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Journal Club

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A Role for AnkyrinG in Maturation and Maintenance of the Nodes of Ranvier

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Review of Saifetiarova et al.

Rapid propagation of action potentials is essential for communication in neural circuits. In vertebrates, the speed of action potentials is increased by axonal myelination. To permit saltatory conduction of action potentials along the axon, the insulating myelin sheath is punctuated by unmyelinated regions, known as nodes of Ranvier, where voltage-gated sodium (NaV) channels are clustered to allow sodium influx from the extracellular space. NaV channels must be precisely and densely clustered along these unmyelinated node regions to ensure that sodium influx is not obstructed by the myelin sheath. As a result, changes in node morphology alter action potential conductance, and such changes have been observed in several diseases, including multiple sclerosis, autism, and epilepsy (Howell et al., 2006; Peñagarikano et al., 2011; Anderson et al., 2012). Thus, understanding how nodes are assembled and maintained over time may prove beneficial for understanding disease pathology.

Several proteins contribute to node assembly and maturation, including the cell-adhesion molecule Neurofascin 186 (NF186), which interacts with the extracellular matrix (Davis and Bennett, 1994), and two cytoskeletal proteins, βIV-Spectrin and AnkyrinG (AnkG), which link other nodal proteins to the actin cytoskeleton. These proteins are transported to the node and clustered via protein–protein interactions. Of these nodal proteins, AnkG is thought to act as a multivalent organizer during the initial assembly of nodes as other important nodal proteins, such as NaV channels (Lemaillet et al., 2003), βIV-Spectrin (Yang et al., 2004), and NF186 (Davis and Bennett, 1994) all have AnkG binding domains and knockdown of AnkG disrupts clustering of these proteins at the developing node (Dzhashiashvili et al., 2007).

Although many studies have focused on the initial recruitment of proteins to and development of the node, the long-term maintenance of nodal proteins is less well characterized and provides technical challenges. For example, the use of a pan-neuronal driver to globally knock out AnkG is lethal just after birth, making analysis of AnkG's function in nodal maintenance, a postnatal phenomenon, difficult. Despite this, studies have suggested an important role for AnkG in nodal maintenance. Studies using shRNA knockdown of NF186 have determined that NF186 is stably maintained within mature nodes with a turnover time ranging from weeks to months (Zonta et al., 2011) but that this turnover time is accelerated in the absence of interactions with AnkG (Zhang et al., 2012) suggesting AnkG's importance in nodal maintenance as well initial assembly. Alternatively, previous studies have suggested that AnkG is dispensable in initial assembly and maintenance as AnkyrinR performs a compensatory role in protein clustering in the sensory neurons and retinal ganglion cells of mice, which have AnkG selectively ablated in these cell types (Ho et al., 2014). Indeed, these mice showed no physical impairments and had normal NaV channel clustering even at 2 years and 18 months of age. Given these conflicting results and the limitations hindering the study of AnkG in long-term maintenance, a temporally restricted knock-out is needed to define the role of AnkG in the assembly, maturation, and maintenance of the nodes.

In a recent paper in *The Journal of Neuroscience*, Saifetiarova et al. (2017) addressed this problem by selectively knocking out AnkG in sensory neurons by administration of tamoxifen in mice expressing Cre recombinase and a floxed AnkG allele. This allowed the authors to eliminate AnkG in spinal cord and sciatic nerve neurons at different developmental stages by administering tamoxifen during appropriate time periods. To ablate AnkG when nodes were initially forming, tamoxifen injections were given to the mother at embryonic day 19 and from P0-P5, which allowed pups to ingest tamoxifen through nursing. Reduced AnkG expression during this period did not af-

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fect the number of nodes formed or eliminate the clustering of βIV-Spectrin, NaV channels, or NF186 at nodes. This indicates that trafficking of nodal proteins to the site of the future node occurs in the absence of AnkG. However, the nodes that formed were morphologically abnormal as nodal proteins were less clustered and more diffuse. The boundaries of the node were also less well defined overall with nodal proteins spilling over into the adjacent paranodes where they would usually be covered by edges of the myelin sheath. These changes occurred rapidly with noticeable loss of nodal proteins as early as P5, progressively worsening and affecting levels of βIV-Spectrin, NaV channels, and NF186 simultaneously, suggesting a complete destabilization of the protein complex at the node. Moreover, these mice exhibited signs of pathology, including tremors and ataxia, and decreased compound muscle action potential amplitude; they did not survive past P40. The rapidity and severity of nodal degeneration indicate that AnkG plays a vital role during maturation of nodes. These data are consistent with the hypothesis that AnkG functions as a molecular scaffold that organizes and stabilizes other nodal proteins. Without AnkG, the tight spatial localization of the proteins and their interactions with each other are disrupted, which impairs propagation of action potentials and eventually leads to neuron degeneration.

In contrast to early loss of AnkG, late depletion through tamoxifen injections to P23-P32 mice resulted in a more gradual nodal degeneration. Moreover, the loss of nodal proteins was sequential, with βIV-Spectrin being lost as soon as 1 month after injection and loss of NaV channels and, to a lesser extent, NF186 occurring much later (10 months after injection). Nevertheless, pathology in these mice eventually progressed similarly to that in mice with early depletion of AnkG, exhibiting ataxia, tremor, and decreased compound muscle action potential amplitude. The protracted degeneration after late AnkG loss suggests that nodal complexes assembled in the presence of AnkG are more resilient to destabilization. However, the fact that AnkG loss in mature mice eventually resulted in degeneration of the nodes means that nodes are actively maintained and require AnkG.

The balance of the evidence thus seems to support essential roles for AnkG in both maturation and long-term maintenance of nodes of Ranvier. In contrast with previous results reported by Ho et al. (2014), that loss of AnkG is compensated for by the upregulation of AnkyrinR, Saifetiarova et al. (2017) showed no evidence of compensation by AnkyrinR or other ankyrins following ablation of AnkG. However, methodological differences could possibly account for the discrepancy, given that Saifetiarova et al. (2017) disrupted AnkG in a more precise and temporally controlled manner through inducible tamoxifen-dependent ablation, whereas Ho et al. (2014) knocked out AnkG at earlier time points.

Saifetiarova et al. (2017) explore late loss of AnkG showing a sequential pattern of destabilization at the node with βIV-Spectrin being lost first, followed by NaV channels, and finally NF186. AnkG and NaV persisting at the node longer than βIV-Spectrin broadly demonstrates that nodal stability results from both external interactions with cell adhesion molecules as well internal interactions with the neuronal cytoskeleton. Additionally, work from the same group, using a similar tamoxifen inducible approach to knock out NF186, revealed that NF186 is extremely stable at the nodes, but its loss eventually leads to the loss of AnkG and other nodal proteins (Taylor et al., 2017). These results show that primary organizers of the node, such as NF186, are in turn stabilized by the proteins they cluster, such as AnkG in a reciprocal process. This suggests a more complicated relationship between AnkG and NF186 that leaves open questions as to which protein is more important for node maturation and maintenance.

In conclusion, by knocking out AnkG both perinatally and in young adult neurons, Saifetiarova et al. (2017) have provided evidence that AnkG functions in both the maturation and maintenance of the nodes of Ranvier in CNS and PNS neurons. In early node development, AnkG loss results in immature nodes that undergo rapid destabilization within a month. Conversely, nodal destabilization after late ablation of AnkG is more gradual, occurring over a 10 month span. These disruptions in nodal stability lead to alterations in the neuron's electrical properties, subsequent axonal ultrastructural changes, and ultimately pathology. While other studies have shown that disrupting nodal organization leads to pathology, the current study provides direct insight into AnkG loss-mediated pathology and demonstrates the importance of nodal maintenance for proper nervous system function. In doing so, Saifetiarova et al. (2017) have provided compelling evidence for AnkG's involvement in nodal maintenance and have forced us to contemplate a role for it that goes beyond assembly.

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