Turning to myelin turnover

Tobias J. Buscham, Maria A. Eichel, Sophie B. Siems, Hauke B. Werner
Department of Neurogenetics, Max Planck Institute of Experimental Medicine, Göttingen, Germany

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Abstract
Neural plasticity in the adult central nervous system involves the adaptation of myelination, including the formation of novel myelin sheaths by adult-born oligodendrocytes. Yet, mature oligodendrocytes slowly but constantly turn over their pre-existing myelin sheaths, thereby establishing an equilibrium of replenishment and degradation that may also be subject to adaptation with consequences for nerve conduction velocity. In this short review we highlight selected approaches to the normal turnover of adult myelin in vivo, from injecting radioactive precursors of myelin constituents in the 1960s to current strategies involving isotope labeling and tamoxifen-induced gene targeting.

Key Words: oligodendrocyte; myelin plasticity and turnover; degradation; tamoxifen; metabolic labeling; isotope labeling; neural plasticity; myelinoid bodies; central nervous system

Introduction
Myelination of axons by oligodendrocytes facilitates rapid and precise nerve impulse propagation in the central nervous system (CNS) of jawed vertebrates (Hartline and Colman, 2007; Nave and Werner, 2014; Weil et al., 2018). The regular structure of the myelin sheath in healthy conditions and its abnormalities in myelin-related disorders are commonly approached by electron microscopy (Möbius et al., 2016). However, the two-dimensional character of electron micrographs provides static images, necessitating careful quantification when assessing changes of myelination during normal development and aging. Indeed, myelination of axonal segments is adapted throughout life (Bergles and Richardson, 2015; Baraban et al., 2016), and it is now widely recognized that the addition of new myelin sheaths by adult-born oligodendrocytes represents a mechanism of plasticity in the mature CNS (O’Rourke et al., 2014; Sampaio-Baptista and Johansen-Berg, 2017). Yet, the maintenance of pre-existing myelin sheaths by mature oligodendrocytes involves slow but constant replenishment and degradation of myelin constituents and thus life-long turnover of myelin sheaths. This was emphasized when the fallout of nuclear bomb tests in the late 1950s and the early 1960s was utilized as a labeling pulse (Yeung et al., 2014). Indeed, measuring the carbon isotope ¹³C in human post-mortem brains allowed calculating the age of oligodendroglial cell bodies and of myelin. Importantly, in the analyzed white matter tract (the corpus callosum), nearly all oligodendrocytes were born before the age of 5 years; only a small proportion was replaced throughout life. Conversely, myelin was entirely replenished during life, indicating a much faster turnover compared to that of the myelinating oligodendrocytes themselves (Yeung et al., 2014). Together, this work has shown that existing oligodendrocytes remodel their myelin sheaths in adult humans in vivo; however, the approach did not allow quantifying a particular turnover rate.

The Medline database was electronically searched for articles from 1954 to 2019 using the following keywords: oligodendrocyte; neural plasticity; myelin turnover; degradation; tamoxifen; metabolic labeling; isotope labeling; neural plasticity; myelinoid bodies; central nervous system.

Radioactive Labeling
In first attempts to approach myelin turnover directly in the 1960s (Hildebrand et al., 1993), radioactively labeled precursors of lipids or proteins were injected into young or adult animals, which were sacrificed at various times after injection to measure the radioactivity in myelin-enriched brain fractions. Plotting the remaining radioactivity against time allowed calculating protein half-lives. However, the reported half-lives of myelin lipids and proteins differed widely across various studies depending on the age at the time of injection and the type of precursor used. Additionally, interpretations at first were difficult owing to the utilization of myelin constituents over the course of long-term ex-
periments and poorly developed protocols for myelin purification (Hildebrand et al., 1993). Notwithstanding that the morphological equivalent of the measured half-lives initially remained speculative, these studies established for the first time that myelin membranes are indeed subject to turnover in the adult brain (Smith, 1968).

Upon the establishment of more reliable myelin purification protocols, proteolipid protein (PLP) and myelin basic protein (MBP) appeared more stable compared to cyclic nucleotide phosphodiesterase (CNP; previously referred to as Wolfgram protein) (Fischer and Morell, 1974). Considering that CNP is a marker of non-compact myelin while PLP and MBP are major constituents of compact myelin, this result implies that non-compact myelin is turned over faster than compact myelin. Indeed, the non-compacted cytosolic channels through myelin, including the nodal and paranodal myelin sub-compartment and the adaxonal myelin layer, differ from compacted myelin membranes not only in their turnover rate but also with respect to protein composition, ultrastructure and metabolic activity (Nave and Werner, 2014).

Metabolic Labeling

More recently, techniques became available that enable systematic measurement of the lifetimes of numerous individual proteins in vivo. Here, metabolic labeling by a dietary labeling pulse using amino acids harboring the stable isotopes 15N or 13C is followed by a period of feeding unlabeled chow and subsequent mass spectrometric analysis (Price et al., 2010; Toyama et al., 2013; Fornasiero et al., 2018). When calculating protein lifetimes from the ratio of labeled and unlabeled amino acids in tryptic peptides, myelin proteins were consistently among the proteins with the longest average lifetimes in rodent brains, in agreement with their localization in a structure that is turned over slowly. Generalized, the lifetime of myelin proteins is approximately as long as that of nuclear histones (Price et al., 2010; Toyama et al., 2013). Yet, considering that the postnatal period of most active developmental myelination involves substantial remodeling of oligodendroglial processes and myelin sheaths (Hines et al., 2015), the measured protein half-lives may not accurately reflect the turnover of myelin in adults if the dietary pulse was applied during juvenile development (Toyama et al., 2013). Importantly, the lifetime of proteins that localize to compact myelin (CLDN11, PLP, MBP, and MOBP) was about twice as long as that of those residing in non-compact myelin (CNP, MAG) (Fornasiero et al., 2018), confirming the previous studies utilizing radioactive precursors (Fischer and Morell, 1974). However, measurements by metabolic labeling so far probably underestimated the lifetimes of myelin in the adult CNS because the analysis of total brains does not allow discriminating between proteins present in the cell bodies of oligodendrocytes from those that have been incorporated into myelin sheaths, which most likely undergo turnover at different rates. Yet, this limitation can easily be overcome in future approaches if purified myelin rather than total brain is assessed.

Tamoxifen-Induced Gene Targeting

Conditional gene targeting in experimental mice enables the tamoxifen-induced deletion of myelin-related genes after developmental myelination has ceased (Leone et al., 2003). This experimental system allows assessing the abundance of a given protein in myelin purified at various timepoints after terminating its replenishment. Notably, if the tamoxifen-induced deletion of genes for crucial transcription factors or signal-transducing proteins interferes with the maintenance of myelin per se (Koenning et al., 2012; Ishii et al., 2014), the resulting severe demyelination prevents conclusions about the normal turnover of myelin in healthy states. However, tamoxifen-induced deletion of several other myelin-related genes does not cause considerable demyelination. For example, the abundance of the myelin septin filaments that stabilize the adaxonal non-compact myelin subcompartment was approximately halved in myelin purified from Sept-8lox/lox;P1pCreERT2 mice 4 weeks after tamoxifen injection into adult mice (Patzig et al., 2016). Conversely, the abundance of the major constituent of compact myelin, PLP, was reduced by half in myelin purified from P1plox/lox;P1pCreERT2 mice (Lüders et al., 2019) not until 6 months after tamoxifen injection (Figure 1). Together, this confirms that in adult mice the turnover of compact myelin is much slower compared to that of non-compact myelin. We note that owing to the experimental design the continued differentiation of oligodendrocyte precursor cells generates a number of adult-born myelinating oligodendrocytes with newly-formed myelin sheaths that contain septins or PLP, respectively. Yet, we believe that this approach reflects comparatively well the half-life of non-compact and compact myelin membranes in adult mice.

Degradation of Myelin Membranes

How is myelin degraded upon its turnover? The existence of myelin turnover implies that new membranes
Increasing amount of degraded myelin causes the formation of microglia (Safaiyan et al., 2016). Interestingly, the mechanisms are not fully understood, it is commonly assumed that myelinoid bodies are mainly cleared via lysosomal degradation in microglia (Safaiyan et al., 2016). Interestingly, the increasing amount of degraded myelin to the formation of lysosomal inclusions, which might result in microglial dysfunction in the aged brain (Safaiyan et al., 2016). Notably, astrocytes in the brain and microvascular endothelial cells in the spinal cord can also engulf myelin debris (Porath et al., 2017; Zhou et al., 2019). Engulfment and lysosomal degradation of myelin debris by microglia, astrocytes or endothelial cells may represent an early response to myelin pathology that subsequently induces recruitment of macrophages and lymphocytes.

Outlook

Taken together, recent technical developments allow assessing the turnover of myelin in vivo. Notwithstanding that these approaches may still over- or underestimate the precise rate of myelin turnover to some degree, it appears feasible to apply these tools to test if the rate of myelin turnover differs between thick and thin sheaths, gray and white matter, upon aging or in myelin-related disorders that involve degeneration and/or regeneration of myelin sheaths. Notably, it is also possible to experimentally stimulate the net growth of myelin membranes by pre-existing oligodendrocytes in adult mice beyond what is required for normal myelin turnover (Goebbels et al., 2010; Gibson et al., 2014), possibly involving local protein translation of the transcript pool enriched in myelin enriched (Thakurela et al., 2016). We speculate that modulating the rate of myelin replenishment and degradation may serve to optimize myelin sheath thickness and thus the speed and precision of nerve impulse propagation (Arancibia-Carcamo et al., 2017), thereby representing a possible mechanism of neuroplasticity. Toward a full understanding of myelin turnover in adults, it will also be relevant to consider genetic tools to entirely inhibit the replenishment or degradation of myelin membranes.

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Figure 1 Turnover of proteolipid protein (PLP) in central nervous system myelin.
Quantitative immunoblot was performed to detect the most abundant structural myelin protein, PLP, in myelin biochemically purified from the brains of Plp\textsuperscript{flox/Y}; Plp\textsuperscript{CreERT2} (iKO) and Plp\textsuperscript{flox/Y} (Ctrl) mice at the indicated time points after tamoxifen injection (pti). The graph shows the abundance of PLP in myelin compared to the mean of the age-matched Ctrl. Note that the abundance of PLP in iKO myelin is halved about 6 months (mo) pti, probably reflecting the turnover of the compact layers of central nervous system myelin. Figure taken from Lüders et al. (2019) with permission.

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