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The Myelin Brake: When Enough is Enough

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Abstract

Myelination by Schwann cells in the peripheral nervous system (PNS) and by oligodendrocytes in the central nervous system (CNS) is tightly regulated by interactions with axons. Various investigations have shed light on the signaling pathways that mediate the production of myelin, but an important question remains; that is, which signals determine when the cell stops myelinating. New studies demonstrate that in Schwann cells, this is controlled by the abundance of Dlg1, which acts to stop active myelination.

A remarkable intercellular communication event that occurs during nervous system development is myelination. The production of myelin by Schwann cells in the peripheral nervous system (PNS) and by oligodendrocytes in the central nervous system (CNS) is tightly regulated by interactions with axons. The complexity of the cellular changes that result from myelination both in Schwann cells and oligodendrocytes and in axons has been the focus of intense investigation. Specializations of the plasma membrane of the glial cell or of the axon result in domains of compact myelin and noncompact myelin such as Schmidt-Lanterman incisures along with nodal, paranodal, juxtaparanodal, and internodal domains of both the myelin and the axonal plasma membrane (1). These interactions have been extensively analyzed, as investigators identified growth factors or other ligands that regulate cell proliferation, migration, or differentiation (2-4); transcription factors that are required for specific stages of cell specification or differentiation (5, 6); epigenetic events that regulate aspects of these interactions (7-11); intracellular signaling pathways that induce differentiation or myelination (12-15); or protein-protein interactions that mediate membrane specializations in one or both cells (16, 17). (These references are broad reviews and do not encompass the extensive literature in this area.)

The production of myelin is strictly regulated, and as noted at least three decades ago, the amount of myelin generated is in proportion to the diameter of the axon (18, 19). Thus, thicker axons have greater amounts of myelin than thinner axons, and that ratio is constant within species. The ratio of axonal diameter and total fiber diameter is defined as the g-ratio, and in normal samples, this ratio is tightly maintained for axons over a wide range of diameters. The internodal length (which is the distance between nodes of Ranvier) also correlates with axonal diameter and myelin thickness. These two elements, internodal length and myelin thickness, are determined by the volume of myelin generated per glial cell, and as demonstrated by Friede and Bischhausen (18), the volume of myelin per internode has a linear relation to the axolemmal surface area that it encompasses. This is an interactive system. In myelinating cultures, the presence of myelin increases the diameter of dorsal root ganglion axons (20). On the other hand, signals from the axons mediate the thickness of the myelin produced by glial cells (21, 22). Thus, a major question in this field has been: How

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does the glial cell generate the correct amount of myelin for the diameter of the axon that it is myelinating?

Signals on axons instruct the myelinating cell, the best established of which in the PNS is neuregulin-1 type III. Birchmeier, Nave, and Salzer's groups have shown that the amount of neuregulin-1 type III on the surface of peripheral axons determines the thickness of myelin (21, 22). Decreased surface abundance of neuregulin-1 type III on axons reduces Schwann cell myelin thickness, and excess surface abundance of neuregulin-1 type III increases myelin thickness. Several laboratories have demonstrated the importance of the Akt/mTOR (mammalian target of rapamycin) pathway in the regulation of myelination (12-15). In oligodendrocytes, overexpression of a constitutively active form of Akt generates a hypermyelination phenotype that is induced by increased mTOR signaling (12, 13). This hypermyelination is pathological, because these mice are apparently unable to stop making myelin and die by ~14 months of age. Induction of myelination in Schwann cells by neuregulin signaling is also regulated by Akt signaling (23). Akt is itself inhibited by the activity of PTEN (phosphatase and tensin homolog deleted on chromosome 10). The conditional deletion of PTEN in glial cells elevates phosphatidylinositol 3,4,5-trisphosphate (PIP3) concentrations in both oligodendrocytes and Schwann cells and releases inhibition of Akt signaling, which generates a hypermyelination phenotype in both the PNS and CNS (15).

Thus, one major positive regulator of PNS myelination is neuregulin-1 type III, which acts through ErbB receptors on Schwann cells apparently to decrease PTEN activity, thereby modulating PIP3 concentrations and increasing Akt signaling (15). However, a crucial question has remained unanswered in the literature: How does the glial cell know when to stop? Arriving at the correct g-ratio of myelin thickness must involve signals that induce myelination and those that stop it. As noted above, overexpression of constitutively active Akt, which promotes myelination, generates an animal that cannot stop making myelin. What normally decreases the activity of Akt when active myelination is finished and the appropriate amount of myelin has been produced?

Cotter *et al.* (24) provide the first identification of a putative myelin "brake." This study developed from the finding that a mutation in the gene encoding myotubularin-related protein 2 (*MTMR2*) induces Charcot-Marie-Tooth disease 4B, a peripheral neuropathy that manifests as hypermyelination by Schwann cells (25, 26). *MTMR2* interacts with mammalian discs large homolog 1 [Dlg1; also known as synapse-associated protein 97 (SAP97)], which contains PDZ (PSD-95, Discs-large, ZO-1)-binding domains involved in protein-protein interactions (27). Dlg1 can act as a scaffolding protein and is involved in cell polarity and membrane protein trafficking, including that of AMPA receptors in neurons (28).

Cotter *et al.* (24) demonstrate that silencing of Dlg1 during PNS myelination generated a hypermyelination phenotype, comparable to that caused by conditional deletion of PTEN or overexpression of Akt in oligodendrocytes. Conversely, overexpression of Dlg1 reduced myelination. Regulation of myelination by Dlg1 appeared to come from its interaction with PTEN. Dlg1 directly interacted with PTEN in Schwann cells and reduced its proteosomal degradation, which stabilized it. This in turn reduced the activity of Akt, a driver of myelination. When Dlg1 was silenced, baseline phosphorylation (and thus activation) of Akt increased, and the increase in phosphorylated Akt after neuregulin treatment was greater in Dlg1-silenced Schwann cells than in those cells with Dlg1.

In addition to inducing PNS myelination, neuregulin also affected this putative myelin brake. In normal cells, neuregulin induced increases in the abundance of Dlg1 by reducing its

ubiquitination and degradation. Increasing amounts of nonubiquitinated Dlg1 led to the stabilization and thus increased amounts of PTEN as myelination increased. Thus, both the positive and negative mediators of active myelination are increasing in Schwann cells as they mature and generate myelin, until the abundance of the negative mediator, the complex of Dlg1 and PTEN, is sufficient to reduce Akt activity and to stop active myelination (Fig. 1).

The identification of at least one protein complex involved in the brake mechanism for active myelination provides us with evidence of how a glial cell stops generating myelin and is an important new direction for research on the interaction of myelinating cells and axons. A balance between the activities of Akt and PTEN (which is stabilized at the protein level by Dlg1), both of which are induced by at least one myelination signal, likely defines the end of active myelination. On the other hand, the question of how the glial cell knows that it has generated the precise amount of myelin needed for an axon of a specific diameter remains under investigation. In the PNS, it might simply be the amount of neuregulin-1 type III on the surface of the axon, which would be increased for an axolemmal domain on a larger axon, thereby inducing increased myelination, and potentially determining the point at which the stabilized Dlg1-PTEN complex begins to inhibit Akt activity. In the CNS, on the other hand, neuregulin-1 type III signaling is insufficient to define the amount of myelin produced by oligodendrocytes (29), although it affects myelination in some regions of the CNS (30). CNS myelination is driven by the activity of Akt (12), although it may also be influenced by other factors, such as polysialylated neural cell adhesion molecule (31, 32) and extracellular matrix molecules and their ligands (4). Perhaps the fact that oligodendrocytes can myelinate multiple axons, whereas Schwann cells ensheath single axons, indicates a requirement for a more complex axonal signaling system for myelination in the CNS. No clear data exist yet on a putative brake in the CNS. However, initial work from the Colognato laboratory suggests a potential inhibitory role for C-terminal Src kinase in CNS myelination (33). This research area is likely to identify important new players in this essential intercellular communication.

References and Notes

1. Trapp, BD.; Kidd, GJ. Myelin Biology and Disorders. Lazzarini, RA., editor. Elsevier; San Diego, CA: 2004. p. 3-27.
2. Barres BA, Raff MC. Axonal control of oligodendrocyte development. *J. Cell Biol.* 1999; 147:1123–1128. [PubMed: 10601327]
3. Dubois-Dalcq M, Murray K. Why are growth factors important in oligodendrocyte physiology? *Pathol. Biol. (Paris)*. 2000; 48:80–86. [PubMed: 10729915]
4. Baron W, Colognato H, French-Constant C. Integrin-growth factor interactions as regulators of oligodendroglial development and function. *Glia*. 2005; 49:467–479. [PubMed: 15578662]
5. Jessen KR, Mirsky R. Signals that determine Schwann cell identity. *J. Anat.* 2002; 200:367–376. [PubMed: 12090403]
6. Wegner M. A matter of identity: Transcriptional control in oligodendrocytes. *J. Mol. Neurosci.* 2008; 35:3–12. [PubMed: 18401762]
7. He Y, Sandoval J, Casaccia-Bonnel P. Events at the transition between cell cycle exit and oligodendrocyte progenitor differentiation: The role of HDAC and YY1. *Neuron Glia Biol.* 2007; 3:221–231. [PubMed: 18634613]
8. Dugas JC, Cuellar TL, Scholze A, Ason B, Ibrahim A, Emery B, Zamanian JL, Foo LC, McManus MT, Barres BA. Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. *Neuron*. 2010; 65:597–611. [PubMed: 20223197]
9. Pereira JA, Baumann R, Normén C, Somandin C, Mieh M, Jacob C, Lüthmann T, Hall-Bozic H, Mantei N, Meijer D, Suter U. Dicer in Schwann cells is required for myelination and axonal integrity. *J. Neurosci.* 2010; 30:6763–6775. [PubMed: 20463238]

10. Yun B, Anderegg A, Menichella D, Wrabetz L, Feltri ML, Awatramani R. MicroRNA-deficient Schwann cells display congenital hypomyelination. *J. Neurosci.* 2010; 30:7722–7728. [PubMed: 20519547]
11. Zhao X, He X, Han X, Yu Y, Ye F, Chen Y, Hoang T, Xu X, Mi QS, Xin M, Wang F, Appel B, Lu QR. MicroRNA-mediated control of oligodendrocyte differentiation. *Neuron.* 2010; 65:612–626. [PubMed: 20223198]
12. Flores AI, Narayanan SP, Morse EN, Shick HE, Yin X, Kidd G, Avila RL, Kirschner DA, Macklin WB. Constitutively active Akt induces enhanced myelination in the CNS. *J. Neurosci.* 2008; 28:7174–7183. [PubMed: 18614687]
13. Narayanan SP, Flores AI, Wang F, Macklin WB. Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination. *J. Neurosci.* 2009; 29:6860–6870. [PubMed: 19474313]
14. Tyler WA, Gangoli N, Gokina P, Kim HA, Covey M, Levison SW, Wood TL. Activation of the mammalian target of rapamycin (mTOR) is essential for oligodendrocyte differentiation. *J. Neurosci.* 2009; 29:6367–6378. [PubMed: 19439614]
15. Goebbels S, Oltrogge JH, Kemper R, Heilmann I, Bormuth I, Wolfer S, Wichert SP, Möbius W, Liu X, Lappe-Siefke C, Rossner MJ, Groszer M, Suter U, Frahm J, Boretius S, Nave KA. Elevated phosphatidylinositol 3,4,5-trisphosphate in glia triggers cell-autonomous membrane wrapping and myelination. *J. Neurosci.* 2010; 30:8953–8964. [PubMed: 20592216]
16. Salzer JL, Brophy PJ, Peles E. Molecular domains of myelinated axons in the peripheral nervous system. *Glia.* 2008; 56:1532–1540. [PubMed: 18803321]
17. Susuki K, Rasband MN. Molecular mechanisms of node of Ranvier formation. *Curr. Opin. Cell Biol.* 2008; 20:616–623. [PubMed: 18929652]
18. Friede RL, Bischhausen R. The precise geometry of large internodes. *J. Neurol. Sci.* 1980; 48:367–381. [PubMed: 7441287]
19. Friede RL, Bischhausen R. How are sheath dimensions affected by axon caliber and internode length? *Brain Res.* 1982; 235:335–350. [PubMed: 7188332]
20. Windebank AJ, Wood P, Bunge RP, Dyck PJ. Myelination determines the caliber of dorsal root ganglion neurons in culture. *J. Neurosci.* 1985; 5:1563–1569. [PubMed: 4009246]
21. Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA. Axonal neuregulin-1 regulates myelin sheath thickness. *Science.* 2004; 304:700–703. [PubMed: 15044753]
22. Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P, Chao MV, Falls DL, Role L, Salzer JL. Neuregulin-1 type III determines the ensheathment fate of axons. *Neuron.* 2005; 47:681–694. [PubMed: 16129398]
23. Maurel P, Salzer JL. Axonal regulation of Schwann cell proliferation and survival and the initial events of myelination requires PI 3-kinase activity. *J. Neurosci.* 2000; 20:4635–4645. [PubMed: 10844033]
24. Cotter L, Özçelik M, Jacob C, Pereira JA, Locher V, Baumann R, Relvas JB, Suter U, Tricaud N. Dlg1-PTEN interaction regulates myelin thickness to prevent damaging peripheral nerve overmyelination. *Science.* 2010; 328:1415–1418. [PubMed: 20448149]
25. Bolino A, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou K, Hausmanowa-Petrusewicz I, Mandich P, Schenone A, Gambardella A, Bono F, Quattrone A, Devoto M, Monaco AP. Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. *Nat. Genet.* 2000; 25:17–19. [PubMed: 10802647]
26. Bonneick S, Boentert M, Berger P, Atanasoski S, Mantei N, Wessig C, Toyka KV, Young P, Suter U. An animal model for Charcot-Marie-Tooth disease type 4B1. *Hum. Mol. Genet.* 2005; 14:3685–3695. [PubMed: 16249189]
27. Saras J, Heldin CH. PDZ domains bind carboxy-terminal sequences of target proteins. *Trends Biochem. Sci.* 1996; 21:455–458. [PubMed: 9009824]
28. Braithwaite SP, Meyer G, Henley JM. Interactions between AMPA receptors and intracellular proteins. *Neuropharmacology.* 2000; 39:919–930. [PubMed: 10727702]
29. Brinkmann BG, Agarwal A, Sereda MW, Garratt AN, Müller T, Wende H, Stassart RM, Nawaz S, Humml C, Velanac V, Radyushkin K, Goebbels S, Fischer TM, Franklin RJ, Lai C, Ehrenreich H,

- Birchmeier C, Schwab MH, Nave KA. Neuregulin-1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system. *Neuron*. 2008; 59:581–595. [PubMed: 18760695]
30. Taveggia C, Thaker P, Petrylak A, Caporaso GL, Toews A, Falls DL, Einheber S, Salzer JL. Type III neuregulin-1 promotes oligodendrocyte myelination. *Glia*. 2008; 56:284–293. [PubMed: 18080294]
31. Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, Zalc B, Lubetzki C. Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97:7585–7590. [PubMed: 10840047]
32. Fewou SN, Ramakrishnan H, Büssow H, Gieselmann V, Eckhardt M. Down-regulation of polysialic acid is required for efficient myelin formation. *J. Biol. Chem.* 2007; 282:16700–16711. [PubMed: 17420257]
33. Tzvetanova, ID.; Colognato, H. personal communication. SUNY Stony Brook.

Fig. 1.

Putting the brakes on myelination. **(A)** During the active phase of myelination of peripheral nerves, axonal expression of neuregulin-1 type III (Nrg1) activates ErbB2 and ErbB3 receptors on Schwann cells and signals through the phosphatidylinositol 3-kinase (PI3K)–Akt pathway to initiate myelin production. Neuregulin also induces an increase in the abundance of Dlg1, presumably by preventing its ubiquitination and subsequent degradation. **(B)** As nonubiquitinated Dlg1 accumulates, it stabilizes PTEN, which reduces its proteosomal degradation, and the number of Dlg1-PTEN complexes increases. The presence of active PTEN reduces Akt activity and terminates active myelination. Dashed lines indicate indirect interactions, and inactive proteins or processes are indicated by lighter colors.