Myelin under construction—teamwork required

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Myelinating glial cells synthesize specialized myelin proteins and deposit them in the growing myelin sheath that enwraps axons multiple times. How do axons and myelinating glial cells coordinate this spectacular cell–cell interaction? In this issue, Trajkovic et al. (p. 937) show that neuronal signaling regulates cell surface expression of the myelin proteolipid protein in cultured oligodendrocytes in unexpected ways that may also contribute to myelination in situ.

Myelination is a stunning example of how multiple cells cooperate to build a complex structure. Understanding how myelinating glia and neurons work together to achieve this feat is thus a challenging and important problem. Trajkovic et al. (p. 937) investigate the regulation of the trafficking of a major myelin protein, proteolipid protein (PLP), to the plasma membrane (PM) of cultured oligodendrocytes (OLs). When initially expressed in cultured OLs, PLP resides in a compartment with characteristics of a late endosome/lysosome (LE/L). Co-culture with neurons leads to an increase of PLP on the PM and a disappearance from the LE/L. This increased surface expression of PLP is due to at least two distinct mechanisms: a decrease in PLP endocytosis from the PM and an increase in exocytosis from the LE/L. The relative contributions of these two mechanisms (and possibly additional ones?) remain open questions for the future.

The cells that produce myelin are highly specialized glial cells, Schwann cells in the peripheral nervous system (PNS) and OLs in the central nervous system (CNS). Myelin consists of many wrappings of glial cell membrane around the axon with little or no cytoplasm left between adjacent wraps. This compact myelin region insulates the axon from the extracellular medium and allows saltatory conduction along axons. Each successive myelin wrap creates at its lateral margins a membrane loop containing some cytoplasm. These so-called paranodal loops make up part of the noncompact myelin. Each paranodal loop forms a specialized cell junction with the axon, the axoglial apparatus. The paranodal loops, in turn, flank Nodes of Ranvier, gaps in the myelin where voltage-gated sodium channels cluster and regenerate the action potential (for review see Sherman and Brophy, 2005).

Myelination is a supreme example of differential protein distribution. During myelination, glia elaborate distinct domains (such as soma and compact and noncompact myelin) with distinct lipids and protein components. At the same time, axonal membrane proteins also accumulate in distinct regions, such that the Node of Ranvier contains different proteins than the paranodal region (underlying the paranodal loops) or the juxtaparanode (flanking the paranode). Much work on who signaled whom, when, and why, revealed that neurons and myelinating glia communicate with each other bidirectionally in multiple ways to orchestrate myelination (Sherman and Brophy 2005). For instance, glial cells signal to neurons to influence axonal diameter, neurofilament spacing, and phosphorylation (Hsieh et al., 1994). Additionally, nodal, paranodal, and juxtaparanodal domains on axons form as a result of interactions with glial cells. Mutations in genes encoding paranodal proteins lead to aberrant paranodal loops and mislocalization of paranodal and juxtaparanodal components in the axon (for review see Poliak and Peles, 2003; Salzer, 2003). Somewhat surprisingly, nodal proteins still cluster in these mice, leading to the suggestion that nodal assembly might be intrinsic to axons or (in the CNS) driven by diffusible glial-derived factors (Kaplan et al., 1997). New work argues that glial cell processes which contact the node itself could direct nodal assembly. In the PNS, the node is contacted directly by microvilli of the myelinating Schwann cell. Mice lacking Schwann cell dystroglycan or laminin have aberrant microvilli and poorly clustered voltage-gated sodium channels (Saito et al., 2003; Occhi et al., 2005). Gliomedin, identified by the Peles lab, is expressed in Schwann cell microvilli and required for clustering of nodal axonal components (Eshed et al., 2005). In the CNS, Colman's group localized the outgrowth-inhibitory molecule Omgp to distinct glial cells that can encircle nodes (Huang et al., 2005). Omgp knock-out mice show wider and disorganized nodes as well as aberrant sprouting of branches from nodes. These findings highlight the importance of node-encircling glial cells for organizing the axon.

Do neurons in turn give instructions to glial cells? Oligodendrocyte precursor cells (OPCs) in the CNS migrate into developing white matter where they differentiate into postmitotic OLs and produce the myelin sheath. The differentiation of OPCs in terms of changes in gene expression and in morphology has been studied extensively in vitro and in vivo (for reviews see

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Abbreviations used in this paper: CNS, central nervous system; LE/L, late endosome/lysosome; OL, oligodendrocyte; OPC, oligodendrocyte precursor cell; PLP, proteolipid protein; PM, plasma membrane; PNS, peripheral nervous system.

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Pfeiffer et al., 1993; Barres and Raff, 1999). Because OPCs differentiate normally in axon-free culture and express myelin components, a role for neurons was not immediately apparent. In vivo, on the other hand, few OLs develop after transection of the optic nerve and subsequently, axons were shown to be required for survival and differentiation of OLs (Barres and Raff, 1999). OPCs and newly born OLs require astrocyte-derived factors such as PDGF, but OLs become dependent on axonal signals later. Axonal signaling to OLs occurs on at least two levels (Barres and Raff, 1999; Coman et al., 2005). Electrical activity (mediated by extrasynaptic release of adenosine [Stevens et al., 2002]) is required for proliferation of OPCs. Additionally, contact-mediated neuronal signals play important roles in OPC and Schwann cell differentiation and myelination (Corfas et al., 2004). Salzer and colleagues recently showed that the levels of neuregulin 1 type III expressed on axons determine the ensheathment fate of axons in the PNS (Taveggia et al., 2005).

Compact myelin has a very specific composition of 70% lipids by dry weight (mostly composed of galactoceramide and cerebroside) with 80% of the protein mass comprised of only two proteins, myelin basic protein MBP and proteolipid protein PLP/DM20 (for review see Kramer et al., 2001). Studies have therefore focused on how OLs synthesize MBP and PLP and incorporate them into the growing myelin sheath. MBP is synthesized on free ribosomes, but its mRNA is localized to the myelin sheath (Colman et al., 1982). PLP on the other hand is a membrane-spanning protein that traverses the ER and Golgi. The role for axonal signaling for production of the myelin sheath is not well understood. For instance, OPCs in culture undergo differentiation and start to synthesize myelin components in the absence of neurons (Pfeiffer et al., 1993). Early reports from cultured rat OLs concluded that PLP was synthesized and incorporated into the PM without neurons (Hudson et al., 1989). Interestingly though, PM expression of PLP could not be detected for many days after intracellular pools of PLP were clearly detectable. The delayed PM expression of PLP raised the possibility that axonal signaling could speed up PM expression.

The paper by Simons and colleagues in this issue demonstrates neuronal control of PLP trafficking (Trajkovic et al., 2006). Primary OLs, as well as two OL cell lines, contain PLP in a LE/L (as well as on the PM). This LE/L pool of PLP persists if neurons are absent from the culture. When OLs are cocultured with neurons, PLP is found with LE/L initially, but later disappears from there and increased amounts can be detected on the PM. When brain sections were costained against lysosomal markers and PLP, high colocalization of PLP with LE/L was detected in P7 mice while in P60 brains PLP did not colocalize with LE/L. Therefore, PLP localizes (at least partially) with LE/L in vivo and disappears from there upon myelination. This finding assuages much of the fear that PLP-containing LE/L are just a culture phenomenon or due to inappropriate expression levels (Kramer et al., 2001; Simons et al., 2002). The authors tested three explanations to account for their observations: increased proteolysis of PLP, decreased endocytosis, and/or increased exocytosis from LE/L. Proteolysis of PLP was found to be unaffected by neuronal coculture. Endocytosis (via a clathrin-independent, cholesterol-dependent, actin-dependent, and RhoA-dependent pathway), on the other hand, was decreased. Using PLP-GFP and lysotracker to mark LE/L in live OLs, the authors also found that the LE/L became much more mobile in the presence of neurons. To determine whether the moving LE/L in cocultured OLs can fuse with the PM and potentially deliver PLP sequestered in LE/L, the authors used total internal reflection fluorescence microscopy (TIRFM) on lysotracker-labeled OLs. Without neurons present, the LE/L was not found within 100 nm of the PM and was therefore invisible to TIRFM. When neurons were present, many LE/L were found near the PM and events suggestive of fusion could be observed at a rate of 1–2 events/min. Lastly, the authors determined that diffusible neuronal factors were sufficient to induce increased PLP surface expression. Addition of a membrane-permeable cAMP analogue to OLs in the absence of neurons led to increased PLP on the surface as well as high mobility of lysotracker pools containing PLP-GFP.

These results suggest that diffusible neuronal factors (currently unknown) could activate cAMP signaling in OLs and regulate endocytosis and exocytosis of PLP. Exocytosis from LE/L is a regulated pathway in other cells as well (Blott and Griffiths, 2002). In OLs, at least some of the PLP could be stored in LE/L until neuronal promyelinating signals are received. Because many proteins arrive in the LE/L from the TGN, it would be interesting to investigate the potential neuronal regulation of PLP sorting events in the Golgi. Although we still await a complete quantitative account of what proportion of PLP is transported where and when, this paper presents an exciting advance in our understanding of the neuronal control of OL membrane traffic.

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