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Nodal Dynamics after *In Vivo* Rescue of βIV Spectrin Expression

Clara Maria Bacmeister and Michael Andrew Thornton
Department of Cell and Developmental Biology, Anschutz Medical Campus, University of Colorado, Aurora, Colorado 80045
Review of Saifetiarova et al.

Precise localization of voltage-gated sodium channels (Na_v) at nodes of Ranvier is required for efficient signal transmission along myelinated axons. By restricting Na_v to gaps in the myelin sheath, the nodal complex focuses the regeneration of action potentials to discrete sites and ensures faithful signal conduction. Disruption of nodal proteins plays a key role in the pathophysiology of demyelinating disorders (Craner et al., 2004) and complex psychiatric disorders like autism spectrum disorder and schizophrenia (Davis et al., 2003; Bi et al., 2012). Despite this, the mechanisms of nodal maintenance and reorganization after destabilization remain unclear. A molecular understanding of these processes may uncover novel therapeutic targets for the treatment of pathologically altered nodes of Ranvier.

A number of proteins contribute to the formation and maintenance of functional nodes of Ranvier. These include the cell adhesion molecule neurofascin-186 (NF186) and the cytoskeletal proteins, ankyrinG (ankG) and βIV spectrin. AnkG binds NF186, βIV spectrin, and Na_v, and, collectively, these interactions play important roles in constructing the nodal complex at appropriate locations along the axon (Sherman et al., 2005; Dzhashiashvili et al., 2007; Yang et al., 2007; Susuki et al., 2013). As the node matures, βIV spectrin anchors the entire nodal complex to the underlying axonal cytoskeleton through association with actin (Berghs et al., 2000) and NF186 associates with components of a glia-secreted extracellular matrix (Salzer, 2003; Sherman et al., 2005). These interactions likely contribute to nodal stability over time (Zhang et al., 2012).

The intricate molecular interactions involved in forming and maintaining the node make it particularly fragile in pathological conditions. To uncover critical factors in the destabilization and restabilization of nodes, Saifetiarova et al. (2018) used spectrin-deficient mice in which the expression of *Sptbn4*—the gene encoding βIV spectrin—was disrupted by an insertion element that could be excised with tamoxifen injections to rescue βIV spectrin expression. In spectrin-deficient mice, ankG, Na_v, and NF186 were still recruited to developing nodes, but they became increasingly destabilized over time and were sequentially lost, beginning with ankG, followed by Na_v and NF186.

The researchers chose two time points for inducing βIV spectrin expression: an "early rescue" time point at 4 months of age, when spectrin-deficient mice exhibit a moderate motor phenotype; and a "late rescue" time point at 7 months of age, when this phenotype has progressed to complete paralysis. Although βIV spectrin rescue induced successful reorganization of nodes in the sciatic nerve at both time points tested, the timing of rescue was critical for nodal restoration in spinal cord axons. A subset of nodes did not reintegrate nodal proteins upon rescue of βIV spectrin expression. This failure to reintegrate was most pronounced in the spinal cord of late-rescue mice, but even in the sciatic nerve—where nodal reorganization was comparatively robust—the intensity of nodal Na_v staining was lower in late-rescue mice than in age-matched controls. By studying the organization of key nodal proteins before and after βIV spectrin expression, Saifetiarova et al. (2018) uncovered time-dependent and region-specific differences in the ability of these proteins to reintegrate at nodes after destabilization.

Long-term destabilization of nodes was coupled with a progressive decline in axonal health, which eventually resulted in permanent nodal disorganization and axonal damage. Sciatic nerve axons resisted degeneration better than spinal cord axons and showed improvements in conduction after βIV spectrin rescue regardless of age. Still, early rescue mice demonstrated increased recovery of motor function and signal conduction compared with late rescue animals, and both rescue groups had decreased life span, incomplete motor recovery, and altered

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Correspondence should be addressed to Clara Maria Bacmeister, Department of Cell and Developmental Biology, Anschutz Medical Campus, University of Colorado, 405 Lincoln Street, Aurora, Colorado 80045. E-mail: clara.bacmeister@ucdenver.edu.
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conductive properties in sciatic nerve axons when compared to controls.

The findings by Saifetiarova et al. (2018) are consistent with previous work showing that CNS and PNS nodes exhibit different susceptibility to disruption. For example, CNS nodes disintegrate more quickly than PNS nodes upon ablation of NF186 in adult mice (Desmazieres et al., 2014), and the timing of simultaneous ablation of ankG and NF186 strongly influences the rate at which Na_v is lost from nodes in spinal cord, but not in sciatic nerve (Taylor et al., 2018). What could be driving this differential stability? One possibility is that NF186 is better stabilized at PNS nodes by extracellular interactions. Schwann cell microvilli recruit Na_v to developing nodes (Eshed et al., 2005), and stimulated emission depletion super-resolution microscopy of adult mouse sciatic nerve revealed the tight periodic association of proteins in Schwann cell microvilli and the axonal cytoskeleton (D'Este et al., 2017). Furthermore, genetic deletion of both gliomedin and NrCAM—two extracellular molecules that interact with Na_v through NF186—leads to the gradual loss of nodal proteins (Amor et al., 2014). The selective deletion of Schwann cell dystroglycan—which may mediate interactions between Schwann cell microvilli and the nodal axolemma—has similar effects (Saito et al., 2003). Perhaps Schwann cell microvilli play an important role in the increased stability of the PNS nodal complex.

Another molecule that may contribute to axon-specific differences in the stability of the nodal complex is αII spectrin. Dorsal root axons of comparable size to those in the sciatic nerve (>2.5 μm diameter) have a higher density of αII spectrin and similar levels of βIV spectrin when compared to smaller-diameter peripheral axons. These large-diameter sensory axons are preferentially degenerated after the loss of αII spectrin (Huang et al., 2017). Together, these results indicate that, in large PNS axons, cytoplasmic αII spectrin may play a heightened role in resisting degeneration, a mechanism that could contribute to greater stability of sciatic nerve nodes and axons when compared with spinal cord in spectrin-deficient mice.

In addition to its role in the nodal complex, βIV spectrin participates in a robust cytoskeletal scaffold that is thought to maintain the structural integrity of the axon initial segment (AIS; Leterrier et al., 2015; Wang et al., 2018). The AIS is involved both in the generation of action potentials and in the maintenance of neuronal polarity and axon trafficking (Hedstrom et al., 2007). Therefore, the disruption and subsequent reorganization of the AIS is another plausible explanation for the axonal degeneration, functional deficits, and recovery seen in spectrin-deficient and rescue mice. In future studies, further characterization of the functional effects of spectrin deficiency and rescue could be accomplished by examining the AIS in central and peripheral axons.

Recent evidence indicates that node length plasticity may be an efficient mechanism for fine-tuning action potential conduction speed in the adult brain (Sampaio-Baptista and Johansen-Berg, 2017). Additionally, high-frequency stimulation results in fast, calpain-dependent paranodal retraction via spectrin breakdown (Huff et al., 2011). Changes in node length could require calpain-dependent cleavage of the spectrin cytoskeleton to change the location of the paranodal junction or to insert or delete nodal membrane. In traumatic and ischemic injuries, calpain-mediated proteolysis of βIV-spectrin and ankG also leads to nodal destabilization (Schafer et al., 2009). Therefore, to maintain the capacity for activity-dependent modulation, CNS nodes may remain more susceptible to spectrin cleavage than PNS nodes, accelerating their disintegration in pathological conditions.

The electrophysiological and behavioral improvements found by Saifetiarova et al. (2018) suggest a remarkable capacity for functional recovery following nodal disorganization. Most current treatments for multiple sclerosis patients target the immune system to decrease inflammation and prevent additional axonal damage, while emerging therapies aim to promote the differentiation and integration of oligodendrocytes into damaged CNS circuits (Trojano and Amato, 2018). Because nodal disorganization contributes to pathology and may exacerbate axonal degeneration, therapies aimed at increasing nodal stability could reduce the severity of a demyelinating event and expedite recovery during remyelination. Furthermore, the decreased nodal reorganization and continued axonal degeneration seen by Saifetiarova et al. (2018) at late-rescue time points suggests nodal restoration and axonal rescue operates within a critical period. If nodes are allowed to disorganize beyond the critical window for restoration, remyelinating therapies may be ineffective in restoring proper conduction along axons. A greater understanding of the region-specific molecular processes driving nodal deorganization and reorganization could enhance our ability to effectively treat demyelinating and neuropsychiatric diseases.

References

Amor V, Feinberg K, Eshed-Eisenbach Y, Vainshtein A, Frechter S, Grumet M, Rosenbluth J, Peles E (2014) Long-term maintenance of Na^+ channels at nodes of Ranvier depends on glial contact mediated by gliomedin and NrCAM. J Neurosci 34:5089–5098. CrossRef Medline

Berghs S, Aggujaro D, Dirkx R Jr, Maksimova E, Stabach P, Hermel JM, Zhang JP, Philbrick W, Slepnev V, Ort T, Solimena M (2000) βIV spectrin, a new spectrin localized at axon initial segments and nodes of Ranvier in the central and peripheral nervous system. J Cell Biol 151:985–1002. CrossRef Medline

Bi C, Wu J, Jiang T, Liu Q, Cai W, Yu P, Cai T, Zhao M, Jiang YH, Sun ZS (2012) Mutations of ANK3 identified by exome sequencing are associated with autism susceptibility. Hum Mutat 33:1635–1638. CrossRef Medline

Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG (2004) Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. Proc Natl Acad Sci U S A 101:8168–8173. CrossRef Medline

Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, Buxbaum J, Haroutunian V (2003) White matter changes in schizophrenia: evidence for myelin related dysfunction. Arch Gen Psychiatry 60:443–456. CrossRef Medline

Desmazieres A, Zonta B, Zhang A, Wu LM, Sherman DL, Brophy PJ (2014) Differential stability of PNS and CNS nodal complexes when neuronal neurofascin is lost. J Neurosci 34:5083–5088. CrossRef Medline

D'Este E, Kamin D, Balzarotti F, Hell SW (2017) Ultrastructural anatomy of nodes of Ranvier in the peripheral nervous system as revealed by STED microscopy. Proc Natl Acad Sci U S A 114:E191–E199. CrossRef Medline

Dzhashiashvili Y, Zhang Y, Galinska J, Lam I, Grumet M, Salzer JL (2007) Nodes of Ranvier and axon initial segments are ankyrin G–dependent domains that assemble by distinct mechanisms. J Cell Biol 177:857–870. CrossRef Medline

Eshed Y, Feinberg K, Poliak S, Sabanay H, Sarig-Nadir O, Spiegel I, Bermingham JR Jr, Peles E (2005) Gliomedin mediates schwann cell-axon interaction and the molecular assembly of the nodes of Ranvier. Neuron 47:215–229. CrossRef Medline

Hedstrom KL, Xu X, Ogawa Y, Frischknecht R, Seidenbecher CI, Shrager P, Rasband MN (2007) Neurofascin assembles a specialized extracellular matrix at the axon initial segment. J Cell Biol 178:875–886. CrossRef Medline

Huang CY, Zhang C, Zollinger DR, Leterrier C, Rasband MN (2017) An αII spectrin-based cytoskeleton protects large-diameter myelinated axons from degeneration. J Neurosci 37:11323–11334. CrossRef Medline

Huff TB, Shi Y, Sun W, Wu W, Shi R, Cheng JX (2011) Real-time cars imaging reveals a calpain-dependent pathway for paranodal myelin retraction during high-frequency stimulation. PLoS One 6:e17176. CrossRef Medline

Leterrier C, Potier J, Caillol G, Debarnot C, Rueda Boroni F, Dargent B (2015) Nanoscale architecture of the axon initial segment reveals an organized and robust scaffold. Cell Rep 13:2781–2793. CrossRef Medline

Saifetiarova J, Shi Q, Paukert M, Komada M, Bhat MA (2018) Reorganization of destabilized nodes of Ranvier in βIV spectrin mutants uncovers critical timelines for nodal restoration and prevention of motor paresis. J Neurosci 38:6267–6282. CrossRef Medline

Saito F, Moore SA, Barresi R, Henry MD, Messing A, Ross-Barta SE, Cohn RD, Williamson RA, Sluka KA, Sherman DL, Brophy PJ, Schmelzer JD, Low PA, Wrabetz L, Feltri ML, Campbell KP (2003) Unique role of dystroglycan in peripheral nerve myelination, nodal structure, and sodium channel stabilization. Neuron 38:747–758. CrossRef Medline

Salzer JL (2003) Polarized domains of myelinated axons. Neuron 40:297–318. CrossRef Medline

Sampaio-Baptista C, Johansen-Berg H (2017) White matter plasticity in the adult brain. Neuron 96:1239–1251. CrossRef Medline

Schafer DP, Jha S, Liu F, Akella T, McCullough LD, Rasband MN (2009) Disruption of the axon initial segment cytoskeleton is a new mechanism for neuronal injury. J Neurosci 29:13242–13254. CrossRef Medline

Sherman DL, Tait S, Melrose S, Johnson R, Zonta B, Court FA, Macklin WB, Meek S, Smith AJ, Cottrell DF, Brophy PJ (2005) Neurofascins are required to establish axonal domains for saltatory conduction. Neuron 48:737–742. CrossRef Medline

Susuki K, Chang KJ, Zollinger DR, Liu Y, Ogawa Y, Eshed-Eisenbach Y, Dours-Zimmermann MT, Oses-Prieto JA, Burlingame AL, Seidenbecher CI, Zimmermann DR, Oohashi T, Peles E, Rasband MN (2013) Three mechanisms assemble central nervous system nodes of Ranvier. Neuron 78:469–482. CrossRef Medline

Taylor AM, Shi Q, Bhat MA (2018) Simultaneous ablation of neuronal neurofascin and ankyrin G in young and adult mice reveals age-dependent increase in nodal stability in myelinated axons and differential effects on the lifespan. eNeuro 5:ENEURO.0138-18.2018. CrossRef Medline

Trojano M, Amato MP (2018) Multiple sclerosis in 2017: progress in multiple sclerosis—from diagnosis to therapy. Nat Rev Neurol 14:72–74. CrossRef Medline

Wang CC, Ortiz-González XR, Yum SW, Gill SM, White A, Kelter E, Seaver LH, Lee S, Wiley G, Gaffney PM, Wierenga KJ, Rasband MN (2018) βIV spectrinopathies cause profound intellectual disability, congenital hypotonia, and motor axonal neuropathy. Am J Hum Genet 102:1158–1168. CrossRef Medline

Yang Y, Ogawa Y, Hedstrom KL, Rasband MN (2007) βIV spectrin is recruited to axon initial segments and nodes of Ranvier by ankyrinG. J Cell Biol 176:509–519. CrossRef Medline

Zhang Y, Bekku Y, Dzhashiashvili Y, Armenti S, Meng X, Sasaki Y, Milbrandt J, Salzer JL (2012) Assembly and maintenance of nodes of Ranvier rely on distinct sources of proteins and targeting mechanisms. Neuron 73:92–107. CrossRef Medline