Netrin-1 marshals mitochondrial movement, morphology, and metabolism in myelin

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Oligodendrocytes are the myelinating cells of the central nervous system (CNS) that ensheath nearby axons to support action potential propagation and axon metabolism. Myelination involves the rapid production of lipid-rich membrane, compaction of the multilamellar myelin sheath, and the resultant restriction of cytoplasm to non-compact compartments. During myelination, septate-like junctions form between the axon and lateral cytoplasmic endings of the myelin sheath at a specialized domain called the paranode (**Figure 1A**). Cytoplasm-filled loops at the paranode (hereon referred to as paranodal loops) host a network of organelles, but their regulation and function are poorly understood. Until recently, it was not at all clear how molecules from the oligodendrocyte cell body were transported across the compacted layers of the myelin sheath to access the cytoplasmic growing edge. In 2014, cytoplasmic channels that traverse the compact myelin sheath were identified in the CNS (Snaidero et al., 2014), opening the door to investigate the function and regulation of the organelles contained within these specialized cytoplasmic compartments. Further, it was shown that active mitochondria in non-compact myelin contribute to the generation of calcium transients that appear to regulate internode remodeling (Battefeld et al., 2019), supporting a functional link between mitochondria and myelin remodeling.

Mitochondria are responsible for numerous critical functions in eukaryotic cells. Fondly known as the "powerhouse of the cell," mitochondria generate energy in the form of adenosine triphosphatase (ATP). Oligodendrocyte mitochondria have a relatively low surface area of inner membrane cristae, a finding that suggests relatively low ATP production by mitochondrial oxidative phosphorylation (OXPHOS) (Rinholm et al., 2016) and supports previous reports that oligodendrocytes are highly glycolytic cells (Fünfschilling et al., 2012). Glycolysis converts glucose into pyruvate that is then shuttled into mitochondria and converted into acetyl-CoA, which may be either consumed in the citric acid cycle to generate ATP or utilized as a precursor for lipid synthesis. Lactate, another product of glycolysis, can also be converted into pyruvate and similarly used by mitochondria to generate acetyl-CoA. Oligodendrocytes generate lactate through glycolysis, and may take up additional lactate released by nearby astrocytes and blood vessels. Notably, the rate of lipid synthesis from lactate is reported to be significantly higher in oligodendrocytes than in neurons or astrocytes (Sánchez-Abarca et al., 2001). These studies suggest that a major role for mitochondria in mature or post-myelinating oligodendrocytes is to support lipid metabolism.

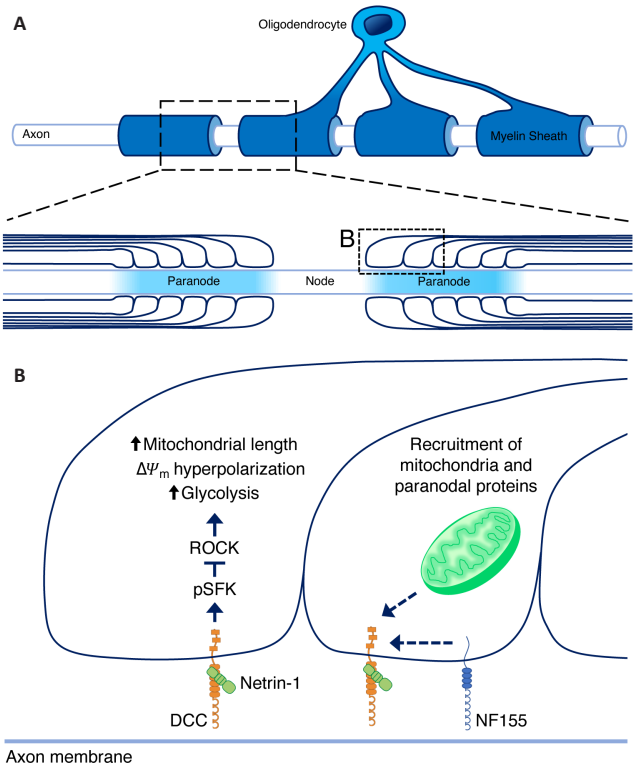

Figure 1 | **Schematic illustrating the potential roles of netrin-1 signaling at paranodal junctions in the central nervous system.**
(A) Ultrastructural organization of oligodendrocyte myelin sheaths. (B) Magnification of oligodendrocyte paranodal loops. Netrin-1 binding to its receptor deleted in colorectal carcinoma (DCC) activates downstream Src-family kinases (SFKs), including Fyn, which inhibits ROCK, resulting in increased mitochondrial length, mitochondrial membrane potential (Δψm) hyperpolarization, and increased glycolysis. We propose that netrin-1 binding to DCC at paranodal junctions contributes to the local recruitment of mitochondria and key paranodal proteins such as neurofascin-155 (NF155).

Currently, little is known about the instructive cues that direct the localization and function of mitochondria in oligodendrocytes. Netrin-1 is a secreted extracellular protein expressed by oligodendrocytes and neurons in the CNS (Lai Wing Sun et al., 2011). In oligodendrocytes, netrin-1 promotes the expansion of myelin-like membranes. Membrane expansion by oligodendrocytes is signaled by netrin-1 binding its receptor deleted in colorectal carcinoma (DCC), activating downstream Src-family kinase (SFK) Fyn and inhibiting ROCK (Rajasekharan et al., 2009). Netrin-1 and the netrin receptor DCC are enriched at CNS paranodes, and loss of their expression in mice results in severe disorganization of paranodal junctions (Lai Wing Sun et al., 2011; Bull et al., 2014), suggesting that netrin-1 signaling pathways are locally activated at paranodes. To investigate local signaling by netrin-1, we applied microbeads coated with netrin-1 protein to oligodendrocytes in culture. These netrin-1-coated beads exhibited SFK-dependent adhesion to the plasma membrane and stimulated rapid local remodeling of the cytoskeleton and myelin (Nakamura et al., 2021). A screen for proteins enriched with the netrin-1-coated beads identified components of mitochondria, tight junctions and lipid rafts (Nakamura et al., 2021). Proteins enriched at paranodes, including DCC, neurofascin 155, and zona occludens-1 were also enriched at netrin-1-coated beads (Nakamura et al., 2021). These findings provide tantalizing evidence that focal netrin-1 signaling is sufficient to direct the organization of mitochondria as well as an adhesive tight-junction-like protein complex in oligodendrocytes (**Figure 1B**).

Can large organelles like mitochondria fit through the narrow cytoplasmic channels that traverse the myelin sheath? Recent studies have visualized remarkably small mitochondria within multiple compartments of non-compacted myelin, including the paranodal loops, inner tongue, and cytoplasmic crossings of the myelin sheath (Rinholm et al., 2016; Battefeld et al., 2019; Nakamura et al., 2021). Surprisingly, oligodendrocyte mitochondria rapidly migrate through the cytoplasmic channels that cross compacted myelin-like membranes which are considerably narrower than the mitochondria themselves (Nakamura et al., 2021). The flexible, rapidly accommodating nature of these channels facilitates organelle trafficking in the oligodendrocyte myelin sheath; however, the mechanisms that permit this migration are not known. In primary cell culture, oligodendrocyte mitochondria migrate toward sites of netrin-1 signaling and remain stationed at sites of adhesion to netrin-1-coated beads (Nakamura et al., 2021). The detected enrichment of

kinesin and dynein motor proteins with netrin-1-coated beads suggests that netrin-1 signaling plays a role in mitochondrial trafficking (Nakamura et al., 2021). *In vivo*, the cytoplasmic channels that cross the myelin sheath close during maturation but can be reopened by increasing PIP3 levels (Snaidero et al., 2014). Netrin-1 activates PI3 kinase in neurons which is required to generate PIP3 (Lai Wing Sun et al., 2011), raising the possibility that netrin-1 may facilitate mitochondrial trafficking in oligodendrocytes by opening cytoplasmic channels in the myelin sheath.

Schwann cells are substantially larger than oligodendrocytes, and paranodes in the peripheral nervous system harbour hundreds to thousands of mitochondria (Rydmark et al., 1998). In stark contrast, 1–2 oligodendrocyte mitochondria were detected in approximately 23% of CNS paranodes, with the remaining 77% containing none (Nakamura et al., 2021). This finding highlights what we anticipate will be a fine-tuned trafficking mechanism that efficiently guides oligodendrocyte mitochondria to specific locations within the complex network of cytoplasmic channels and compartments in the myelin sheath. Focal netrin-1 signaling at the paranode may serve to recruit and retain mitochondria at the paranode; however, it remains to be determined what other factors coordinate with netrin-1 to regulate mitochondrial trafficking. For instance, we detected the inositol 1,4,5-trisphosphate receptor calcium channel as a significant hit associated with netrin-1-coated beads cultured with oligodendrocytes (Nakamura et al., 2021), but it is not known if netrin-1 stimulates intracellular calcium release in oligodendrocytes or if calcium regulates mitochondrial docking in oligodendrocytes, as it does in neurons. Unlike in neurons, mitochondrial movement increases in oligodendrocytes in response to glutamate activation of NMDA receptors, indicating unique differences in the regulation of mitochondrial trafficking by intracellular calcium between neurons and oligodendrocytes (Rinholm et al., 2016).

The adaptation of mitochondrial shape and size to the bioenergetic needs of the cell facilitates energy dispersion and the maintenance of healthy mitochondrial DNA. In yeast and mammalian cells, the expansive coverage that a fused network of mitochondria provides allows for the dispersion of metabolic energy throughout the cell. In mouse models of demyelination, injections with P110, an inhibitor of dynamin-related protein 1-driven mitochondrial fission in all cells, improve oligodendrocyte survival and attenuates demyelination (Luo et al., 2017). Mitochondrial elongation also promotes the formation of inter-organelle contact sites that mediate calcium transfer and lipid synthesis by increasing mitochondrial surface area. In oligodendrocytes exposed to exogenous netrin-1 for 2 hours, mitochondrial length significantly increases via SFK activation and ROCK inhibition – an effect paralleled by an increase in Mfn2 protein levels (Nakamura et al., 2021). At this timepoint, hyperpolarization of the mitochondrial membrane potential ($\Delta\psi m$) was detected, reflecting a larger proton gradient across the inner mitochondrial membrane, but the rates of glycolysis and OXPHOS were unchanged (Nakamura et al., 2021). When netrin-1 exposure was increased to 5 days, a time point when oligodendrocytes have increased expansion of their myelin-like membranes in response to netrin-1 (Rajasekharan et al., 2009), glycolysis was significantly increased while OXPHOS remained unchanged (Nakamura et al., 2021). These findings indicate that mitochondrial length is not strictly correlated with the level of OXPHOS in oligodendrocytes, since netrin-1-induced mitochondrial elongation was uncoupled from an increase in OXPHOS. A rise in basal glycolysis and the consequential increase in the levels of pyruvate, lactate, and acetyl-CoA may support a higher rate of lipid synthesis required for increased myelin production. Concurrently, this may also support lactate being shuttled from the oligodendrocyte to the axon to metabolically support action potential firing (Fünfschilling et al., 2011). Neuronal activity stimulates the release of netrin-1 by dendrites (Glasgow et al., 2018). If axons similarly secrete netrin-1, this may stimulate nearby oligodendrocytes to enhance glycolysis and increase lactate production to support membrane expansion and local axon metabolism. In the future, it will be interesting to determine how netrin-1 signaling may facilitate communication between the oligodendrocyte and axon.

In summary, we propose that localized extracellular netrin-1 functions as an instructive cue that directs the organization of paranodal proteins, the underlying cytoskeleton, and the recruitment and function of mitochondria in oligodendrocytes. Netrin-1 signaling stimulates a metabolic shift in oligodendrocyte mitochondria that enhances glycolysis and supports myelin remodeling. Oligodendroglial paranodes and mitochondria are susceptible to damage in multiple sclerosis, a neurodegenerative disease in which the immune system attacks CNS myelin sheaths. Understanding how mitochondria are regulated in oligodendrocytes will provide insight into how their activity can be targeted to promote myelin synthesis, remodeling, and repair in neurodegenerative diseases like multiple sclerosis.

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