Axon degeneration: Linking axonal bioenergetics to myelin

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The mechanisms by which axonal degeneration occurs, even in the presence of apparently normal myelin sheaths, remain unknown. In this issue, Yin et al. (2016. *J. Cell Biol.* https://doi.org/10.1083/jcb.201607099) study mutant mice in which proteolipid protein is replaced by the peripheral myelin protein P_0 and describe a number of early axonal abnormalities, which together suggest that aberrant mitochondrial energy metabolism precedes axonal degeneration.

Axons, the long cellular projections of neurons extending from the cell body all the way to the distal synapse, are essential for neuronal wiring. This arrangement is facilitated by the intimate association of axons with Schwann cells or oligodendrocytes. These glial cells form compact myelin sheaths around axons in the peripheral nervous system (PNS) or the central nervous system (CNS). Myelin sheaths enable saltatory, and therefore greatly accelerated, conduction of electrical impulses and have been proposed to support axonal integrity. Still, because of their incredible length—several meters for certain axons in larger vertebrate species—axons are at continuous risk of damage. Axons also have an extraordinary demand for adenosine triphosphate to support intensive energy-consuming processes, including axonal transport and generation of ion gradients. The question of how these seemingly fragile cellular processes are maintained throughout life has puzzled neuroscientists for many decades.

Even focal axonal damage in the CNS can result in irreversible interruption of axonal continuity and produce impairment of fundamental neuronal functions such as sensation, ambulation, memory, and cognition. In fact, axon degeneration is an early event and pathological hallmark in a broad range of acquired and hereditary neurodegenerative disorders, especially those primarily affecting the myelin sheaths of axons (Taveggia et al., 2010; Beirowski, 2013). Moreover, the demise of axons and not the degeneration of myelin in these conditions is the most important predictor of morbidity, despite that demyelination can be the most prominent histopathological feature. This is probably most pronounced in multiple sclerosis, where there is significant evidence that axon degeneration may sometimes parallel or even precede the onset of demyelination (Trapp and Nave, 2008).

It is intuitive that the immune-mediated attack on the myelin sheath may result in neurotoxicity harmful for axons. However, it is now also clear that axon degeneration can occur in the absence of demyelination when individual molecules in myelin sheaths are missing (Nave and Trapp, 2008). For example, deletion of the proteolipid protein (PLP) from myelin sheaths in the CNS leads to a progressive axonopathy in both humans and rodent models (Garbern et al., 2002). Mice deficient for PLP display grossly normal myelin sheaths that are slightly thin and uncompacted. Nonetheless, they subsequently develop a late-onset axonopathy mimicking the loss of long CNS axons that occurs in patients suffering from hereditary spastic paraplegia type 2 (HSP2) or the leukodystrophy Pelizaeus-Merzbacher disease. Both neurodegenerative conditions are caused by mutations in the *PLP1* gene coding for PLP.

Further support for dissociation of axonal degeneration from changes in myelin comes from mutant mice, P_0-CNS, in which the myelin compaction defect secondary to PLP ablation has been largely rescued. Although the functionally related P_0 glycoprotein is expressed in Schwann cells in the PNS, these transgenic mice also express P_0 in oligodendrocytes (Yin et al., 2006). Similar to PLP in the CNS, P_0 promotes membrane compaction in myelin sheaths in the PNS. Strikingly, P_0-CNS mice, which are the subject of study in this issue by Yin et al. show accelerated axonopathy in the form of axonal transport deficits and axonal swellings, despite the stabilization of myelin (Yin et al., 2006). These findings support the model that PLP exerts axon-supportive functions independent from myelination, and those functions cannot be substituted by the related protein, P_0.

What is the etiology of axonal degeneration in mutant oligodendrocytes or their PLP-deficient myelin sheaths? To answer this question, one needs to expand the traditional view of glia as mere myelin insulators of axons. Several recent studies have provided evidence for another key function of myelinating glia: metabolic support of axons. According to this concept, metabolic deficits in Schwann cells or oligodendrocytes, or impaired metabolite transport, are thought to account for axonal degeneration (Lee et al., 2012; Beirowski et al., 2014). In fact, energy-rich products of glycolysis (pyruvate) or fermentation (lactate) in oligodendrocytes appear to support CNS axons when shuttled into them via monocarboxylate transporters (Fünfschilling et al., 2012; Lee et al., 2012). Modeled after intercellular lactate shuttling proposed to operate in various tissues, these intermediates may then be used by axonal mitochondria for ATP production.

Impairment of metabolic exchange between axons and glia in mice lacking a single myelin protein like PLP could sug-

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gest a direct role for the myelin protein in metabolic coupling, or could be an indirect effect of the myelin protein loss of function. In this respect, it is important to note that compact myelin sheaths contain an elaborate system of channel-like structures (broadly known as noncompact myelin), which contain conduits of glial cytoplasm that are likely essential for conveying metabolites from glia to axons. In addition, these channels are also likely required for transport of the molecular machinery that enables metabolite exchange at axo–glial junctions (i.e., monocarboxylate transporters). Indeed, based on indirect experimental evidence, abnormalities in these structures have been hypothesized in PLP-deficient mice (Edgar and Nave, 2009). Moreover, P_0-CNS white matter fibers show substantially shorter myelin sheath internodes and formation of altered noncompact myelin in the form of Schmidt-Lanterman incisures that are normally formed exclusively in the PNS (Yin et al., 2006, 2008). Thus, it is tempting to hypothesize that the metabolic exchange system between oligodendrocytes and axons may be compromised in P_0-CNS mutants, resulting in deficient axonal bioenergetics.

Such bioenergetic deficits might be expected to manifest early as attenuated axonal transport resulting in organelle accumulation at juxtaparanodal axon sites. This is because "slow" organelles would not be able to pass the narrow nodes of Ranvier (Fig. 1; constriction is exaggerated for clarity). Accumulation of organelles including mitochondria would then lead to axonal swelling because of the space restraints. Indeed, such pathological features have been well documented in PLP-deficient mice as well as in P_0-CNS mutants (Griffiths et al., 1998; Edgar et al., 2004; Yin et al., 2006). Notably, this pattern of axon pathology is remarkably similar to the axonal swellings in CNS axons undergoing an energetic crisis during early Wallerian degeneration (a program of axonal autodestruction) after physical separation from the neuronal cell body (Beirowski et al., 2010).

Yin et al. (2016) do not address compromised axon–glia communication but focus on the downstream bioenergetic consequences in axons resulting from this putative impairment of metabolic coupling in P_0-CNS mice. Applying elegant serial block-face EM (SBEM) reconstructions of optic nerves, which enables 3D assessment of cytoskeleton and organelle shape and distribution, the authors demonstrated marked mitochondrial accumulations in swollen juxtaparanodal axon segments of 1-mo-old P_0-CNS mice. At this age, only a small amount of axonal degeneration is observed in P_0-CNS optic nerves. The authors found that the increase in mitochondrial content was caused by elevated mitochondrial numbers accompanied by drastic changes in mitochondrial shape with a shift toward a much more rounded appearance and reduced volume of individual mitochondria. Moreover, they observed alterations in the ultrastructure of the mitochondrial matrix. These changes were accentuated in the distal juxtaparanodal axon segment (on the opposite side of each node of Ranvier from the cell body), suggesting a potential problem of retrograde axonal transport (Griffiths et al., 1998). Together, these results suggested altered mitochondrial motility and structural adaptations at sites of energetic depletion in mutant axons.

To correlate these static observations with direct assessment of mitochondrial dynamics, Yin et al. (2016) subsequently performed time-lapse imaging of mitochondrial transport in P_0-CNS Purkinje cell axons using cerebellar organotypic slice cultures. The mean velocity of the motile mitochondrial fraction was severely reduced in P_0-CNS preparations as compared with controls with an accentuation of retrograde transport deficits.

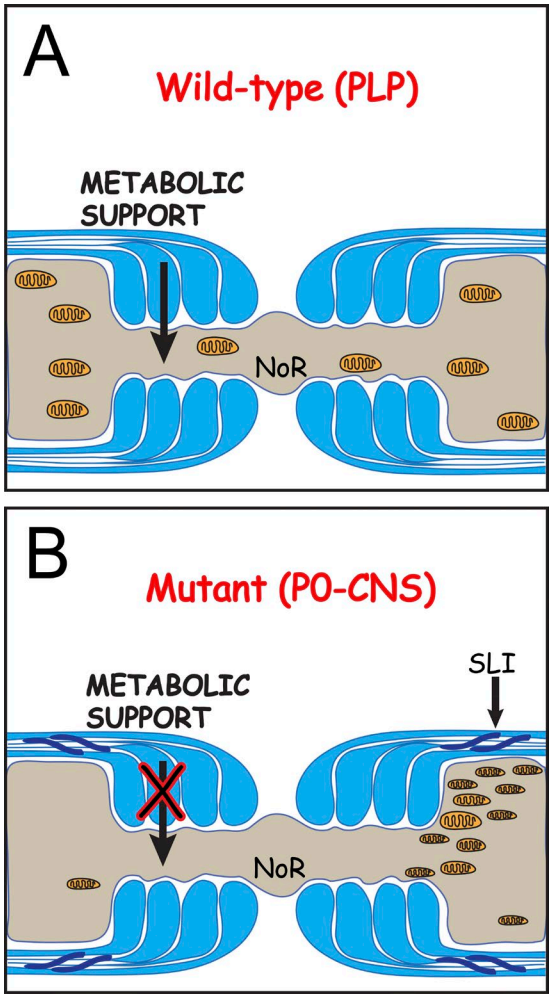

Figure 1. **Loss of oligodendrocytic metabolic support is associated with abnormalities in axonal mitochondria in mutant P_0-CNS optic nerves.** (A and B) Schematic illustrating a model for oligodendrocytic metabolic support for axons in a normal optic nerve (A) and in a mutant P_0-CNS optic nerve (B). Note the axonal narrowing at nodes of Ranvier (NoR; narrowing exaggerated for clarity) that motile mitochondria must traverse. (B) Metabolic support (large arrow) is compromised (red X) in the absence of PLP in P_0-CNS optic nerves. They manifest shorter myelin internodes, leading to an increased number of nodes of Ranvier (not depicted), and altered noncompact myelin (Schmidt-Lanterman incisures [SLI]). Altered noncompact myelin spaces may perturb metabolic coupling between glia and the axon. As a consequence, axonal transport is delayed, leading to accumulations of mitochondria at paranodal and juxtaparanodal areas. Note that accumulation is accentuated at the distal juxtaparanode (on the side of the node of Ranvier further from the cell body). Secondary adaptations include reduced mitochondrial volume (presumably by the induction of mitochondrial fission), changes in mitochondrial matrix ultrastructure, and reduced association of mitochondria to the axonal SER system (not depicted), causing calcium overload and eventual axonal destruction.

This resulted in a preferential standstill of mitochondrial movement at the distal juxtaparanodal region in P_0-CNS fibers, thus explaining the organelle accumulations at these sites. These results are reminiscent of the deficits observed in PLP-deficient mice in which retrograde transport in optic nerve axons has been studied by measurements of dynein/dynactin levels and injections of cholera toxin B (CTB; Edgar et al., 2004).

Are these abnormalities in mitochondrial motility associated with reduced axonal energy content as a prelude to axonal degeneration? To answer this, Yin et al. (2016) used a functional

approach. Electrophysiological activity analysis as a surrogate for axonal energy content after induction of metabolic stress (oxygen-glucose deprivation) demonstrated that optic nerve axons from 1-mo-old P_0-CNS mice showed an accelerated loss of electrical function and recovered more slowly after stress. This can be best explained by a reduced ATP content in P_0-CNS axons. In agreement, the authors showed reduced ATP concentrations in optic nerve extracts using a bioluminescence assay. In the future, it will be interesting to study ATP levels specifically in the axonal compartment within this model.

To further probe the mechanisms for compromised mitochondrial dynamics, Yin et al. (2016) next studied the ultrastructural organization and biochemical features of microtubules, the tracks that carry mitochondria as they move along axons. No loss of microtubules or abnormalities in their orientations along axons could be detected in optic nerves from young P_0-CNS mutants. However, in 6-mo-old mutants, an age with more prominent axonal degeneration, microtubule orientation at paranodes/juxtaparanodes was disorganized, and microtubule length was significantly shorter as visualized by EM tomography. This was paralleled by abnormal acetylation of α-tubulin, which, together with β-tubulin, are the polymerizing constituents of microtubules. Tubulin acetylation is considered a marker for microtubule stability and regulates the anchoring of molecular motors for mitochondria. To test whether the microtubule binding protein, tau, participates in these abnormalities, Yin et al. (2016) studied its phosphorylation status in P_0-CNS samples and found prominent hyperphosphorylation, likely mediated by stimulation of glycogen synthase 3 (GSK3) signaling. Tau has a critical role in the regulation of microtubule dynamics, and also binds to motor proteins. Collectively, these data suggest early energetic depletion in P_0-CNS axons that leads to later disruption of the axonal cytoskeleton and their associated motors with involvement of upstream regulators of microtubule stability. Future studies will be needed to address the mechanistic relationship between axonal energy status and features such as microtubule acetylation, its polymerization, and the association with tau and motor proteins.

The abnormal mitochondrial dynamics raised the possibility that the interaction of mitochondria with other organelles in the axon is compromised. The tethering of mitochondria to specialized sites of the ER (i.e., the mitochondria-associated ER membrane [MAM]) plays a significant role for the maintenance of intracellular calcium homeostasis. Importantly, intraaxonal calcium overload is a convergent step of distinct pathways that lead to axon degeneration (Beirowski et al., 2010). Yin et al. (2016) extended their beautiful SBEM reconstructions to visualize MAM associations with a novel kind of mitochondrial outer membrane extension and found an 86% reduction in the associations between mitochondrial membranes and the axonal smooth ER (SER) tubular system in axons from 1-mo-old P_0-CNS optic nerves. Furthermore, they noted a rather fragmented geometry of the SER tubules in P_0-CNS axons. Thus, it is likely that calcium homeostasis is perturbed in P_0-CNS axons, although this aspect was not examined in this study.

Altogether, these results from a mouse model with grossly normal myelin, yet accelerated axonal degeneration, begin to shed light on how bioenergetic deficits can elicit complex alterations in axonal structure, ultimately leading to axonal loss. This paper also has important implications for our understanding of disease pathogenesis. Because the features of damage in P_0-CNS axons are very similar to those of older PLP-null mice (Griffiths et al., 1998; Edgar et al., 2004; Yin et al., 2006, 2016), the mechanisms of axonal degeneration in P_0-CNS mice are likely to be relevant to the pathogenesis of HSP2. Confirmation in older PLP-null optic nerves of reduced ATP concentration, microtubular disorganization, and altered MAM associations will strengthen the relevance. In addition, future studies are needed to demonstrate that P_0-CNS axons are indeed deprived of metabolic support by their oligodendrocytes. Although a formidable task, conducting careful structural and functional studies of axon–glia communication through noncompact myelin sites in this model may be a viable starting point. The results will provide a mechanistic framework integrating both glial and axonal energy metabolism and may reveal therapeutic targets for the many neurodegenerative conditions with prominent axonal degeneration.

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References

Beirowski, B. 2013. Concepts for regulation of axon integrity by enwrapping glia. *Front. Cell. Neurosci.* 7. http://dx.doi.org/10.3389/fncel.2013.00256

Beirowski, B., A. Nógrádi, E. Babetto, G. Garcia-Alias, and M.P. Coleman. 2010. Mechanisms of axonal spheroid formation in central nervous system Wallerian degeneration. *J. Neuropathol. Exp. Neurol.* 69:455–472. http://dx.doi.org/10.1097/NEN.0b013e3181da84db

Beirowski, B., E. Babetto, J.P. Golden, Y.J. Chen, K. Yang, R.W. Gross, G.J. Patti, and J. Milbrandt. 2014. Metabolic regulator LKB1 is crucial for Schwann cell-mediated axon maintenance. *Nat. Neurosci.* 17:1351–1361. http://dx.doi.org/10.1038/nn.3809

Edgar, J.M., and K.A. Nave. 2009. The role of CNS glia in preserving axon function. *Curr. Opin. Neurobiol.* 19:498–504. http://dx.doi.org/10.1016/j.conb.2009.08.003

Edgar, J.M., M. McLaughlin, D. Yool, S.C. Zhang, J.H. Fowler, P. Montague, J.A. Barrie, M.C. McCulloch, I.D. Duncan, J. Garbern, et al. 2004. Oligodendroglial modulation of fast axonal transport in a mouse model of hereditary spastic paraplegia. *J. Cell Biol.* 166:121–131. http://dx.doi.org/10.1083/jcb.200312012

Fünfschilling, U., L.M. Supplie, D. Mahad, S. Boretius, A.S. Saab, J. Edgar, B.G. Brinkmann, C.M. Kassmann, I.D. Tzvetanova, W. Möbius, et al. 2012. Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature.* 485:517–521.

Garbern, J.Y., D.A. Yool, G.J. Moore, I.B. Wilds, M.W. Faulk, M. Klugmann, K.A. Nave, E.A. Sistermans, M.S. van der Knaap, T.D. Bird, 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.* 125:551–561. http://dx.doi.org/10.1093/brain/awf043

Griffiths, I., M. Klugmann, T. Anderson, D. Yool, C. Thomson, M.H. Schwab, A. Schneider, F. Zimmermann, M. McCulloch, N. Nadon, and K.A. Nave. 1998. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science.* 280:1610–1613. http://dx.doi.org/10.1126/science.280.5369.1610

Lee, Y., B.M. Morrison, Y. Li, S. Lengacher, M.H. Farah, P.N. Hoffman, Y. Liu, A. Tsingalia, L. Jin, P.W. Zhang, et al. 2012. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature.* 487:443–448. http://dx.doi.org/10.1038/nature11314

Nave, K.A., and B.D. Trapp. 2008. Axon-glial signaling and the glial support of axon function. *Annu. Rev. Neurosci.* 31:535–561. http://dx.doi.org/10.1146/annurev.neuro.30.051606.094309

Taveggia, C., M.L. Feltri, and L. Wrabetz. 2010. Signals to promote myelin formation and repair. *Nat. Rev. Neurol.* 6:276–287. http://dx.doi.org/10.1038/nrneurol.2010.37

Trapp, B.D., and K.A. Nave. 2008. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 31:247–269. http://dx.doi.org/10.1146/annurev.neuro.30.051606.094313

Yin, X., R.C. Baek, D.A. Kirschner, A. Peterson, Y. Fujii, K.A. Nave, W.B. Macklin, and B.D. Trapp. 2006. Evolution of a neuroprotective function of central nervous system myelin. *J. Cell Biol.* 172:469–478. http://dx.doi.org/10.1083/jcb.200509174

Yin, X., G.J. Kidd, K.A. Nave, and B.D. Trapp. 2008. P_0 protein is required for and can induce formation of Schmidt-Lantermann incisures in myelin internodes. *J. Neurosci.* 28:7068–7073. http://dx.doi.org/10.1523/JNEUROSCI.0771-08.2008

Yin, X., G.J. Kidd, N. Ohno, G.A. Perkins, M.H. Ellisman, C. Bastian, S. Brunet, S Baltan, and B.D. Trapp. 2016. Proteolipid protein–deficient myelin promotes axonal mitochondrial dysfunction via altered metabolic coupling. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201607099