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CELL SCIENCE AT A GLANCE

Myelination at a glance

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ABSTRACT

The myelin sheath is a plasma membrane extension that is laid down in regularly spaced segments along axons of the nervous system. This process involves extensive changes in oligodendrocyte cell shape and membrane architecture. In this Cell Science at a Glance article and accompanying poster, we provide a model of how myelin of the central nervous system is wrapped around axons to form a tightly compacted, multilayered membrane structure. This model may not only explain how myelin is generated during brain development, but could also help us to understand myelin remodeling in adult life, which might serve as a form of plasticity for the fine-tuning of neuronal networks.

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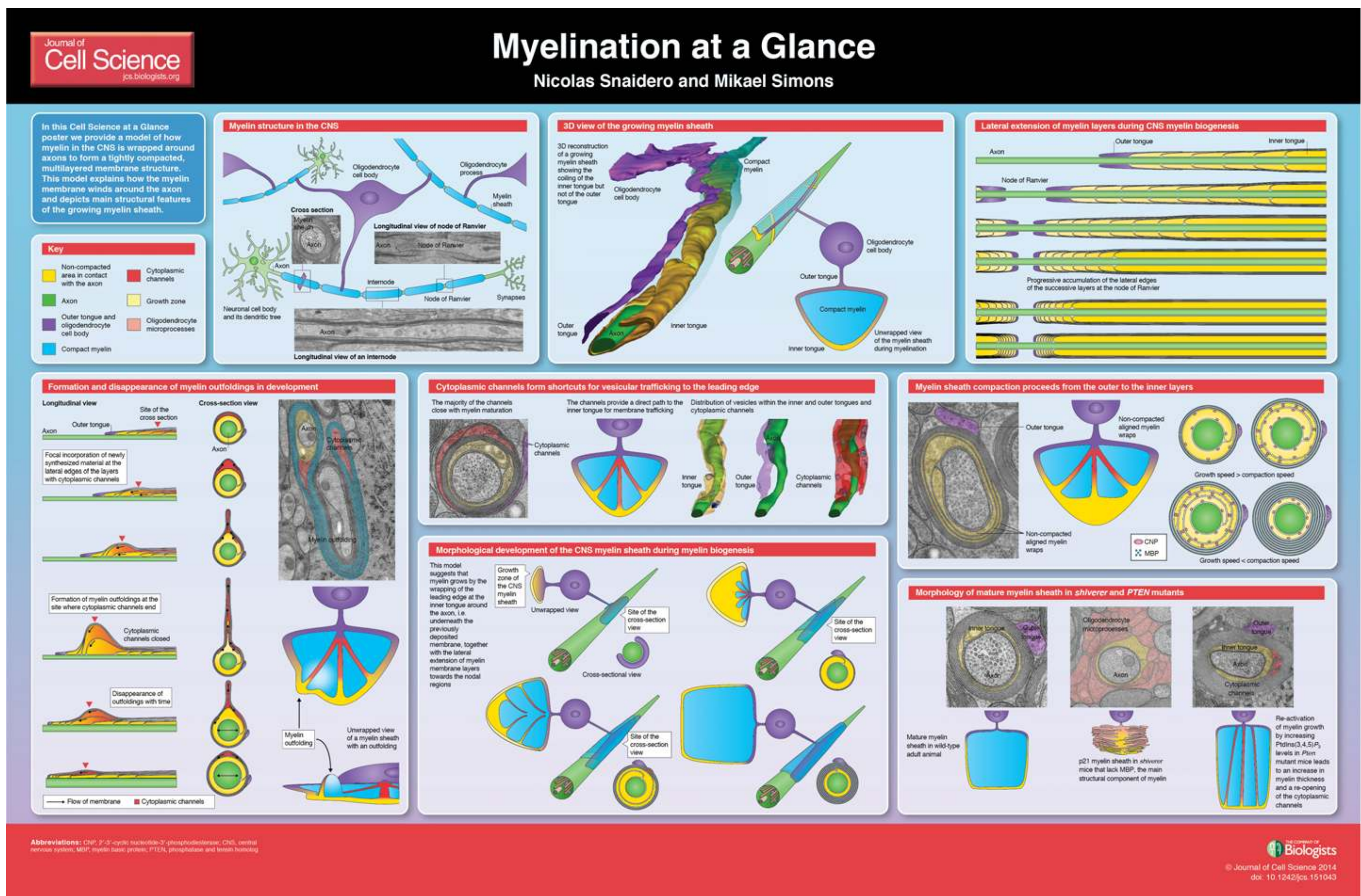
Introduction

With the growing complexity of the nervous system, there is a relative increase in the white matter of vertebrates. In humans, around 40% of the brain contains white matter comprising densely packed fibres, of which myelin is a main component (50–60% dry weight of the white matter) (Morell and Norton, 1980). Considering the limited amount of space available in the human skull, it is clear that myelin, occupying ~20% of it, must be of vital importance. The fundamental differences between unmyelinated and myelinated axons are best illustrated when comparing their performances. To conduct with a speed of 25 m/sec, an unmyelinated giant squid axon must have a diameter of ~500 μm, whereas a myelinated mammalian axon with a diameter of only few μm can conduct with the same speed using 5000 times less energy (Ritchie, 1982).

The term 'myelin' was first coined by Rudolf Virchow in 1864, and was named after the Greek word 'marrow' (myelos), because it



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is particularly abundant in the core, or marrow, of the brain. He assumed that myelin was secreted by neurons, but almost a century later the improved histological staining procedures by Pio del Rio-Hortega revealed that myelin is formed by oligodendrocytes. In contrast to the peripheral nervous system (PNS), where Schwann cells establish a one-to-one connection with the axon, the challenge of studying the central nervous system (CNS) was to indentify the thin branches of the oligodendrocytes that connect the cell body with the myelin sheath. Depending on the brain region, the number of these processes differs dramatically. Whereas cells in the cortex and corpus callosum can myelinate up to 80 internodes (myelin segments) on different small-diameter axons, many oligodendrocytes that myelinate larger calibre axons have fewer processes but longer internodes and thicker myelin sheaths (Murray and Blakemore, 1980; Matthews and Duncan, 1971), (Hildebrand et al., 1993). For example, some oligodendrocytes in the spinal cord generate myelin only around one single large axon, with up to 150 lamellae (or layers) and with an internode length of 1500 μm (Remahl and Hildebrand, 1990), whereas cells in the corpus callosum and cortex form between 30 and 80 internodes ranging from 20 to 200 μm in length with up to 60 different lamellae (Matthews and Duncan, 1971; Chong et al., 2012). There is almost a linear relationship between axon diameter, number of lamellae and internodal length; for example, with the increase in the diameter of the axon from 1 to 15 μm, the length of the internode rises from 100 to 1500 μm (Murray and Blakemore, 1980; Hildebrand and Hahn, 1978). The estimated surface area of myelin formed by one oligodendrocyte reaches 20×10^5 μm^2, which makes these cells the most powerful membrane producers in our body (Pfeiffer et al., 1993). Strikingly, recent live-imaging analysis of myelination in zebrafish has shown that its biogenesis occurs at a much faster rate than previously estimated. Oligodendrocytes (at least in zebrafish) make new myelin sheaths during a period of just five hours (Czopka et al., 2013). Thus, previous estimates of the amount of myelin produced by oligodendrocytes during the active phase of myelination (~5000 μm^2 surface area per day and 10^5 molecules per minute) (Pfeiffer et al., 1993) have to be corrected by almost two orders of magnitude.

Myelination occurs relatively late in development in a defined temporal sequence. In mice, it starts at birth in the spinal cord and is almost completed at postnatal day 60 (P60) in most brain regions (Baumann and Pham-Dinh, 2001). In humans, the peak of myelination occurs during the first year of life, but continues into young adulthood, especially in some cortical areas of the brain (Fields, 2008). Within a specific region, the largest axons are always the first to acquire myelin. For example, myelination starts at P1 in the thickest fibre tracts of the cuneate fasciculus within the mouse spinal cord, whereas the smallest axons become myelinated after P20 (Hildebrand et al., 1993).

In the PNS, only axons with a diameter of 1 μm or more are myelinated, but there is no strict size relationship for myelination in the CNS. Oligodendrocytes are able to myelinate axons of a diameter larger than 0.2 μm, but between diameters of 0.2 μm and 0.8 μm, both myelinated and unmyelinated axons are found (Remahl and Hildebrand, 1982; Waxman and Bennett, 1972). Thus, it appears that size cannot be the sole criteria to explain how oligodendrocytes select the axons. However, when oligodendrocytes are cultured together with axon-mimicking inert polystyrene fibres of different diameters, there is a size-dependent ensheathment of fibres with a diameter of 0.4 μm or more (Lee et al., 2012). Thus, at least in the diameter range of 0.2 μm to 0.8 μm, repulsive and/or instructive factors on axons must operate *in vivo* in order to control myelination.

Myelin plasticity and remodeling

Because the decision of whether an axon becomes covered with myelin has dramatic functional consequences for how neurons transmit their signals, myelination is likely to have a role in modulating network activity in the brain (Fields, 2008). Indeed, there is new evidence that myelin is dynamically regulated by experience both during development and in adult life (Makinodan et al., 2012; Liu et al., 2012; Mangin et al., 2012). It appears that the extent of myelin sheath formation may serve as a form of plasticity to adapt brain function to environmental stimuli. It is also possible that more subtle structural changes in myelin, for example, variations in myelin thickness or internodal length, participates in the timing of conduction velocity in neurons. The ratio of the inner axonal diameter to the total outer diameter (g-ratio) is used as a structural index of optimal axonal myelination. Theoretical considerations have shown that axons have an optimal g-ratio of 0.6 (Waxman and Bennett, 1972; Chomiak and Hu, 2009). If myelin thickness deviates from this value – with higher or lower g-ratios – conduction velocity drops. Similar considerations apply for the internodal length (Waxman, 1997; Wu et al., 2012). During the course of evolution, most myelinated fibres have developed sheaths with radial and longitudinal dimensions close to their calculated optimum for maximal conduction speed. However, in many areas of the brain there is a need for neurons not only to conduct as quickly as possible, but also synchronise conduction velocities. For example, if axons of different lengths have to discharge synchronously, differences in myelin thickness and/or internodal length might help to couple the activity of these neurons to each other.

Myelination has been thought to occur relatively stereotypically, according to a predefined genetic program, strictly as a developmental process (Baumann and Pham-Dinh, 2001). However, it now appears that myelin biogenesis contributes to brain plasticity being modifiable by experience and various environmental factors (Fields, 2008). Furthermore, myelination is not limited to early development but occurs throughout adulthood. Remarkably, a recent study conducted in W. D. Richardson's laboratory demonstrated that there is a significant fraction of adult-born oligodendrocytes that are actively engaged in forming myelin sheaths (Young et al., 2013). In fact, virtually all oligodendrocyte precursor cells continue to divide in adult mice with a cell-cycle time of ~20–40 days (Young et al., 2013; Simon et al., 2011; Kang et al., 2010). A significant number (~30–40%) survives long term, differentiating into mature oligodendrocytes and forming new myelin sheaths. However, these cells form myelin with slightly different properties. Adult-born oligodendrocytes generate a higher number of internodes, but with much shorter intermodal length. For example, oligodendrocytes that occur between P30 and P60 make on average ~21 internodes with a length of ~76 μm, whereas cells that occur after P120 generate ~77 internodes with a mean length of 22 μm. Thus, myelination should not be regarded as a process strictly restricted to development anymore but as an activity that reshapes the CNS into old age.

To integrate the concept of myelin plasticity into the fine-tuning of neuronal networks, we have to understand how oligodendrocytes form myelin, how they select axons for myelination, and how they regulate myelin thickness and intermodal length.

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Wrapping

The now-classic work of Betty Ben Geren, using electron microscopy to examine the peripheral nervous system in chicken, revealed that myelin is not axon derived but, rather, a continuous membranous extension of Schwann cells in the PNS (Ben Geren, 1954). To determine how the myelin spiralling occurs, Bunge and colleagues followed the motion of the Schwann cell nucleus during myelination (Bunge et al., 1989). If the outer myelin tongue (or edge) moves around the axon, the Schwann cell body would need to follow this movement. However, as the nuclear motion did not correlate with the wrapping process, the authors concluded that there must be an active progression of the inner lip of the myelin membrane, which continuously moves underneath the growing sheath. This 'jelly roll' (also known as 'carpet crawler') model of spiral wrapping is generally accepted for the PNS.

Analysis of myelin wrapping in the CNS was lagging behind owing to the difficulties in accessing and fixing the tissue, and the oligodendroglial architecture with its relatively thin processes connecting the cell body with the myelin sheath. However, with progress of sample preparation and fixation conditions, it was possible to recognise glial cytoplasm in the inner and outer tongues of myelin connected to the oligodendroglial processes (Maturana, 1960; Peters, 1960a; Peters, 1960b). Today – by using high-pressure freezing followed by freeze substitution, which results in preservation of tissue architecture close to its native state – the myelin sheath and its cytoplasmic-rich areas appear much better preserved, suggesting that some structures collapse or are less recognisable when chemical fixation is used (Möbius et al., 2010).

Several models of CNS myelin biogenesis have been proposed. Some of them deviate substantially from the jelly roll model of the PNS. According to one early theory, CNS myelin forms by coalescence of intracytoplasmic membranes (De Robertis et al., 1958), whereas others have suggested that myelin is formed by the fusion of many different glial processes from one or different oligodendrocytes (Luse, 1956). This patchwork model of myelin biogenesis was recently re-adopted and modified (Ioannidou et al., 2012). Using time-lapse light microscopy, the authors observed that – in the early stages of myelination – there were gaps between oligodendroglia processes that appeared to be filled in later.

Once this first initial ensheathing contact with the axon is made, it is generally accepted that the myelin membrane advances in a spiralling motion. However, the mode of progression has been a matter of debate (Bauer et al., 2009). According to the jelly roll model, the glial membrane extends along the entire axonal segment (the future internode) before it makes one turn and moves underneath the growing sheet (Bunge et al., 1961). Subsequently, the inner tongue continuously moves underneath the previously generated layers of myelin membrane, much like in the PNS. However, in the CNS, the number of myelin layers can vary along the length of an intermodal region and, as visualised by light microscopy, a coil with an average periodicity of 5.7 to 7 μm appears along the internodal dimension (Pedraza et al., 2009; Sobottka et al., 2011; Butt and Berry, 2000). This has led to the suggestion that myelin thickening is achieved by the addition of new layers on top of the inner ones in a 'croissant-like' manner (Sobottka et al., 2011), or that myelin twists as a coil across the axon in a corkscrew motion ('yo-yo' or 'serpent' model) (Pedraza et al., 2009). In the latter model, myelin growth begins with a single glial process that, after making axonal contact, spirally encircles the future internode. Once the appropriate number of turns has been generated, the individual membrane layers grow laterally and glide over each other. This model is not based on the extension of one inner tongue, but on lateral growth of several layers of membrane.

The application of new technologies, such as serial block face imaging by focused ion beam milling coupled to scanning electron microscopy (FIB-SEM), together with cryopreparation methods, such as high-pressure freezing, has made it possible to follow myelin formation during its development in a large volume close to its native state (Snaidero et al., 2014). This analysis reveals that myelin is not a serpent of overlapping membrane sheets, but appears as a single flat extension of membrane of a triangular shape with the outermost layer in direct contact with the cell body and the innermost layer with the shortest lateral width in contact with the axon. This model suggests that myelin grows by the wrapping of the leading edge at the inner tongue around the axon, i.e. underneath the previously deposited membrane, together with the lateral extension of myelin membrane layers towards the nodal regions. The lateral cytoplasmic-rich membranous pockets of each myelin layer are always in close contact with the axonal surface, giving rise to the coiling helical pattern previously described (Pedraza et al., 2009; Sobottka et al., 2011; Butt and Berry, 2000). These cytoplasmic-rich lateral edges move towards the future node where they align and position as paranodal loops. During this lateral extension, the glial membrane appears to be attached to the axon by the formation of the axoglial adhesion complex consisting of contactin-1 (CNTN1) and the contactin-associated protein (CNTNAP1) on the axon, and the 155 kDa isoform of neurofascin (NFASC) on the glial, paranodal loops (Pedraza et al., 2009; Zonta et al., 2008). Interestingly, in mice that lack neurofascin, caspr or contactin-1 the lateral migration of the myelin layers is retarded (Zonta et al., 2008; Susuki et al., 2013; Çolakoğlu et al., 2014). Thus, the axoglial adhesion complex is not only important for node formation, but also to some extent for promoting the lateral extension of myelin layers towards the node.

Myelinic channels and outfoldings

If the advancing innermost tongue is responsible for radial growth of myelin, the newly synthesised membrane has to be transported all the way through the developing myelin sheath. There appears to be an elaborate system of cytoplasmic-rich (myelinic) channels within compacted myelin that provide a helical path for the transport of membrane to the growth zone (Velumian et al., 2011; Nave, 2010). These myelinic channels, reminiscent of Schmidt-Lanterman incisures (myelin incisures of PNS myelin), contain microtubules and vesicular carriers in order to deliver membrane from the biosynthetic pathway to the leading edge at the inner tongue. Since these channels tend to shrink and collapse in chemically fixed and dehydrated tissue, they have been difficult to detect. Another reason for being overlooked is that they are mainly found in developing myelin sheaths but largely disappear when myelination is completed (Snaidero et al., 2014).

For the lateral growth of the myelin layers, which is responsible for the longitudinal extension of the internode, membrane needs to be transported to the lateral cytoplasmic-rich membranous pockets of each myelin layer (the future paranodal loops). Thus, the trafficking pathways of membrane required for radial and longitudinal growth are spatially separated within the growing myelin sheath. Whereas a large fraction of the myelinic channels, which are a prerequisite for radial growth, close after myelination is terminated, the paranodal loops remain open and connected to the biosynthetic transport routes of the oligodendrocyte. Hence, at later stages of development, e.g. to adjust internodal length to the

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further elongation of the axon during the growth of the organ, they may represent preferential delivery sites for membrane needed for the longitudinal extension of the myelin sheath.

By using three-dimensional electron microscopy reconstructions of developing myelin sheaths it was possible to show that some myelinic channels end within myelin outfoldings (Snaidero et al., 2014). As these myelin outfoldings are frequently associated with different myelin diseases including dysmyelinating neuropathies (Pereira et al. 2012; Bolino et al., 2004), they have been mainly regarded as pathological features. However, early in development myelin outfoldings appear in almost every growing myelin sheath, suggesting that they are a physiological structure and part of normal myelin development. They seem to form by the preferential delivery of membrane through myelinic channels into the inner tongue, resulting in focal accumulation of excess membrane that extends outwards. When myelinic channels close, these outfoldings might slowly spread out owing to the fluidity of the membrane and the absence of fully formed radial components that stabilise the myelin sheath in the adult. It is possible that the outfoldings serve as membrane reservoirs to allow the completion of the lateral growth of the layers and secure unperturbed radial growth of the myelinated axon itself, which would explain their gradual disappearance later in life. This model does not imply that all myelin membrane components are inserted at the leading edge of the growing myelin sheath. It is possible that a fraction of molecules, in particular lipids, are also added at the outer layers of the emerging sheath before spreading inwards (Gould, 1977; Gould and Dawson, 1976). Myelin should, therefore, not be regarded as a rigid structure but, rather, as a fluid in which lipids and small proteins diffuse freely, enabling the dynamic and plastic design of the developing myelin sheath.

Compaction

The compaction of the cytoplasmic leaflets of the myelin bilayer is achieved by myelin basic protein (MBP) and starts early in development after only a few wraps (Readhead et al., 1987). Whereas membrane growth occurs close to the axon at the innermost region of the myelin sheath, compaction starts in the outermost layers and progresses inward. The spatial segregation and the coordinated regulation of myelin growth and compaction are important to prevent premature compaction of the growing zone. It is not clear how the initial nucleation sites for membrane compaction are set. One possibility is that compaction starts at the site of local *MBP* mRNA translation. However, this is unlikely because MBP has been proposed to be synthesised in the innermost layers close to the axon (Ainger et al., 1997; Colman et al., 1982; Trapp et al., 1987; Wake et al., 2011; Laursen et al., 2011; White et al., 2008). Another explanation is that compaction is simply not fast enough to keep up with the rapid extension of the innermost tongue during myelin growth and, therefore, lags behind.

To provide directionality in the compaction process and exclude the formation of uncompacted pockets in the myelin sheath, it is crucial to limit compaction to a single site within the myelin sheath. This is most easily achieved by making the nucleation step rate limiting, similar to the formation of amyloid fibrils. One way to impede nucleation is to deposit a spacer into the growing myelin sheath that keeps the inner leaflets of two myelin layers apart; 2′-3′-cyclic nucleotide-3′-phosphodiesterase (CNP) appears to be one protein that fulfils this function (Snaidero et al., 2014).

In the absence of CNP, myelin compaction proceeds faster and extends to the inmost layers of the myelin sheath (Snaidero et al., 2014), whereas overexpression of CNP occurs in areas that lack myelin compaction (Gravel et al., 1996; Yin et al., 1997). Thus, it is possible that MBP is synthesised within the innermost tongue – regulated in part by axonal signals (Wake et al., 2011) – followed by its diffusion backwards towards the outer layers when compaction is initiated. Once MBP is bound to the two adjacent cytoplasmic surfaces of the myelin bilayer, it rapidly polymerises into a fibrous network that provides the force and the basis for unidirectional membrane compaction (Aggarwal et al., 2011; Aggarwal et al., 2013).

Whereas the cytoplasmic leaflets are held tightly together by MBP, the extracellular leaflets of two adjacent leaflets are attached by much weaker interactions (Bakhti et al., 2013). One reason why oligodendrocytes use weak forces for the alignment of extracellular membrane surfaces is the way myelin is generated and wrapped (Bakhti et al., 2013). Because the inner tongue needs to wind multiple times around the axon, the newly synthesised membrane bilayers have to glide along each other to prevent constriction of the axon. This slippage of myelin layers (Hirano and Dembitzer, 1967) requires dynamic and weak connections at the extracellular site of the myelin layers.

Regulation

The amount of myelin has to be adjusted to the size and requirement of axons. To make this adjustment, neurons have to control signalling pathways that drive myelination in oligodendrocytes. An in-depth discussion of these signals is beyond our scope but we will briefly consider them in the context of myelin growth and wrapping (for a more detailed review, see Mitew et al., 2013; Piaton et al., 2010; Taveggia et al., 2010; Simons and Lyons, 2013; Fancy et al., 2011; White and Krämer-Albers, 2014). To generate myelin, the distinct but interconnected processes of compaction and radial and longitudinal growth need to be tightly coordinated. For example, when myelin compaction is impaired – as it is in *shiverer* mice (a natural mouse mutant that lacks MBP) – the growing myelin sheath becomes unstable and cannot grow beyond a few wraps (Inoue et al., 1981; Roach et al., 1983). Even if these different processes are closely coupled, there are a number of studies that point to distinct mechanisms in their regulation. We will divide the phenotypes of mouse mutants that have provided key insights into myelin growth regulation into different categories.

In the first category are overgrowth phenotypes that lead to both focal hypermyelination (e.g. myelin outfoldings) and a general increase in myelin thickness. One example is mice in which phosphatidylinositol (3,4,5)-trisphosphate [PtdIns (3,4,5)P_3] levels are specifically elevated through genetic disruption of phosphatase and tensin homolog (*Pten*) in oligodendrocytes. These *Pten* mutants show both, an increase in the overall delivery of myelin membrane to the inner tongue at the leading edge as well as accelerated focal delivery of membrane along myelinic channels to specific areas at the leading edge leading to the outfoldings (Goebbels et al., 2010).

In the second possible category, mice show an increase in myelin thickness that is not accompanied by areas of focal hypermyelination. This occurs when constitutively active AKT kinase, a downstream target of PtdIns (3,4,5)P_3, is expressed in oligodendrocytes (Flores et al., 2008). The differences in the *Pten* mutant are best explained by postulating that PtdIns (3,4,5)P_3 – in addition to AKT – regulates effectors that specially determine the abundance and/or size of the myelinic channels or the transport within them and/or the specific insertion of membrane at their target

site. Apart from the PI3K–AKT–mTOR pathway, ERK/MAPK signalling has emerged as an important pathway in determining overall myelin thickness (Ishii et al., 2012; Furusho et al., 2012; Fyffe-Maricich et al., 2011).

The third class of phenotype is that in which mutation leads to focal outfoldings and hypomyelination. An interesting example is the ablation of *Cdc42* or *Rac1* in oligodendrocytes, which results in the formation of numerous myelin outfoldings with abnormal accumulation of cytoplasm but with reduced overall myelin thickness (Thurnherr et al., 2006). One interpretation of this phenotype is that the protruding forces that drive the wrapping of the membrane around the axon are impaired, whereas production of myelin membrane, its transport along myelinic channels and its insertion at the inner tongue is unaffected. Finally, it may be that the ratio between radial and longitudinal growth is altered. There is at least the theoretical possibility that both processes are specifically regulated – as demonstrated in the PNS, where the initial elongation of Schwann cells along the axon can define the future intermodal length (Cotter et al., 2010; Simpson et al., 2013).

Concluding remarks

In this review we have highlighted key aspects of myelin membrane growth and incorporated them into a model that may explain how myelin is generated when a multilamellar membrane stacks around an axon. However, even so progress has been made, the picture is still incomplete. One of the key unanswered questions is how oligodendrocytes recognise and form stable contacts with those axons that need to be myelinated. What does the remarkable finding that oligodendrocytes are able to wrap their membrane around inert polystyrene fibres *in vitro* mean for myelination *in vivo*? Is myelin, indeed, only controlled by physical parameters or are instructive molecular cues required *in vivo*? What is the force that drives myelin around the axon? How is the amount of myelin adjusted to the requirement of the axon? Once formed, how stable is myelin? How much turnover and remodelling occurs in the adult organism? All these exciting questions are still left to be answered. It is clear that they can only be solved by an integrative and multidisciplinary approach in different model systems. The hope is that answers to these questions will not only deepen our knowledge in normal myelination as it occurs during development, but also help to understand how we can repair myelin in diseases.

Competing interests
The authors declare no competing interests.

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