Sorting and trafficking of proteins in oligodendrocytes during myelin membrane biogenesis

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Chapter 6

Summary and Perspectives

Summary

During myelin formation oligodendrocytes (OLGs) utilize basic mechanisms of membrane trafficking, commonly seen in eukaryotic cells, as outlined in the introductory chapter (*Chapter 1*). However, whether specific transport pathways, unique to myelin biogenesis are involved and if so, how such pathways might be regulated in the biogenesis and maintenance of the myelin sheath, has been largely unexplored thus far. Such insight is of major relevance in order to device strategies for exogenous manipulation to stimulate and/or promote de novo biogenesis of myelin sheath assembly at pathological conditions that lead to demyelination, as in the case of multiple sclerosis (MS). In addition, the special morphological features of OLGs, consisting of a cell body, bounded by a plasma membrane, and myelin 'protruding' from that cell body, yet maintaining a highly specific and quite distinct membrane composition when compared to the plasma membrane, raises many fundamental questions of great interest to current cell biology. Accordingly, a major purpose of the work described in this thesis was to acquire insight into the sorting and trafficking of structural myelin components such as PLP and MBP, and to reveal regulatory mechanisms, instrumental in promoting myelin sheath biogenesis. As noted, we believe that this kind of insight is a prerequisite for a prosperous advancement in developing therapeutic approaches for a disease as complex as MS.

The trafficking and sorting of myelin constituents was primarily studied in vitro, using isolated OLG precursor cells from newborn rats. This approach also allowed us to study myelin biogenesis as a function of cellular development. In *chapter 2*, molecular parameters that govern the targeting and incorporation of myelin proteins and model molecules into myelin membranes are investigated. Since we specifically took into account the polarized nature of OLGs, based on previous work, we investigated whether the presence of a tyrosine residue in the C-terminal part of membrane proteins, acting as a basolateral sorting signal in polarized epithelial cells, sufficed to target model proteins to the myelin sheet. The data support the concept that the myelin membrane displays typical basolateral-like features, and in terms of targeting depends on typical basolateral signals, while myelin-directed trafficking is regulated by protein kinase C activity in a manner that bears similarity to basolateral membrane–directed trafficking in epithelial cells. *Chapter 3* describes the role of syntaxins 3 and 4 in OLG polarity and their potential ability to regulate trafficking of myelin specific proteins. For the first time we demonstrate a functional role of these SNAREs in OLGs and moreover, their distinct activity at either the

plasma membrane (syntaxin 3) or sheet (syntaxin 4), which is entirely in line with the polarized properties of these membrane domains in OLGs. In *chapter 4*, we investigated the role of exogenous factors on the regulation of myelin biogensis. To this end, OLGs were grown on different substrates of extracellullar matrix (ECM) to analyze whether extracellular signals are affecting the transport of PLP, which is used as a molecular marker for myelin assembly. Our data indicate that sheet-directed transport of PLP in OLGs relies on a transcytotic transport mechanism, in which PLP, early after biosynthesis, integrates into Triton X-100 resistant microdomains for transport to the plasma membrane of the OLG cell body, sulfatide seeming to play an important role in this process. Following transport to the OLG plasma membrane, the protein is internalized and subsequently transported to the myelin sheet. In *chapter 5* the role of MAL in the regulation of sorting and transport of PLP is examined. A GFP-MAL construct was overexpressed in proliferating OLG progenitor cells and the expression and localization of MAL as well as its effect on transport of myelin specific proteins was studied during different stages of OLG development. In this chapter it is shown that in GFP-MAL (over)expressing cells neither proliferation nor differentiation was affected. Whereas the expression and distribution of CNP, a myelin marker for non-vesicular transport, or transport of MBP mRNA, were not modified in GFP-MAL overexpressing cells, the expression level and transport of PLP was blocked. These data suggest that MAL is a negative regulator of myelin sheet –directed vesicular transport of PLP.

Future Perspectives

In this thesis molecular parameters were identified that regulate transport to the myelin sheet, representing part of the complex mechanism of myelination. Importantly, further evidence was provided supporting the polarized properties of myelin and plasma membrane in oligodendrocytes, bearing similarity to the typical polarized features seen in epithelial cells. However, several important issues obviously need further investigation. In chapter 2 we demonstrated that basolateral trafficking in OLGs appeared to be dependent on a tyrosine signal in the C-terminal membrane domains of transmembrane proteins. Thus, the question should now be addressed whether this tyrosine signal and/or other basolateral signals, are also operating in the sorting and transport of myelin specific proteins, particularly in proteins like MAG and PLP, containg tyrosine and dileucine residues, respectively. However, in this respect it cannot be excluded that the role of

basolateral and apical sorting signals in myelin protein transport and myelin assembly may well depend on signals more complex than a single amino acid residue. Indeed, although the regulation of the sorting and transport pathway of myelin specific PLP during myelin development is still largely obscure, our data in chapter 3 suggest that PLP is sorted and transported to the plasma membrane prior to sorting and transport to the myelin sheet, indicating at least dual signalling entities. Moreover, the transcytotic mechanism as observed partly relies on the t-SNARE syntaxin 3. It will be of interest to determine the precise mechanism as to how and where syntaxin 3 regulates the route and docking of PLP to and at the myelin sheet. Of particular relevance are the effects of extracellular matrix signals on the development of a proper myelin sheet. It is anticipated that a better characterization of the involvement of these signals will shed light on the mechanism by which syntaxin 3 is involved in determining and defining the route of PLP transport to the myelin sheet. Further work is also needed to determine how syntaxin 3 and syntaxin 4 differentially regulate the synthesis and/or processing of myelin proteins, including PLP and MBP, respectively. Particularly the observation that syntaxins might be able to regulate transport of mRNA (of MBP) as well will have an impact of general cellbiological significance. Specifically, it will be of interest to determine whether syntaxin 4 actually associates with MBP mRNA in some sort of complex. RNAi knock down approaches will enable to study this in more detail. In respect to this, overexpression and RNAi knock down experiments can be used to study the v- and t-SNARE combinations, required for the different pathways involved in the assembly of the myelin membrane. In this process it will also be important to elucidate the role of the myelin lipids, GalCer and sulfatide. In this regard our initial results, described in chapter 4, indicate that sulfatide is involved in early (i.e., plasma membrane-directed) transport of PLP. Additional work will be needed to firmly support these preliminary yet highly interesting observations.

Finally, elucidation of the segregation of myelin proteins during development will be of help to provide further insight into the process of myelination during neural tissue development. This knowledge will be imperative in the search of therapies in demyelinationg diseases, either by preventing a worsening in their development or by promoting the recovery from a condition of demyelination.