Chapter 7

Summary and Future Perspectives & Nederlandse Samenvatting

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Summary

Oligodendrocytes (OLGs) are specialized, myelin forming cells of the central nervous system . One OLG can myelinate more than 50 axons at a time, thereby providing rapid and efficient saltatory nerve conduction. To reach a myelin forming stage, oligodendrocyte progenitor cells (OPCs) need to undergo a series of tightly regulated developmental and morphological changes. During early development OPCs proliferate and migrate, properties that cease to function upon further differentiation, which leads to myelin membrane biosynthesis [1]. Signaling molecules present in the extracellular environment contribute to OLG development in a spatial, sequential and transient manner [2]. Upon demyelination of an axon, this myelination machinery resumes its function, as only adult OPCs, and not mature OLGs, are able to enwrap denuded axons by functional myelin sheaths [3,4]. However, in the case of demyelinating diseases, such as multiple sclerosis (MS), the myelination machinery malfunctions, and loss of OLGs or the presence of quiescent OPCs, unable to differentiate and synthesize myelin sheaths, results in severe demyelination, ultimately leading to secondary axonal loss [3,5]. Remyelination failure in MS is likely a result of dramatic changes in the external environment, among others due to the persistent presence of signaling molecules, like pro-inflammatory cytokines such as TNFα [6,7], and the presence of deleterious extracellular matrix (ECM) molecules, such as fibronectin [8,9] (**chapter 1**). In order to elucidate the underlying mechanism(s) of remyelination failure, extensive knowledge of the functioning of the myelination machinery is required. Therefore, to improve our knowledge of OLGs and myelination, it is crucial to comprehend the exact role of structural myelin lipids and proteins, as well as the functioning of the extracellular environment such as ECM and soluble signals, which was the focus of the studies described in this thesis.

Along their differentiation, OLGs synthesize myelin specific proteins and lipids in a sequential manner. When compared to other membranes, the composition of myelin membranes is unique with its high lipid to protein ratio (70:30) [10,11]. Almost one third of the myelin lipid pool consists of the galactolipids, galactosylceramide (GalC) and sulfatide. Furthermore, OLGs express a specific repertoire of myelin proteins of which proteolipid protein (PLP) and myelin basic protein (MBP) are the major ones. Due to their polarized nature, OLGs exploit a polarized trafficking machinery to transport their cargo to the final destination, myelin membranes being served by a basolateral route [10,12]. Besides the trafficking machinery, the organization of myelin lipids and proteins within the membrane is also crucial for myelin to enable salutatory nerve conduction. For example, galactolipids are important constituents

of membrane microdomains, so called 'lipid rafts', which play an important role in OLG development and differentiation [13]. In addition to that, the localization behavior of myelin proteins within these membrane microdomains is also essential for myelin assembly (**chapters 3 and 4,** [10]) . All these aspects and the continuous reorganization of myelin suggest that myelin membranes display a dynamic structure. For example, the sequential surface expression of myelin lipids might alter the fluidity of myelin membranes, which leads to changes in the lateral organization and mobility of the myelin proteins. In order to obtain more detailed insight into the dynamics of myelin proteins in the cell body plasma membrane or myelin membranes, different methodologies such as non-invasive optical microscopic biophysical techniques like fluorescence correlation spectroscopy (FCS), and raster image correlation spectroscopy (RISC) might be of use [15–17]. In **chapter 2**, we provided insight into how these biophysical methodologies can be applied in the myelin field. Furthermore, we provided information on the availability of different model systems such as OLG cell lines and model membrane systems like large and giant unilamellar vesicles (LUVs/ GUVs) [18,19]. These kinds of model membranes can be composed with a minimum number of elements such as lipids only or a joint set of lipids and proteins, taking into account the appropriate physiological ratios. In this manner, answers to lipid specific questions, i.e., the effect of fatty acyl chain length of galactolipids on membrane domain formation [20] can be investigated, or alternatively, provide information about specific myelin lipid-protein interactions [21].

The major myelin protein PLP is very important for the integrity of the myelin membrane as it regulates the close apposition of the extracellular leaflet of the myelin membranes [22,23]. PLP employs a vesicular transport machinery to reach the myelin membrane and as shown in **chapter 3**, vesicular transport of PLP is regulated in line with the polarized nature of OLGs; i.e., the myelin membrane is the target of a basolateral-like trafficking mechanism, whereas the cell body plasma membrane is a target of an apical-like mechanism [10,24,25]. We showed that PLP, prior to its incorporation into CHAPS-resistant membrane microdomains in the basolateral myelin membrane, is transported to the apical-like plasma membrane of the OLG cell body in a syntaxin-3 (t-SNARE) dependent manner, displaying resistance to the detergent TX-100. Our data further revealed a sulfatide-mediated shift of PLP from TX-100-resistant microdomains to CHAPS-resistant-microdomains at the cell surface. This apical-to basolateral transcytotic transport route of PLP to the myelin membrane could be mimicked in the polarized liver cell line, HepG2 cells. Interestingly, upon transcytotic transport of PLP to the myelin membranes, we showed that the conformation of the second extracellular loop of PLP is altered in a sulfatide-dependent manner.

After pinpointing the role of sulfatide in transcytotic PLP transport and the

role of PLP's lateral organization within the membrane, we further examined the link between this lateral organization and dynamic behavior (**chapter 4**). A CHAPS extraction study performed in an OLN-93 cell line that allows selective expression of GalC alone or GalC and sulfatide, revealed that upon transient transfection with PLP, the presence of sulfatide increases PLP's CHAPS insoluble membrane association. In relation to this observation, a pronounced sulfatide-mediated decrease in the lateral mobility of PLP was detected. A similar sulfatide-induced increase in the association with CHAPS-resistant membrane microdomains, along with a decreased lateral mobility of PLP in the presence of sulfatide was observed, when the cells were grown on laminin-2, a physiological substrate that promotes myelin membrane formation and harbors binding sites for sulfatide [26–29]. In contrast, on fibronectin, a pathological substrate, which inhibits myelin membrane formation and impairs remyelination in MS lesions [8,9,30], PLP was not present in these CHAPS-resistant membrane microdomains, which was accompanied by a dramatic increase in its lateral mobility.

In parallel, the lateral membrane organization and mobility of 18.5-kDa MBP, the other major myelin protein, was examined in **chapter 4**. The presence of GalC, but not sulfatide, increased the CHAPS-resistant microdomain association of MBP, which correlated with an increase in the lateral mobility of the protein. Unlike PLP, MBP's lateral mobility did not display differences in relation to different ECM coatings. Indeed, previous studies showed that the lateral organization of myelin-localized MBP is likely driven by secreted neuronal signals [31]. Therefore, under pathological conditions, rather than ECM proteins, soluble signals in the extracellular milieu might affect the lateral organization of MBP.

In MS lesions the expression of pro-inflammatory cytokines such as TNFα are upregulated [32]. In this context, in **chapter 5**, the effect of TNFα on MBP in myelin forming OLGs was examined. Rather surprisingly, exposure to relatively low levels of TNFα reduced the length of myelin segments, produced in myelinated cultures. In vitro, this was reflected by a marked and reversible redistribution in the localization of MBP protein, i.e., from myelin sheets towards primary processes, mediated by activation of TNF receptor 1 (TNFR1) . Notably, cell survival, MBP protein and mRNA levels, and the localization of MBP mRNA remained unaltered upon TNFα treatment. Similar changes in the localization of the phosphorylated form of MBP were observed, with a concomitant decrease of phosphorylation. Interestingly, the underlying mechanism for the TNF α-mediated redistribution of MBP appeared to be related to a disorganized actin cytoskeleton, which likely induced a shift of MBP from actin-dependent to actin-independent membrane microdomains. This might interfere with the barrier function of MBP, given a similar redistribution of PLP towards primary

processes, and a relocalization of CNP towards the myelin sheets. Hence, these findings suggest that TNFα might influence the maintenance of myelin membranes in an MBP- and actin-dependent manner.

The MBP protein family consists of different isoforms [33], as a result of an alternative splicing of one MBP transcript, which is generated from a gene complex called Golli (Gene in the Oligodendrocyte Lineage) consisting of 11 exons [34]. Among these isoforms, the postnatal MBP isoforms of 14 and 18.5 kDa are lacking exon-II, and localize to the myelin membrane, playing a role in myelin membrane compaction and acting as a molecular barrier for the entry of proteins with large cytoplasmic tails to the myelin sheath [34,35]. In contrast, the postnatal exon-II positive MBP isoforms, i.e., 17 and 21.5 kDa in rat, localize to the nucleus and cytoplasm and their expression peaks during early OLG development [36,37]. Their function is however less clear. Therefore, in chapter 6, we investigated the function of exon-II positive MBP isoforms. We employed galactolipid deficient OLN-93 cells, which are not able to synthesize the major postnatal MBP isoforms. Interestingly, our results revealed that OLN-93 cells endogenously express an MBP isoform with an apparent Mw of 16 kDa, which we identified as the exon-II-positive embryonic isoform of MBP (e-MBP). e-MBP displayed a similar nuclear and cytoplasmic localization pattern as postnatal exon-II positive MBPs. When cell proliferation was inhibited, e-MBP was excluded from the nucleus, whereas upon reestablishment of the proliferative conditions, e-MBP relocalizes to the nucleus, suggesting active nucleocytoplasmic shuttling in response to proliferation. Interestingly, e-MBP was also expressed in non-CNS cell lines, including HepG2, HeLa and HEK293 cells. Direct evidence for a role of e-MBP in proliferation was obtained upon down regulation of MBP, which markedly decreased the proliferation of the cell lines. Furthermore, live cell imaging and FRAP (Fluorescensce recovery after photobleaching) analysis with RFP-tagged exon-II positive postnatal 21.5-kDa MBP revealed that at proliferative conditions, 21.5-kDa MBP-RFP localized mainly in the nucleus, whereas it was excluded from the nucleus when proliferation was inhibited. Interestingly, the nuclear export blocking agent Leptomycin-B (LMB) prevented nuclear export of MBP. Thus, 21.5-kDa MBP actively shuttles between cytoplasm and nucleus upon mitogenic modulation. Hence, the exon-II containing MBP isoforms might be crucial players in cell proliferation during embryonic development and, after birth in OPC proliferation.

In conclusion, the work presented in this thesis provided new insight into mechanisms related to myelin biogenesis, highlighting major roles of the myelin proteins PLP and MBP and the major myelin lipids GalC, sulfatide. Furthermore, we provided evidence that detailed knowledge of membrane microdomain association of myelin proteins improve our understanding on myelin biogenesis in both health

and disease. The obtained knowledge will help to better understand why myelin biogenesis or myelin maintenance fails in MS, adding to the elucidation of potential repair mechanisms, which are still largely unknown in MS.

Future Perspectives

In MS, de novo myelin membrane synthesis fails, which is likely due to an altered extracellular environment [8,38]. Interestingly, previous studies suggest that the biochemical structure of myelin galactolipids and myelin proteins are altered in MS, e.g., the extent of hydroxylation of sulfatide and deamination of MBP are increased[34,39]. Given the importance of sulfatide in the timing of PLP transport, and MBP in myelin biogenesis and maintenance (this thesis), these changes in structural myelin elements might provide novel insight as to why myelin biogenesis fails in MS. Thus, it will be interesting to further investigate the effect of the biochemical structure of myelin lipids in model membranes such as GUVs (see chapter 2). For example, one can make use of myelin membranes obtained from MS patients or alternatively synthetic lipids where hydroxylation levels are different, to reconstitute GUVs that mimic the conditions in MS. Furthermore, the data described in chapters 3 and 4 provide a better understanding of the role of myelin galactolipids, especially sulfatide on transcytotic transport, localization and dynamics of the major myelin protein PLP. The work presented here would benefit from further investigations; i.e., it is not known yet how PLP transport, localization and dynamics are affected in MS. In that regard, dynamics studies relying on bleaching experiments, such as FRAP analyses, may provide detailed insight on the direction of PLP transport in the presence of different extracellular signals; i.e., the presence of neuronal-conditioned medium or the presence of fibronectin to elucidate whether PLP transport is perturbed in MS. Chapter 4 investigated the lateral mobility and membrane microdomain association of PLP and MBP as a function of the lipid environment, i.e., by selectively expressing GalC and/or sulfatide in OLN-93 cells. As galactolipids together with cholesterol play a role in membrane domain formation and since cholesterol levels are altered in MS [40,41], it will be interesting to analyze the effect of the cholesterol pool on raft partitioning and dynamic behavior of PLP and MBP, and their relevance to myelin biogenesis. To determine the lateral mobility of PLP and MBP, we made use of biophysical techniques FCS and RICS; however more advanced biophysical techniques such as spot variation FCS (discussed in chapter 2) enable to directly evaluate whether changes in the mobility of PLP are a consequence of its localization in distinct membrane microdomains.

Similarly, the persistent presence of pro-inflammatory cytokines in MS may interfere with OLG polarity, myelin biogenesis and maintenance. In that context, our investigations on the cellular localization of MBP and its membrane microdomain association, in response to TNFα and a disorganized actin cytoskeleton, provided some insight as to why myelin segments in myelinated cultures are shortened as compared to those in untreated cultures. However, we still do not know the exact underlying mechanism of this myelin segment shortening and its pathological and/or physiological relevance, if any. Additional experiments on the TNFR1 might provide some clues. TNFR1 localizes to membrane microdomains upon TNFα stimulation [42,43]. Therefore, it would be of interest to determine whether the effect of TNFα is mediated by TNFR1 in membrane microdomains, and whether TNFR1 membrane microdomain association is altered in MS. In addition, further investigations are necessary to elucidate why the actin cytoskeleton disorganizes and how this is linked to MBP and microdomain association. Proteins potentially involved might be Rac and Rho, since these proteins are known to regulate cytoskeletal dynamics. Besides, in MS, the post-translational modifications of MBP change dramatically. For example, MS patients have increased levels of deiminated MBP, and decreased levels of phosphorylated MBP [33]. Therefore, it will be of further interest to study the effect of TNFα on MBP post-transitional modifications.

In Chapter 6, we proposed a novel function for exon-II containing MBP isoforms in cell proliferation. As these MBP isoforms regulate cell proliferation, it will be of interest to investigate whether these isoforms are re-expressed upon demyelination and contribute to remyelination, and whether this is valid in MS lesions. In addition, it is important to clarify the underlying mechanism of how exon-II containing MBPs regulate cell proliferation. By nuclear export blocking experiments with LMB, we suggested that the shuttling between nucleus and cytoplasm of MBP can be nuclear export signal (NES) dependent. However, to our knowledge, MBP does not contain NES, indicating LMB affects MBP indirectly, i.e., via another protein. In that regard, it is crucial to identify the interaction partners of nuclear MBP to unravel the underlying mechanism of the shuttling process. Our preliminary data suggest a direct interaction between e-MBP and p27 under proliferating conditions, whereas e-MBP exits the nucleus before p27. Furthermore, the involvement of post-translational modifications of MBP, such as phosphorylation, in the shuttling , as has been reported for other nuclear proteins [44], will also be of interest for future research.

Netherlandse Samenvatting

Oligodendrocyten (OLGs) zijn cellen van het centrale zenuwstelsel die gespecialiseerd zijn in het maken van myeline, een vetachtig isolerend laagje van membranen rondom axonen. Dit myeline maakt een sprongsgewijze, en daardoor snelle en efficiënte, zenuwgeleiding mogelijk. Om het myeline-vormend stadium te bereiken moeten voorlopercellen van oligodendrocyten (OPCs) een reeks van nauwkeurig gereguleerde ontwikkelingsstadia en morfologische veranderingen ondergaan. Tijdens de vroege ontwikkeling vermenigvuldigen ('prolifereren') en verplaatsen ('migreren') de OPCs zich, eigenschappen die verdwijnen tijdens de verdere ontwikkeling ('differentiatie'), welke uiteindelijk zal leiden tot de vorming ('biogenese') van myeline [1]. Lokale en tijdelijk aanwezige signaalmoleculen in de extracellulaire omgeving dragen bij aan deze strak gereguleerde uitrijping van OPCs [2]. Bij afbraak van myeline ('demyelinisatie') zal de machinerie voor myelinisatie opnieuw in werking moeten worden gezet, omdat alleen OPCs en niet volwassen OLGs in staat zijn om de 'kale' axonen opnieuw te omhullen met een functioneel laagje myeline ('remyelinisatie') [3,4]. In het geval van de ziekte multiple sclerosis (MS) is de (re)myelinisatie machinery echter ontregeld. Het verlies aan OLGs en de aanwezigheid van latente OPCs die niet kunnen differentiëren, leiden tot permanente demyelinisatie in MS en uiteindelijk als secundair effect tot het verlies van axonen [3,5]. Het falen van remyelinisatie in MS is waarschijnlijk het gevolg van veranderingen in de externe omgeving van de aangetaste gebieden ('laesies'), onder andere vanwege de blijvende aanwezigheid van normaliter tijdelijke signaalmoleculen, zoals pro-inflammatoire cytokines, waaronder TNFα [6,7], en extracellulaire matrix (ECM) moleculen, zoals fibronectine [8,9] (**hoofdstuk 1**). Om het onderliggende mechanisme(n) van het falen van remyelinisatie op te helderen, is uitgebreide kennis van de werking van de myelinisatie machinerie vereist. Om onze kennis van OLGs en myelinisatie te kunnen verbeteren is het cruciaal om de precieze rol van structurele myeline lipiden en eiwitten te ontrafelen, alsmede de werking van de extracellulaire omgeving zoals de ECM en oplosbare signalen. Dit was dan ook de focus van de studies zoals deze beschreven zijn in dit proefschrift.

Tijdens de differentiatie synthetiseren OLGs myeline eiwitten en lipiden in een nauwgezette volgorde. Door de relatief hoge lipide-eiwit verhouding (70:30) is de samenstelling van myeline membranen, in vergelijking tot andere membranen, uniek [10,11]. Bijna een derde van de myeline lipide fractie bestaat uit de galactolipiden galactosylceramide (GalC) en sulfatide. Daarnaast brengen OLGs myeline-specifieke eiwitten tot expressie, waarvan proteolipid protein (PLP) en myeline

basic protein (MBP) de voornaamste zijn. OLGs zijn gepolariseerde cellen en maken gebruik van verschillende transport routes om eiwitten en lipiden naar de juiste membraandomeinen te vervoeren. Transport naar de groeiende myeline membranen verloopt via een basolaterale route [10,12]. Daarnaast is ook de rangschikking van myeline eiwitten en lipiden in het membraan cruciaal voor het vormen van functioneel myeline en het bereiken van sprongsgewijze zenuwgeleiding. Zo zijn de galactolipiden belangrijke componenten van microdomeinen, de zogenaamde 'lipid rafts', in het membraan, welke een belangrijke rol spelen in de ontwikkeling van OLGs [13]. Bovendien is de aanwezigheid van specifieke myeline eiwitten in deze membraan microdomeinen essentieel voor myeline vorming [10, **hoofdstuk 3,4**]. Al deze aspecten en de voortdurende reorganisatie van myeline suggereren dat myeline membranen dynamisch zijn. Zo kan de rangschikking of plaatselijke oppervlakte expressie van myeline lipiden de vloeibaarheid van de membranen veranderen, wat vervolgens leidt tot veranderingen in de laterale organisatie en mobiliteit van myeline eiwitten. Om meer gedetailleerd inzicht te krijgen in de dynamiek van myeline eiwitten enerzijds in het plasma membraan van het cellichaam, en anderzijds in het myeline membraan, kan het gebruik van niet-invasieve optische microscopische technieken, zoals fluorescentie correlatie spectroscopie (FCS) en rasterafbeelding correlatie spectroscopie (RISC) nuttig zijn [15-17]. In **hoofdstuk 2** hebben we inzicht gegeven in hoe deze biofysische technieken op het gebied van myeline kunnen worden toegepast. Verder is de beschikbaarheid van verschillende modelsystemen beschreven, zoals OLG cellijnen en modelmembraan systemen, bijvoorbeeld grote en zeer grote unilamellaire blaasjes (LUVs/GUVs [18,19]. Dit soort modelmembranen kunnen worden samengesteld met een minimum aan elementen zoals lipiden alleen of een geselecteerde set van lipiden en eiwitten, rekening houdend met de juiste fysiologische verhoudingen. Op deze manier kan antwoord worden gekregen op lipide-specifieke vragen. Zo kan bijvoorbeeld het effect van de lengte van de vetzuurketens van galactolipiden op de vorming van membraan microdomeinen worden onderzocht [20] of informatie worden verkregen over specifieke interacties tussen myeline eiwitten en lipiden [21].

Het myeline eiwit PLP is een essentieel eiwit voor het in stand houden van de integriteit van myeline, omdat het de toenadering van de buitenzijden van de lipide bilaag, dus van de twee verschillende omwindingen, faciliteert [22,23]. PLP wordt via vesiculair transport naar het myeline membraan getransporteerd, en zoals weergeven in **hoofdstuk 3**, is dit transport in lijn met het gepolariseerd karakter van OLGs gereguleerd; het myeline membraan is het doelwit van basolateraal transport, terwijl het plasma membraan van het cellichaam het doelwit is van apicale transport mechanismen[10,24,25]. We hebben aangetoond dat PLP,

voorafgaand aan de inbouw in CHAPS-resistente membraan microdomeinen in het myeline membraan, eerst wordt getransporteerd naar het plasma membraan van het cellichaam via een syntaxin-3 (t-SNARE)-afhankelijke route, waarbij het is ingebouwd in TX-100-resistente microdomeinen. De resultaten laten verder een sulfatide-gemedieerde verschuiving van PLP van TX-100-resistente membraan microdomeinen naar CHAPS-resistente membraan microdomeinen op het celoppervlak zien. De apicale-basolaterale transcytotische route van PLP naar het myeline membraan kan worden nagebootst in de gepolariseerde levercellijn HepG2. Tevens hebben wij kunnen aantonen dat de conformatie van de tweede extracellulaire lus van PLP verandert in aanwezigheid van sulfatide.

Na de rol van sulfatide in het transcytotische transport van PLP en de verandering in de laterale organisatie van PLP in het membraan te hebben vastgesteld, hebben we in **hoofdstuk 4** naar een mogelijk verband gezocht tussen deze laterale organisatie en het dynamisch gedrag van PLP. Een extractie studie met CHAPS in een OLG cellijn, OLN-93, waarin een selectieve expressie van GalC alleen en GalC en sulfatide mogelijk is, is gebleken dat na een transiente transfectie met PLP, sulfatide de CHAPS onoplosbaarheid van PLP verhoogd. Tevens werd een sulfatide-gemedieerde afname van de laterale beweeglijkheid van PLP gedetecteerd. Een vergelijkbare sulfatide-geïnduceerde toename in de associatie met CHAPS-resistente membraan microdomeinen, samen met een verminderde beweeglijkheid van PLP in het membraan, werd ook waargenomen wanneer de cellen werden gekweekt op laminine-2, een fysiologisch ECM substraat dat myelinisatie stimuleert en tevens bindingsplaatsen voor sulfatide bevat [26–29] . Daarentegen bleek PLP op fibronectine, een pathologisch ECM substraat dat de vorming van myeline membranen remt en remyelinisatie in MS laesies [8,9,30] schaadt, niet aanwezig te zijn in deze CHAPS-resistente membraan microdomeinen, wat gepaard ging met een toename van de laterale beweeglijkheid van PLP.

In **hoofdstuk 4** werd tevens de laterale organisatie en de mobiliteit van een andere belangrijke myeline eiwit, 18,5 kDa MBP, in het membraan onderzocht. De aanwezigheid van GalC, maar niet van sulfatide, verhoogde de aanwezigheid van 18,5 kDa MBP in CHAPS-resistente membraan microdomeinen, wat correleerde met een verhoging van de laterale beweeglijkheid van het eiwit. In tegenstelling tot PLP bleek de laterale mobiliteit van 18,5 kDa MBP niet te verschillen in cellen die gekweekt werden op laminine-2 en fibronectine. Inderdaad, eerdere studies hebben aangetoond dat de laterale mobiliteit van MBP in myeline waarschijnlijk gereguleerd wordt door oplosbare signalen [31]. Daarom zullen tijdens pathologische condities oplosbare signalen in de extracellulaire omgeving, en niet ECM eiwitten, de laterale organisatie van MBP beïnvloeden.

In MS laesies is de expressie van pro-inflammatoire cytokines, zoals TNFα, verhoogd [32]. In deze context werd in **hoofdstuk 5** het effect van TNFα op MBP in myeliniserende kweken onderzocht. Verrassenderwijs bleek dat blootstelling aan relatief lage hoeveelheden van TNFα de lengte van de myeline segmenten kleiner maakte. In OLG monokweken kwam dit tot uiting in een duidelijke en omkeerbare herverdeling in de lokalisatie van MBP, dat wil zeggen van myeline membranen naar primaire uitlopers. Dit werd gemedieerd door TNF receptor 1 (TNFR1). De overleving van de OLG, de hoeveelheid aan MBP eiwit en RNA, en de lokalisatie van MBP mRNA bleven onveranderd na behandeling met TNFα. Onze resultaten laten verder zien dat de TNFα-gemedieerde herverdeling van MBP gerelateerd was aan een dysorganisatie van het actine cytoskelet, wat gepaard ging met een verschuiving van MBP van actine-afhankelijke naar actine-onafhankelijke membraan microdomeinen. Dit verstoort waarschijnlijk de barrière functie van MBP aangezien een soortgelijke herverdeling van PLP naar primaire uitlopers werd waargenomen en een herlokalisatie van CNP, een ander OLG specifiek eiwit, in de richting van myeline membranen. Deze bevindingen suggereren dat de permanente aanwezigheid van TNFα het in stand houden van myeline membranen verstoort, op een MBP- en actine-afhankelijke manier.

De MBP eiwit familie omvat verschillende isoformen [33] als gevolg van alternatieve splicing van een MBP transcript dat is gegenereerd uit een uit 11 exonen bestaand gen complex genaamd Golli (Gene in Oligodendrocyte Lineage) [34]. Zo ontbreekt exon-II in de postnatale 14 en 18,5 kDa MBP isoformen. Deze isoformen bevinden zich voornamelijk in het myeline membraan waar ze een rol spelen in de ‘verdichting’ van het myeline membraan aan de cytoplasmatische zijde en daarnaast ook optreden als een moleculaire barrière voor de opname van eiwitten met grote cytoplasmatische staarten in het myeline membraan [34,35]. Daarentegen zijn de postnatale exon-II positieve 17 en 21,5 kDa MBP isoformen gelokaliseerd in de kern en het cytoplasma [36,37]. De expressie van deze isoformen piekt tijdens de vroege ontwikkeling van OLGs, maar de functie van deze isoformen is echter nog onduidelijk. Daarom is in **hoofdstuk 6** de functie van deze exon-II bevattende isoformen onderzocht. Hiertoe is gebruik gemaakt van de galactolipide deficiënte OLN-93 cellen die niet in staat zijn om postnatale MBP isoformen te produceren. Uit de resultaten bleek dat OLN-93 cellen wel een MBP isoform van ongeveer 16 kDa tot expressie brengen, welke wij geïdentificeerd hebben als een exon-II positieve embryonale isoform van MBP (e-MBP). e-MBP verschijnt in een vergelijkbaar lokalisatie patroon als postnatale exon-II positieve MBP isoformen; voornamelijk in de kern en het cytoplasma. Als de proliferatie van de cellen werd geremd, werd e-MBP uitgesloten van de kern, terwijl bij het herstel van proliferatie e-MBP weer terugkeert in de kern.

Dit suggereert een actief pendelen tussen het cytoplasma en de kern als reactie op proliferatie. Opvallend is dat e-MBP ook tot expressie komt in niet-OLG cellijnen, zoals HepG2, HeLa en HEK293 cellen. Direct bewijs voor een rol van e-MBP in cel proliferatie werd verkregen na downregulatie van MBP middels shRNA, waardoor de proliferatie in alle geteste cellijnen minder werd. Bovendien lieten imaging studies met levende cellen en FRAP (fluorescence recovery after photobleaching)-analyse met fluorescent-gelabeld exon-II positief postnataal 21,5 kDa MBP zien, dat onder prolifererende condities 21,5 kDa MBP hoofdzakelijk in de kern was gelokaliseerd, terwijl het werd uitgesloten van de kern als de proliferatie werd geremd. Leptomycin-B (LMB), een remmende stof voor het transport van eiwitten uit de kern, verhinderde ook de export van MBP uit de kern. Kortom evenals e-MBP pendelt 21,5 kDa MBP actief tussen het cytoplasma en de kern als reactie op mitogene modulatie. Waarschijnlijk zijn de exon-II bevattende MBP isoformen cruciale spelers in de proliferatie van cellen tijdens de embryonale ontwikkeling en na geboorte voor OPCs.

Kortom, het werk zoals beschreven in dit proefschrift heeft nieuw inzicht verschaft in mechanismen die verband houden met de aanmaak van myeline, en laten een belangrijke rol voor de myeline eiwitten PLP en MBP en de typische myeline galactolipiden, GalC en sulfatide zien. Verder is in dit proefschrift bewijs geleverd dat gedetailleerde kennis van de associatie van myeline eiwitten met membraan microdomeinen belangrijke nieuwe inzichten geeft in de vorming van myeline membranen tijdens gezonde en ziekte-gerelateerde omstandigheden. De verkregen kennis draagt bij aan het beter begrijpen waarom de (her)aanmaak en het onderhoud van myeline faalt in MS, en biedt daardoor perspectief voor het verder ontwikkelen van therapeutische mogelijkheden voor een ziekte waarvan het ontstaan nog grotendeels onbekend is.

References

[1] S.E. Pfeiffer, A.E. Warrington, R. Bansal, The oligodendrocyte and its many cellular processes, Trends Cell Biol. 3 (1993) 191–197.

[2] D. Clemente, M.C. Ortega, C. Melero-Jerez, F. de Castro, The effect of glia-glia interactions on oligodendrocyte precursor cell biology during development and in demyelinating diseases, Front. Cell. Neurosci. 7 (2013).

[3] R.J.M. Franklin, C. ffrench-Constant, Remyelination in the CNS: from biology to therapy, Nat. Rev. Neurosci. 9 (2008) 839–855.

[4] K.M. Young, K. Psachoulia, R.B. Tripathi, S.-J. Dunn, L. Cossell, D. Attwell, et al., Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling, Neuron. 77 (2013) 873–885.

[5] A. Compston, A. Coles, Multiple sclerosis, Lancet. 372 (2008) 1502–1517.

[6] D.S. Titelbaum, A. Degenhardt, R.P. Kinkel, Anti-Tumor Necrosis Factor Alpha-Associated Multiple Sclerosis, Am. J. Neuroradiol. 26 (2005) 1548–1550.

[7] T.B. Martins, J.W. Rose, T.D. Jaskowski, A.R. Wilson, D. Husebye, H.S. Seraj, et al., Analysis of proinflammatory and anti-inflammatory cytokine serum concentrations in patients with multiple sclerosis by using a multiplexed immunoassay, Am. J. Clin. Pathol. 136 (2011) 696–704.

[8] J.M.J. Stoffels, J.C. de Jonge, M. Stancic, A. Nomden, M.E. van Strien, D. Ma, et al., Fibronectin aggregation in multiple sclerosis lesions impairs remyelination, Brain J. Neurol. 136 (2013) 116–131.

[9] J.M.J. Stoffels, C. Zhao, W. Baron, Fibronectin in tissue regeneration: timely disassembly of the scaffold is necessary to complete the build, Cell. Mol. Life Sci. CMLS. 70 (2013) 4243–4253.

[10] W. Baron, D. Hoekstra, On the biogenesis of myelin membranes: sorting, trafficking and cell polarity, FEBS Lett. 584 (2010) 1760–1770.

[11] S. Aggarwal, L. Yurlova, M. Simons, Central nervous system myelin: structure, synthesis and assembly, Trends Cell Biol. 21 (2011) 585–593.

[12] N. Snaidero, W. Möbius, T. Czopka, L.H.P. Hekking, C. Mathisen, D. Verkleij, et al., Myelin membrane wrapping of CNS axons by PI(3,4,5)P3-dependent polarized growth at the inner tongue, Cell. 156 (2014) 277–290.

[13] N. Jackman, A. Ishii, R. Bansal, Oligodendrocyte Development and Myelin Biogenesis: Parsing Out the Roles of Glycosphingolipids, Physiology. 24 (2009) 290–297.

[14] O. Maier, W. Baron, D. Hoekstra, Reduced raft-association of NF155 in active MS-lesions is accompanied by the disruption of the paranodal junction, Glia. 55 (2007) 885–895.

[15] E. Gielen, J. Vercammen, J. Sýkora, J. Humpolickova, M. Vandeven, A. Benda, et al., Diffusion of sphingomyelin and myelin oligodendrocyte glycoprotein in the membrane of OLN-93 oligodendroglial cells studied by fluorescence correlation spectroscopy, C. R. Biol. 328 (2005) 1057–1064.

[16] E. Gielen, N. Smisdom, M. vandeVen, B. De Clercq, E. Gratton, M. Digman, et al., Measuring diffusion of lipid-like probes in artificial and natural membranes by raster image correlation spectroscopy (RICS): use of a commercial laser-scanning microscope with analog detection, Langmuir ACS J. Surf. Colloids. 25 (2009) 5209–5218.

[17] E. Gielen, N. Smisdom, B. De Clercq, M. Vandeven, R. Gijsbers, Z. Debyser, et al., Diffusion of myelin oligodendrocyte glycoprotein in living OLN-93 cells investigated by raster-scanning image correlation spectroscopy (RICS), J. Fluoresc. 18 (2008) 813–819.

[18] N. Kahya, D. Scherfeld, P. Schwille, Differential lipid packing abilities and dynamics in giant unilamellar vesicles composed of short-chain saturated glycerol-phospholipids, sphingomyelin and cholesterol, Chem. Phys. Lipids. 135 (2005) 169–180.

[19] M.J. Hope, M.B. Bally, G. Webb, P.R. Cullis, Production of large unilamellar vesicles by a rapid extrusion procedure: characterization of size distribution, trapped volume and ability to maintain a membrane potential, Biochim. Biophys. Acta. 812 (1985) 55–65.

[20] S.N. Pinto, L.C. Silva, A.H. Futerman, M. Prieto, Effect of ceramide structure on membrane biophysical properties: The role of acyl chain length and unsaturation, Biochim. Biophys. Acta BBA - Biomembr. 1808 (2011) 2753–2760.

[21] J.M. Boggs, P.M. Yip, G. Rangaraj, E. Jo, Effect of Posttranslational Modifications to Myelin Basic Protein on Its Ability to Aggregate Acidic Lipid Vesicles†, Biochemistry (Mosc.). 36 (1997) 5065–5071.

[22] J.M. Greer, M.B. Lees, Myelin proteolipid protein--the first 50 years, Int. J. Biochem. Cell Biol. 34 (2002) 211–215.

[23] M. Bakhti, N. Snaidero, D. Schneider, S. Aggarwal, W. Möbius, A. Janshoff, et al., Loss of electrostatic cell-surface repulsion mediates myelin membrane adhesion and compaction in the central nervous system, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 3143–3148.

[24] K. Trajkovic, A.S. Dhaunchak, J.T. Goncalves, D. Wenzel, A. Schneider, G. Bunt, et al., Neuron to Glia Signaling Triggers Myelin Membrane Exocytosis from Endosomal Storage Sites, J. Cell Biol. 172 (2006) 937–948.

[25] R. White, E.-M. Krämer-Albers, Axon-glia interaction and membrane traffic in myelin formation, Front. Cell. Neurosci. 7 (2014) 284.

[26] P.C. Buttery, C. ffrench-Constant, Laminin-2/integrin interactions enhance myelin membrane formation by oligodendrocytes, Mol. Cell. Neurosci. 14 (1999) 199–212.

[27] S.J. Chun, M.N. Rasband, R.L. Sidman, A.A. Habib, T. Vartanian, Integrin-linked kinase is required for laminin-2–induced oligodendrocyte cell spreading and CNS myelination, J. Cell Biol. 163 (2003) 397–408.

[28] D.D. Roberts, C.N. Rao, J.L. Magnani, S.L. Spitalnik, L.A. Liotta, V. Ginsburg, Laminin binds specifically to sulfated glycolipids, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 1306–1310.

[29] H. Colognato, P.D. Yurchenco, Form and function: the laminin family of heterotrimers, Dev. Dyn. Off. Publ. Am. Assoc. Anat. 218 (2000) 213–234.

[30] Z. Sisková, V.W. Yong, A. Nomden, M. van Strien, D. Hoekstra, W. Baron, Fibronectin attenuates process outgrowth in oligodendrocytes by mislocalizing MMP-9 activity, Mol. Cell. Neurosci. 42 (2009) 234–242.

[31] D. Fitzner, A. Schneider, A. Kippert, W. M|[ouml]|bius, K.I. Willig, S.W. Hell, et al., Myelin basic protein-dependent plasma membrane reorganization in the formation of myelin, EMBO J. 25 (2006) 5037–5048.

[32] A. Caminero, M. Comabella, X. Montalban, Tumor necrosis factor alpha (TNF-α), anti-TNF-α and demyelination revisited: an ongoing story, J. Neuroimmunol. 234 (2011) 1–6.

[33] J.M. Boggs, Myelin basic protein: a multifunctional protein, Cell. Mol. Life Sci. CMLS. 63 (2006) 1945–1961.

[34] G. Harauz, J.M. Boggs, Myelin management by the 18.5-kDa and 21.5-kDa classic myelin basic protein isoforms, J. Neurochem. 125 (2013) 334–361.

[35] S. Aggarwal, L. Yurlova, N. Snaidero, C. Reetz, S. Frey, J. Zimmermann, et al., A size barrier limits protein diffusion at the cell surface to generate lipid-rich myelin-membrane sheets, Dev. Cell. 21 (2011) 445–456.

[36] E. Barbarese, J.H. Carson, P.E. Braun, Accumulation of the four myelin basic proteins in mouse brain during development, J. Neurochem. 31 (1978) 779–782.

[37] L. Pedraza, Nuclear transport of myelin basic protein, J. Neurosci. Res. 50 (1997) 258–264.

[38] J. van Horssen, C.D. Dijkstra, H.E. de Vries, The extracellular matrix in multiple sclerosis pathology, J. Neurochem. 103 (2007) 1293–1301.

[39] B.N. Marbois, K.F. Faull, A.L. Fluharty, S. Raval-Fernandes, L.H. Rome, Analysis of sulfatide from rat cerebellum and multiple sclerosis white matter by negative ion electrospray mass spectrometry, Biochim. Biophys. Acta. 1484 (2000) 59–70.

[40] R.W.R. Baker, H. Sanders, R.H.S. Thompson, K.J. Zilkha, Serum cholesterol linoleate levels in multiple sclerosis, J. Neurol. Neurosurg. Psychiatry. 28 (1965) 212–217.

[41] C.E. Teunissen, C.D. Dijkstra, C.H. Polman, E.L.J. Hoogervorst, K. von Bergmann, D. Lütjohann, Decreased levels of the brain specific 24S-hydroxycholesterol and cholesterol precursors in serum of multiple sclerosis patients, Neurosci. Lett. 347 (2003) 159–162.

[42] D.F. Legler, O. Micheau, M.-A. Doucey, J. Tschopp, C. Bron, Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation, Immunity. 18 (2003) 655–664.

[43] J.R. Muppidi, J. Tschopp, R.M. Siegel, Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction, Immunity. 21 (2004) 461–465.

[44] N. Ishida, T. Hara, T. Kamura, M. Yoshida, K. Nakayama, K.I. Nakayama, Phosphorylation of p27Kip1 on serine 10 is required for its binding to CRM1 and nuclear export, J. Biol. Chem. 277 (2002) 14355–14358.

Abbreviations

Approx, approxemately
BC, bile canaliculus
BSA, bovine serum albümin
BrdU, 5-Bromo-2'-deoxy-uridine
cAMP, Cyclic adenosine monophosphate
CDK, cyclin-dependent kinase
CGT, ceramide glucosyltransferase
CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate
CNP, 2', 3'-cyclic-nucleotide 3'-phosphodiesterase
CNS, central nervous system;
CRM1, chromosome region maintenance1;
CN, cytoplasm nucleus;
CSPGs, chondroitin sulfate proteoglycans
CST, cerebroside sulfotransferase
Ctrl, control
DIV, days in vitro
FCS, fetal calf serum;
FCS, fluorescence correlation spectroscopy;
FCCS, fluorescence cross-correlation spectroscopy;
FGF, fibroblast growth factor;
Fn, fibronectin
FRAP, fluorescence recovery after photobleaching;
FRET, Förster resonance energy transfer
e-MBP, embryonic myelin basic protein;
EM, electron microscopy
ECM, extracellular matrix
GalC, galactosylceramide
GD3, ganglioside 3
GFP, green fluorescent protein;
Golli, gene in the oligodendrocyte lineage;
GUVs, giant unilamellar vesicles;
GSL, glycosphingolipids
HD, high density;
IFNγ, interferon gama
IGF-1, insulin like growth factor 1
IL1β, interleukin 1 beta
IPL, intraperiod line
LD, low density;
Ld, liquid disordered
LDH, lactate dehydrogenase
LMB, leptomycin B;
Ln2, laminin-2
Lo, liquid ordered
LUVs, large unilamellar vesicles
mAb, monoclonal antibody;
MAG, myelin associated glycoprotein
MBP, myelin basic protein;
MDCK, Madin-Darby canine kidney
MOG, myelin oligodendrocyte glycoprotein;
MS, multiple sclerosis
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NC, nucleus cytoplasm;
NES, nuclear export signal;
NF155, neurofascin155
NGS, normal goat serum
N&B, number and brightness analyses
OLGs, oligodendrocytes
OPC, oligodendrocyte progenitor cell;
pAb, polyclonal antibody;
PCH, photon counting histogram
PBS, phosphate-buffered saline;
PDGF, platelet-derived growth factor;
PIE-FI, pulsed interleaved excitation fluctuation imaging;
PFA, paraformaldehyde;
PNS, post-nuclear supernatant
PSF, point spread function
RER, rough endoplasmic reticulum
RICS, raster image correlation spectroscopy
PI, phosphatidylinositol
PIP2, phosphatidylinositol 4,5-Biphosphate
PLL, poly-L-lysine;
PLP, proteolipid protein
PMD, Pelizaeus-Merzbacher disease
PS, phosphatidylserine
RFP, red fluorescent protein;
RT, room temperature;
SD, standard deviation
SEM, standard error of the mean
SF, serum-free
s-FCS, scanning fluorescence correlation spectroscopy
SPLSs, supported bilayers
sv-FCS, spot variation fluorescence correlation spectroscopy
S3, syntaxin 3
qPCR, Real-Time quantitative polymerase chain reaction
TLC, thin layer chromatography
TNF1, TNF receptor 1
TNFα, tumor necrosis factor α
2C1D, 2 components 1 dimension;
2C2D, 2 components 2 dimensions

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