Cellular/Molecular

Differential Contribution of Cadm1–Cadm3 Cell Adhesion Molecules to Peripheral Myelinated Axons

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Cell adhesion proteins of the Cadm (SynCAM/Necl) family regulate myelination and the organization of myelinated axons. In the peripheral nervous system (PNS), intercellular contact between Schwann cells and their underlying axons is believed to be mediated by binding of glial Cadm4 to axonal Cadm3 or Cadm2. Nevertheless, given that distinct neurons express different combinations of the Cadm proteins, the identity of the functional axonal ligand for Cadm4 remains to be determined. Here, we took a genetic approach to compare the phenotype of Cadm4 null mice, which exhibit abnormal distribution of Caspr and Kv1 potassium channels, with mice lacking different combinations of Cadm1–Cadm3 genes. We show that in contrast to mice lacking the single Cadm1, Cadm2, or Cadm3 genes, genetic ablation of all three phenocopies the abnormalities detected in the absence of Cadm4. Similar defects were observed in double mutant mice lacking Cadm3 and Cadm2 (i.e., Cadm3−/−/Cadm2−/−) or Cadm3 and Cadm1 (i.e., Cadm3−/−/Cadm1−/−), but not in mice lacking Cadm1 and Cadm2 (i.e., Cadm1−/−/Cadm2−/−). Furthermore, axonal organization abnormalities were also detected in Cadm3 null mice that were heterozygous for the two other axonal Cadms. Our results identify Cadm3 as the main axonal ligand for glial Cadm4, and reveal that its absence could be compensated by the combined action of Cadm2 and Cadm1.

Key words: axon-glia interaction; cell adhesion; myelin; node of Ranvier; Schwann cells; SynCAM

Significance Statement
Myelination by Schwann cells enables fast conduction of action potentials along motor and sensory axons. In these nerves, Schwann cell-axon contact is mediated by cell adhesion molecules of the Cadm family. Cadm4 in Schwann cells regulates axonal ensheathment and myelin wrapping, as well as the organization of the axonal membrane, but the identity of its axonal ligands is not clear. Here, we reveal that Cadm mediated axon-glia interactions depend on a hierarchical adhesion code that involves multiple family members. Our results provide important insights into the molecular mechanisms of axon-glia communication, and the function of Cadm proteins in PNS myelin.

Introduction
Myelinating Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the CNS are engaged in a continuous dialogue with the axons they ensheathe. Axon-glia contact not only affects myelin membrane wrapping, but also controls the precise placement of ion channels along the axolemma, and the generation of specific axoglial contact sites that are required for the formation of a functional myelin units (Stadelmann et al., 2019; Wilson et al., 2020). Such interactions are mediated by distinct cell adhesion systems that are present at the axon-glial interface at the nodes of Ranvier, the paranodal junction (PNJ), and along the internodes below the compact myelin (Rasband and Peles, 2015). Recent studies revealed that accurate myelination in the CNS involves the coordinate action of both paranodal and internodal adhesion systems (Djannatian et al., 2019; Elazar et al., 2019a).

Intercellular adhesion mediated by members of the Cadm family, also known as Nectin like (Necls) and synaptic adhesion molecules (SynCAMs), regulates different developmental aspects of the nervous system, including axon guidance (Niederkofler et al., 2010; Frei et al., 2014), synapse formation and plasticity (Biederer et al., 2002; Robbins et al., 2010; Perez de Arce et al., 2015; Ribic et al., 2019), and myelination (Maurel et al., 2007; Spiegel et al., 2007; Park et al., 2008; Golan et al., 2013; Chen et al., 2016; Elazar et al., 2019a,b). Not surprisingly, variations in Cadm genes are associated with diverse neurologic and mental health-related conditions (Day et al., 2016; Pasman et al., 2018; Xu et al., 2018; Dohnn and Saporta, 2020). The Cadm proteins belong to a small group of the immunoglobulin superfamily cell adhesion molecules that contains four different members...
Cadm1–Cadm4, Mandai et al., 2015). They are transmembrane glycoproteins that contain three immunoglobulin-like (Ig) domains in their extracellular region and a short intracellular domain which mediates their interactions with protein 4.1 and PDZ-domain proteins (Zhou et al., 2005; Hoy et al., 2009; Einheber et al., 2013; Rademacher et al., 2016; Meng et al., 2019). Cadms mediate Ca\(^{2+}\)-independent cell-adhesion by binding homophilically, as well as heterophilically to other members of the family (Kakunaga et al., 2005; Fogel et al., 2007; Spiegel et al., 2007; Liu et al., 2019). Cadm1 (Ncd2), Cadm 2 (Ncd3), and Cadm3 (Ncd1; Kakunaga et al., 2005; Fogel et al., 2011), but not Cadm4 (Ncd4; Liu et al., 2019), also form homophilic and heteromeric cis dimers on the cell membrane. In addition, the interaction between Cadm family members is modulated by differential glycosylation (Fogel et al., 2007, 2010; Galuska et al., 2010). In the PNS, Cadm proteins are differentially expressed in Schwann cells and axons, i.e., Cadm1, Cadm2, and Cadm3 are mostly found in neurons, whereas Cadm4 and to a much lesser extent Cadm1, are present in myelinating Schwann cells (Maurel et al., 2007; Spiegel et al., 2007). Cadm4 is present at the internodal Schwann cell-axon interface, directly apposing Cadm2 and Cadm3 in the axolemma. The extracellular domain of Cadm4 preferentially binds to Cadm2-expressing and Cadm3-expressing cells, as well as to cultured sensory neurons, but not to Schwann cells (Spiegel et al., 2007), suggesting that in the PNS, axon-glia interaction is primarily mediated by binding of glial Cadm4 to axonal Cadm2 and Cadm3 (Maurel et al., 2007; Spiegel et al., 2007). Similarly, in the CNS, genetic deletion of Cadm2 and Cadm3 completely abolished the binding of the extracellular domain of Cadm4 to spinal cord neurons (Elazar et al., 2019b). However, since both sensory (Zheng et al., 2019) and motor (Maciel et al., 2018) neurons express different combinations of Cadm genes, the identity of the functional axonal ligand for Cadm4 is presently unclear. Given that loss-of-function phenotypes of receptors and of their ligands are often similar, we compared between Cadm4 null mice and mice lacking each of the other single Cadm genes, as well as mice lacking different allelic Cadm combinations. This genetic analysis identified Cadm3 as the main axonal ligand for Cadm4 in the PNS, and revealed a compensatory contribution of the two other axonal Cadms.

Materials and Methods

Animal handling and generation of knock-out mice

All experiments were performed in compliance with the relevant laws and institutional guidelines and were approved by the Weizmann’s Institutional Animal Care and Use Committee. Mice were kept in Weizmann’s animal facility, in aseptic SFP conditions until used for experiments. Knock-out mice for Cadm1, Cadm2, Cadm3, and Cadm4 were generated as described previously (Fujita et al., 2005, 2006; Golan et al., 2013). Cadm1KO, Cadm2KO, and Cadm3 KO mice were crossed to generate triple KO (Cadm123 KO) mice. Genotyping was done by PCR of genomic DNA using the following primers: Cadm1WT allele, GATGTTGCTGACCTAGGACAGGTC and GAGGTGATTAACACGCTGAGCGCAAT; targeted, GATGTTGCTGACTTAGGAAAGCTGGTC and TCGAGGAGCCAGGACTCCTGGT10GAC; Cadm2WT allele, AGGCACGTGCTGACAGTGC and ATTCGACCCGATTTCC; targeted, AGGCACAAATGTTGCAGCCA and CAAGGTAGACGCTGGCCAC; Cadm3WT allele, GCCCTGACCTGAAAAACGCGGAC and CGAGGTCGTTGTGCTTGGTT; targeted, GCCCTGACTGAAACGGCGAC and CTGGTCGTCACCACCGGATTG; Cadm4WT allele, CGAGGTTCCTGGATTTGCAG; Cadm4KO allele, GCCAGGGGAGGTGTCAGTG.

Immunofluorescent staining

Teased sciatic nerves were prepared and immunolabeled as previously described (Eshed-Eisenbach et al., 2020). Briefly, sciatic nerves were dissected and immersed in 4% paraformaldehyde for 20 min at room temperature (RT). Tearing of sciatic nerves was done by sheath- ing followed by teasing on SuperFrost Plus slides (Menzel-Gäser, Thermo Scientific), air dried overnight, and then kept frozen at −20°C till used. Teased peripheral nerves were all stained according to the following methodology. When required, samples were postfixed for 7 min using cold methanol (−20°C) following by consecutive PBS washes. Blocking and permeabilization were done by incubation for 1 h in 5% normal goat serum and 0.5% Triton X-100, in PBS at RT. Primary antibodies were diluted in 5% normal goat serum and 0.1% Triton X-100, in PBS and incubated overnight at 4°C. Secondary antibodies were incubated for 40 min at RT in 5% normal goat serum and 0.1% Triton X-100, in PBS. Samples were mounted with Fluoromount-G (SouthernBiotech). Images were taken using Nikon eclipse 90i microscope or 3DHistech Panoramic Midi II scanner.

Antibodies

The following primary antibodies were used: rat anti-neurofilament (NF-H; MAB5468, Millipore Bioscience Research Reagents), chicken anti-NF-H (AB_2313552 AVES labs), rabbit anti-Caspr (Peles et al., 1997), rabbit anti-Kv1.2 (Ogawa et al., 2008), mouse anti-Kv1.2 (K14/16; NeuroMab), rat anti-NrCAM (Lustig et al., 2001). Fluorophore-coupled antibodies included the following: 488-coupled anti-rabbit was purchased from Invitrogen; Cy3-coupled anti-rabbit, Cy3-coupled anti-mouse, and Cy5-coupled anti-rat were obtained from Jackson ImmunoResearch.

Electronic microscopy

Mice were scarified and sciatic nerves were exposed and fixed by continuous dripping of fresh fixative containing 4% PFA, 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Nerves were then excised and incubated overnight in the same fixative at RT and stored at 4°C. Nerves were processed as previously described (Elazar et al., 2019a). Samples were examined using a FEI Tecnai T12 transmission electron microscope equipped with a XF416 TVIP camera. In parallel, the resin embedded sections were stained with 1% toluidine blue and then analyzed using a 3DHistech Panoramic Midi II scanner.

Quantification and statistical analysis

Samples from at least three animals were used per genotype in all quantitation performed on teased sciatic nerves. Statistical significance was determined using Student’s two-tailed t test. Graphs were prepared using GraphPad Prism software. Images were taken using Nikon eclipse 90i microscope or 3DHistech Panoramic Midi II scanner.

Results

Loss of Cadm4, but not other Cadms, disrupts axonal organization

We previously found that genetic deletion of Cadm4 results in pronounced disorganization of myelinated axons in the PNS, as reflected by the disruption of Caspr-labeled paranodal junctions, and clustering of Kv1.2 potassium channels away from the juxtaparanodal region (JXP) (Fujita et al., 2005, 2006; Golan et al., 2013). To examine whether one of the neuronal Cadms (i.e., Cadm1–Cadm3) serves as an axonal ligand for glial Cadm4, we compared the distribution of Caspr and Kv1.2 in sciatic nerve of Cadm4 null mice, to mice lacking either Cadm1, Cadm2, or Cadm3 (Fig. 1). Similar to wild-type mice, all the last three single Cadm mutants exhibited normal accumulation of Caspr and Kv1.2 channels at the paranodal junction and the juxtaparanodal region, respectively. In contrast, and as expected, Cadm4 null mice displayed prominent disruption of the axonal organization (Fig. 1).

Triple mutant mice lacking Cadm1–3 exhibit diverse axonal organization defects

The observation that Cadm1−/−, Cadm2−/−, or Cadm3−/− null mice do not display similar abnormalities as detected in the
absence of Cadm4, suggests that the axonal Cadms compensate for each other, or alternatively, that other non-Cadm proteins serve as a ligand for glial Cadm4. To further examine these possibilities, we generated mutant mice lacking all three axonal Cadm proteins (Cadm1–/–/Cadm2–/–/Cadm3–/–). Similar to the single nulls (Fujita et al., 2005, 2006; Golan et al., 2013), homozygous Cadm1–/–/Cadm2–/–/Cadm3–/– mice display no apparent neurologic abnormalities and exhibit PNS myelin that was morphologically indistinguishable from wild-type animals (Fig. 2A). Nevertheless, immunolabeling of teased sciatic nerves isolated from these mice revealed clear abnormalities in the distribution of Caspr and Kv1.2 channels compared with each of the single mutants (Fig. 2B). While in the single mutants Kv1.2 potassium channels were restricted to the juxtaparanodal region and the mesaxon, in Cadm1–/–/Cadm2–/–/Cadm3–/– mice they were located away from this site and often showed elongated, diffuse or irregular appearance (Fig. 2B, arrows). The triple mutant also displayed aberrant distribution of Caspr away from the paranodal junction and almost complete absence of this protein from the inner mesaxon, i.e., the site where the innermost Schwann cell membrane is attached to the axolemma. As depicted in Figure 2C, Caspr abnormalities could be divided into three major subgroups, including aberrant extension beyond the paranodal junction in what appeared as unwinding paranodal loops, abnormal accumulation in patches away from the paranodal junction, and diffuse Caspr immunoreactivity along the internodes. The abnormalities observed for Kv1.2 channels included the presence of gaps within the juxtaparanodal region, the appearance of irregular membrane clusters, and their focal accumulation along the internodes (Fig. 2C), which all resemble the abnormalities reported for mice lacking Cadm4 in Schwann cells (Fujita et al., 2005, 2006; Golan et al., 2013). Quantitative analysis showed that 63 ± 0.07% of nodal environs of Cadm1–/–/Cadm2–/–/Cadm3–/– mice displayed abnormal distribution of Kv1.2 channels compared with 2 ± 0.004% in Cadm1–/–/–, 3 ± 0.002% in Cadm2–/–, and 9 ± 0.02% in Cadm3–/– (Fig. 2D). Abnormal Caspr immunoreactivity was detected in 70 ± 0.02% of the sites in Cadm1–/–/Cadm2–/–/Cadm3–/– mice compared with 7 ± 0.01% in Cadm1–/–, 5 ± 0.02% in Cadm2–/–, and 17 ± 0.05% in Cadm3–/– (Fig. 2E).

**Cadm1–/–/Cadm2–/–/Cadm3–/– triple nulls phenocopy mice lacking Cadm4**

We next compared between Cadm1–/–/Cadm2–/–/Cadm3–/– and Cadm4–/– null mice. Immunolabeling of teased sciatic nerves isolated from adult mice using antibodies to Caspr and Kv1.2 channels revealed the presence of remarkably similar abnormalities in both genotypes, but not in wild-type mice (Fig. 3A). Quantitative analysis showed no significant difference in appearance of these proteins in the two genotypes (Fig. 3B,C). Cadm1–/–/Cadm2–/–/Cadm3–/– mice displayed abnormal distribution of Kv1.2 channels in 74 ± 0.07% and 81 ± 0.02% in Cadm1–/–/Cadm2–/–/Cadm3–/– and Cadm4–/– mice, respectively (Fig. 3B). Likewise, we noted abnormal distribution of Caspr in 93 ± 0.01% of the paranodes compared with 89 ± 0.02% in Cadm4–/– null mice (Fig. 3C). These results demonstrate that deletion of all three neuronal Cadm proteins phenocopies mice lacking Cadm4, indicating that Cadm1–Cadm3 serve as the exclusive axonal ligand for glial Cadm4.

**Cadm3 is the main functional axonal ligand for glial Cadm4**

The observation that Cadm1–/–/Cadm2–/–/Cadm3–/– but not the single genes mutants phenocopies Cadm4–/– null mice, supports the notion that axonal Cadms compensate for each other. To further corroborate the existence of such a functional redundancy, we examine the distribution of Caspr in mice lacking different combinations of Cadm1–Cadm3 genes (Fig. 4A). We found that double mutant mice lacking Cadm3 and Cadm2 (i.e., Cadm3–/–/Cadm2–/–) or Cadm3 and Cadm1 (i.e., Cadm3–/–/Cadm1–/–) display abnormal distribution of Caspr at the paranodes corresponding to unusual “opening” of the paranodal loops, as well as intermittent accumulation of Caspr along the internodes. In contrast, no such abnormalities were detected in double mutant mice lacking both Cadm1 and Cadm2 (i.e., Cadm1–/–/Cadm2–/–), indicating that Cadm3 is a primary axonal ligand for glial Cadm4. Quantification of the results revealed that abnormal distribution of Caspr was detected at 55 ± 0.04% and 81 ± 0.1% of the sites in Cadm3–/–/Cadm1–/– and Cadm3–/–/Cadm2–/– mice, respectively, which was similar to mice lacking all three neuronal Cadms (71 ± 0.02%; Fig. 4B).
detected Caspr abnormalities in only 7 ± 0.01% of the sites in Cadm1<sup>−/−</sup>/Cadm2<sup>−/−</sup> mice, and 7 ± 0.01% in wild-type animals. Comparable results were obtained for Kv1.2 as well (WT 3 ± 0.01%, Cadm1<sup>−/−</sup>/Cadm2<sup>−/−</sup> 3 ± 0.01%, Cadm3<sup>−/−</sup>/Cadm2<sup>−/−</sup> 66 ± 0.1%, Cadm3<sup>−/−</sup>/Cadm1<sup>−/−</sup> 46 ± 0.1%, Cadm1<sup>−/−</sup>/Cadm2<sup>−/−</sup>/Cadm3<sup>−/−</sup> 77 ± 0.04%; Fig. 4C). These experiments identify Cadm3 as the main axonal ligand for Cadm4 in the PNS, and revealed a compensatory contribution of the two other axonal Cadms. They also suggest that Cadm-mediate axon-glia interactions involve multiple family members. To further explore this point, we asked whether deletion of Cadm3 along with a 50% reduction in the expression of the other two genes, will result in a phenotype. As depicted in Figure 5A,D, Cadm3<sup>−/−</sup> null mice that were heterozygous for Cadm1 and Cadm2 (Cadm1<sup>+/−</sup>/Cadm2<sup>+/−</sup>/Cadm3<sup>−/−</sup>) exhibit abnormal localization of Caspr. As expected, no such abnormalities were detected in Cadm1<sup>−/−</sup> or Cadm2<sup>−/−</sup> null mice that were heterozygous for the other two neuronal Cadm genes (Fig. 5B–D). Quantitative analysis revealed that 85 ± 0.01% of nodal environs of Cadm3<sup>+/−</sup>/Cadm1<sup>+/−</sup>/Cadm2<sup>−/−</sup> mice displayed abnormal distribution of Caspr compared with 6 ± 0.03% in Cadm1<sup>−/−</sup>/Cadm2<sup>−/−</sup>/Cadm3<sup>−/−</sup>, and 14 ± 0.04% in Cadm2<sup>−/−</sup>/Cadm1<sup>−/−</sup>/Cadm3<sup>−/−</sup> (Fig. 5D). Taken together, our results demonstrate that Cadm3 is the main axonal ligand for glial Cadm4 and...
that its absence can be compensated by the combined action of Cadm1 and Cadm2.

**Discussion**

Given that loss-of-function of receptors and their cognate ligands often results in similar phenotypes, we took a comparative genetic approach to identify the axonal Cadm ligand for Schwann cell Cadm4. We reasoned that this approach would be useful since members of this family are co-expressed in sensory and motor neurons (Maciel et al., 2018; North et al., 2019; Sharma et al., 2020) and are engaged in homophilic and multiple heterophilic interactions (Fogel et al., 2007; Maurel et al., 2007; Spiegel et al., 2007). Our results show that in peripheral myelinated neurons, Cadm3 is the chief axonal ligand for glial Cadm4. They further reveal a compensatory contribution of Cadm1 and Cadm2. Such redundancy may explain the discrepancy between results obtained from single gene deletion experiments, in which myelination is normal or only mildly affected (Park et al., 2008; Golan et al., 2013; Zhu et al., 2013), to those affecting/involving a few members of the family such as gain of function experiments (Elazar et al., 2019b), the use of dominant negative proteins (Hughes and Appel, 2019), ligand-receptor interference by soluble Cadm’s extracellular domains (Spiegel et al., 2007) and or deletion of Cadm4 together with other axoglial adhesion systems (Djennatian et al., 2019; Elazar et al., 2019a).

The distribution of ion channels along myelinating axons strongly depends on Schwann cells and represents a sensitive indicator of myelin abnormalities (Arroyo et al., 2004). The precise positioning of these channels at the axolemma is regulated by their association with axoglial CAMs and cytoskeleton-linker proteins (Horresh et al., 2010; Einheber et al., 2013; Amor et al., 2017; Brivio et al., 2017). It was previously shown that genetic deletion of Cadm4 in Schwann cells results in abnormal organization of the axonal membrane, reflected by aberrant localization of Caspr and Kv1.2 channels along the internodes (Golan et al., 2013). A similar phenotype was also detected in mice lacking protein 4.1G, which binds Cadm4 and regulates its expression at the axon–glial interface (Ivanovic et al., 2012). The observation that similar axonal organization defects are obtained by deletion of axonal Cadms compared with glial Cadm4, indicates that the function of Cadm4 in shaping the axonal membrane is mediated by binding to Cadm3–Cadm1. We propose that similar to the role Cadms are playing in CNS synapse (Fogel et al., 2007; Perez de Arce et al., 2015), binding of glial Cadm4 to the axonal Cadms forms an adhesion scaffold that contributes to the organization of the axonal membrane by recruiting intracellular linker proteins and cytoskeletal components. In support of this view, binding of Cadm3 to Cadm4 recruits the polarity protein Par3 to the axoglial interface (Meng et al., 2019). In addition, deletion of protein 4.1B, which binds members of the Cadm proteins (Hoy et al., 2009; Yang et al., 2011), results in reduced axonal expression of Cadm3, Cadm1, and αII spectrin in myelinated DRG axons (Einheber et al., 2013).

Axoglial adhesion mediated by Cadm4 regulated myelination in both CNS and PNS (Maurel et al., 2007; Spiegel et al., 2007; Golan et al., 2013; Elazar et al., 2019a,b). Here, we found that Cadm3 is the main axonal ligand for glial Cadm4. This conclusion is supported by previous observations showing that (1) Cadm3 is localized at the axoglial interface in myelinated peripheral nerves (Kakunaga et al., 2005; Maurel et al., 2007; Spiegel et al., 2007), and is found together with Cadm4 in axoglial-some-enriched fraction of sciatic nerves (Eichel et al., 2020); (2) the extracellular domain of Cadm4 binds to neurons expressing Cadm3 and Cadm2 and not to Schwann cells or oligodendrocytes (Maurel et al., 2007; Pellissier et al., 2007; Spiegel et al., 2007; Elazar et al., 2019b); (3) Cadm4 binding is abolished in cultured spinal cord neurons isolated from double Cadm2−/−/Cadm3−/− null mice (Elazar et al., 2019b); and (4) Cadm3 showed preferential binding to Cadm4 and Cadm1 (Fogel et al., 2007; Liu et al., 2019). Further support comes from experiments showing that while increased expression of Cadm4 lacking its intracellular domain (Cadm4(DCT)) in oligodendrocytes inhibits myelination, this effect could be rescued when Cadm4(DCT) OPCs were co-cultured with Cadm2−/−/Cadm3−/− neurons (Elazar et al., 2019b).

In contrast to genetic ablation of Cadm3 alone, deletion of Cadm3 together with either Cadm2 or Cadm1 resulted in an axonal organization defect, suggesting that Cadm1 and Cadm2...
cooperate with each other generating a complex that could functionally substitute for Cadm3. Furthermore, the observation that deletion of Cadm3 together with only one allele of both Cadm1 and Cadm2 resulted in a phenotype, indicating that the function of the axonal Cadms is dose dependent. The involvement of Cadm1 was surprising as previous studies demonstrated that Cadm4 binds Cadm3 and Cadm2 but not Cadm1 (Maurel et al., 2007; Pellissier et al., 2007; Spiegel et al., 2007). Its contribution could point to a role for Cadm1 in regulating the binding of Cadm2 and Cadm3 to Cadm4 through the formation of cis-heterodimers. In support of this idea, it was previously demonstrated that Cadm1-Cadm2 form cis-heterodimers (Shingai et al., 2003) that modulate trans interactions (Frei et al., 2014). Such a mode of interaction may also explain why co-deletion of Cadm3 and Cadm1 resulted in a somewhat milder phenotype than the one obtained in mice lacking Cadm3 and Cadm2 (Fig. 4C, note the trend although it is not statistically significant). Our findings also have clinical implication as mutations in the extracellular domain of human CADM3 gene that replace a tyrosine with a cysteine residue (Y172C) results in axonal Charcot-Marie-Tooth neuropathy (Dohrn and Saporta, 2020). Notably, while the Y172C mutation did not seem to directly affect Cadm3 binding, it lies within the second Ig domain, an area that could change some of the regulatory cis-mediated trans interactions of the protein (Liu et al., 2019).

The redundant function of Cadm1–Cadm3 in myelinated axons described here is also relevant for the roles Cadm (SynCAM) proteins play during other neurodevelopmental processes such as the establishment of CNS synapses and axon guidance (Frei and Stoecilli, 2017). Different combinations of Cadm1–4 were found in synaptic sites by biochemical fractionation as well as proteomic identification of the synaptic cleft proteins (Fogel et al., 2007; Loh et al., 2016; Cijsouw et al., 2018). Notably, the role of Cadm proteins in synapse formation of hippocampal neurons was masked by overlapping functions of these gene family members, and was only revealed by multiple gene deletions (Fowler et al., 2017) Binding and coimmunoprecipitation experiments suggested that the binding between Cadm1 and Cadm2, as well as between Cadm3 and Cadm4 provide a synaptogenic adhesive code (Fogel et al., 2007). Such a code would be affected by the presence of other members of the Cadm family that are present at the presynaptic or postsynaptic membranes, as revealed by recent proteomic approaches (Cijsouw et al., 2018). Whether synaptogenic and myelinogenic...
Cadm-dependent adhesion code share molecular similarities will be an interesting topic to explore in future studies.

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