CA, Lazzarini RA. 1987. Aberrant splicing of proteolipid protein mRNA in the dysmyelinating jimpy mutant mouse. *Proc. Natl. Acad. Sci. USA* 84:1454–58

Hudson LD, Puckett C, Berndt J, Chan J, Gencic S. 1989. Mutation of the proteolipid protein gene PLP in a human X chromosome-linked myelin disorder. *Proc. Natl. Acad. Sci. USA* 86:8128–31

Inoue Y, Nakamura R, Mikoshiba K, Tsukada Y. 1981. Fine structure of the central myelin sheath in the myelin deficient mutant Shiverer mouse, with special reference to the pattern of myelin formation by oligodendroglia. *Brain Res.* 219:85–94

Inoue K. 2005. PLP1-related inherited dysmyelinating disorders: Pelizaeus-Merzbacher disease and spastic paraplegia type 2. *Neurogenetics* 6:1–16

Inoue K, Tanabe Y, Lupski JR. 1999. Myelin deficiencies in both the central and the peripheral nervous systems associated with a SOX10 mutation. *Ann. Neurol.* 46:313–38

Ip CW, Kroner A, Bendszus M, Leder C, Kobsar I, et al. 2006. Immune cells contribute to myelin degeneration and axonopathic changes in mice overexpressing proteolipid protein in oligodendrocytes. *J. Neurosci.* 26:8206–16

Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, et al. 2006. Astrocytes promote myelination in response to electrical impulses. *Neuron* 49:823–32

Jessen KR, Mirsky R. 2005. The origin and development of glial cells in peripheral nerves. *Nat. Rev. Neurosci.* 6:671–82

Johnston AW, McKusick VA. 1962. A sex-linked recessive form of spastic paraplegia. *Am. J. Hum. Genet.* 14:83–94

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.* 16:7920–29

Kagitani-Shimono K, Mohri I, Oda H, Ozono K, Suzuki K, et al. 2006. Lipocalin-type prostaglandin D synthase (beta-trace) is upregulated in the alphaB-crystallin-positive oligodendrocytes and astrocytes in the chronic multiple sclerosis. *Neuropathol. Appl. Neurobiol.* 32:64–73

Kassmann CM, Lappe-Siefke C, Baes M, Brugger B, Mildner A, et al. 2007. Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes. *Nat. Genet.* 39:969–76

Kidd GJ, Hauer PE, Trapp BD. 1990. Axons modulate myelin protein messenger RNA levels during central nervous system myelination in vivo. *J. Neurosci. Res.* 26:409–18

Kirkpatrick LL, Witt AS, Payne HR, Shine HD, Brady ST. 2001. Changes in microtubule stability and density in myelin-deficient shiverer mouse CNS axons. *J. Neurosci.* 21:2288–97

Klämbt C, Hummel T, Granderath S, Schimmelpfeng K. 2001. Glial cell development in *Drosophila*. *Int. J. Dev. Neurosci.* 19:373–78

Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, et al. 1997. Assembly of CNS myelin in the absence of proteolipid protein. *Neuron* 18:59–70

Kumar S, Kahn MA, Dinh L, de Vellis J. 1998. NT-3-mediated TrkC receptor activation promotes proliferation and cell survival of rodent progenitor oligodendrocyte cells in vitro and in vivo. *J. Neurosci. Res.* 54:754–65

Lai C, Brow MA, Nave KA, Noronha AB, Quarles RH, et al. 1987. Two forms of 1B236/myelin-associated glycoprotein, a cell adhesion molecule for postnatal neural development, are produced by alternative splicing. *Proc. Natl. Acad. Sci. USA* 84:4337–41

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, et al. 2003. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nat. Genet.* 33:366–74

Lassmann H, Bartsch U, Montag D, Schachner M. 1997. Dying-back oligodendrogliopathy: a late sequel of myelin-associated glycoprotein deficiency. *Glia* 19:104–10

Lee J, Gravel M, Zhang R, Thibault P, Braun PE. 2005. Process outgrowth in oligodendrocytes is mediated by CNP, a novel microtubule assembly myelin protein. *J. Cell Biol.* 170:661–73

Lee J, O'Neill RC, Park MW, Gravel M, Braun PE. 2006. Mitochondrial localization of CNP2 is regulated by phosphorylation of the N-terminal targeting signal by PKC: implications of a mitochondrial function for CNP2 in glial and nonglial cells. *Mol. Cell Neurosci.* 31:446–62

Leimeroth R, Lobsiger C, Lussi A, Taylor V, Suter U, Sommer L. 2002. Membrane bound neuregulin1 type III actively promotes Schwann cell differentiation of multipotent progenitor cells. *Dev. Biol.* 246:245–58

Li C, Tropak MB, Gerlai R, Clapoff S, Abramow-Newerly W, et al. 1994. Myelination in the absence of myelin-associated glycoprotein. *Nature* 369:747–50

Li S, Jiang Q, Stys PK. 2000. Important role of reverse $Na^{(+)}$-$Ca^{(2+)}$ exchange in spinal cord white matter injury at physiological temperature. *J. Neurophysiol.* 84:1116–19

Linker RA, Maurer M, Gaupp S, Martini R, Holtmann B, et al. 2002. CNTF is a major protective factor in demyelinating CNS disease: a neurotrophic cytokine as modulator in neuroinflammation. *Nat. Med.* 8:620–24

Magistretti PJ. 2006. Neuron-glia metabolic coupling and plasticity. *J. Exp. Biol.* 209:2304–11

Marcus J, Honigbaum S, Shroff S, Honke K, Rosenbluth J, Dupree JL. 2006. Sulfatide is essential for the maintenance of CNS myelin and axon structure. *Glia* 53:372–81

Marrosu MG, Vaccargiu S, Marrosu G, Vannelli A, Cianchetti C, Muntoni F. 1998. Charcot-Marie-Tooth disease type 2 associated with mutation of the myelin protein zero gene. *Neurology* 50:1397–401

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.* 103:2439–48

Maurel P, Einheber S, Galinska J, Thaker P, Lam I, et al. 2007. Nectin-like proteins mediate axon Schwann cell interactions along the internode and are essential for myelination. *J. Cell Biol.* 178:861–74

Maurel P, Salzer JL. 2000. Axonal regulation of Schwann cell proliferation and survival and the initial events of myelination requires PI 3-kinase activity. *J. Neurosci.* 20:4635–45

McFerran B, Burgoyne R. 1997. 2′,3′-Cyclic nucleotide 3′-phosphodiesterase is associated with mitochondria in diverse adrenal cell types. *J. Cell Sci.* 110:2979–85

McPhilemy K, Griffiths IR, Mitchell LS, Kennedy PG. 1991. Loss of axonal contact causes down-regulation of the PLP gene in oligodendrocytes: evidence from partial lesions of the optic nerve. *Neuropathol. Appl. Neurobiol.* 17:275–87

McTigue DM, Horner PJ, Stokes BT, Gage FH. 1998. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J. Neurosci.* 18:5354–65

Meier C, Bischoff A. 1975. Oligodendroglial cell development in jimpy mice and controls. An electron-microscopic study in the optic nerve. *J. Neurol. Sci.* 26:517–28

Merzbacher L. 1909. Gesetzmaessigkeiten in der Vererbung und Verbreitung verschiedener hereditaer-familiaerer Erkrankungen. *Arch. Rass. Ges. Biol.* 6:172–98

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, et al. 2004. Axonal neuregulin-1 regulates myelin sheath thickness. *Science* 304:700–3

Misgeld T, Kerschensteiner M, Bareyre FM, Burgess RW, Lichtman JW. 2007. Imaging axonal transport of mitochondria in vivo. *Nat. Methods* 4:559–61

Montag D, Giese KP, Bartsch U, Martini R, Lang Y, et al. 1994. Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. *Neuron* 13:229–46

Moore MW, Klein RD, Farinas I, Sauer H, Armanini M, et al. 1996. Renal and neuronal abnormalities in mice lacking GDNF. *Nature* 382:76–79

Morrissey TK, Levi AD, Nuijens A, Sliwkowski MX, Bunge RP. 1995. Axon induced mitogenesis of human Schwann cells involves heregulin and p185erbB2. *Proc. Natl. Acad. Sci. USA* 92:1431–35

Murray MA. 1968. An electron microscopic study of the relationship between axon diameter and the initiation of myelin production in the peripheral nervous system. *Anat. Rec.* 161:337–52

Mutsaers SE, Carroll WM. 1998. Focal accumulation of intra-axonal mitochondria in demyelination of the cat optic nerve. *Acta Neuropathol.* 96:139–43

Nave KA, Boespflug-Tanguy O. 1996. Developmental defects of myelin formation: from X-linked mutations to human dysmyelinating diseases. *Neuroscientist* 2:33–43

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. USA* 83:9264–68

Nave KA, Salzer JL. 2006. Axonal regulation of myelination by neuregulin 1. *Curr. Opin. Neurobiol.* 16:492–500

Nave KA, Sereda MW, Ehrenreich H. 2007. Mechanisms of disease: inherited demyelinating neuropathies—from basic to clinical research. *Nat. Clin. Pract. Neurol.* 3:453–64

Nixon RA. 1982. Increased axonal proteolysis in myelin-deficient mutant mice. *Science* 215:999–1001

Ogata T, Iijima S, Hoshikawa S, Miura T, Yamamoto S, et al. 2004. Opposing extracellular signal-regulated kinase and Akt pathways control Schwann cell myelination. *J. Neurosci.* 24:6724–32

Paratcha G, Ledda F, Ibanez CF. 2003. The neural cell adhesion molecule NCAM is an alternative signaling receptor for GDNF family ligands. *Cell* 113:867–79

Paratore C, Goerich DE, Suter U, Wegner M, Sommer L. 2001. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. *Development* 128:3949–61

Pelizaeus F. 1885. Ueber eine eigentumliche Form spastischer Lahmung mit Cerebralerscheinungen auf hereditarer Grundlage (Multiple Sklerose). *Arch. Psychiat. Nervenkr.* 16:698–710

Peters A, Palay SL, Webster H deF. 1991. *The Fine Structure of the Nervous System*. New York: Oxford Univ. Press. 3rd ed.

Pichel JG, Shen L, Shang HZ, Granholm AC, Drago J, et al. 1996. Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* 382:73–76

Raine CS, Cross AH. 1989. Axonal dystrophy as a consequence of long-term demyelination. *Lab. Invest.* 60:714–25

Rasband MN, Tayler J, Kaga Y, Yang Y, Lappe-Siefke C, et al. 2005. CNP is required for maintenance of axon-glia interactions at nodes of Ranvier in the CNS. *Glia* 50:86–90

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.* 49:1355–60

Readhead C, Schneider A, Griffiths I, Nave KA. 1994. Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. *Neuron* 12:583–95

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* 389:725–30

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* 42:149–55

Rosenberg SS, Ng BK, Chan JR. 2006. The quest for remyelination: a new role for neurotrophins and their receptors. *Brain Pathol.* 16:288–94

Rosenbluth J. 1980. Central myelin in the mouse mutant shiverer. *J. Comp. Neurol.* 194:639–48

Rosenbluth J, Nave KA, Mierzwa A, Schiff R. 2006. Subtle myelin defects in PLP-null mice. *Glia* 54:172–82

Rosenfeld J, Friedrich VL Jr. 1983. Axonal swellings in jimpy mice: Does lack of myelin cause neuronal abnormalities? *Neuroscience* 10:959–66

Roy K, Murtie JC, El-Khodor BF, Edgar N, Sardi SP, et al. 2007. Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. *Proc. Natl. Acad. Sci. USA* 104:8131–36

Sahenk Z, Nagaraja HN, McCracken BS, King WM, Freimer ML, et al. 2005. NT-3 promotes nerve regeneration and sensory improvement in CMT1A mouse models and in patients. *Neurology* 65:681–89

Saher G, Brugger B, Lappe-Siefke C, Mobius W, Tozawa R, et al. 2005. High cholesterol level is essential for myelin membrane growth. *Nat. Neurosci.* 8:468–75

Salzer JL, Williams AK, Glaser L, Bunge RP. 1980. Studies of Schwann cell proliferation. II. Characterization of the stimulation and specificity of the response to a neurite membrane fraction. *J. Cell Biol.* 84:753–66

Sanchez MP, Silos-Santiago I, Frisen J, He B, Lira SA, Barbacid M. 1996. Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* 382:70–73

Sandell JH, Peters A. 2003. Disrupted myelin and axon loss in the anterior commissure of the aged rhesus monkey. *J. Comp. Neurol.* 466:14–30

Saugier-Veber P, Munnich A, Bonneau D, Rozet JM, Le Merrer M, et al. 1994. X-linked spastic paraplegia and Pelizaeus-Merzbacher disease are allelic disorders at the proteolipid protein locus. *Nat. Genet.* 6:257–62

Scherer SS, Vogelbacker HH, Kamholz J. 1992. Axons modulate the expression of proteolipid protein in the CNS. *J. Neurosci. Res.* 32:138–48

Schneider A, Montague P, Griffiths I, Fanarraga M, Kennedy P, et al. 1992. Uncoupling of hypomyelination and glial cell death by a mutation in the proteolipid protein gene. *Nature* 358:758–61

Schroering A, Carey DJ. 1998. Sensory and motor neuron-derived factor is a transmembrane heregulin that is expressed on the plasma membrane with the active domain exposed to the extracellular environment. *J. Biol. Chem.* 273:30643–50

Schweigreiter R, Roots BI, Bandtlow CE, Gould RM. 2006. Understanding myelination through studying its evolution. *Int. Rev. Neurobiol.* 73:219–73

Senderek J, Hermanns B, Lehmann U, Bergmann C, Senderek J, et al. 2000. Charcot–Marie–Tooth neuropathy type 2 and P0 point mutations: two novel amino acid substitutions (Asp61Gly; Tyr119Cys) and a possible "hotspot" on Thr124Met. *Brain Pathol.* 10:235–48

Sendtner M, Carroll P, Holtmann B, Hughes RA, Thoenen H. 1994. Ciliary neurotrophic factor. *J Neurobiol.* 25:1436–53

Shah NM, Marchionni MA, Isaacs I, Stroobant P, Anderson DJ. 1994. Glial growth factor restricts mammalian neural crest stem cells to a glial fate. *Cell* 77:349–60

Sherwood CC, Stimpson CD, Raghanti MA, Wildman DE, Uddin M, et al. 2006. Evolution of increased glia-neuron ratios in the human frontal cortex. *Proc. Natl. Acad. Sci. USA* 103:13606–11

Simons M, Krämer EM, Thiele C, Stoffel W, Trotter J. 2000. Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. *J. Cell Biol.* 151:143–54

Sinha K, Karimi-Abdolrezaee S, Velumian AA, Fehlings MG. 2006. Functional changes in genetically dysmyelinated spinal cord axons of shiverer mice: role of juxtaparanodal Kv1 family K^+ channels. *J. Neurophysiol.* 95:1683–95

Smith KJ, Blakemore WF, Murray JA, Patterson RC. 1982. Internodal myelin volume and axon surface area. A relationship determining myelin thickness? *J. Neurol. Sci.* 55:231–46

Southwood CM, Garbern J, Jiang W, Gow A. 2002. The unfolded protein response modulates disease severity in Pelizaeus-Merzbacher disease. *Neuron* 36:585–96

Spencer PS, Sabri MI, Schaumburg HH, Moore CL. 1979. Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? *Ann. Neurol.* 5:501–7

Spiegel I, Adamsky K, Eshed Y, Milo R, Sabanay H, et al. 2007. A central role for Necl4 (SynCAM4) in Schwann cell-axon interaction and myelination. *Nat. Neurosci.* 10:861–69

Sporkel O, Uschkureit T, Bussow H, Stoffel W. 2002. Oligodendrocytes expressing exclusively the DM20 isoform of the proteolipid protein gene: myelination and development. *Glia* 37:19–30

Springer JE, Seeburger JL, He J, Gabrea A, Blankenhorn EP, Bergman LW. 1995. cDNA sequence and differential mRNA regulation of two forms of glial cell line-derived neurotrophic factor in Schwann cells and rat skeletal muscle. *Exp. Neurol.* 131:47–52

Stecca B, Southwood CM, Gragerov A, Kelley KA, Friedrich VL Jr, Gow A. 2000. The evolution of lipophilin genes from invertebrates to tetrapods: DM-20 cannot replace proteolipid protein in CNS myelin. *J. Neurosci.* 20:4002–10

Stevens B, Fields RD. 2000. Response of Schwann cells to action potentials in development. *Science* 287:2267–71

Stevens B, Porta S, Haak LL, Gallo V, Fields RD. 2002. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. *Neuron* 36:855–68

Stys PK, Waxman SG, Ransom BR. 1992. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and $Na^{(+)}$-Ca^{2+} exchanger. *J. Neurosci.* 12:430–39

Sussman CR, Vartanian T, Miller RH. 2005. The ErbB4 neuregulin receptor mediates suppression of oligodendrocyte maturation. *J. Neurosci.* 25:5757–62

Suter U, Scherer SS. 2003. Disease mechanisms in inherited neuropathies. *Nat. Rev. Neurosci.* 4:714–26

Suter-Crazzolara C, Unsicker K. 1994. GDNF is expressed in two forms in many tissues outside the CNS. *NeuroReport* 20:2486–88

Takahashi R, Yokoji H, Misawa H, Hayashi M, Hu J, Deguchi T. 1994. A null mutation in the human CNTF gene is not causally related to neurological diseases. *Nat. Genet.* 7:79–84

Taniike M, Mohri I, Eguchi N, Beuckmann CT, Suzuki K, Urade Y. 2002. Perineuronal oligodendrocytes protect against neuronal apoptosis through the production of lipocalin-type prostaglandin D synthase in a genetic demyelinating model. *J. Neurosci.* 22:4885–96

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, et al. 2005. Neuregulin-1 type III determines the ensheathment fate of axons. *Neuron* 47:681–94

Thippeswamy T, McKay JS, Morris R, Quinn J, Wong LF, Murphy D. 2005. Glial-mediated neuroprotection: evidence for the protective role of the NO-cGMP pathway via neuron-glial communication in the peripheral nervous system. *Glia* 49:197–210

Trapp BD, Andrews SB, Cootauco C, Quarles R. 1989. The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. *J. Cell Biol.* 109:2417–26

Trapp BD, Bernier L, Andrews SB, Colman DR. 1988. Cellular and subcellular distribution of 2′,3′-cyclic nucleotide 3′-phosphodiesterase and its mRNA in the rat central nervous system. *J. Neurochem.* 51:859–68

Trapp BD, Nave KA. 2008. Axonal degeneration in multiple sclerosis. *Annu. Rev. Neurosci.* 31:247–69

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. 1998. Axonal transection in the lesions of multiple sclerosis. *N. Engl. J. Med.* 338:278–85

Trupp M, Rydén M, Jörnvall H, Funakoshi H, Timmusk T, et al. 1995. Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. *J. Cell Biol.* 130:137–48

Ueda H, Levine JM, Miller RH, Trapp BD. 1999. Rat optic nerve oligodendrocytes develop in the absence of viable retinal ganglion cell axons. *J. Cell Biol.* 146:1365–74

Van Vught PW, Van Wijk J, Bradley TE, Plasmans D, Jakobs ME, et al. 2007. Ciliary neurotrophic factor null alleles are not a risk factor for Charcot-Marie-Tooth disease, hereditary neuropathy with pressure palsies and amyotrophic lateral sclerosis. *Neuromuscul. Disord.* 17:964–67

Vartanian T, Fischbach G, Miller R. 1999. Failure of spinal cord oligodendrocyte development in mice lacking neuregulin. *Proc. Natl. Acad. Sci. USA* 96:731–35

Vartanian T, Goodearl A, Viehover A, Fischbach G. 1997. Axonal neuregulin signals cells of the oligodendrocyte lineage through activation of HER4 and Schwann cells through HER2 and HER3. *J. Cell Biol.* 137:211–20

Vega C, Martiel JL, Drouhault D, Burckhart MF, Coles JA. 2003. Uptake of locally applied deoxyglucose, glucose and lactate by axons and Schwann cells of rat vagus nerve. *J. Physiol.* 546:551–64

Vogel US, Thompson RJ. 1988. Molecular structure, localization, and possible functions of the myelin-associated enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase. *J. Neurochem.* 50:1667–77

Voyvodic JT. 1989. Target size regulates calibre and myelination of sympathetic axons. *Nature* 342:430–33

Watanabe M, Sakurai Y, Ichinose T, Aikawa Y, Kotani M, Itoh K. 2006. Monoclonal antibody Rip specifically recognizes 2′,3′-cyclic nucleotide 3′-phosphodiesterase in oligodendrocytes. *J. Neurosci. Res.* 84:525–33

Weinberg HJ, Spencer PS. 1976. Studies on the control of myelinogenesis. Evidence for neuronal regulation of myelin production. *Brain Res.* 113:363–78

Weiss MD, Hammer J, Quarles RH. 2000. Oligodendrocytes in aging mice lacking myelin-associated glycoprotein are dystrophic but not apoptotic. *J. Neurosci. Res.* 62:772–80

Werner HB, Kuhlmann K, Shen S, Uecker M, Schardt A, et al. 2007. Proteolipid protein is required for transport of sirtuin 2 into CNS myelin. *J. Neurosci.* 27:7717–30

Wilkins A, Majed H, Layfield R, Compston A, Chandran S. 2003. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. *J. Neurosci.* 23:4967–74

Willem M, Garratt AN, Novak B, Citron M, Kaufmann S, et al. 2006. Control of peripheral nerve myelination by the beta-secretase BACE1. *Science* 314:664–66

Windebank AJ, Wood P, Bunge RP, Dyck PJ. 1985. Myelination determines the caliber of dorsal root ganglion neurons in culture. *J. Neurosci.* 5:1563–69

Yin X, Baek RC, Kirschner DA, Peterson A, Fujii Y, et al. 2006. Evolution of a neuroprotective function of central nervous system myelin. *J. Cell Biol.* 172:469–78

Yin X, Crawford TO, Griffin JW, Tu P, Lee VM, et al. 1998. Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. *J. Neurosci.* 18:1953–62

Yin X, Kidd GJ, Pioro EP, McDonough J, Dutta R, et al. 2004. Dysmyelinated lower motor neurons retract and regenerate dysfunctional synaptic terminals. *J. Neurosci.* 24:3890–98

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. Neurosci. Res.* 50:238–47

Yool DA, Klugmann M, McLaughlin M, Vouyiouklis DA, Dimou L, et al. 2001. Myelin proteolipid proteins promote the interaction of oligodendrocytes and axons. *J. Neurosci. Res.* 63:151–64

Zanazzi G, Einheber S, Westreich R, Hannocks MJ, Bedell-Hogan D, et al. 2001. Glial growth factor/neuregulin inhibits Schwann cell myelination and induces demyelination. *J. Cell Biol.* 152:1289–99

Annual Review of Neuroscience

Volume 31, 2008

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Neuroscience* articles may be found at http://neuro.annualreviews.org/