**SPOTLIGHT**

# Healthy attachments: Cell adhesion molecules collectively control myelin integrity

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**Many cell adhesion molecules are present along myelinated axons and in myelinating glia, but functional interactions among these proteins have not been fully elucidated. In this issue, Elazar et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201906099) report that distinct adhesion proteins act in coordination to ensure accurate myelination.**

The multilayered myelin sheath surrounds axons in the vertebrate nervous system and permits rapid nerve impulse propagation at minimal metabolic cost. Specialized glial cells—oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS)—iteratively wrap their membrane to generate the multilamellar myelin sheath. Myelinated axons are organized into several discrete domains (Fig. 1): voltage-gated sodium channels cluster at nodes of Ranvier, the gaps between myelin segments, which are flanked by paranodal loops. Potassium channels are found at juxtaparanodes, and the internode represents the long stretch of myelinated axon between two nodal regions (1). This organization permits rapid impulse propagation from node to node via saltatory conduction, and previous work has identified a number of cell adhesion molecules (CAMs) present along myelinated axons and/or in myelinating glia.

Synaptic CAMs (SynCAMs), or Nectin-like (Necl) molecules, belong to the immunoglobulin superfamily of CAMs and are well known for their roles at the synapse (3). Several SynCAM family members have also been implicated in myelination, including CAM1 (Cadm1/SynCAM1/Necl2), which is present in oligodendrocytes; Cadm2 (SynCAM2/Necl3) and Cadm3 (SynCAM3/Necl1), which are present in axons; and Cadm4 (SynCAM4/Necl4), which is present in both oligodendrocytes and neurons (4). At paranodes, glial neurofascin 155 forms a complex with axonal contactin and Caspr (1), while myelin-associated glycoprotein (MAG) localizes with Cadm4 along internodes and is also enriched at paranodes (Elazar et al., in this issue). Although disruption of individual CAMs can impair myelination (e.g., 5), none are essential for myelination.

Given the presumed importance of axon–glial adhesion for myelination, why would the deletion of individual CAMs lead to, at worst, mild myelination defects? One reason may be compensatory mechanisms from other CAMs. To test this, Elazar et al. (2) generated double $Cadm4^{-/-}/Mag^{-/-}$ and $Cadm4^{-/-}/Caspr^{-/-}$ mutants to disrupt adhesion at paranodes and internodes simultaneously. Ultrastructural analyses of different CNS regions demonstrated a significant increase in the number of axons surrounded by multiple myelin sheaths in both double mutants that were often interspersed with noncompact cytoplasmic pockets (Fig. 1). Examination of paranodes showed that these structures were aberrantly localized either above or below compact myelin (Fig. 1), and these ultrastructural abnormalities were accompanied by motor coordination defects.

What mechanisms underlie these unique myelin perturbations? Given that Cadm4 and Mag are found along internodes and Mag and Caspr localize to paranodes, Elazar et al. propose a model in which the coordination between internodal and paranodal CAMs are critical for proper constraint of myelin growth along the axons such that simultaneous disruption results in myelin sheath overgrowth. This model is consistent with previous studies that show expression of Cadm4's extracellular domain restricts myelin sheath growth in the CNS (6) and expression of the cytoplasmic domain of Cadm4 inhibits Schwann cell myelination (7).

The significantly increased percentage of axons surrounded by multiple myelin sheaths in double mutants underscores the importance of proper axon–glial adhesions for correct myelination. However, only ~15% of total axons were observed with this phenotype, raising the question of whether only a subset of axons are affected. One possibility is that transmission EM underestimates the prevalence of this phenotype because only a single section of an internode can be examined in a given micrograph. Indeed, in this and other studies, three-dimensional reconstruction of serial sections can provide a clearer picture of the extent of myelin pathologies (5). On the other hand, it is also possible that some myelinated axons are more sensitive to the regulation of Cadm4/Mag/Caspr levels than others. Considering recent work to define oligodendrocyte heterogeneity (e.g., 8), it is also formally possible that subpopulations of oligodendrocyte lineage cells exist with different CAM repertoires required for proper axon–glial interactions.

What is the cell biological basis for the ultrastructural defects observed in internode/paranode double CAM mutants? Neighboring myelin sheaths may be disinhibited following CAM loss and overgrow into adjacent territory. Paranodal loops may also detach and migrate to incorrect regions. Three-dimensional reconstruction of serial EM sections suggests that overgrowth of neighboring myelin sheaths is at least partly

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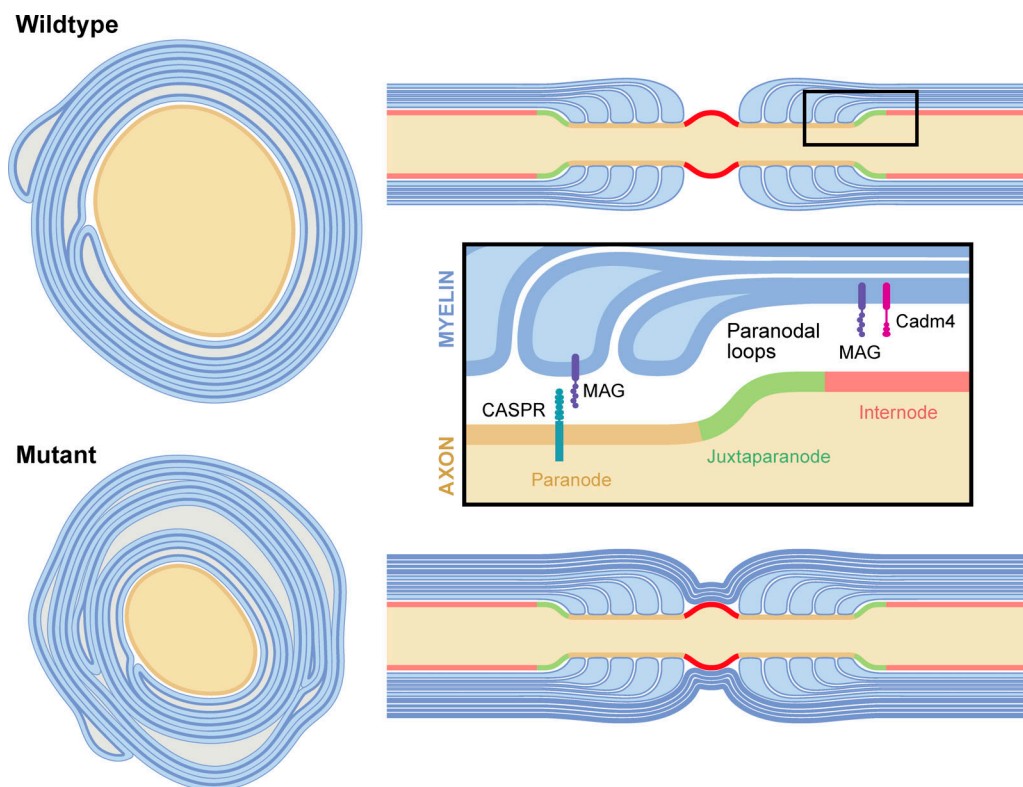Correspondence to Kelly R. Monk: monk@ohsu.edu.

**Figure 1.** **Domains of myelinated axons and adhesion mutant phenotypes.** Top: Wild-type myelinated axon shown in cross-section (left) and longitudinal (right) views. Myelin, blue; axon, beige. On the right, the axonal domains are depicted (red, node of Ranvier; tan, paranode; green, juxtaparanode; pink, internode), as are the three CAMs studied in the work by Elazar et al. (2). Bottom: In double *Cadm4/Mag* and *Mag/Caspr* mutants, myelin ultrastructure is disrupted. In the cross section, the myelin sheath appears split, with aberrant regions of noncompact cytoplasm. In the longitudinal section, paranodal loops are observed under (depicted) or above compact myelin. Image credit: Lori Vaskalis, Vollum Institute, Oregon Health and Science University, Portland, OR.

responsible. Larger-scale reconstructions would be technically challenging; therefore, future studies that employ time-lapse imaging in mouse or zebrafish might be especially useful to understand the mechanical underpinnings of the interesting phenotypes observed in double mutants. Given that Cadm4 is expressed in both neurons and oligodendrocytes, future cell type–specific studies may be warranted. Moreover, myelin ultrastructural abnormalities in double mutants were observed as early as 3 wk of age, suggesting that the myelin sheath grows abnormally from the onset of myelination. Thus, it may be worthwhile to temporally delete Cadm4/Mag and/or Mag/Caspr to determine if there is any effect on the maintenance of myelin. It would also be interesting to delete other paranodal and nodal CAMs to determine if the phenotypes observed are generally caused by simultaneous disruption of these two domains versus a specific function for the CAMs disrupted by Elazar et al. (2). Finally, although Cadm4, Mag, and Caspr are

all present in myelinated axons in the PNS, myelin abnormalities were only observed in the CNS. Work to understand why Schwann cell myelin is unaffected thus warrants future study.

The multi-myelin sheath caused by deletion of *Cadm4/Mag* or *Cadm4/Caspr* is an interesting phenomenon distinct from previously described pathologies like myelin outfoldings (e.g., 5) and bears some similarity to the "split" myelin sheaths observed in a rat model of neuromyelitis optica (9). Split myelin with ultrastructural similarities to *Cadm4/Mag* and *Cadm4/Caspr* mutants has also been observed in aged primate cortex (10). Thus, it would be interesting to examine these models for levels of paranodal and nodal CAMs to determine if their perturbation is correlated with the appearance of split myelin. Given that paranodes are thought to be particularly vulnerable in demyelinating disease like multiple sclerosis (11) and both MAG and CASPR expression and localization are perturbed in multiple sclerosis tissue (12, 13), the new study

from Elazar et al. may shed new light on our understanding of pathological changes that occur in demyelinating disease.

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