A unified cell biological perspective on axon–myelin injury

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Demyelination and axon loss are pathological hallmarks of the neuroinflammatory disorder multiple sclerosis (MS). Although we have an increasingly detailed understanding of how immune cells can damage axons and myelin individually, we lack a unified view of how the axon–myelin unit as a whole is affected by immune-mediated attack. In this review, we propose that as a result of the tight cell biological interconnection of axons and myelin, damage to either can spread, which might convert a local inflammatory disease process early in MS into the global progressive disorder seen during later stages. This mode of spreading could also apply to other neurological disorders.

Pathology of the axon–myelin unit in multiple sclerosis (MS)

Immune cells enter the peripheral nervous system (PNS) and central nervous system (CNS) in several neurological conditions of infectious or autoimmune origin. These immune invaders interact with the target tissue, which can result in damage of neural cells. The predominant resident target is often used to classify the resulting disease: for instance, myelin and axons are targeted in the case of demyelinating and axonal polyneuropathies, respectively (Köller et al., 2005; Kuwabara and Yuki, 2013). Yet, on biopsy, many demyelinating polyneuropathies present with mixed myelin and axon pathology (Bosboom et al., 2001), with the latter serving as an important predictor of disease outcome (Bouchard et al., 1999). The intertwined nature of axon and myelin pathology becomes even more apparent in MS, a common inflammatory disease of the CNS. MS has been classically regarded as a primarily demyelinating disorder. However,

recent work indicates that axon injury is already prominent in the earliest stages of MS (Trapp et al., 1998; Kuhlmann et al., 2002; Singh et al., 2013). The finding that damage can be initiated in axons that are still myelinated, both in experimental and human neuroinflammatory lesions (Nikić et al., 2011), further indicates that axons can—at least in some cases—be a primary target of the inflammatory attack. Hence, primary and secondary immune targets are difficult to differentiate with certainty. This is even more accentuated in progressive MS, which is characterized by a spread of neurodegeneration into both gray and white matter (Lassmann et al., 2012) and the parallel expansion of myelin damage, leading to confluent areas of subpial demyelination in the cortices of progressive MS patients (Bø et al., 2003; Kutzelnigg et al., 2005). Overall, the neuroglial conundrum is best illustrated by the fact that neuronal, and not glial damage, is the best predictor of long-term outcome, even if demyelination is the most prominent histopathological feature of the MS lesion (Bjartmar et al., 2000; De Stefano et al., 2001; Lubetzki and Stankoff, 2014). Together, these findings indicate that neuronal and glial pathology in inflammatory conditions should not be regarded as separate entities but rather as highly interdependent entry points into damage of a common target, the axon–myelin unit.

In this review, we bring together findings from the fields of axon and myelin biology to develop an integrated view of neuroinflammatory axon–myelin pathology. In particular, we discuss the commonalities and differences in the way axons and glial cells degenerate to find out which mechanistic concepts can be transferred from one cell type to the other. We further explore the interdependence of axons and myelin to better understand how glial dysfunction might cause axonal damage and vice versa. Finally, we suggest that the special geometry and spatial relation of axons and oligodendrocytes help to explain the spreading of pathology in advanced stages of MS.

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Abbreviations used in this paper: CNS, central nervous system; MBP, myelin basic protein; MS, multiple sclerosis; PNS, peripheral nervous system.

THE JOURNAL OF CELL BIOLOGY

Cell biology of the axon–myelin unit

One of the most striking features of the axon–myelin unit is the close association of two plasma membrane surfaces over extensive areas. In general, plasma membrane interactions are prevented by repulsive forces generated by steric and electrostatic repulsion of large and negatively charged oligosaccharide polymers present at the cell surface. In most cases, membranes are, therefore, only closely connected to each other within tiny regions by anchoring junctions that are strong enough to overcome the repellent forces of the cell surface. The advantage of this general arrangement is that the majority of the plasma membrane surface remains exposed to the extracellular space and diffusible signals, whereas cell–cell interactions are confined to specialized signaling hubs. Axons in contrast, require a special arrangement of their membrane surface to allow the fast saltatory conduction of action potentials. Whereas saltatory conduction avoids the need to constantly regenerate the impulses along the axonal surface, it comes at a price. First, the axon becomes dependent on oligodendrocytes for communication with the external environment. Thus, the axon is not only electrically but also metabolically isolated (Nave, 2010). Second, there is a massive redistribution of proteins from the internodal membrane toward the nodes of Ranvier, which are small (∼1 µm) myelin-free gaps on the axonal surface between two neighboring internodes that remain in contact with extracellular space. Because this particular molecular composition of the nodal region is likely of major relevance for the emergence of axon–myelin pathology, we briefly summarize some of its key features.

When myelin sheath biogenesis is completed, the firmly attached lateral edges of the myelin layers come closely together to form the paranodal loops (Fig. 1). These form septate junctions with the axonal surface, held together by the adhesion proteins Caspr and contactin on the axonal side and neurofascin-155 on the glial surface (Fig. 1; Salzer et al., 2008). Paranodes are further strengthened by a submembranous cytoskeleton consisting of ankyrinB, αII-spectrin, and βII-spectrin (Zhang et al., 2013). Juxtaparanodal junctions, which include a complex formed by TAG-1 on the glial surface and Caspr2 on the axonal side (Traka et al., 2003), further help to maintain the domain structure at the nodes and the flanking juxtaparanodes. Within the limited space of a node of Ranvier, large multimolecular complexes are formed consisting of sodium channels, cell adhesion molecules (neurofascin-186), cytoskeleton proteins (βIV-spectrin), scaffolding proteins (ankyrinG), and extracellular matrix proteins (brevican, versican, and Bral1; Sherman et al., 2005; Feinberg et al., 2010; Susuki et al., 2013). In addition to sodium channels, specific types of voltage-gated K$^+$ channels (KCNQ2 and KCNQ3) also localize to the nodes. Such a dense concentration of molecules, which among other functions maintain structural integrity and mediate ion fluxes, onto relatively small areas at the cell surface might also carry a substantial risk and predispose the nodes of Ranvier as "hot spots" for the initiation of axonal damage (Arancibia-Carcamo and Attwell, 2014).

A large amount of the axonal membrane in the internodal region (i.e., between the two paranodal domains) is not in direct contact with the extracellular environment but faces the insulating myelin sheath. Because space at the nodes is limited, it is unlikely that nodes of Ranvier harbor all essential surface molecules, such as transporters and ion channels, that are required for maintaining axonal integrity and communication with the extracellular environment. Instead, communication may occur in part via myelinating oligodendrocytes. Indeed, oligodendrocytes provide nutritional support (by virtue of lactate transport) to associated axons (Fünfschilling et al., 2012; Lee et al., 2012). In addition, glia-derived exosomes have been recently shown to participate in a novel mode of neuron–glia communication (Frühbeis et al., 2013). This on-demand supply of glial support nicely illustrates the close functional coupling of glia and axons, which is essential for proper function of the axon–myelin unit.

Cellular mechanisms of axon degeneration

More than for most other cells, geometry matters to neurons—the right number, length, and shape of axons, dendrites, and synapses all are important for neuronal function. As a consequence, neuronal damage is a complex process. It can start at different sites and comes in morphologically and mechanistically different flavors (Coleman et al., 2005). This has long been known by neuropathologists and is reflected in the commonly used classification of axon loss as either following a pattern of (Wallerian) degeneration, a fast fragmentation process that is presumably initiated at the cut site in the axon and spreads centrifugally, or a pattern of dying-back pathology, which is generally considered to start at the synapse and involves the slow centripetal retraction of the axon (Fig. 2 A; Luo and O'Leary, 2005). Beyond these classical categories, other more focal forms of compartmentalized neurite loss have been described, ranging from synaptosis (Gillingwater and Ribchester, 2001) to spontaneous pruning of axon branches (Martin et al., 2010) and localized axosome shedding (Bishop et al., 2004). Before considering the relation of the distinct patterns of axon loss to neuroinflammatory disease processes, we would like to briefly outline the current knowledge of the mechanism underlying axon loss (for a detailed review of the molecular mechanisms, see, e.g., Coleman and Freeman, 2010; Wang et al., 2012).

Wallerian degeneration (Fig. 2 A, left) is undoubtedly the most thoroughly investigated form of axon loss—and indeed, ongoing research is revealing the molecular pathways that result in the removal of severed axon segments. The starting point for this mechanistic deconstruction of Wallerian axon dismantling has been the serendipitous identification of a spontaneous mouse mutant with profoundly delayed Wallerian degeneration (WLD^S [Wallerian degeneration slow]; Lunn et al., 1989). Molecular genetic analysis of this mutant has resulted in the surprising identification of enzymes of the NAD biosynthetic pathway as central players in axon degeneration (Coleman et al., 1998; Conforti et al., 2000), even though the details of the underlying molecular mechanisms that actually result in axon fragmentation remain to be elucidated. Importantly, the phylogenetic conservation of WLD^S sensitivity has allowed identification of Wallerian-like degeneration events in "screenable" organisms, such as *Drosophila melanogaster* (Hoopfer et al., 2006; MacDonald et al., 2006). This has paved the way for new investigations that have identified additional components of endogenous axon-dismantling pathways, such as dual leucine zipper kinase–Wallenda (Miller
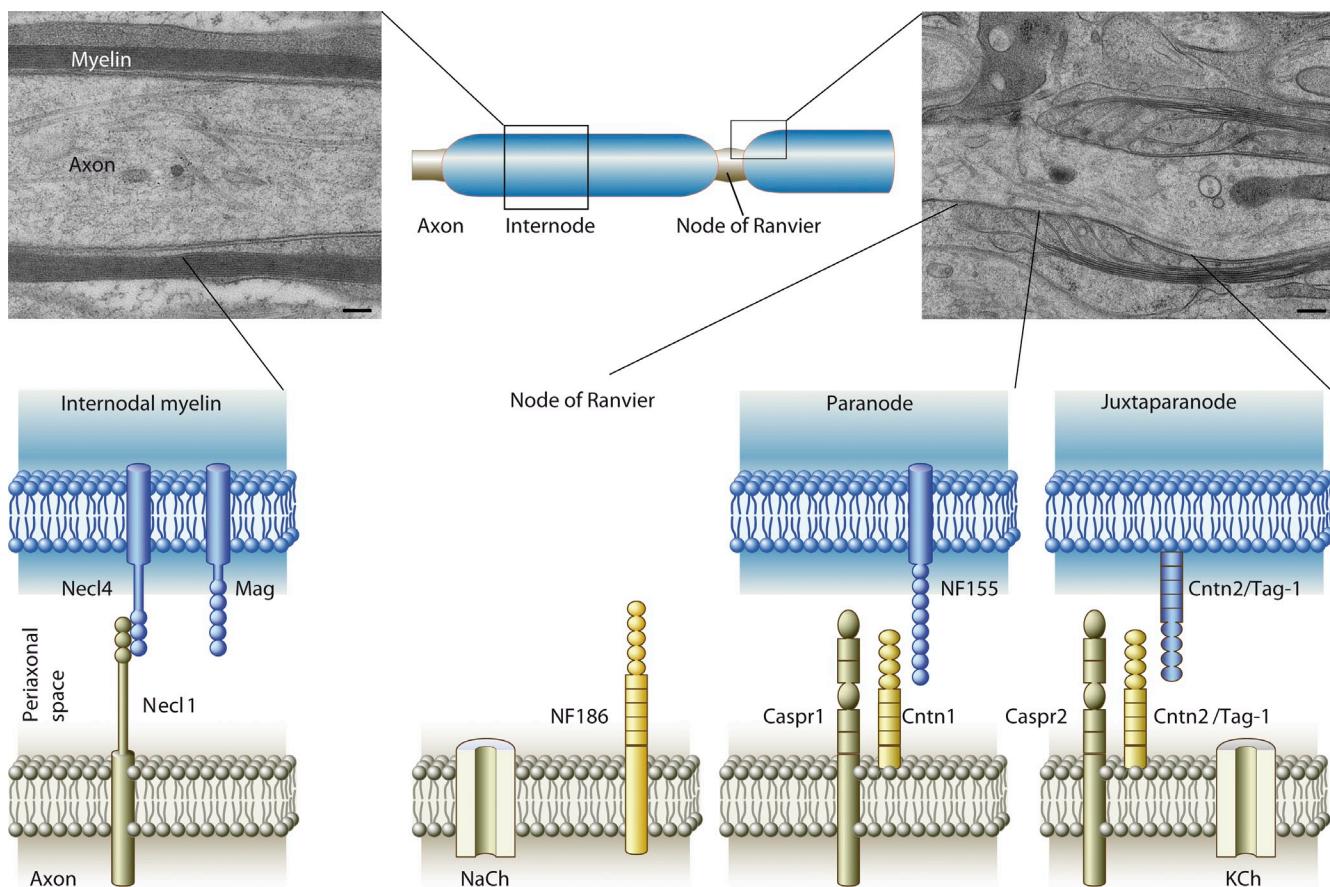
Figure 1. **Molecular composition of the axon–myelin unit.** Schematic diagram and electron microscopy images showing the different domains of a myelinated axon. Each of these domains is characterized by the expression of specific proteins: the node of Ranvier contains voltage-gated Na$^+$ channels (NaCh), the paranode is the region where myelin is tightly attached to the axon by a complex formed by neurofascin-155 (NF155), contactin-1 (Cntn1), and contactin-associated protein (Caspr1), and the juxtaparanode where most voltage-gated K$^+$ channels (KCh) are found is separated from the paranodes by a complex formed by contactin-associated protein 2 (Caspr2) and contactin-2 (Cntn2). The internodal region contains several cell adhesion molecules (Nectin-like molecule 1 [Necl1], Nectin-like molecule 4 [Necl4], and myelin-associated glycoprotein [Mag]). Bars, 80 nm.

et al., 2009), ZNRF1 (Wakatsuki et al., 2011), sterile α motif–containing and armadillo motif–containing protein (Sarm1; Osterloh et al., 2012), and the E3 ubiquitin ligase Phr1 (Xiong et al., 2012; Babetto et al., 2013), the abolition of which results in a WLD^S-like phenotype. Although the molecular principles of Wallerian degeneration are emerging, some of the characteristic cell biological features of Wallerian degeneration remain to be explained. These include the lag phase, a characteristic property of Wallerian degeneration that precedes onset of fragmentation after axon transection (Vargas and Barres, 2007). The significance and cause of this lag is not yet fully understood—for instance, synaptic versus axonal compartments seem to differ in this respect for unexplained reasons (Gillingwater and Ribchester, 2003). Perhaps the most prevailing explanation is the idea of an axon-intrinsic default destruction mechanism (Raff et al., 2002). This view is obviously inspired by models of cellular suicide and surmises that distal axon segments contain destructive machinery that is held at bay by a continuous supply of inhibitory factors provided through axonal transport. This model predates identification of the WLD^S mutation (Lubińska, 1982), which provides an obvious set of potential protectors. Indeed, Gilley and Coleman (2010) have shown that among the three murine nicotinamide mononucleotide adenylyl transferases that

are related to the enzyme mutated in WLD^S mice, Nmnat2 has the shortest lifetime and is associated with motile vesicles, suggesting that its continuous supply acts as a tonic endogenous survival signal for axons. This transport model provides a conceptual framework for the observed lag phase and explains why disruption of microtubule transport can substitute for transection as the trigger for Wallerian degeneration. Although the transport hypothesis is attractive, other explanations obviously exist—for example, the emergence of positive destruction signals, which could be as simple as an increasing load of ions that may activate self-destruction via bioenergetic deprivation (Mishra et al., 2013).

In contrast to Wallerian degeneration, which is typically initiated in the axonal compartment, the centripetal spread from the synapses (Fig. 2 A, center) is part of the defining criteria of die back; however, many other mechanistic aspects of this widespread pattern of axon loss remain obscure. In classical pathological parlance, die back suggests a mechanism in which the most distal structures would be deprived of a shared resource (Schaumburg et al., 1974). Die-back patterns of axon loss have been described prominently in toxic and degenerative forms of axon loss (Fischer et al., 2004; Schaefer et al., 2005). In these disease processes, distal axonal segments disappear
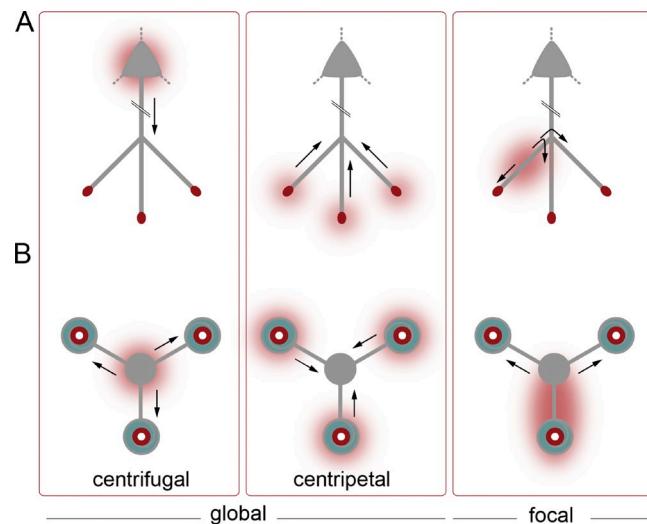
Figure 2. **Patterns of axon and myelin injury.** (A, left) Axonal damage can either follow a classical centrifugal Wallerian pattern emanating from the cell body or an axonal transection. (middle) In contrast, an axonal die-back pattern originates at or near the synaptic compartment and spreads centripetally. (right) Finally, focal forms of axon injury have been described, e.g., during neuroinflammatory attack. (B) Similarly, in oligodendrocytes, injury can start at the soma (left), at the myelin sheaths (oligodendrocytic die back; middle), or focally at a single myelin-bearing process (right).

first, whereas proximal axons and somata are relatively spared. A progressive retrograde spread of pathology is then believed to result in progressive denervation and eventually neuronal cell death. As most (but not all; compared with toxic sensory neuropathies) axons bear synapses at their distal-most tips, early synaptic loss is often equated with a dying-back pattern of axon loss. Given the geometry of this form of axon loss and the fact that cytoskeleton-disrupting drugs can induce a dying-back pattern, axonal transport is believed to also play a central role in this form of axon loss. In contrast to Wallerian degeneration, such transport deficits would be more subtle and chronic—and the relevant cargoes remain unidentified.

Although Wallerian degeneration and dying back represent the best-characterized forms of axon loss, other patterns exist—some resemble Wallerian degeneration (e.g., the developmental pruning of long spinal axons, which appears to involve fragmentation but is not blocked efficiently by WLD^S; Hoopfer et al., 2006), whereas others look like axonal die back (the distal-to-proximal shedding of axonal particles seen during developmental branch loss in the PNS; Bishop et al., 2004). Others, however, seem to differ entirely—and these might be the most relevant forms of axon loss for inflammation-mediated CNS injury. Indeed, the role of Wallerian-like mechanisms in MS and its animal models remains unclear (Coleman et al., 2005), in part because the WLD^S mutation might also have effects on nonneural cells (Kaneko et al., 2006; Chitnis et al., 2007; Dziedzic et al., 2010). Hence, even though disconnected axon fragments undoubtedly undergo Wallerian degeneration also in neuroinflammation (Dziedzic et al., 2010), WLD^S-mediated protection against inflammatory axon damage appears to be hard to prove. Similarly, although chronic axonal dystrophy seems likely to occur in advanced stages of MS (Schirmer et al., 2011; Lassmann et al., 2012), it is not fully resolved, whether

this results in a genuine dying-back pattern or early synapse loss. Indeed, our own recent work suggests that nonclassical axon loss mechanisms might be at work, which are less global than either Wallerian degeneration or a dying-back type of dystrophy. Our in vivo observations in murine MS models have revealed the focal emergence of swellings that are predilection sites for subsequent axon breakage (Nikić et al., 2011). This form of focal axonal degeneration (Fig. 2 A, right) does not conform to the morphological and dynamic characteristics that we have previously observed in the same axon population during Wallerian degeneration and Wallerian-like acute axonal degeneration after trauma (Kerschensteiner et al., 2005) and, hence, is likely distinct in molecular terms (albeit this still has to be formally proven). Interestingly, focal axonal degeneration seems to preferentially emerge at nodes of Ranvier but, at the same time, does not seem to be promoted by demyelination (Nikić et al., 2011), suggesting that it is the special metabolic demand on nodes, rather than their lack of a myelin sheath, that renders this site susceptible. Importantly, demise is not an invariable outcome for these predamaged axons, as the focal axonal degeneration cascade seems to be reversible even in relatively advanced stages of morphological, subcellular, and functional disruption. Thus, the somewhat surprising identification of a subacute form of axon loss that appears to be dependent on inflammation, but not on demyelination, begs a more detailed discussion of how oligodendrocyte and axon pathology might intersect in the human disease, such as MS, in which both are dominant features.

Mechanisms of oligodendrocyte degeneration
Oligodendrocyte injury can occur by a diverse range of insults, among which inflammation ranks prominently. Given the geometry of the oligodendrocyte, the consequences of damage not only depend on the nature of the attack but also on the site where oligodendrocytes are targeted. If sufficiently strong injury occurs at the level of the cell body, pathology is likely to spread centrifugally to all myelin segments that are connected to the oligodendrocyte (Fig. 2 B). Depending on the brain region, the consequences can be dramatic: whereas some oligodendrocytes in the spinal cord generate myelin only around one large axon, a single cell in the cortex and corpus callosum can myelinate ≤80 internodes (Chong et al., 2012). This suggests that injury could radiate out substantially, but to understand how fast such spread might occur, we need to know how long myelin internodes are able to survive without being connected to an oligodendrocyte. Recently, using in vivo pulse–chase labeling of proteins synthesis with ^{15}N isotopes, followed by mass spectrometry, myelin proteins were found to be among the longest lived proteins in the mouse (Toyama et al., 2013). Considering that myelin is, in contrast to most membranes, a highly stable system, which is close to equilibrium at steady state (Aggarwal et al., 2011), it is possible that myelin internodes can continue to exist for a long time without being associated with an oligodendrocyte. Although we know only little about the relative kinetics of oligodendrocyte and myelin injury, some clues have come from oligodendrocyte ablation experiments (Traka et al., 2010; Ghosh et al., 2011; Pohl et al., 2011; Locatelli et al., 2012; Oluich et al., 2012). When the diphtheria toxin receptor is

targeted to oligodendrocytes in transgenic mice, the subsequent injection of diphtheria toxin, which acts as an RNA translation inhibitor, allows specific ablation of oligodendrocytes. The first signs of cell death are found ~1 wk after the toxin injection, when the cell bodies appear damaged with shrunken nuclei and condensed cytoplasm. Myelin vacuolization (i.e., the loss of the typical dense packing of myelin layers) starts only about 3 wk after injection, and fully demyelinated axons are seen after another 2 wk, confirming that myelin might be rather long lived even if oligodendrocyte viability is compromised. Such a centrifugal or "inside-out" pattern of oligodendrocyte death, which progresses from the soma toward the myelin sheaths, is likely relevant for several insults, including viral infection, genetic defects (e.g., lysosomal storage diseases), ischemia, and also a subgroup of MS patients (Rodriguez, 1992; Rodriguez and Scheithauer, 1994; Lucchinetti et al., 2000).

However, in the majority of MS patients, the damage is thought to occur at the level of the myelin sheath and to spread centripetally or "outside in." When considering an autoimmune attack on myelin, we need to distinguish the different subcompartments that exist on the surface of a myelin sheath and hence can be targeted. The largest fraction of the myelin sheath surface is tightly connected to the underlying myelin membrane stack, is lipid rich, and contains only very few surface proteins, among which the proteolipid protein and myelin-oligodendrocyte glycoprotein are the most abundant. These proteins are obvious targets for autoantibodies, and indeed, high levels of antibodies to myelin-oligodendrocyte glycoprotein have been described in pediatric cases of autoimmune demyelination, both in acute disseminated encephalomyelitis and in MS (Pröbstel et al., 2011). The noncompacted regions of myelin comprise the outer "tongue" (or "lip") of the myelin membrane and the paranodal loops at the edges of each myelin internode. These areas are protein rich and composed of an entirely different set of proteins than compacted myelin, some of which are specifically targeted in autoimmune diseases. An interesting finding in this respect is the identification of autoantibodies in MS against neurofascin, a protein concentrated at the paranodes and at the node of Ranvier (Mathey et al., 2007). Furthermore, contactin-2/TAG-1, a protein localized at the juxtaparanodal domain, has recently also been identified as an MS autoantigen targeted by T cells and autoantibodies (Derfuss et al., 2009). Interestingly, disintegration of the axon and glial connections at the paranodal junction is also observed after mild ischemia in mice (Reimer et al., 2011). It is possible that the myelin sheath is under particularly high tension at the paranodes, which may explain why this is a site of preferential damage.

A common structural feature of myelin in demyelinating diseases is the fragmentation of the membrane stacks. How is myelin membrane architecture distorted in diseases? Recently, we provided evidence that the interaction of myelin basic protein (MBP) with the cytoplasmic leaflet of the myelin bilayer triggers polymerization of MBP into a fibrous network that holds myelin lamellae together (Aggarwal et al., 2013). A transition of MBP back from a condensed to a dispersed phase may therefore trigger acute myelin disassembly. This could, for example, occur by an increase in Ca^{2+}, which may impact anchoring of MBP to the head group of phosphoinositol 4,5-bisphosphate and phosphatidylserine in the lipid bilayer (Musse et al., 2008; Nawaz et al., 2009). Even disturbing local ionic strength (other than calcium) and pH may be sufficient to reduce the adhesion of MBP to the myelin lamellae and thereby decrease myelin stability.

Myelin damage does not only occur from the outside of the sheath but can also start at the innermost tongue of myelin. This form of oligodendrocyte cell death has been termed dying-back oligodendropathy, as it is initiated in the most distal area of the cell and spreads retrogradely. The first evidence for such a process came from mice fed with cuprizone (a copper chelator), in which degenerative changes were first observed in the inner tongue of the myelin sheath that extended back to the cell body 3–4 wk later (Ludwin and Johnson, 1981). Similar alterations have been detected in some brain biopsies of MS patients and in Theiler's virus–induced encephalomyelitis (Rodriguez, 1992). The molecular changes underlying dying-back oligodendropathy have so far remained obscure. Recent analyses using high-pressure freezing electron microscopy have shown that the region of the myelin sheath close to the axonal surface is filled with organelles and appears to be metabolically more active than previously thought (Möbius et al., 2010; Snaidero et al., 2014). Much like the synapses of an axon, the innermost tongue of myelin might thus be particularly vulnerable to metabolic disturbances, possibly because of its high energy demand and its long distance from the cell body. An alternative possibility is that disruptions of adhesions between the inner tongue and the axon could trigger a pathology that subsequently spreads backward. Evidence for such a scenario comes from aged mice deficient for the adhesion molecule myelin-associated glycoprotein, which show the first signs of damage within the periaxonal inner tongue reminiscent of a dying-back oligodendropathy (Lassmann et al., 1997; Weiss et al., 2000).

The axon–myelin unit as a target of inflammatory attack

To understand axonal pathology in demyelinating disease, it is important to look at myelin and the underlying axon as a closely connected functional unit and ask how damage of one component affects survival and well-being of the other. Large-scale loss of the insulating function of myelin results in a drop of nerve conduction speed and possibly conduction block. To compensate, demyelinated axons start to express Na^+ channels along the entire axon. However, these changes come at the cost of increased energy demand to maintain ion gradients. Considering the already high energy expenditure of a neuron, which has been estimated to be approximately five billion ATP molecules per second in a cortical neuron (Zhu et al., 2012), it is likely that loss of myelin pushes the energy requirement of a neuron to an upper limit. This situation has been termed virtual hypoxia, which stands for a mismatch of energy demand and supply eventually leading to axon damage (Stys, 2005; Trapp and Stys, 2009). According to this model, the ATP supply of denuded axons is not sufficient for efficiently operating Na^+/K^+-ATPase pumps and hence to clear the increased axonal Na^+ load. If Na^+ rises above a critical concentration, the Na^+/Ca^{2+}-ATPase will start to operate in reverse mode, which leads to accumulation of intra-axonal Ca^{2+} and the

activation of Ca^{2+}-dependent degradation pathways (Fig. 3). Although the increased energy demand of an axon per se may not be sufficient to trigger axonal degeneration, it is likely that energy deprivation renders the neurons more vulnerable to stress. A "second hit"—for example, the induction of mitochondrial damage by reactive nitrogen and oxygen species or other inflammatory mediators—would exacerbate the energy mismatch and could be sufficient to induce axonal degeneration.

In addition to increased energy demand, demyelination likely also leads to the loss of trophic and metabolic support of the axon (Fig. 3). Indeed, myelin is not simply an inert insulator but contains metabolically active noncompacted regions. In this context, it is interesting that there are several mouse mutants that have minimal pathology within compacted regions of myelin but show disturbed organization of cytoplasm-rich areas. One example is 2′,3′-cyclic nucleotide 3′-phosphodiesterase 1–deficient mice, which show normal myelination by itself but have pathology in noncompacted myelin compartments at the paranodal domains (Lappe-Siefke et al., 2003; Rasband et al., 2005). Whereas the structural changes in the compacted myelin remain small, these mice develop a swelling of the inner tongue (Edgar et al., 2009) and late-onset, chronic progressive neurodegeneration (Lappe-Siefke et al., 2003). Axonal swellings, transections, and an impairment of axonal transport that occur in these mice are highly reminiscent of the changes found in the CNS of patients suffering from MS. Such mouse mutants indicate that oligodendrocyte-derived metabolic support is required for axonal homeostasis. Indeed, recent data suggest that oligodendrocytes and axons are metabolically coupled and that oligodendrocytes provide lactate via MCT1 (monocarboxylate transporter 1) into the periaxonal space (Fünfschilling et al., 2012; Lee et al., 2012). Disruption of this mechanism by deletion of MCT1 causes neurodegeneration. Although loss of metabolic support is an attractive hypothesis to explain how pathology may spread from glia to the axons, it is unlikely to be the only mechanisms. Gain of toxic function provides an additional explanation (Fig. 3). However, currently, it is mostly unknown which prodegenerative signals might be transferred from glia to neurons and how they act. There are a few exceptions: For example, psychosine is a cytotoxic sphingolipid that is generated in oligodendrocytes during globoid cell leukodystrophy (Krabbe's disease) and spreads into axons where it causes damage (Cantuti Castelvetri et al., 2013). Other neurotoxic lipid species are acylcarnitines, intermediates of fatty acid β-oxidation, which are generated in Schwann cells after mitochondrial dysfunction (Viader et al., 2013). Furthermore, dying oligodendrocytes are a major source of iron, which can exert toxicity if Fe^{2+} is oxidized by hydrogen peroxide to Fe^{2+} (Fenton reaction) and neurotoxic reactive hydroxyl radicals and hydroxyl anions are generated (Hametner et al., 2013). This mechanism might be of particular importance in advanced stages of MS, in which accumulating iron can amplify oxidative stress and contribute to progressive neurodegeneration.

In summary, myelin damage has severe consequences for the axonal partner that go beyond the loss of a protective insulation and likely include an increased energy demand and lack of metabolic and trophic support as well as the exposure to myelin-derived neurotoxic mediators. Whether similar detrimental consequences also occur if the sequence of damage is reversed is currently less well understood. However, an interesting example of how damage signals could be transferred from neurons to glia comes from the PNS. Here, it has been shown that Schwann cells "sense" axon damage and respond by activating multiple signaling pathways, including extracellular signal–regulated kinase–MAPK, JNK–c-Jun, Notch, and JAK-STAT (Janus kinase–signal transducers and activators of transcription; Harrisingh et al., 2004; Arthur-Farraj et al., 2006; Napoli et al., 2012). These signals not only induce cell activation but also trigger myelin fragmentation into ovoid structures in an active process that requires actin polymerization in the cytoplasmic clefts (so-called Schmidt–Lanterman incisures; Jung et al., 2011). Hence, at least in the PNS, it appears that once a destructive pathway is induced in one cell, this signal can be transferred to the other to orchestrate a mutual breakdown. Whether glial and neuronal fates are similarly coupled in the CNS remains to be explored. However, one may suspect that the consequences of cell loss on one side of the axon–myelin divide should be more variable in the CNS than in the PNS, as they likely depend on the specific stoichiometry of the axon–oligodendrocyte relation that varies in different parts of the brain and spinal cord.

Outlook: Spreading as a consequence of axon–myelin interdependence

So how do the mechanisms that mediate axon degeneration and myelin loss intersect? Can some of the mechanistic concepts that have emerged on one side of the axon–myelin unit be transferred to other? Interestingly, despite the clearly distinct roles of neurons and oligodendrocytes, both cell types display structural similarities. Both cell bodies need to support disproportionately large peripheral compartments—axons and synapses in the case of neurons and myelin sheaths in the case of the oligodendrocyte—via a relatively small and vulnerable-appearing set of conduits (Fig. 4). Both cells thus have to deal with the same challenge, namely how to best support their vast cellular periphery from the soma. In neurons, fast and slow axonal transport processes that shuttle organelles and substrates between soma and synapses have evolved to meet this challenge (Hirokawa et al., 2010). It will be interesting to better understand the equivalent transport processes in oligodendrocytes and along their myelin sheaths (Simons et al., 2012). Structural and functional similarities between axons and the oligodendrocyte also suggest that shared mechanisms might mediate the removal of these cells or their degenerating appendages. Disturbances in organelle transport have, for example, not only been linked to dying-back neuropathies but also to the induction of active axonal self-destruction during Wallerian degeneration (Coleman et al., 2005). Although some evidence already suggests the presence of a dying-back pathology in oligodendrocytes (Rodriguez, 1992; Aboul-Enein et al., 2003), it is currently unclear whether active programs exist that could mediate dismantling of oligodendrocyte processes. Likewise, it is interesting to speculate whether the stereotypic pruning of axonal connections during development leads to a similarly stereotypic removal of myelin sheaths that supported these early connections—a notion that seems at least possible, as transient myelination events have
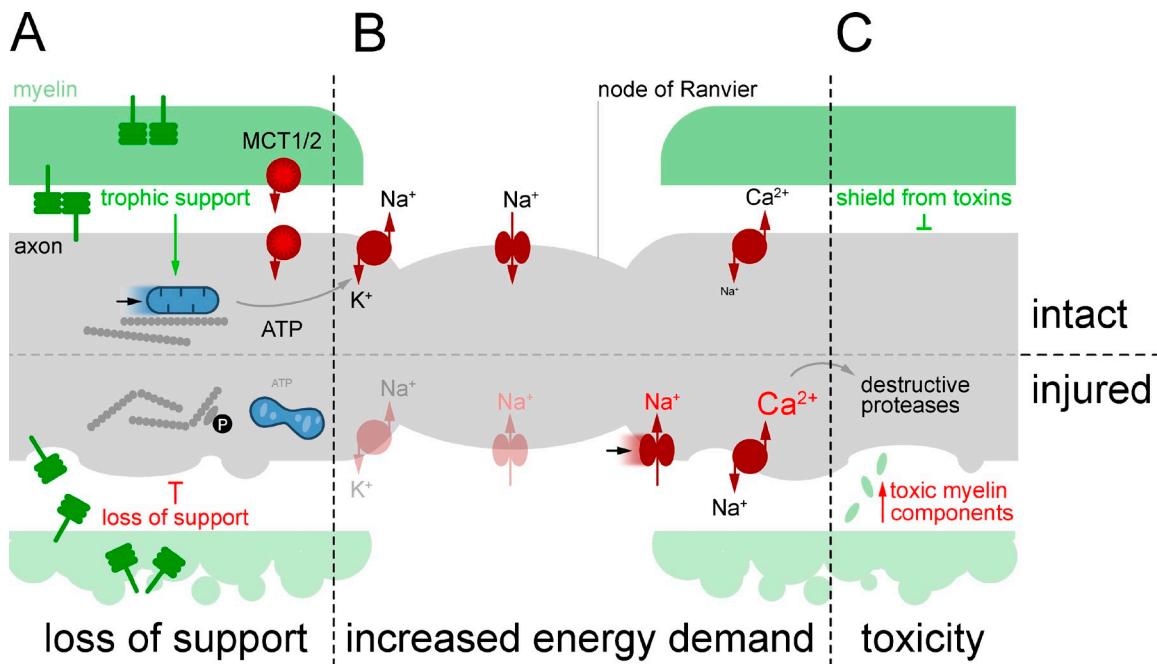
Figure 3. **Mechanisms of coupling injury within the axon–myelin unit.** (A–C) Proposed mechanisms of injury that affect the axon–myelin unit involve several nonexclusive scenarios, including loss of trophic or metabolic support (A), increased energy demand as a result of loss of myelin or compromised membranes (B), and gain of toxic functions, either within the axon or emanating from the myelin (C). P, phosphorylation.

been observed (Berthold and Nilsson, 2002; Czopka et al., 2013; Liu et al., 2013) and myelin can in rare cases be found around axon branches destined for elimination.

The interdependence of neuronal and glial health should also have major consequences for the way pathology spreads through the nervous system. Depending on the location of the primary insult, pathology could either originate in oligodendrocytes and extend to axons or advance in reverse direction. In this context, it is interesting to consider how the special geometry and stoichiometry of the axon–oligodendrocyte interaction may affect the pattern in which pathology spreads (Fig. 5). If the primary insult targets an axon, pathology may spread to the myelin sheaths covering the axon and further to the oligodendrocyte soma. As one oligodendrocyte is connected to several axons, a focal trigger may initiate a chain reaction within the entire nerve tract. Such a longitudinal spread of pathology along one axonal tract could, for example, explain how alterations progress from the brain to the spinal cord or vice versa. Moreover, myelinated

axons that enter gray matter areas, for example, in the superficial layers of cortex, could transfer pathology from the white to the gray matter. Wallerian degeneration would be a classical example of such a longitudinal spreading mechanism, and indeed, a recent histopathological study indicates that (at least part) of the widespread microglial activation that is found in progressive stages of MS is related to the phagocytosis of axonal and glial debris that results from Wallerian degeneration of axons (Singh et al., 2013). If the primary insult targets oligodendrocytes, this would likely impede the function of several axons (≤80 in the CNS; Chong et al., 2012) that are myelinated by the same oligodendrocyte. Pathology would in this case spread in a more transversal pattern (Fig. 5). In contrast to the longitudinal spread, this pattern would only be found in the CNS, as a result of the one-to-one relation of axons and myelinating Schwann cells in the PNS. Despite the very different points of initiation, both spreading modes could ultimately result in rather similar and diffuse patterns of axon–myelin damage. The degree to which this happens
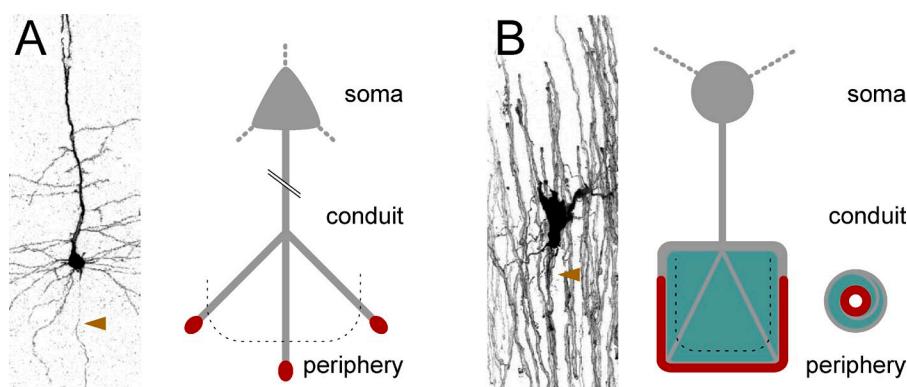
Figure 4. **Topologic similarities between neurons and oligodendrocytes.** (A and B) As extended, process-bearing cells, neurons (A) and oligodendrocytes (B) show similarities in their topology, including a trophic center, the soma, which is connected with a metabolically highly active periphery (dark red; synapses for neurons and the inner myelin tongue for oligodendrocytes) through a rather thin and vulnerable conduit (a neuron's axon and oligodendrocytic processes, respectively). Oligodendrocytes carry a large additional membrane compartment, compacted myelin (teal)—which in the main drawing is shown in a hypothetical "unrolled" state and also schematized in the cross section. Arrowheads point to the axon (A) and an oligodendrocyte process (B).
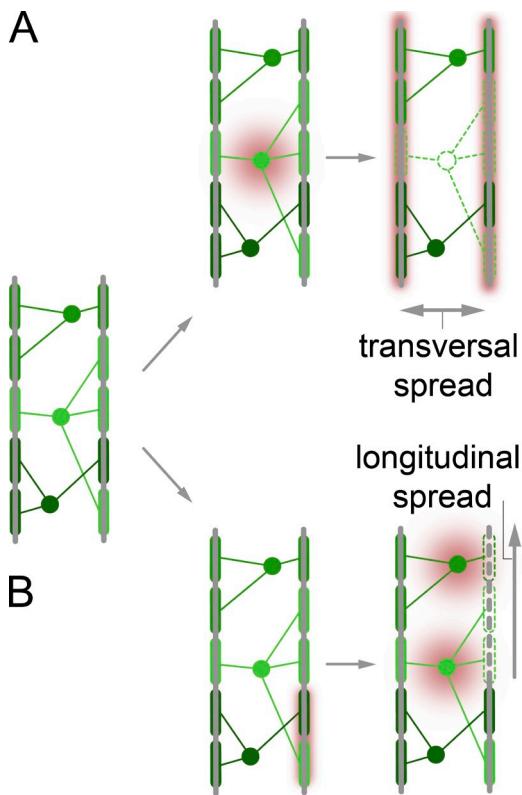
Figure 5. **Mechanisms of spread of axon–myelin injury.** (A and B) The axon–myelin unit imposes a tight coupling of neuronal and oligodendrocytic health. This could result in different patterns of spreading pathology—e.g., a transversal pattern (A), in which primary loss of an oligodendrocyte injures all of the axons it subserves, versus a longitudinal pattern (B), in which Wallerian degeneration affects all oligodendrocytes distal from a primary axonal lesion. Gray, axons; green, oligodendrocytes; red haze, site of injury.

depends on how compartmentalized injury can be within a cell and how severely loss of one partner of the axon–myelin unit affects the other partner. For example, if one myelin sheath is targeted, does the pathology spread to the other myelin sheaths of the same cell? How many myelin sheaths can an oligodendrocyte lose before a global response ensues? Similarly, the loss of how many internodes can an axon tolerate—not only acutely in terms of nerve conduction but also chronically with regards to metabolic support? Interestingly, a recent study suggests that many individual cortical axons are not homogenously myelinated but rather show a patchwork pattern of myelinated and nonmyelinated segments (Tomassy et al., 2014). Remarkably, many of these fundamental cell biological questions are currently open. With the advent of in vivo imaging of the axon–myelin unit both in mice (Nikić et al., 2011; Hughes et al., 2013; Romanelli et al., 2013; Schain et al., 2014) and zebrafish (Kirby et al., 2006; Czopka et al., 2013), many of them can now be addressed. Likewise, it will be important to better understand how other cellular components of the nervous system modulate the spread of axon–myelin changes. Here, it is interesting to note that oligodendrocytes are interconnected with gap junctions not only with each other but also with astrocytes (Orthmann-Murphy et al., 2007), which in turn form a functional network along which physiological and pathological signals, including calcium waves, can travel through the CNS (Kuchibhotla et al., 2009; Han et al., 2013).

Signal propagation along glial or neuroglial networks is likely to be further influenced by microglia cells that rapidly react to nervous system pathology (Davalos et al., 2005) and that, as a result of their pleiotropic functions (Nayak et al., 2014), might both accelerate or limit the spread of neuroglial pathology, depending on the disease context (Miron et al., 2013).

Overall, the interdependence of the cellular partners of the axon–myelin unit has the potential to explain how pathological alterations can expand through the nervous system and across gray and white matter boundaries. The spreading hypothesis might thus help to understand how, in a disease such as MS, focal lesions present in the early relapsing-remitting stage of the disease can, over time, lead to widespread gray and white matter changes that characterize the advanced stages of the disease (Lassmann et al., 2012). It should be noted, however, that transition of a focal or multifocal to a diffuse disease process likely requires a disease-specific susceptibility of the axon–myelin unit or multiple rounds of damage, as it is not observed after typical focal and isolated insults are caused, e.g., by trauma or ischemia. Here, the preexisting damage of the axon–myelin unit in MS that can result from the long-standing inflammatory process might play a key role. Spreading of pathology along the axon–myelin unit, however, is likely not exclusive to MS but might also contribute to the dissemination of pathology in classical neurodegenerative diseases, such as Alzheimer's disease, in which myelin damage has been observed both in patients with mild cognitive impairment and late-onset Alzheimer's disease (Bartzokis, 2011; Carmeli et al., 2013).

Clearly, further work is necessary to reveal the signals that mediate communication, metabolic exchange, and structural integrity in the axon–myelin unit. With additional insights into the specific vulnerability of the axon–myelin unit, we will hopefully better understand how distinct signaling disturbances can result in characteristic patterns of neuroglia damage and pathology progression in various CNS disorders.

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