Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) in press
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2300776

The structure of myelin in the central nervous system and another possible driving force for its formation—myelin compaction

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Abstract: Myelin formation is considered the last true "invention" in the evolution of vertebrate nervous system cell structure. The rapid, jumping pulse propagation achieved by myelin enables the high conduction speed that is the basis of human movement, sensation and cognitive function. As a key structure in the brain, white matter is the gathering place of myelin. However, with age, white matter-associated functions become abnormal, and a large number of myelin sheaths show degenerative changes, causing serious neurological and cognitive disorders. Despite the extensive time and effort invested in exploring myelination and its functions, numerous unresolved issues and challenges persist. In-depth exploration of the functional role of myelin may bring new inspiration for the treatment of central nervous system (CNS) diseases and even mental illnesses. In this study, we conducted a comprehensive examination of the structure and key molecules of the myelin in the CNS, delving into its formation process. Specifically, we propose a new hypothesis regarding the source of power for myelin expansion in which membrane compaction may serve as a driving force for myelin extension. The implications of this hypothesis could provide valuable insights into the pathophysiology of diseases involving myelin malfunction and open new avenues for therapeutic intervention in myelin-related disorders

Key words: Myelin; Central nervous system; White matter; Myelin compaction

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1 Introduction

Myelin is a multilayered sheath that envelops the outside of nerve cell axons. In the CNS of mammals from marsupials to humans, myelin sheaths are derived from oligodendrocytes (OLs), which are derived from morphologically complex precursor cells (oligodendrocyte precursor cells (OPCs) or NG2 cells). OPCs are a type of glial cell that exists in the CNS and originates from neural stem cells (NSCs). They are the largest dividing population of neural cells, distributed evenly and accounting for an average of 5% to 10% of all CNS cells (Tepavcevic and Lubetzki, 2022). Mature OLs create myelin by wrapping their own cell membranes around axons in a spiral shape, ultimately becoming multilayered sheaths that cover long axon segments. The fundamental function of myelin is saltatory conduction from one node of Ranvier to the next, thereby increasing nerve impulse conduction velocity (Zalc et al., 2008). High conduction speed is the basis of motor, sensory and cognitive functions. Therefore, the formation of myelin is regarded as the last true “invention” in vertebrate evolution in the structure of nervous system cells (Nave, 2010).

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Received Oct. 26, 2023; Revision accepted Feb 22 2024;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

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White matter is the primary component of the CNS in humans (accounting for 60% of brain volume) and is composed of a large number of myelinated nerve fibers (Krafft et al., 2012). In contrast, gray matter is composed of a large number of nerve cell bodies and synapses, and contains less myelin than white matter. Due to its location at the end of arterial circulation, white matter is particularly susceptible to reduced blood flow and oxygenation. Consequently, with age, a significant number of myelin sheaths undergo degenerative changes that lead to severe neurological and cognitive impairments (Liu et al., 2017). Since OLs are primarily responsible for synthesizing myelin, changes in OLs and their precursor cells can significantly affect the structure and function of myelin. The degeneration of OLs and their precursor cells increases with age. Additionally, aging is accompanied by chronic systemic low-grade inflammation caused by a sustained antigen load that mainly stimulates the innate immune system to produce pro-inflammatory cytokines (Correale and Ysrraelit, 2022). White matter aging is also associated with higher inflammatory activity of microglia and astrocytes, mitochondrial dysfunction, and iron deposition in the CNS leading to oxidative damage (Correale and Ysrraelit, 2022). In this article, we elucidate the structure and function of myelin and describe the process of myelin formation to lay a foundation for a better understanding of myelin-related diseases. Finally, we introduce the epidemiology of myelin-related diseases and describe their potential mechanisms of action.

2 Structure and function of myelin

2.1 Major dense line

Alternating major dense lines and intraperiod lines are the two characteristic periodic morphological features of the myelin sheath. The major dense lines, three nanometers in width, are formed by the tightly packed intracellular (cytoplasmic) surfaces located between the inner membranes of the lipid bilayers (Aggarwal et al., 2011; Kister and Kister, 2022). This unique structure is stabilized by multiple adhesive mechanisms, in which myelin basic protein (MBP) is essential. MBP is the second most abundant protein in the CNS myelin, accounting for 30% of the mass of myelin protein in the CNS. MBP tightly binds the two negatively charged cytoplasmic leaflets of the myelin membrane that form the major dense lines, and is referred to as an “executive” molecule of the myelin membrane, playing a key role in the formation of compacted myelin (Moscarello, 1997) (Fig. 1A). MBP is essentially an intrinsically disordered protein; however, upon binding to membranes, MBP adopts α-helical and β-sheet structures (Harauz et al., 2004) (Fig. 1B). Membrane binding alters the properties of MBP, promoting self-interaction into a densely packed protein phase that occupies the major dense lines and tightly binds the cytoplasmic surface of the bilayer together to limit the entry of most proteins into the myelin sheaths (Raasakka et al., 2017; Snaidero et al., 2017). Force-distance measurements indicate that maximal adhesion force and minimal cytoplasmic spacing occur when each negative lipid in the membrane can bind to a positively charged arginine or lysine residue on MBP (Min et al., 2009). In addition to its structural importance (through interactions with lipids and myelin-associated proteins), MBP may play a regulatory role in myelination because it participates in pathways that promote both neuron survival and death, such as binding to MAGEB2/D2 proteins associated with neurotrophin receptor p75NTR1 (Smirnova et al., 2021).

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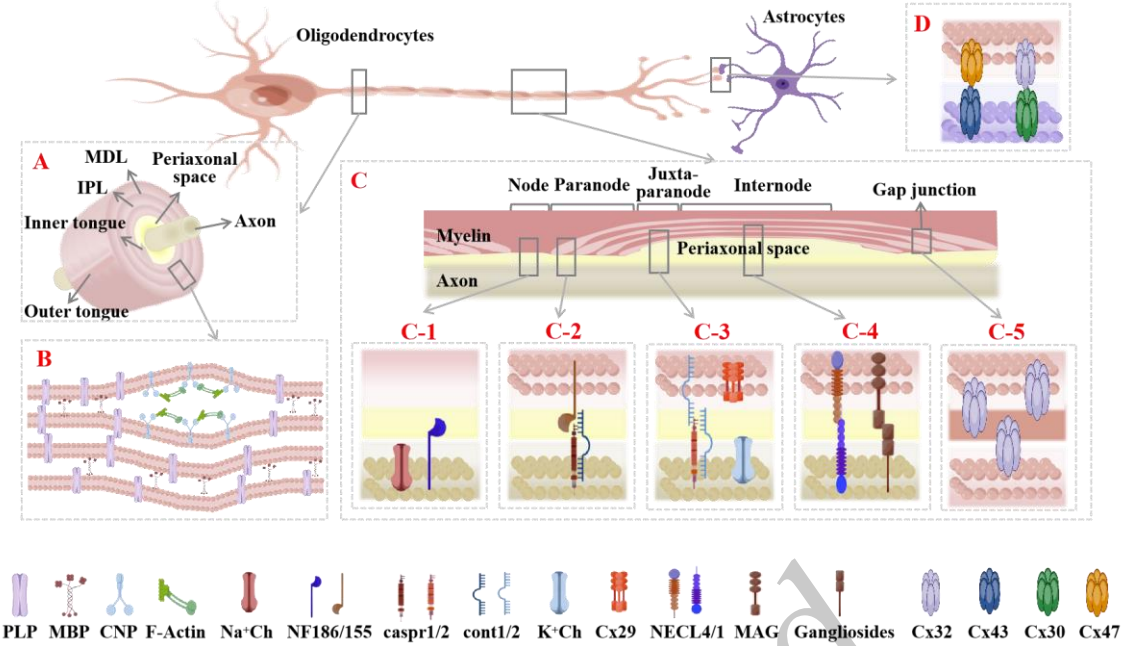

Fig. 1 Schematic diagram of myelin structure in the CNS. A: longitudinal section of myelin; B: intracellular lipid bilayer (marked in purple), cell membrane (marked in pink); C: cross section of myelin, where C-1 is a node, C-2 a paranode, C-3 a juxtaparanode, C-4 an internode, and C-5 a gap junction in an oligodendrocyte; D: Gap junction in oligodendrocyte and astrocyte networks.

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Both an excess and a deficiency of MBP can cause the repulsion of myelin bilayers (this pertains only to the cytoplasmic sides of myelin bilayers) and may lead to the destruction of myelin (demyelination). At higher MBP concentrations, there is an abundance of MBP, and presumably, excess positive charge at the interface, which not only causes the bilayers to repel each other electrostatically, but also leads to swelling of the water gap, resembling demyelination in vivo (Min et al., 2009). When there is an overabundance of MBP, a phase transition is observed across highly swollen bilayers, indicating the formation of a weak, dilute, yet extensive gel-like structure of MBP rather than simple electrostatic repulsion. This gel exhibits a phase transition upon compression, allowing it to collapse when subjected to a compressive pressure of ≈ 1 MPa (10 atm). When the swelling pressures are not too severe, the bilayers can be pressed together again to almost the same separation, within 0–2 nm, as the separation for adhesive, nonswelling systems, even from distances as large as 80 nm (Min et al., 2009). Shiverer mice, which lack expression of MBP, form less myelin and it is less stable than myelin in wild-type mice. The myelin formed by shiverer mice consists of loosely compacted layers and is at most four layers thick. Although some basic structural elements of the myelin remain, such as radial components and axon-glial connections, most of the axonal surface is completely devoid of myelin (Brady et al., 1999; Snaidero et al., 2014). In contrast to mice lacking basic components of paranodal axo-glial cell junctions (such as Caspr, contactin-1, and NF155), shiverer mice do not exhibit severe axonal degeneration (Sherman et al., 2005; Pillai et al., 2009). This may be because non-myelinated nerve fibers in shiverer mice are still able to propagate action potentials, albeit in an immature continuous manner, whereas mice lacking paranodal structures suffer from conduction block caused by current leakage (McNamara et al., 2023).

2.2 Intraperiod line

An intraperiod line is formed by the outer surface of the myelin membrane lipid bilayer. The intraperiod line is wider than the main dense lines when observed using electron and X-ray microscopy (Karthigasan and Kirschner, 1988; Kirschner et al., 1989; Snaidero et al., 2014). According to most scholars, the intraperiod line measures about four to five nanometers (nm), whereas the main dense line is about three nm wide (Raasakka et

al., 2019; Kister and Kister, 2022). The values calculated by Min et al. (2009) based on the cable theory equation set, align closely with these measurements (Fig. 1A, 1B). Proteolipid protein (PLP) is the most abundant myelin protein in the CNS, accounting for 38% of the total myelin protein mass. PLP is a highly conserved hydrophobic protein composed of 276 amino acids, and contains four transmembrane segments, two disulfide bonds, and covalently binds with lipids (at least six palmitic acid groups in mammals) (Weimbs and Stoffel, 1992). PLP exists in two isoforms: a larger isoform weighing 30 kDa and a shorter isoform (DM20) weighing 26 kDa, which lacks a specific sequence of 35 amino acids (Ruskamo et al., 2022). PLP and DM20 have strong lipophilicity and are expected to contain fatty acid portions connected by several cysteines. PLP interacts with the membrane and has high affinity for CH-rich lipid rafts. PLP and DM20 play important roles in forming and maintaining the axon integrity of myelin periodic lines. One key function of PLP is to bind myelin lamellae together and enhance compaction of myelin lipids, thereby increasing the physical stability of myelin and supporting axon-myelin metabolism (Ruskamo et al., 2022). Therefore, a high level of PLP is required in the myelin to maintain its integrity. A 50% decrease in PLP content can lead to ultrastructural and axonal pathological changes in myelin, including outfolding of myelin, splitting of lamellae, and the formation of axonal spheroids (Luders et al., 2019).

The adhesion function of PLP has been a topic of controversy. In Pelizaeus-Merzbacher patients, a more severe clinical phenotype results from mutated PLP than from an absence of PLP (Stadelmann et al., 2019). Several studies have shown that PLP-deficient mice exhibit moderate phenotypic changes, including slightly reduced or delayed formation of myelin with small-diameter axons, an increased number of cytoplasmic channels in compact myelin, insufficiently compacted myelin, and axonal damage (Ruskamo et al., 2022). The lack of PLP also damages the abundance of cholesterol in the myelin, axonal integrity and motor ability (Luders et al., 2019). However, in mice completely knocked out for PLP, these severe phenotypes have not been confirmed. Instead, the existence of compact myelin sheaths has been demonstrated, which are even more tightly compressed and periodically reduced (Klugmann et al., 1997). Recent studies have found that PLP does indeed have adhesion; however, even without PLP, the extracellular outer surfaces of the myelin layer membrane will stick together. Therefore, it is not the expression of PLP but rather the loss of repulsion between adjacent membrane bilayers that may drive the compaction of myelin at the site of PLP (Bakhti et al., 2013). All these results suggest that while PLP is not necessary for myelin formation, it is necessary for axonal integrity. Furthermore, when PLP is overexpressed in transgenic mice the CNS exhibits poor myelin development and demyelination occurs, and disease severity is correlated with PLP expression levels (Kagawa et al., 1994). It is unclear why abnormal levels of PLP lead to cell death; however, changes in the proportion of myelin membrane components may lead to accumulation of toxic substances inside cells (Stadelmann et al., 2019).

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2.3 Non-compact myelin

When myelin basic protein (MBP) binds to the surface of two adjacent cytoplasmic membranes, it drives the membrane zipper on the cytoplasmic surface of the myelin bilayer. This process causes cytoplasmic solute extrusion and forms a narrow and dense protein phase. To provide directionality during compaction and exclude the formation of isolated un-compacted cytoplasmic pockets in the myelin, there must be a mechanism to counteract MBP-mediated membrane compaction. 2’,3’-cyclic nucleotide 3’-phosphodiesterase (CNP) appears to be a protein that accomplishes this function (Snaidero et al., 2014; Snaidero et al., 2017) (Fig. 1B). CNP is abundant in the CNS, where it makes up 4% of the total protein of the myelin sheath (Gravel et al., 2009). It is located in OL cell bodies and non-compact myelin, i.e., tongue-in, tongue-out protrusions and paranodal loops (Edgar et al., 2009). During development, CNP is highly upregulated in OLs before myelination and remains constant throughout life, playing a crucial role in the formation and lifelong maintenance of myelin (Lee et al., 2005). First, CNP can directly bind to the actin cytoskeleton to form a robust structure that can counteract the adhesion force exerted by MBP molecules (Snaidero et al., 2017). Second, as a dual-function protein, both a phosphodiesterase and a membrane-binding MAP, CNP can act as a linker protein between microtubules and cell membranes to regulate microtubule dynamics (Bifulco et al., 2002). During myelin formation, microtubules

provide transport pathways for vesicles and mbp mRNA (Muller et al., 2013). Studies have also shown that CNP binds to poly (A) (+) mRNA in vivo and inhibits in vitro translation in a dose-dependent manner, possibly regulating mRNA expression in OLs of the CNS (Gravel et al., 2009).

In the absence of CNP, myelin compaction proceeds more rapidly (Snaidero et al., 2014). Moreover, CNP-deficient mice exhibit progressive axonal pathology characterized by axonal swelling and spheroid formation (Lappe-Siefke et al., 2003), with a lower number of cytoplasmic channels within their myelin sheaths (Snaidero et al., 2017). When MBP levels are reduced, cytoplasmic channels are restored in CNP-deficient mice, and the pathology of large-caliber axons is reduced (Snaidero et al., 2017), indicating that MBP has an antagonistic effect on CNP. Similarly, overexpression of CNP occurs in areas lacking compacted myelin (Gravel et al., 1996) and increases the amount of cytoplasm within the myelin. Therefore, CNP plays a crucial role in generating myelin and maintaining axonal physiology.

2.4 Radial components

In the CNS, the adhesion force between adjacent myelin layers converges at the radial component (RC). The normal CNS myelin compaction along the outer surface of the lamella is believed to be maintained by the interaction between PLP molecules in contiguous layers and the binding at the junction of the RC. One of the components of these tight connections is claudin11 (Cldn11), which is an abundant myelin protein. The RC consists of multiple layers, with most RC elements located in the outer and inner tongues of the myelin. RC elements must be interconnected in some way between myelin layers to explain their formation and maintenance in register, even across different myelin sheaths (Rosenbluth et al., 2006). Claudin-11 forms a tight junction barrier that enhances the insulating properties of CNS myelin , but this enhancement is typically significant only for small axons with thin sheaths and is negligible for large axons (Devaux and Gow, 2008) .

Initial research discovered that mice deficient in Cldn11 lacked RCs and exhibited mild tremors, gait abnormalities, motor defects, and electrophysiological abnormalities. These abnormalities included a 50% reduction in conduction velocity in small-diameter axons (Gow et al., 1999). Subsequent studies confirmed this finding. Devaux and Gow (2008) found that electrophysiological analysis of Cldn11-deficient mice showed that the conduction velocity (CV) in small-diameter fibers was severely slowed down. However, the length and thickness of the myelin were normal. These abnormalities were not caused by disruption of myelin structure or distribution of ion channels in axons, but by changes in the biophysical properties of myelin. Specifically, changes in the function of the myelin barrier suggest that the main function of claudin-11 is to form a diffusion barrier rather than to provide mechanical stability. Supporting this idea, Denninger et al. (2015) found that myelin lacking claudin-11 had stronger permeability to water and small permeants while maintaining the integrity of the myelin structure. However, other studies have found that when both claudin-11 and PLP genes are knocked out, mice have severe neurological dysfunction, significantly abnormal myelin compaction, and smaller axon diameters. Interestingly, when either of these genes is knocked out, expression of the other protein increases. These studies suggest that claudin-11 and PLP serve fundamental structural functions in preserving normal compact myelin and there is redundancy in their functions (Chow et al., 2005). Claudin-11 may have more functions in forming barriers, enhancing the insulation performance of CNS myelin, and maintaining the normal physiological characteristics of axons, and relatively less effect on the structure of myelin.

2.5 Gap junctions

Gap junctions (GJs) are channels that directly connect the cytoplasm of coupled cells. They participate in intercellular substance and energy exchange, and information transmission. Their main function is to mediate transmission of chemical signals between cells and electrical signals across synapses in neurons. GJs allow ions (Ca2+, Na+, K+), glutamate (Glu), glutathione, cyclic adenosine monophosphate (cAMP), inositol triphosphate (IP3), ATP and more to pass through (Lapato and Tiwari-Woodruff, 2018). They play an important role in many aspects of cell metabolism including proliferation and differentiation as well as energy transport and maintenance of tissue homeostasis (Fig. 1C). In recent years, GJ intercellular communication (GJIC) channels have

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been widely studied as emerging membrane protein channels. Almost all differentiated cells in vertebrates communicate through GJs, including red blood cells, smooth muscle cells, epithelial cells, and neurons. Various diseases of the CNS such as demyelinating diseases, epilepsy, gliomas, and cerebral ischemia-hypoxia injury have been confirmed to be related to GJIC (Hou Ana et al., 2020). GJ proteins are a multi-gene family-encoded class of membrane proteins consisting of four transmembrane domains connected by two extracellular loops. Six connexin proteins oligomerize to form a hemichannel that allows the diffusion of ions and small molecules through two hemichannels on adjacent cell membranes that form a GJ. In the CNS, OLs express Cx29, Cx32, and Cx47; astrocytes express Cx26, Cx30, and Cx43; microglia express Cx32a, Cx36, and Cx43a; and neurons express Cx30.2, Cx31.1, Cx32, Cx36, Cx40, Cx45 and Cx50 (Lapato and Tiwari-Woodruff, 2018). Communication between glial cells in the CNS is essential for the formation of normal myelin. The absence or disruption of these connections may lead to severe demyelinating diseases and early death.

Cx47 and Cx32 are GJ proteins that have been shown to form functional GJs such as Cx47/Cx47 and Cx47/Cx43, which promote the diffusion of nutrients, ions, secondary messengers, and small molecules between cells. Cx47 plays a critical role in the formation and repair of myelin in the CNS. On the other hand, Cx32 forms mainly Cx32/Cx32 channels and plays a key role in maintaining the structure and function of myelin, related mainly to the degree of demyelination. The GJs between OLs/astrocytes are composed mainly of Cx47/Cx43 and Cx32/Cx30 channels, with Cx47/Cx43 channels located mainly near the OL cell body and Cx32/Cx30 channels located mainly in the outer layer of the myelin (Maglione et al., 2010) (Fig. 1D). Studies have shown that the absence of Cx47 and Cx32 leads to a slowdown in mouse myelin development, while the simultaneous absence of both leads to severe myelin abnormalities and death within four months after birth (Tress et al., 2011). Clinically, mutations in the Cx47 promoter lead to Pelizaeus-Merzbacher-like disease (Gotoh et al., 2014), while changes in Cx32 lead to symptoms very similar to Charcot-Marie-Tooth disease (Sargiannidou et al., 2015) . However, the precise roles of these GJ proteins are still unclear. GJs deserve further in-depth study because they make it possible for cells to metabolically transport, spatially buffer, and electrically couple with each other.

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2.6 Axon-myelin junctions

The connection between the axon and myelin is composed of three main parts: the paranode, juxtaparanode, and internode. During the formation of myelin, glial cells also push axonal proteins to reorganize into specific regions. It is noteworthy that the formation of myelin causes voltage-gated sodium channels to accumulate at the nodes of Ranvier (the gaps between the myelin), which are about 1 μm long and correspond to less than 1% of the myelinated axon area (Fig. 1C-1). In the CNS, node assembly begins with axoglial junctions formed at paranodes, which act as diffusion barriers (Fig. 1C-2). The paranodal junction is the closest and parallel part between the axon and myelin glial cells. As a result of its circumferential wrapping, the outer, uncompacted edge of the glial cell exhibits a series of loops invaginating the axon (Salzer and Zalc, 2016). At paranodes, axonal protein contactin, contactin-associated protein (Caspr), and glial molecule NF155 form a cell adhesion complex. One characteristic of paranodal connections is the regular interval transverse diaphragm emanating from the axon. Paranodal connections act as membrane diffusion barriers, promoting voltage-gated sodium channels to accumulate at nodes and separating them from potassium channels (Kv1.1 and Kv1.2) in juxtaparanodes (Salzer and Zalc, 2016) (Fig. 1C-3).

The loss of paranodal junctions results in the mislocalization of juxtaparanode KV1 channels to paranodal regions and impairs conduction. In addition to their role as a diffusion barrier, paranodal junctions facilitate nerve conduction by enclosing the myelin on the axon, preventing nodal action currents from being shunted below the myelin, while still leaving a narrow channel connecting the internodal periaxonal space and the perinodal space. In juxtaparanodes near the paranodal region, axonal Caspr2, Tag-1 and glial cell Tag-1 form a cell adhesion complex that initiates the assembly of Kv1 channel clusters. However, the interaction between Caspr2/Tag-1 and KV1 channels remains unclear. The special structural domains of these ion channels and cell adhesion molecules are further stabilized by submembrane cytoskeletal complexes in nodes, paranodes, and

juxtaparanodes. For more information on this topic, please refer to Susuki et al. (2016), Fehmi et al. (2018) and Pinatel and Faivre-Sarrailh (2020).

Myelin-associated glycoprotein (MAG) plays a crucial role in internodes. It is produced by myelinating glial cells and is located around the entire circumference of the axonal myelin (Fig. 1C-4). MAG has a high expression level (1%) in the CNS and exists as two isoforms (L-MAG and S-MAG). L-MAG is produced almost exclusively in the CNS and is the major variant during early development and active myelin formation (Campagnoni, 1988; Trapp, 1990). S-MAG is the major subtype in adult CNS and all age groups of PNS (Harry and Toews, 1998). Although MAG has long been considered an axon growth inhibitor along with nogo and OMgp (McKerracher and Rosen, 2015), its effect on axon growth is bidirectional; it promotes axon growth in young neurons and inhibits growth in older neurons, which is an age- and neuron-type-dependent switch. MAG can regulate axonal stability and integrity and protect axons under pathological conditions. Deletion of MAG leads to accelerated axonal loss in experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis). This suggests that MAG has different roles in axonal responses to injury and disease: in addition to its well-known role in growth inhibition, MAG can also promote axon growth and/or protect axons from further degeneration in the adult CNS (Geoffroy and Zheng, 2014). However, further research is needed on the role of MAG and its receptors in myelin development.

3 Formation and regeneration of myelin

3.1 Maturation of oligodendrocytes

OLs are the sole myelin-forming cells in the CNS and are a major type of glial cell in the brain. They play a crucial role in promoting the propagation of action potentials and supporting the integrity of neurons and axons while providing metabolic and nutritional support for axons. OLs are believed to be derived from OPCs, which differentiate into pre-OLs before finally maturing into myelin Ols (Fig. 2).

OPCs are generated in the ventral forebrain where three distinct groups of OPCs have been identified. These groups are generated in a continuous wave-like pattern with a clear spatiotemporal pattern and migrate from their birthplace to settle in the brain. Although they have different origins, all OPC cell lineages converge at the transcriptional level, which may reflect their contribution to myelin formation. During migration, some neural cells promote transient cell-cell interactions that allow them to play a greater role after integration into the cortical network (Lepiemme et al., 2022). OPCs constantly extend and retract during migration and settlement into their final positions. These dynamic and exploratory processes are repulsive to other OPCs, thus determining the uniform density and spacing of these cells (Hughes et al., 2013). After OPCs enter their designated area, some remain in a precursor state while others differentiate into OLs that form myelin.

In rodents, OPCs proliferate and migrate mainly in the postnatal stage, around the second day after birth. Late-stage OPCs account for most of the OL lineage cells, with a small number of immature OLs also present. Mature OLs appear on the seventh day after birth and initiate CNS myelination. Myelination in most brain regions is almost complete by around 60 days after birth (Snaidero et al., 2014). In humans, OPCs are first observed in the forebrain at around 10 weeks of gestation. Although OPCs and late-stage OPCs remain the main cell types during this stage, immature OLs are observed between 18 and 28 weeks. Therefore, the OL lineage develops between 18-28 weeks, similar to rodents at postnatal day 2. In human development, myelin basic protein (MBP)-positive OLs are initially observed at around 28 weeks, similar to rodents on postnatal day 7 (Barateiro et al., 2016).

Mature OPCs undergo different stages of morphological differentiation and express many cell-specific marker proteins and lipids. Although some molecules have opposing functions in OPC proliferation and OL differentiation, the physiological processes they control are not opposite. Transient expression of signaling molecules that drive OPC proliferation and prevent OL differentiation or maturation is necessary for early OPC content control in the CNS. Continuous transient expression of signaling molecules that drive OL differentiation

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or maturation and prevent OPC proliferation is necessary for myelin formation or remyelination (Wang et al., 2022). The balance between OPC proliferation, OL differentiation, and maturation is delicate and complex. Once myelination begins, immature OLs must be properly positioned because mature OLs appear to have little ability to migrate to new locations or delay myelin synthesis. Once myelination begins, signaling pathways dependent on PtdIns (3,4,5)P3/Akt/mTor and ERK1/2 are critical for driving myelination until mature myelin with optimal g-ratios is produced (Simons and Nave, 2015). For more detailed information on the growth, development, and migration of OPCs, please refer to Kang and Yao (2022).

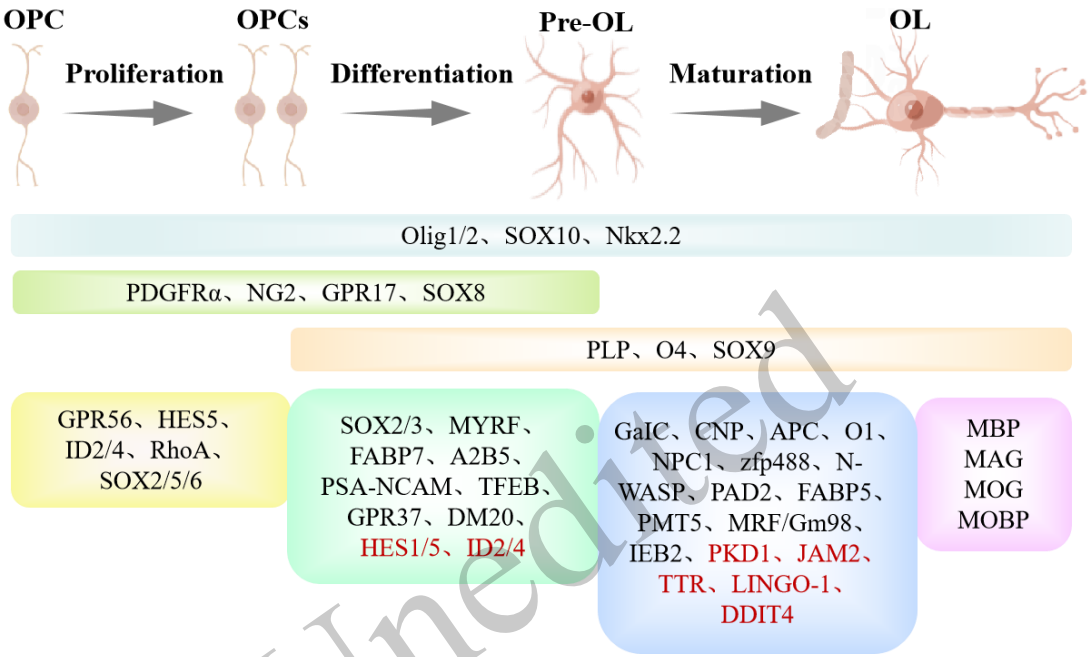

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Fig. 2 The main markers of different stages of oligodendrocyte development (proliferation, differentiation, and maturation). For details, please refer to Simons and Nave (2015), Kuhn et al. (2019), Kang and Yao (2022) and Wang et al. (2022). Genes marked in black are markers of OPC proliferation, OL differentiation and maturation, while those marked in red are inhibitors of OL differentiation or maturation.

3.2 Recognition and adhesion of axons

During the initial period of myelination, the mechanism by which OLs select axons and their subdomains for myelination remains elusive (Fig. 3). A recent study has demonstrated that myelin formation can be initiated by the axon diameter alone without any axonal signals (Mayoral et al., 2018). Another study has shown that OLs can wrap around artificial glass or polymer matrices and sense the diameter of microfibers, thereby increasing the length of the sheath as the fiber grows (Lee et al., 2012; Bechler et al., 2015). These findings suggest that physical properties such as axon diameter play a crucial role in initial axon selection. However, some axons in the brain between 0.2 and 0.8 μm have myelin while others do not (Waxman and Bennett, 1972), indicating that axon diameter is not the sole determinant of myelin formation. Recent studies have found that the blocking of action potentials in zebrafish spinal cords leads to incorrect axon targeting and insufficient myelination (Hines et al., 2015). In addition, OLs can myelinate axons (Rosenberg et al., 2008) fixed with paraformaldehyde (PFA) and nanofibers (Lee et al., 2013) in the absence of neural activity. Furthermore, some scholars have found that OLs on the axons in rodents extend or retract according to permissive or repulsive cues, respectively (Thomason et al., 2020). These findings suggest that correct axon targeting is likely to depend on multiple cues, including axon diameter, neural activity, and permissive or repulsive cues on OLs. However, which factors specifically determine axon selection and how they relate to each other are still unknown.

3.3 The growth of myelin and axon wrapping

Upon finding the target axon, OLs undergo membrane polarization which leads to the accumulation of myelin-specific proteins and lipids in the myelin (Fig. 3). During development, the myelin membranes of OLs can grow up to 5,000 μm^2 per day (Pfeiffer et al., 1993). A single OL can myelinate up to 50 axons (de Faria et al., 2021; Pajevic et al., 2023) while mature myelin consists of up to 160 membrane layers (Ruskamo et al., 2022). Several models were initially proposed for the wrapping of myelin such as the "croissant-like" model (Sobottka et al., 2011) and the "yo-yo" model (Pedraza et al., 2009). However, with the advent of three-dimensional electron microscopy techniques, high-pressure freezing techniques for fixed tissues, live-imaging combined with electron microscopy techniques, and combining 3D reconstruction by electron microscopy with live-imaging techniques, new models have been proposed for the wrapping of myelin (Kerman et al., 2015). The innermost layer of the myelin is the shortest and extends laterally while the top of the myelin gradually increases with each wrapping. This means that myelin grows through two different but coordinated movements: the leading edge at the inner tongue wraps around the axon (i.e., beneath previously deposited membranes) and extends laterally toward the node. Thus, each myelin layer remains in close contact with the axonal surface and moves forward in a continuous spiral manner toward future nodes where they align and position as paranodal loops. Cytoplasmic channels are present in compact myelin sheath to provide short connections between outer and inner tongues that allow newly synthesized membrane components to be transported from cell bodies to inner tongues. Most of these channels close with ongoing development but residual cytoplasmic channels persist throughout the mature compact myelin sheath (Snaidero et al., 2014; Edgar et al., 2021). The cytoplasmic channels appear to be dynamic and have the potential to reopen after the developmental program is terminated (Snaidero et al., 2014).

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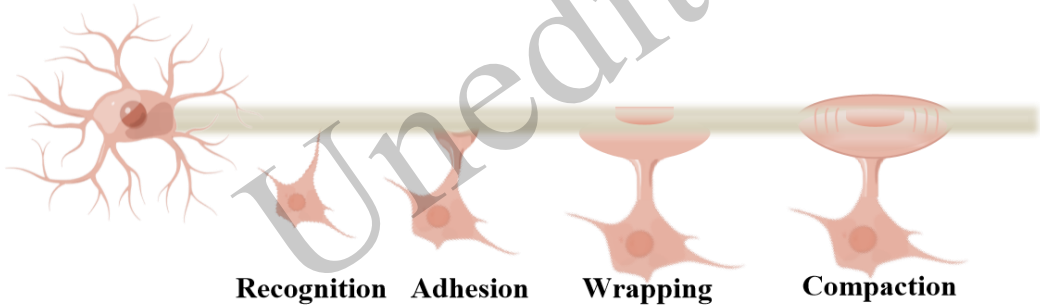

Fig. 3 Schematic of CNS myelination.

3.4 Myelin compaction

Myelin compaction typically occurs after a period of wrapping and simultaneously with wrapping. Although the specific mechanism regulating compaction initiation and corresponding sites is not yet clear, it is clear that compaction on the cytoplasmic surface starts from the outermost layer of myelin phospholipids and progresses inward. During initial formation, myelin is rich in negatively charged phospholipids (PIP2, PIP3). MBP has a high affinity for negatively charged phospholipids, thereby pulling two bilayers together to play a “zipper” role (Snaidero et al., 2014). However, MBP is synthesized in the distal process (i.e., closest to the axon) (Laursen et al., 2011), while myelin compaction occurs at the proximal end. This phenomenon is similar to PLP because PLP is synthesized on ribosomes in the endoplasmic reticulum and then transported to the myelin membrane through a series of steps (Kippert et al., 2007). One possible explanation is that compaction starts from the site where MBP mRNA is translated locally and then moves inward after binding with other proteins (Laursen et al., 2011). The inner tongue may contain necessary substances produced by MBP, but it cannot provide conditions for its compaction because it needs further expansion and extension. This also explains why

compaction always starts after several layers of wrapping. To maintain this rate of membrane compaction and exclude the formation of unconsolidated areas, CNP plays an important role. Unconsolidated areas are rich in CNP; therefore, unconsolidated wrapping decreases in the absence of CNP (Snaidero et al., 2014). CNP may have additional functions as myelin sheaths lose some cytoplasmic channels when it is absent (Snaidero et al., 2014), and the amount of cytoplasm in the myelin increases when it is overexpressed (Gravel et al., 1996; Snaidero et al., 2017). This also means that during the growth of the myelin, the rapid extension of the innermost part of the myelin and the accumulation of CNP between these newly synthesized layers prevent MBP from being compressed prematurely at the front (Snaidero et al., 2014). Under this mechanism, MBP synthesis sites may occur in the innermost tongue, and then MBP spreads to the outer layer where the lamellae are closer together and begins to compact (Stadelmann et al., 2019).

3.5 Node formation

During the lateral extension of myelin sheaths, a special structure called a Ranvier node is formed when two myelin sheaths meet. Myelin phospholipids are electrical insulators. When the membrane at the node is excited, the low capacitance of the sheath causes the axonal potential to “jump” from one node of Ranvier to the next, a process known as saltatory conduction. Due to the low capacitance of the sheath, little energy is required for the remaining membrane between depolarized nodes, which leads to the transmission of action potentials (Seidl, 2014). The propagation of action potentials depends on rapid depolarization and repolarization of the axolemma; these actions are performed by Na+ and K+ channels that aggregate at nodes. Na+ channels act as pore-forming protein subunits that regulate ion flux across membranes and together with K+ channels regulate neuronal excitability. In addition to ion channels, Ranvier nodes are rich in cytoskeletal and scaffolding proteins such as ankyrin G (ankG) and βIV spectrin, which together link Na+ and K+ channels to underlying cytoskeletons. These scaffolds also connect ion channels to a diverse and rich extracellular matrix through CAM (neural cell adhesion molecule NF186) and NrCAM’s 186 kDa subtype (Rasband and Peles, 2015). However, there is still no consensus on the specific assembly process of Ranvier nodes in the CNS. Three potential mechanisms have been proposed: (1) aggregation of NF186 by ECM derived from glial cells; (2) restriction of nodal protein mobility through paranodal axoglial barriers; (3) stabilization of Na channels through axonal CS. For more information, please refer to Susuki et al. (2013).

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4 Another driving force for myelin extension - myelin compaction

However, the molecular mechanism that drives lateral extension of the inner tongue is not well understood. One hypothesis is that actin polymerization at the leading edge drives the process of wrapping, whereas actin depolymerization reduces surface tension, thereby promoting membrane diffusion (Nawaz et al., 2015). Actin polymerization provides power for OL process extension in an Arp2/3-dependent manner while actin depolymerization drives myelin phospholipid wrapping (Zuchero et al., 2015).

In addition to the aforementioned possibilities, we propose a novel hypothesis that myelin compaction provides the driving force for the expansion of myelin. To illustrate this concept, consider filling an elastic plastic container with water and applying pressure to one side. The water will be forced to spread in the opposite direction due to pressure, leading to the expansion of the container. The lateral expansion of myelin is similar. Due to the "zipper" effect of MBP, excess cytoplasm and membrane-binding proteins are restricted from entering the dense myelin layer and therefore are squeezed into the uncompacted area (Raasakka et al., 2017). Due to the increase in uncompacted area content, the myelin membrane is forced to expand and extend in other directions (Fig. 4). That is, the force generated by the polymerization of MBP is used to propel the myelin membrane forward, similar to how actin polymerization drives the leading edge in migratory cells (Bakhti et al., 2014).

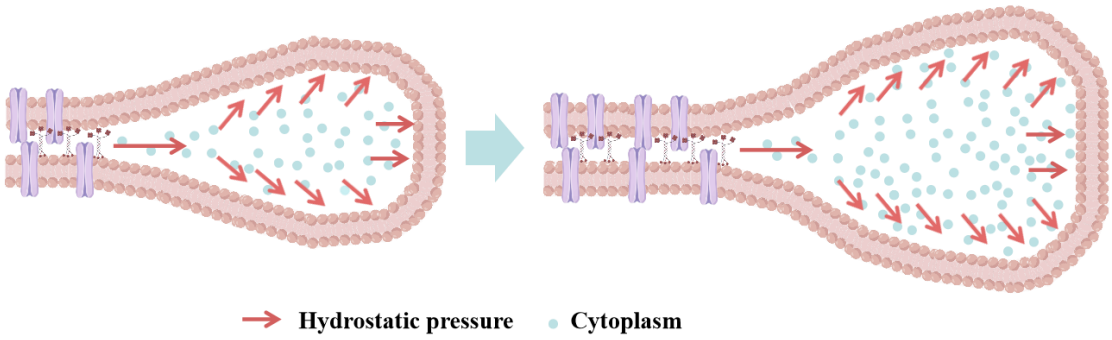

Fig. 4 The force generated by the polymerization of MBP is used to propel the myelin membrane forward.

The high lateral mobility of myelin lipids makes this hypothesis feasible. Strictly speaking, it reduces the adhesiveness of the inner tongue as it crawls under the formed myelin. Notably, myelin has a very high lipid content when compared to other plasma membranes, accounting for 70-75% of its dry weight, and an unusual lipid composition with a molar ratio of about 2:2:1:1 for cholesterol, phospholipids, galactolipids, and plasmalogens, respectively (Norton and Poduslo, 1973). Ceramide galactosyltransferase deficient mice do not synthesize galactocerebroside and sulfatide, but microscopic and morphometric analyses revealed slightly thinner sheaths in the ventral region of the spinal cord although the myelin had a normal ultrastructural appearance (Coetzee et al., 1996). However, cholesterol seems to be a rate limiting factor in myelin formation, as demonstrated in mutated mice and zebrafish lacking an essential enzyme for cholesterol biosynthesis (Saher et al., 2005; Mathews et al., 2014). Squalene synthase activity is the first step in synthesizing cholesterol (a major lipid component essential for myelin fluidity). OL specific deletion of squalene synthase results in a significant decrease in myelin formation rate (Saher et al., 2005).

Furthermore, as mentioned above, shiverer mice, which lack MBP expression, form less myelin and less stable myelin than wild-type mice. The myelin formed by shiverer mice consists of loosely compacted layers and is at most four layers thick in early development (Roach et al., 1985; Snaidero et al., 2014). Although some basic structural elements of the myelin remain, such as radial components and axon-glial connections, most of the axonal surface is completely devoid of myelin (Rosenbluth, 1980). This is very different from the 160 layers found in normal conditions. This implies that the absence of MBP does not affect the structural integrity of the myelin but rather results in a loss of extension power, leading to a decrease in the number of layers. Strangely, on day 21, there was no statistically significant difference in the amount of myelinated axons with noncompacted layers between wild-type and shiverer heterozygote (Shiv ±) mice optic nerves, although Shiv ± mice had more (Snaidero et al., 2014). This indicates that MBP regulates the speed of compaction in early development, while in the later stages, the speed may be driven by hydrostatic pressure built up by MBP (Stadelmann et al., 2019).

5 Conclusions

Despite advancements in modern technology, our understanding of myelin development in the CNS remains limited. In the human CNS, myelin proteomics has revealed the presence of over 800 proteins (Gargareta et al., 2022), whereas in zebrafish, a significantly higher number (over 1000) have been identified (Siems et al., 2021), but current research is focused mainly on high-content proteins. There has been little research on trace proteins, and their role in myelin formation, growth, functional maintenance, and repair remains unknown. Determining whether these trace proteins play a decisive role in myelin formation and growth poses new challenges for future research. In addition to its own structure and function, the mechanism of myelin injury remains to be further studied. Neuroinflammation has been shown to cause changes in myelin. Oligodendrocytes, astrocytes, and microglia are the main glial cells in the CNS. The crosstalk between these cells and their

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effect on the formation and destruction of myelin is still unknown. Although we still face important challenges, as the mechanism of myelin formation becomes clearer, the role of myelin in CNS diseases and even mental disorders may be revealed. This will help the development of new treatments for related diseases.

Acknowledgments

This research was funded by National Natural Science Foundation of China (No. U21A20400) and natural science foundation of Beijing (No. 8217153264) and Key project of Beijing University of Chinese Medicine (No. 2022-JYB-JBZR-004).

Author contributions

Writing—original draft preparation: QS and SM. Writing—article revision: QG, TX. Writing—review and editing: FF, and XQ. Drawing—editing of picture: YY and ZJ. All authors have read and agreed to the published version of the manuscript.

Compliance with ethics guidelines

Qi Shao, Simin Chen, Tian Xu, Yuyu Shi, Zijin Sun, Qingguo Wang, Xueqian Wang, and Fafeng Cheng declare that they have no conflict of interest, and all followed out policy concerning Informed Consent as shown on Springer.com.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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