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Between the Sheets: A Molecular Sieve Makes Myelin Membranes

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Myelin is a lipid-rich, spiraled membrane structure that allows for rapid propagation of action potentials through axons. In this issue, Aggarwal et al. (2011) present evidence that myelin basic protein, essential for myelination by oligodendrocytes, regulates the biosynthesis of myelin membranes by restricting diffusion of membrane-bound proteins into compact myelin.

The insulating myelin sheath is required in vertebrates for fast conduction of action potentials, and the loss of myelin causes severe pathology as seen in multiple sclerosis (for reviews see Lazzarini, 2004; Sherman and Brophy, 2005; Hartline & Colman, 2007). In the central nervous system (CNS), myelination is accomplished by oligodendrocytes (OLs), glial cells that wrap axons with a specialized membrane through largely unknown mechanisms. The final product of myelination is a highly polarized structure composed of (1) tightly packed spiraling layers of membrane that lack cytoplasm, called *compact myelin*, and (2) cytoplasmic *noncompact* regions connecting the OL cell body to the axonal side of the wraps and the Nodes of Ranvier. Unlike other biological membranes, compact myelin is made largely of lipids and is enriched in several proteins, including myelin basic protein (MBP). How this unique composition is generated is unknown. In this issue of *Developmental Cell*, Aggarwal and colleagues (2011) provide evidence that MBP acts as a molecular sieve to exclude most membrane-bound proteins from areas of the membrane where it is present, thereby polarizing the membrane.

Mechanistic studies of myelination are challenging due to the small dimensions and tight packing of myelin in vivo. Aggarwal and colleagues (2011) overcome this problem by using primary cultured OLs in vitro, which they show can adopt a flat morphology akin to an unrolled myelin sheath. Although cultured OLs grow in the absence of axons or other in vivo signals, they still polarize into compact myelin-like membrane sheets (containing MBP, proteolipid protein, and the myelin lipid GalC) and noncompact-like regions (containing proteins like CNPase, organelles, secretory vesicles, and a cytoskeleton). Surprisingly, despite their ~40–50 nm thickness, the membrane sheets also exclude cytoplasmic proteins like GFP. The authors use this system to tease apart how OLs polarize to form membrane sheets that, like true compact-myelin, exclude most proteins.

The first clue to how OLs polarize came when the authors compared the localization of a membrane-targeted reporter protein between wild-type cells and cells isolated from *shiverer* mice that do not express MBP. In wild-type cells, this reporter is excluded from membrane sheets, but it robustly accumulates in the membrane sheets of *shiverer* OLs. A cytoplasmic version of the reporter does not localize to sheets in either wild-type or *shiverer* OLs, suggesting that protein entry into membrane sheets requires membrane binding and can be blocked by MBP. MBP is a small (14–22.5 kDa), highly-charged protein that is expressed in vertebrates mainly by OLs and is thought to interact with the cytoplasmic face of the plasma membrane. The importance of MBP for myelination is underscored by severe dysmyelination in *shiverer* mice that lack it, which causes motor defects (including the shivering phenotype for which the mutant is named) and premature death (Roach et al., 1983; Lazzarini, 2004). When the authors compared endogenous myelin protein localization in wild-type and *shiverer* OLs, they found that noncompact myelin proteins like CNPase aberrantly enter membrane sheets in *shiverer* cells. Importantly, this is recapitulated in myelin in vivo—CNPase mislocalizes throughout both compact and noncompact myelin in the optic nerve of *shiverer* mutants. More generally, the overall protein to lipid ratio of myelin purified from *shiverer* mice is significantly higher than wild-type myelin.

The next step forward came when the authors found that tagging of the cytoplasmic sides of compact myelin proteins with fluorescent reporters prevents accumulation of these proteins (e.g., proteolipid protein, the major transmembrane protein in compact myelin) in wild-type membrane sheets; normal localization is restored in *shiverer* mutants. In contrast, tagging the extracellular side of these proteins does not block entry into sheets. The authors surmised that cytoplasmic domain size is the key factor regulating MBP-dependent protein exclusion from membrane sheets. Indeed, analysis using a series of cytoplasmic domain truncation mutants showed that, consistent with MBP forming a size barrier, the larger the cytoplasmic domain of a myelin protein, the more pronounced its exclusion from membrane sheets. MBP effectively blocks entrance of membrane-bound proteins with cytoplasmic domains with more than 20–30 amino acids, independent of their charge. These findings also hold up in myelin in vivo, as tagging proteolipid protein with cytoplasmic GFP causes it to be mistargeted out of compact myelin (which also raises an important caveat for using tagged myelin proteins in future in vivo studies).

Finally, Aggarwal and colleagues developed a reconstituted membrane system by using purified proteins and lipid vesicles to study the mechanism of how MBP polarizes membrane-bound proteins. Using the interface between supported lipid bilayers and giant unilamellar vesicles to mimic the cytoplasmic faces

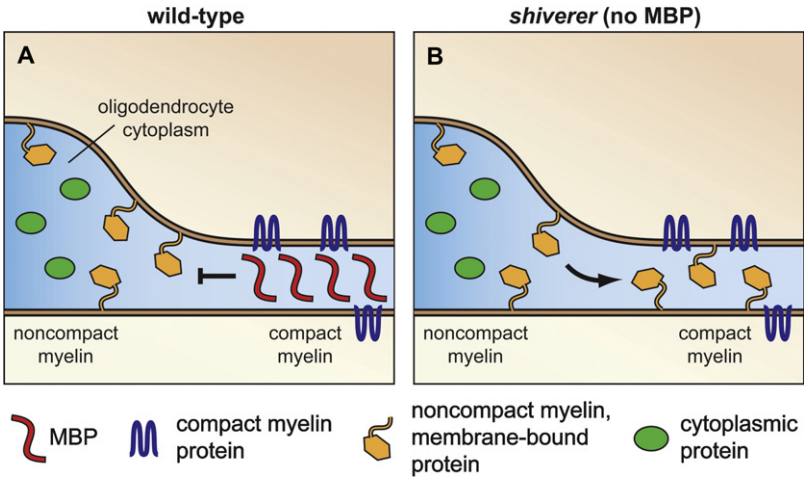

Figure 1. Mechanism Driving Establishment of Polarity in Myelin Membranes
(A) Myelin basic protein (MBP) resides in compact myelin and blocks the entry of membrane-bound proteins with large cytoplasmic domains (e.g., CNPase), thus establishing asymmetry between compact and noncompact myelin. (B) In the absence of MBP, these proteins can invade compact myelin.

of the membrane sheets of an OL, they found that purified MBP protein drives the spreading of the vesicles on top of the lipid bilayer and the exclusion of membrane-bound GFP from this region. This only occurs in the presence of MBP and is not merely due to the crosslink of the two membranes together. Further experiments suggest that binding to both membranes induces a conformational change in MBP that may lead to its multimerization, although the extent to which multimerization requires association with membranes remains unknown. Taken with the findings presented above, these results suggest that MBP is both necessary and sufficient to polarize myelin membranes (Figure 1).

Aggarwal et al. (2011) clearly demonstrate the role for MBP in polarizing myelin membranes, and open the door for future work. As this study makes use of a model membrane that does not entirely recapitulate all the molecular features of myelin (for example, myelin sheets in vitro are 40–50 nm thick, whereas a single wrap of compact myelin in vivo is closer to 10–15 nm thick), an important next step will be to test whether this mechanism is used to generate polarity in wrapped myelin sheaths, either in vivo or in myelinating cocultures with neurons. In addition, MBP protein is very heterogeneous, with several splice variants of different sizes, and multiple reported posttranslational modifications. It will thus be of interest to test how these diverse variants of MBP affect myelin biosynthesis.

Among the unanswered questions for future work is how MBP contributes to the compaction of myelin after it wraps around axons. Based on the defects in myelin ultrastructure in *shiverer* mice, MBP has been suggested to help the zippering-up of the cytoplasmic sides of myelin membranes. Indeed, electron micrographs of CNS myelin reveal that instead of tightly packed intracellular membrane faces, *shiverer* myelin is largely noncompact and still filled with cytoplasm, among other defects (Privat et al., 1979; Rosenbluth, 1980). However, Aggarwal et al. (2011) showed that in vitro, even myelin sheets without MBP contain little cytoplasm and thus exclude cytoplasmic proteins like GFP. It would thus be of interest to test whether these proteins are also excluded from compact myelin in vivo in *shiverer* mutants where myelin wraps appear to contain cytoplasm. If so, the in vitro system developed here could be used to address how this MBP-independent exclusion of cytoplasmic proteins occurs.

It is a formal possibility that polarity mechanisms used in other biological systems such as directed vesicular trafficking or septin-based diffusion barriers (Oh and Bi, 2011) also contribute, alongside MBP, toward the establishment of a polarized myelin membrane. Indeed, septins are known to be upregulated in OLs during myelination (Buser et al., 2009). Directly testing the potential involvement of these processes in myelin polarity is an interesting avenue for further exploration. Clearly, we are still a long way from fully understanding the cell biological mechanisms driving myelination, but Aggarwal and colleagues (2011) shed light on one crucial aspect: MBP regulates which proteins are present between the sheets.

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