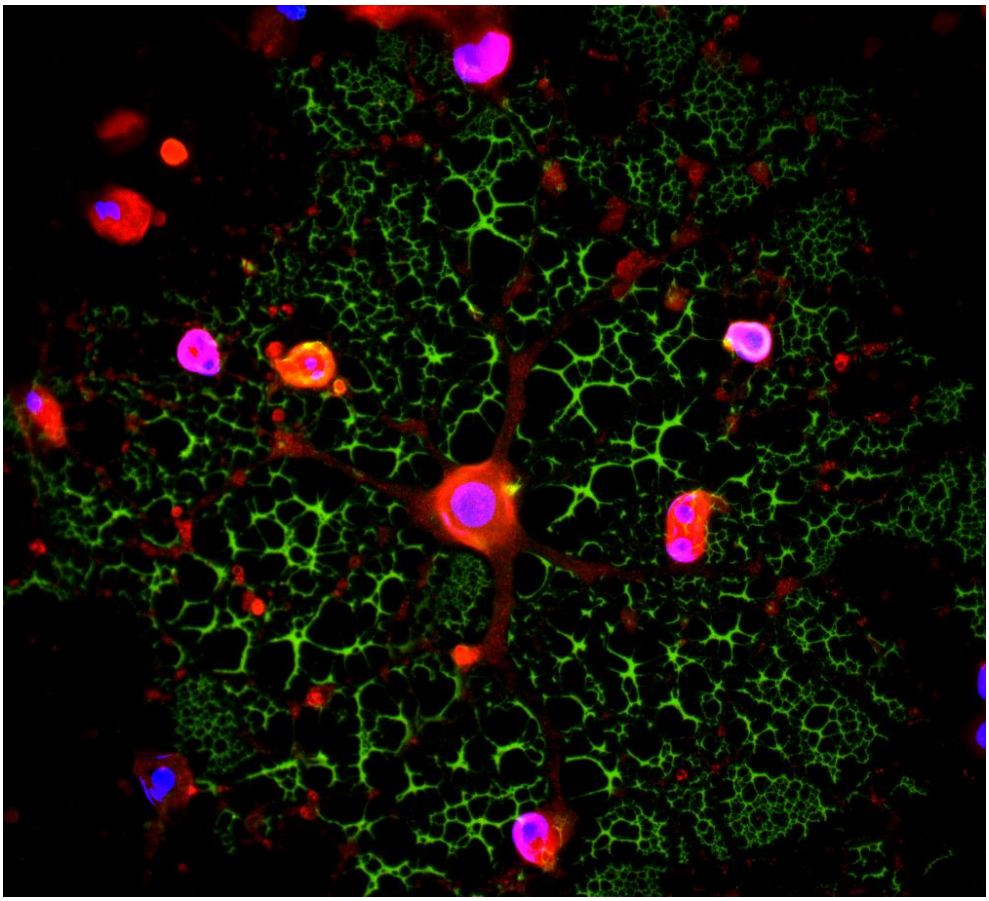
Extracellular factors regulating MBP expression in oligodendrocytes

And how it's deregulated in multiple sclerosis



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* **Figure on front page:** Oligodendrocyte *in vitro* stained for MBP and MBP-mRNA. Green: MBP, red: MBP-mRNA, blue: nucleus. MBP-mRNA is visible in the processes and MBP expression in the myelin-like membranes.

Abstract

Multilayered myelin sheaths surround axons in the CNS and are required for saltatory conduction. Persistent disturbance of myelin formation leads to several brain diseases, including multiple sclerosis (MS). In MS, an inflammation-mediated event results in demyelination, and ultimate failure of remyelination and axonal loss cause disease progression. The myelin sheath extends from processes of oligodendrocytes. The most prominent and crucial protein in myelin biogenesis is myelin basic protein (MBP). MBP facilitates compaction by stabilizing the inner leaflets of the membranes. To prevent ectopic expression, MBP is transported to the processes as mRNA in specific granules where it is translated 'on site'. Myelin biogenesis is exogenous regulated by many inhibiting or stimulating factors that can be divided in contact-dependent factors and soluble factors. In the present report, these factors are described in association to MBP expression. The regulation during developmental myelination is compared with the regulation in MS lesions, followed by suggestions for therapeutic targets that aim for enhanced and correct MBP expression and thereby remyelination in MS.

Introduction

Oligodendrocytes (OLG) are the cells that myelinate multiple axons of neurons in the central nervous system (CNS). Myelin facilitates saltatory conduction along axons and provides axonal protection. Myelin sheaths extend from OLG processes, enwrap axons several times, and contain hundreds of proteins and lipids. The most prominent proteins present in myelin are myelin basic protein (MBP) and proteolipid protein (PLP). The presence of MBP is crucial for myelin formation, whereas absence of PLP results in less stable myelination of axons (over time) (1).

MBP is a highly adhesive protein facing the cytosolic membrane surface, where it facilitates myelin membrane compaction. Premature expression of MBP will likely result in premature compaction at unwanted sites. Therefore, MBP mRNA is transported to the processes where it is translated locally (1). Furthermore, MBP mRNA transport allows local protein synthesis and in addition effectively provides a way to rapid respond to external stimuli (2). In fact, a single mRNA can give rise to multiple copies of a protein, particularly effective for cells with distinct membrane domains separated by large distances.

Before OLG can form myelin sheaths, OLG precursor cells (OPC) have to differentiate to pre-myelinating oligodendrocytes and then into myelin producing cells. These transitions are regulated and marked by the appearance or disappearance of specific proteins and lipids, both in time and order. Together with the fact that not all axons are myelinated simultaneously, this makes myelin sheath formation a highly regulated event. Moreover, persistent perturbation of myelin formation leads to several brain diseases, including multiple sclerosis (MS). In MS, an inflammation mediated event results in demyelination, and ultimate failure of remyelination and axonal loss cause disease progression. Within MS lesions the recruitment of OPC appears to be normal (1, 3). This suggests that remyelinating failure in MS lies within the differentiation of OLG, which is inextricably linked to the expression of MBP, i.e. transport and ‘on site’ translation of MBP mRNA.

OLG differentiation is regulated by all kinds of factors, i.e., signals derived from astrocytes, axons or other environmental cues. It is not well known, which factors or missing factors cause remyelination to be insufficient in MS. Since MBP is crucial for myelin biogenesis, it is interesting to know how MBP mRNA transport and translation is regulated by intracellular and extracellular factors. The focus will lie, however, on the exogenous regulation of this protein, and how this might be misregulated in MS lesions.

MBP expression during developmental myelination

A prerequisite for remyelination is that an OPC has to be recruited to the lesion site where remyelination is needed, followed by differentiation to a pre-myelinating OLG. The pre-myelinating OLG is recognized by the prominent expression of galactosylceramide (GalCer), a galactosphingolipid that is particularly enriched in myelin. After the pre-myelinating state, an OLG further mature into a stage in

which myelin sheaths are formed, as presented schematically in figure 1. Mature OLG abundantly express myelin specific proteins including the most prominent ones, MBP and PLP.

Although myelin membranes are continuous with the cell body, the composition of the plasma membrane surrounding the cell body and primary processes differ significantly, indicating that OLG are polarized cells. This polarized character is also reflected by a different content between compact and noncompact myelin, the latter representing merely paranodal regions (4).

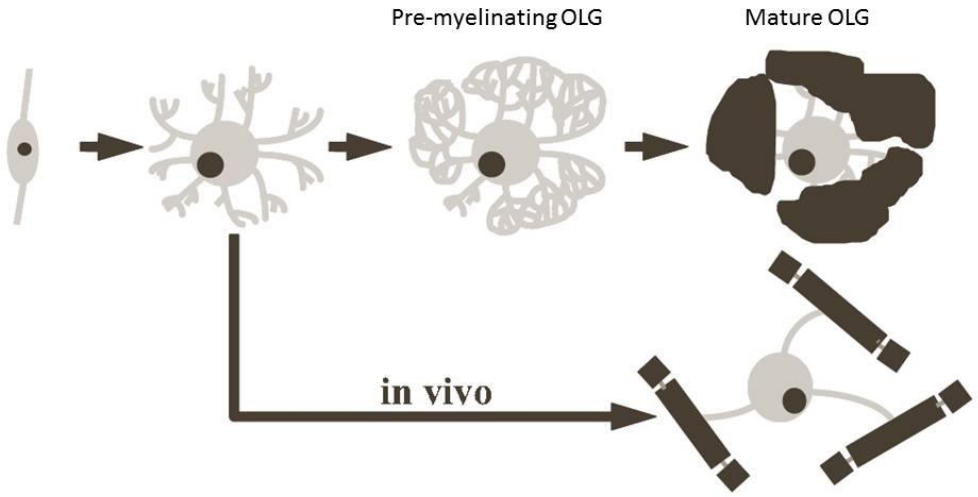

Figure 1. Schematic development of an OLG *in vitro*. An OLG develops from a bipolar stage into a complex network with multiple processes. The pre-myelinating state is recognized by the expression of GalCer, the mature OLG forms myelin-like membraness (black) and abundantly expresses the myelin specific proteins MBP and PLP. The OLG *in vivo* enwraps multiple axons. (adapted from Maier et al. 2005 (5))

PLP and MBP are required for the formation of compact myelin. PLP is transported in vesicles to the myelin sheaths, where it stabilizes the outer cellular leaflets of the multilayered membrane upon compaction (1). MBP facilitates compaction by stabilizing the inner leaflets of the membranes. To avoid “glue-ing” of intracellular membranes, and to prevent “premature compaction”, MBP is present in the myelin sheaths in mature OLG as a consequence of mRNA translation ‘on site’. Thus the transport of mRNA transport consists of export from the nucleus, assembly into transport granules, trafficking and anchoring to the target site. The final step is the activation of translation (1) that leads to MBP expression. Notably, MBP mRNA consists of different isoforms that are distinguished by the exclusion or inclusion of exon 2. The mRNAs lacking exon 2 are transported to the myelin membrane, the mRNA containing exon 2 localizes to the nucleus and cell body cytoplasm (1). Importantly, MBP is the only indispensable factor crucial for myelin membrane synthesis, therefore correct transport and translation is a requisite.

In the nucleus, MBP pre-mRNAs are sorted by binding of a subset of heterogenous nuclear ribonucleoproteins (hnRNPs), resulting in determination of the mRNA’s fate, i.e., the location in the

cytosol, translation efficiency and the level of translation quantity (1). In OLG hnRNP A2 binding consists of a trans-acting factor that binds to a cis-acting factor in the 3'UTR (untranslated region) in the MBP mRNA (6). Analogous to the interaction of transcription factors with DNA promoter elements, these cis-elements in mRNAs are recognized by specific trans-acting factors (i.e. RBPs) that are essential for their transport to cytoplasmic regions (7). More precisely, the binding element in the 3'UTR of MBP is an A2 response element (A2RE), complementary to the hnRNP A2 (figure 2).

Upon binding of 'hnRNP-complex', the mRNA is assembled into a ribonucleoprotein (RNP) complex called 'a granule' and transported in a translationally dormant state. hnRNP E1 binding to hnRNP A2 ensures transport in a translationally silenced state. In contrast to the specific mRNA sorting in the nucleus, granule assembly in the cytoplasm is randomly further assembled (figure 2, step 2). The granule contains besides mRNA and hnRNPs, also the translation machinery and components for protein synthesis (8). Recently new constituents of the granule have been identified hnRNP K and hnRNP F. hnRNP K, a multifunctional protein, binds directly to MBP mRNA and translocates in granules from the nucleus to the myelin sheaths during differentiation. Knockdown of hnRNP K inhibits MBP synthesis during myelination but not the transport of the protein along the primary processes. There was an abnormal accumulation of mRNA at process branch point in hnRNP K knockdown OLGs, which implies that hnRNP K plays a role in the final steps of targeting MBP mRNA to the sheath and in the initiation of local translation (9). White et al. (2011) show that hnRNP F is involved in posttranscriptional regulation of MBP expression. Distinct levels of hnRNP F appear to be required for normal synthesis, as this is affected by knockdown and overexpression of hnRNP F (10).

Transport of the granule along the OLG processes is in a microtubule-kinesin dependent manner. Docking of the granule is poorly understood, but likely the actin cytoskeleton plays an important role in the anchoring. Kinesin motor protein Kif 1b appears to be required to localize MBP mRNA to the processes and to prevent the ectopic production of myelin membranes (11). Thus, zebrafish mutants in the Kif1b results in less myelin surrounding an axon, as mRNA accumulates in the cell body and less mRNA reach the processes. Interestingly, zebrafish Kif1b and human Kif1b show great homology and disruption in this motor protein is associated with a susceptibility to MS (11). Since the granule is not a membrane bound system, it is likely that mechanisms involved in vesicular transport regulation are not involved. However, the SNARE machinery, normally involved in vesicular docking, is also involved in MBP mRNA transport. Thus, when the t-SNARE syntaxin-4, predominantly found in the myelin-like membranes, is overexpressed in primary OLG, MBP mRNA transport and MBP synthesis is blocked (4).

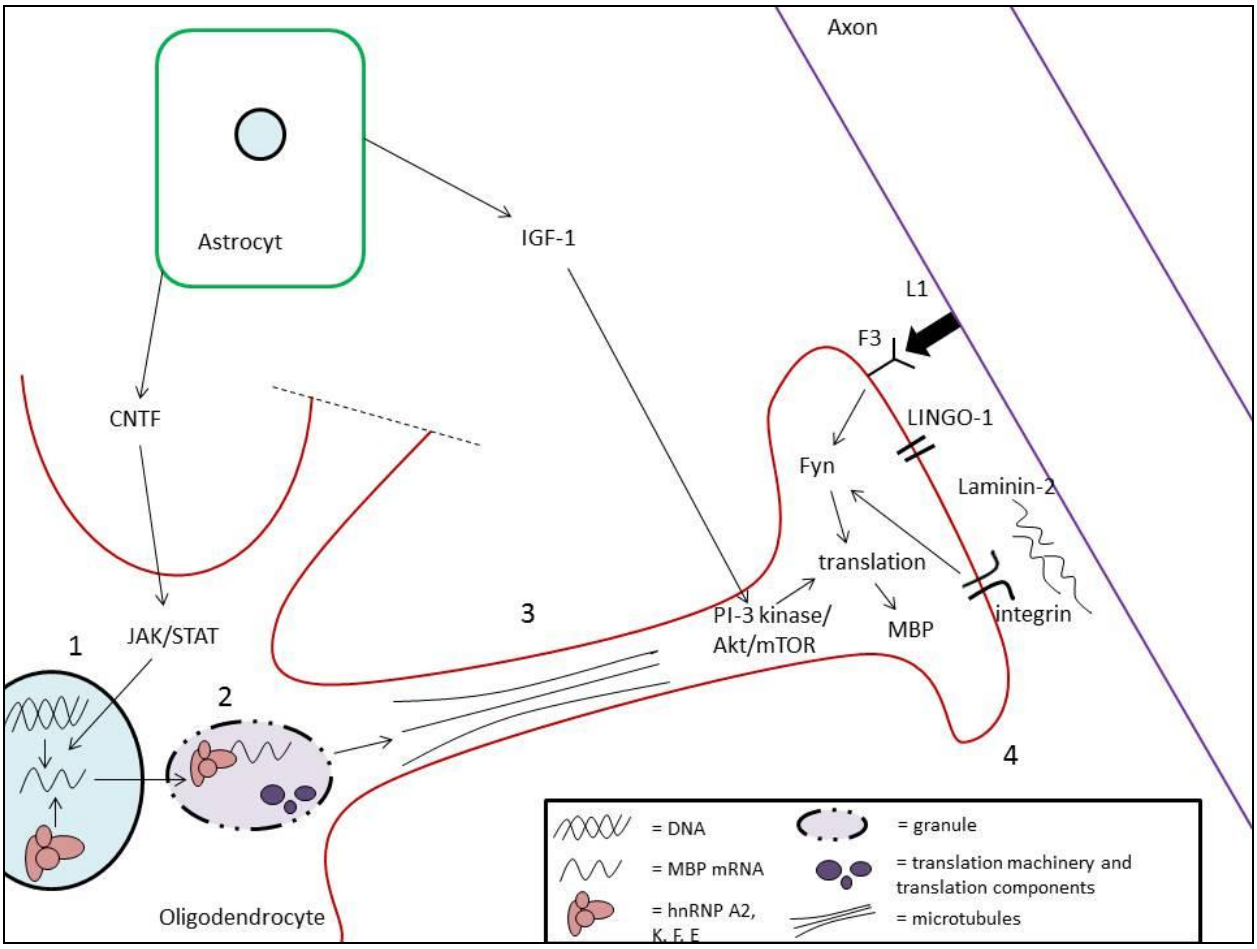

Figure 2. A schematic overview of the extracellular factors involved in MBP expression in oligodendrocytes. Regulation can take place in 4 stadia: (1) transcription of the mbp gene, (2) granule assembly in the cytoplasm, (3) transport of the granule in a microtubule-dependent manner and (4) the translation of the MBP mRNA 'on site'. The central mediator Fyn and the PI-3 kinase/Akt/mTOR pathway also schematically represented and how they are regulated extracellularly by axons and astrocytes.

Once the granule is on the site of action, phosphorylation of hnRNP A2 by Fyn is required for MBP mRNA translation. Activation of the Src-family non-receptor tyrosine kinase Fyn leads to phosphorylation of hnRNP A2, resulting in a conformation change and disconnection of hnRNP E1 and release from the granule, enabling MBP mRNA translation "on site" and immediate incorporation of MBP protein in myelin membranes (10, 12).

Regulation of MBP transport, and hence expression of MBP protein, can take place at many stages (figure 2), i.e., during (1) transcription and nucleus export, (2) granule assembly, (3) granule transport, and (4) anchoring and translation. Since axonal cues influence OLG maturation, including initiation of expression and trafficking of the myelin proteins (1), and given the function of MBP, a regulatory role in MBP transport is anticipated.

Exogenous factors influencing MBP expression during developmental myelination

Myelin biogenesis is exogenous regulated by many inhibiting or stimulating factors, that can be divided in contact-dependent factors and soluble factors. The contact-dependent factors can be subdivided by cell-cell and cell-ECM (extra cellular matrix) interactions. A subset of these regulating factors will subsequently be discussed in relation to MBP expression.

Contact-dependent factors

Myelination is precisely regulated concordant with axonal maturation and together with the fact that not all axons are simultaneously myelinated, makes it seem likely that axon-derived signals regulate myelination (12). Many signals and interactions are known to contribute to the formation of myelin sheaths, but their exact role in MBP transport and expression is largely unknown or are only just explored. A central intracellular mediator in the differentiation of OLG and MBP expression is the Src family non-receptor tyrosine kinase Fyn (figures 2 and 3). Fyn kinase is upregulated in OLG that actively myelinate and mice deficient in the Fyn exhibit severe hypomyelination in the forebrain, underscoring the importance of Fyn in myelination (12). Activated Fyn phosphorylates hnRNP A2 in granules that have reached the axon-OLG contact site. Klein et al. (2002) proposed a role for Fyn kinase in stabilization and recruitment of the microtubules toward the axon-glia site, by binding of tau and tubulin (13). Since granules are transported in a microtubule-mediated manner, local Fyn activation could recruit microtubules that direct MBP mRNA to this location. Furthermore upon Fyn-mediated phosphorlylation of hnRNP A2, hnRNP E1, which is essential for suppressing of MBP mRNA translation, is released, allowing MBP expression. Fyn kinase activation is initiated upon binding of axonal L1 to OLG F3/contactin, ultimately leading to translation of MBP. Interestingly, *in vitro* L1 is down-regulated on myelinated axons, suggesting a role in myelination (14). Futhermore F3 -/- mice show myelination abnormalities (15), further corroboration a functional role of L1-F3 interaction in myelination.

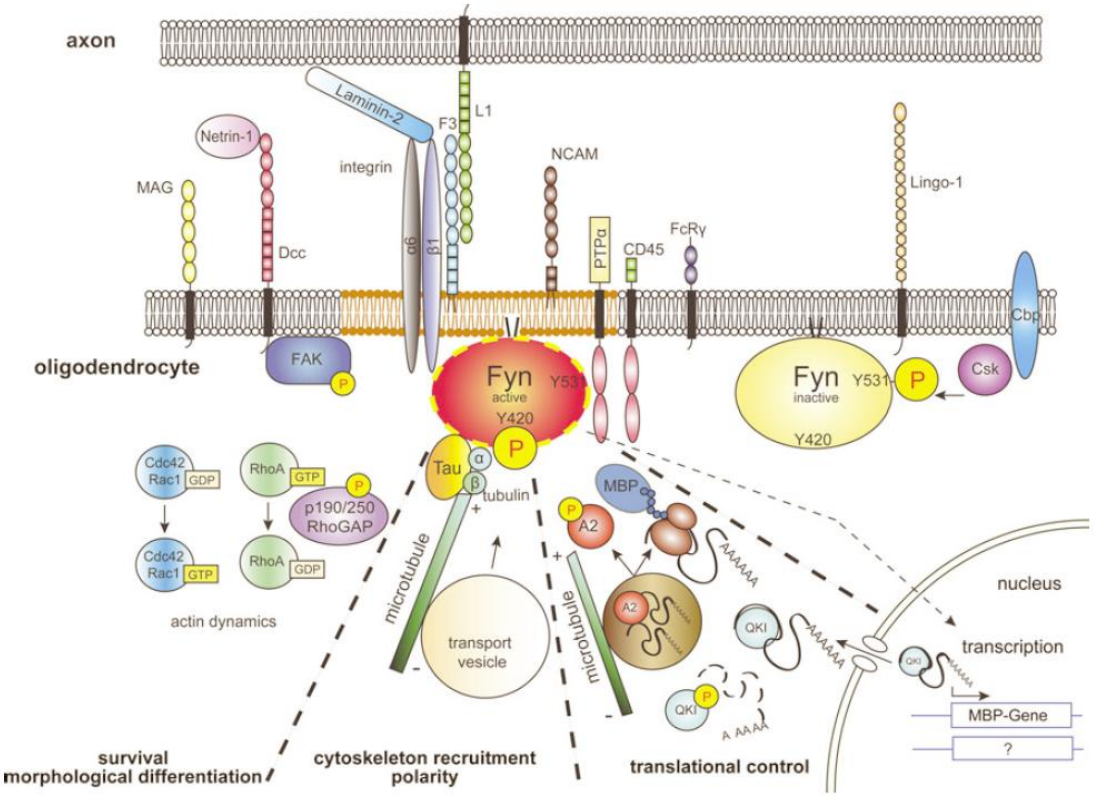

Figure 3. A schematic representation of central mediator Fyn. Fyn regulates three major signaling pathways, together regulating MBP expression and myelin formation. In this context the translation control and the extracellular factors involved in this process is most important. Fyn phosphorylates hnRNP A2, enabling MBP mRNA translation. (Adapted from Krämer-Albers and White 2011(16))

The adhesive interaction between axonal L1 and OLG F3, is not the only interaction that activates Fyn kinase, also a role of ECM molecules have been suggested. In epithelial cells ECM attachment is required in the onset of polarity development that might also be necessary for OLG polarity development. Laminin is likely an ECM molecule candidate, given its expression in developing axonal tracts and myelination defects in mice and humans upon its deficiency (17). Integrins, a family of heterodimeric transmembrane receptors, are associated with ECM transactions. Intracellular integrin signaling is often regulated by Src kinases, including Fyn, and small GTPases: Rac, Rho and Cdc42 (18). Rho inactivation enhances MBP expression, which is likely mediated by adhesive axo-glia interactions. Axon ECM protein laminin-2 has been implicated in initiation of myelination by binding to laminin receptor α6β1, one of the limited set of integrins an OLG expresses (17) (figure 3). The work of Colognato et al. (2004) show that Fyn associates with integrin α6β1 and that differentiation of OLG grown on laminin-2 requires Fyn. MBP expression is strongly reduced in Fyn depleted OLG that differentiate on laminin-2 (19). Integrin α6β1 is associated and also colocalizes with granule component hnRNP K. The implicated role for hnRNP K, in the final steps of MBP mRNA targeting to the sheath and in the initiation of local translation, is therefore also associated with integrins. Mice that lack integrin α6β1 ligand laminin α2, that functions

upstream of Fyn, show hypomyelination (20) and delayed OLG differentiation (21). However, recent research argues against such a role for hnRNP K upstream of Fyn during myelination, as hnRNP K knockdown showed no morphological abnormalities in culture (9). Difference between the integrin and hnRNP K knockdown experiment can be explained by the implication that hnRNP K functions in the final steps of targeting MBP mRNA to the myelin membrane and local translation initiation (see above). Although hnRNP A2 and –K both are required for MBP expression, their function during transport of MBP mRNA differs. Local signals are necessary to coordinate the release of mRNA from the granules and integrins can fulfill such a role.

Functions of Rho are also observed in the myelination inhibiting effect of LINGO-1, the LRR- and Ig domain-containing Nogo receptor-interacting protein. LINGO-1 decreases Fyn kinase activity in OLG, resulting in enhanced Rho activity with subsequent decrease in MBP expression (22), which is possibly related to reduced transport of granules into the sheaths (1). During developmental myelination, loss of LINGO-1 function leads to increased process length, branching, myelin sheet formation and myelination, whereas LINGO-1 overexpression leads to inhibition of OLG differentiation and myelination. Mi et al. (2005) investigated the downstream signaling molecules, Fyn and RhoA, to understand the molecular mechanism of LINGO-1 (22). Fyn kinase ultimately downregulates the activity of RhoA, resulting in OLG differentiation. Consistent with previous mentioned observations regarding Fyn activation, LINGO-1 antagonists increase the expression and phosphorylation status of Fyn and decrease the amount of activated RhoA-GTP. Results of Mi et al (2005) show, by quantifying MBP expression, that endogenous LINGO-1 inhibits myelination and MBP expression and antagonists of LINGO-1 reverse this inhibiting effect (22). This reduction is due to a decrease in Fyn kinase activity resulting in enhanced Rho activity (22, 23).

Taken together, Fyn kinase is a central player in contact-dependent MBP mRNA translation and ultimately in MBP expression, given its role in the phosphorylation of hnRNP A2 leading to MBP mRNA translation. Fyn is also involved in the intracellular signaling pathway of L1-F3/contactin interaction, can bind to integrins and LINGO-1, acting as an upstream mediator of Rho. Hence, a tight regulation of stimulating and inhibiting factors orchestrates axonal myelination, by regulating the expression of MBP. Thus, the function of the contact-dependent factors in Fyn activation and MBP expression as discussed here is largely known, whereas the role of adhesive factors on the transport of the MBP mRNA containing granule to the site of translation is still largely unknown.

Soluble factors

Next to the contact-dependent influences on OLG differentiation and MBP expression, there are several soluble factor involved in these processes. Soluble factors can be derived from axons or astrocytes. Stankhoff et al. (2002) show that astrocyte derived ciliary neurotrophic factor (CNTF) family strongly promotes MBP expression and thereby myelin formation by activating the 130 kDa glycoprotein Janus kinase (gp130-JAK) pathway (figure 2) (3). This demonstrates a novel role for CNTF in OLG, promoting myelination and possibly remyelination. Activation of JAK ensures docking of Src homology 2 domains of

a variety of proteins, like signal transducer and activator of transcription (STAT) proteins (24). Creating transgenic mice offers an easy quantifiable activity of the MBP gene, and revealed a promyelinating effect by CNTF (3). In fact, an increase in myelin formation induced by CNTF is a specific effect on myelin synthesis and not a result of an increase in OLG proliferation and survival. However, their conclusions are based on experiments in which the OLG are in culture with astrocytes and neurons, so CNTF could act directly on OLG or via the other cells. Nonetheless the promyelinating effect of the transgenic mice were confirmed by direct quantification of the extend of axon wrapping. Another crucial signaling pathway in OLG differentiation, next to Fyn kinase, is the PI-3 kinase/ Akt/mTOR signaling pathway (25). When this pathway is over activated, myelin sheath thickness is severely increased (25). CNTF has been described to activate phosphatidylinositol 3 (PI3) kinase signaling as well (26).

Members of the insulin-like growth factor (IGF) family can also stimulate OLG differentiation. Overexpression of IGF-1 in transgenic mice shows increased brain size and myelin content, whereas IGF-1 KO mice have smaller brains, lower number of OLG and suffer from hypomyelination (23, 27). Ye et al. (2002) show a decrease in myelination during early postnatal weeks in IGF-1 KO mice, i.e., a decrease in MBP mRNA and protein expression (28). Meanwhile the adult IGF-1 KO mice show no difference with their littermate controls, indicating a crucial role for IGF-1 during early development (28). IGF binds specific to receptor tyrosine kinases (RTKs), which activate the PI3K/mTOR/Akt pathway (figure 2), regulating the transition from late progenitor to immature OLG.

Unfortunately, there is not much known about how MBP mRNA transport is extracellulary regulated in OLG, in contrast to mRNA transport in neurons. In neurons, as OLG also polarized cells, mRNAs of several proteins are also transported to dendrites or axons, and translated ‘on site’. Localized translation of mRNAs and thereby expression of specific proteins requires, just like in OLG, a coordinated effort by the cell body to target the mRNAs, together with the necessary translational machinery, into dendrites or axons and the control of the individual mRNA translation (7). Dendritic synthesis of transcripts, such as αCaMKII mRNA and β-actin mRNA, can be stimulated by multiple pathways that are regulated by soluble factors that are receptor coupled. NMDA receptor activation stimulates granule movement into the dendrites (29), activation of metabotropic glutamate receptors promotes the localization of specific mRNAs (29) and activation of BDNF receptors also contributes to synthesis of specific proteins (2, 30). Schratt et al. (2004) suggest that synaptic function is modulated by BDNF, a neurotrophic factor like CNTF, and also acting through a PI-3 kinase-mTOR signaling pathway, thereby activating local translation of synaptic proteins (30). There are also several lines of evidence indicating localized mRNA translation by Src-dependent phosphorylation. Huttelmaier et al. (2005) show that the zipcode binding protein is released by Src-dependent phosphorylation, allowing translational activation of the mRNA (31), which is confirmed in an independent study by Sasaki et al. (2010). Furthermore, they suggest that it’s likely that Fyn kinase could possibly phosphorylate ZBP1, further suggesting similar mechanism for regulating local mRNA translation in neurons and OLG (32).

Taken together, the PI-3 kinase/ Akt/mTOR signaling pathway is activated by CNTF and IGF-1, and their activation increase myelin sheath thickness and regulate transition from progenitor to immature OLG. CNTF promotes MBP expression by activating JAK/STAT proteins. Meanwhile IGF-1 fulfills a crucial role during early development. Several links can be made between the regulation of mRNAs in OLG and the regulation in neurons, since not much is known about the OLG regulation it could be helpful to look at how it is regulated in neurons. Especially with regard to MS, if regulation of localized mRNAs in neurons show therapeutic options, the comparison could be very beneficial for the regulation of MBP mRNA transport in OLG. For example, OLG also express NMDA receptors (33), which together with activation of glutamate receptors might also stimulate MBP mRNA granule transport.

Regulation of MBP expression in MS lesions

Remyelination after demyelination most likely resembles a rerun of the developmental myelination program, the so called recapitulation hypothesis (34). Remyelination is however insufficient in a MS lesion. The extracellular environment in a MS lesion, containing several regulating factors, differs from the local environment during developmental myelination. The signaling environment becomes deregulated, resulting in the presence of inhibiting factors or lack of differentiation inducing signals, which likely perturb MBP expression, thereby causing remyelination failure in MS (23). These factors can be subdivided in reappearance of factors that play a role in MBP expression during developmental myelination, and factors that are not present during development, but present in a MS lesion due to the chronic inflammatory environment.

Several contact-dependent and soluble factors involved during the development of the CNS should also be (temporally) present in a MS lesion. The adhesive factor LINGO-1 negatively regulates OLG differentiation during development. Mi et al (2007) show that loss of LINGO-1 function leads to recovery from simulated demyelination, forming a therapeutic target for reversing disease progression (35). Functional recovery in mice suffering from experimentally induced autoimmune encephalomyelitis (EAE), a widely accepted animal model for MS, is induced by loss of function by lingo-1 gene knockout, and antibody antagonists of LINGO-1 (35).

CNTF also appears to have a protective role in EAE, reflecting an inflamed nervous system. Cntf-/- mice show increased OLG apoptosis, indicating that CNTF protects mature OLG from cell death in demyelinating CNS disease. Also the myelin integrity and function are impaired in Cntf KO mice (36). Unfortunately the effect of CNTF on MBP expression in MS lesions is still unknown.

Mason et al. (2003) observed that remyelination is inadequate after a cuprizon-induced demyelination in mice lacking IGF-1 signaling, likely due to a lack in oligodendrocyte progenitor proliferation and/or survival (37). Furthermore, IGF-1 is upregulated in several remyelinating lesion models, i.e., in cuprizone-, EAE-, and lysolecithin- induced demyelination and is essential for proper remyelination, as measured by MBP expression (37).

The presence and function of the two previously discussed contact-dependent factors important for developmental myelination (figure 2) laminin-2 and L1, in MS lesions remains still to be established.

Besides the factors discussed in preceding chapters, there are some other determinants that might be interesting for MS. Polysialylated neural cell adhesion molecule (PSA-NCAM, figure 3) expressed on axons during development acts as an inhibitor of myelination, and is re-expressed upon demyelination. The persistent presence of this inhibitor in MS lesions could participate in disease progression by inhibiting remyelination (38). Its role in regulating MBP expression has not been investigated yet.

One of the factors that is abundantly expressed in MS lesions, but not present during developmental myelination is fibronectin (39). OLG grown on fibronectin express MBP, although myelin-like membrane formation is strongly reduced (39). Some cells were able to extent MBP-positive myelin-like membranes on fibronectin, likely the transport of the MBP mRNA granule and the translation is less ECM dependent than other ECM required trafficking routes like vesicular PLP transport (1). Interestingly, in cells that do not make myelin-like membranes, MBP is ectopically expressed in the cell body in clusters, as observed in kif1b mutants (11).

High molecular weight hyaluronan, a glycosaminoglycan produced by astrocytes that accumulates in MS lesions, is also implicated as an inhibitor of OLG differentiation. Back et al. (2005) show that in the presence of hyaluronan, the MBP expression was impaired (40). Also accumulated myelin debris, resulting from disintegrated myelin sheaths, negatively regulates MBP expression and thereby remyelination (41). This is associated with a poor macrophage response and with a clearance of myelin debris that is delayed (41).

In addition to primary (inflammation-mediated) demyelination, a pathological feature of MS is damage to and loss of axons. In fact, axonal loss increases during the progression of MS, and several lines of evidence suggest a link between axonal loss and to the failure of remyelination (23). Indeed, the lacking of myelin around the axons is a potential explanation for the degeneration or the inflammation in the surrounding tissue (42). However, whether the problem lays within the OLG differentiation, i.e., lack of MBP mRNA transport or protein expression, or the (chronic) inflammatory reaction, a therapy has to be developed that promote remyelination and preserve axons. Current MS therapies target the immune response, which does not stimulate the MBP expression or OLG differentiation.

How could MBP expression be promoted and therefore remyelination in MS lesions?

A detailed understanding of the mechanisms of CNS myelination and OLG differentiation in the healthy and MS situation is key in developing therapy for demyelinating diseases. Insufficient remyelination in demyelinated lesions causes functional problems as the disease progresses. The exact reason for remyelination to fail in MS is still unclear, although some factors present in a MS lesion might contribute to remyelination failure. Undoubtly, correct MBP mRNA transport and regulated expression are crucial

for remyelination (43). The discussed factors in this report, among others, have their individual influence on the regulation of MBP mRNA transport and translation. These factors cause myelination during development and remyelination following demyelination. Nonetheless, in MS this process regulation is aberrant from the healthy situation upon demyelination.

Current MS therapy focuses mainly on immunosuppression without effect on myelin repair. However, the persistent lack of MBP mRNA transport, translation and ensheathment results in demyelinated axons will ultimately lead to disease progression. Therefore, enhancing these regulatory processes could form a therapeutic window for MS. Fyn kinase and the PI-3 kinase/ Akt/mTOR signaling pathway are central in MBP expression and OLG differentiation, for that reason proper activation of these mediators in MS should be one of the therapeutic goals.

The role of contact-dependent factor LINGO-1 is of particular interest for MS therapy. LINGO-1 is selectively expressed during development, thereby regulating the timing of OLG differentiation and the onset of myelination. The upregulated expression of LINGO-1 in MS suggests a suppressing role in remyelination. A block of this inhibitory effect likely promotes MBP expression and OLG differentiation. A therapy with antibodies against LINGO-1 is currently in clinical phase I. A problem during the investigation of the suitability of the anti-LINGO-1 therapy was the low percentage of the injected dose found in the brain of mice (44). However the exposure level, achieved by passive diffusion, appeared to be sufficient for robust myelin formation. The regulation of the L1-F3 interaction and laminin-2-integrin interaction in MS lesions is not well studied. During developmental myelination both factors stimulate Fyn kinase activity and ultimately increase MBP expression. Therefore, it might be interesting to investigate whether (over)stimulating these pathways in MS also enhances MBP expression.

Soluble factor CNTF appears to have a protective effect in inflamed neuronal regions. To apply the findings of Linker et al. (2002) to therapeutic approaches, direct application of CNTF and thereby enhancing the levels of CNTF, might be a possibility. However, systemically administered CNTF accumulates in the liver and only minute amounts reach the CNS (36). For that reason more research has to be performed on how CNTF levels can be increased in the CNS. Another soluble factor discussed is IGF-1, which is crucial during the early stages of development and is upregulated in remyelinating models. For MBP expression to occur properly, IGF-1 signaling is required (37). MS therapy focused on proper IGF-1 signaling might be a way to retain normal MBP mRNA transport and expression and ultimately remyelination.

MS lesion involved factors that are aberrant from the healthy situation could also be one of the causes of remyelination to fail in MS. For instance fibronectin and high molecular weight hyaluronan that accumulates in a MS lesion. Also the remainders of disintegrated sheaths, myelin debris, should be cleared in lesions. For MS therapy the solution for these problems might lie in the work of macrophages. Enhancing their activity might result in better clearance of these MBP expression impeding factors (41).

Other interesting MS therapy that might be considered is to enhance (re)myelination by promoting the MBP mRNA transport. Neurons express NMDA receptors, and upon activation, mRNA containing granules move into the dendrites. Since mRNA transport in OLG is still poorly understood and given that OLG also express NMDA receptors, it might be possible that granule movement in OLG can also be stimulated by NMDA signaling. Recent research in the role of NMDA receptors in OLG development did not show a major role for NMDA receptors on OLG development or EAE (33). However, their role in granule movement is largely unknown.

Although the research in mechanisms behind MS moved significantly forward in the last decade and a lot of papers have been published, there is still a lot unclear why proper MBP expression and remyelination fails in MS. Current investigations and interpretations are mainly based on findings in animal models and at the moment access to human tissue will be crucial. The genetic background of MS is also interesting to analyse. Bringing together the knowledge of biology and genome-wide-association studies might be a powerful tool in clarifying the underlying mechanisms for remyelination failure in MS. In the present report, several possibilities and suggestions towards MS therapy have been discussed. Thus, more research on the mechanisms behind MS in relation to MBP transport and expression is required, for example by continuing the studies in transcriptional, translational regulation and granule-related activities during development, upon demyelination at healthy conditions and in MS situations.

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