Fyn kinase targets in oligodendroglial physiology and myelination

Dissertation zur Erlangung des Grades

„Doktor der Naturwissenschaften“

am Fachbereich Biologie

der Johannes Gutenberg-Universität

in Mainz

von

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Geb. am 06.04.1978 in Heidelberg

Mainz, Oktober 2011

Dekan:

1. Berichterstatterin:

2. Berichterstatter:

Tag der mündlichen Prüfung: 21.12.2011

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2 INTRODUCTION

2.1 The vertebrate central nervous system

The central nervous system (CNS) of vertebrates comprises the brain and the spinal cord (Figure 2-1). All other regions of the nervous system are called the peripheral nervous system (PNS). In cross sections of brain or spinal cord, one can easily distinguish grey matter from white matter areas (Figure 2-2), the former harbouring cell bodies and unmyelinated processes of neural cells (dendrites and axons) and the latter predominantly containing myelinated nerve fibres.

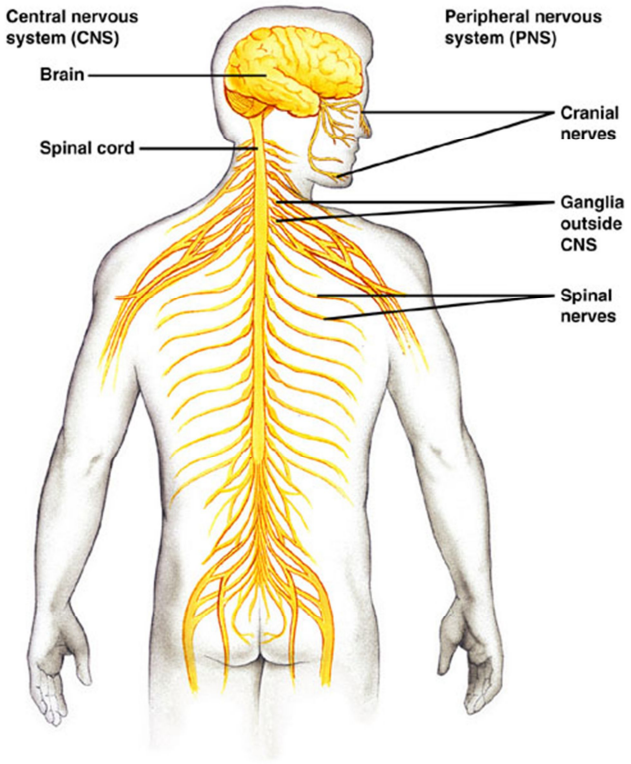


Figure 2-1 The vertebrate nervous system

Drawing of the human nervous system consisting of central (CNS) and peripheral (PNS) parts. Modified from (Campbell and Reece, 2002).

The brain integrates sensory information from the environment and from inside the body coming in over the spinal cord or directly over the cranial nerves. It generates responses to this information which are conducted to muscles, (sensory) organs and glands to trigger appropriate behaviour and regulation of body functions, respectively. Certain stimuli evoke “simple” responses like the patellar reflex, which are processed by the spinal cord independent of the brain.

During evolution, the emergence of bigger and more complex nervous systems with an increasing degree of cephalisation allowed for the development of more sophisticated behaviour and of (higher) cognitive functions. The human brain with its extraordinary complexity and capabilities has enormous requirements for energy. While it counts for only about 2% of the total body mass, it consumes about 20% of the body’s energy (Herculano-Houzel, 2011).

2.2 Cell types in the CNS

Neurons and glial cells are the main cell types in the CNS (Figure 2-3). Glial cells can be divided into microglia and macroglia and the latter subdivided into astrocytes, oligodendrocytes and ependymal cells.

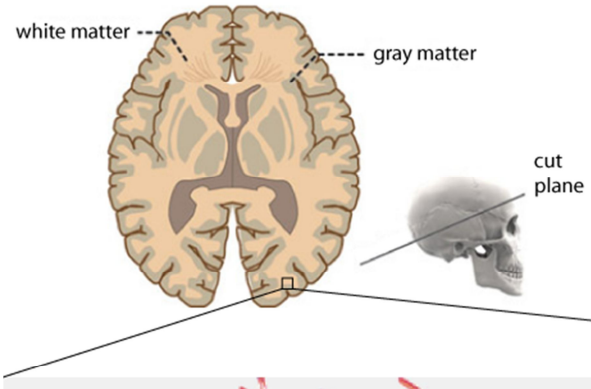


Figure 2-2 Grey and white matter areas in the human brain

Grey matter contains cell bodies and unmyelinated processes while regions with high myelin content appear white in CNS cross sections. Modified from (www.disaboom.com).

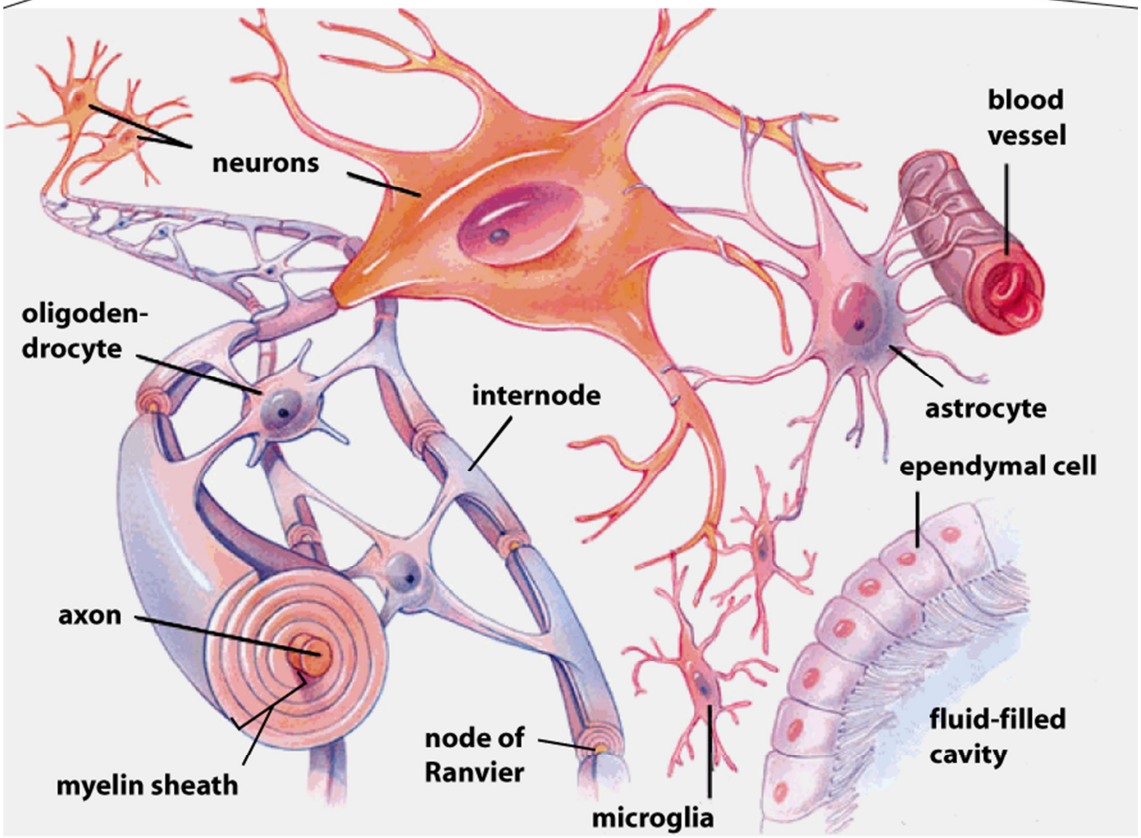


Figure 2-3 Cell types in the CNS

The central nervous system mainly consists of neurons and glial cells. The latter can be divided into astrocytes, oligodendrocytes, ependymal cells and microglia. As depicted here, the different cell types form a complex network with specialised interactions, contributing with their individual capabilities to ensure CNS function. Oligodendrocytes for example wrap around large axonal processes with specialised domains of their membrane. The resulting myelin sheath (internode regions) is periodically interrupted by short unmyelinated tracts (nodes of Ranvier) allowing rapid “saltatory” signal conduction (section 2.3). Modified from (Shier et al., 1999).

Neurons are the basic functional units in the nervous system, responsible for the processing of information. Generally, one can distinguish two different kinds of neuronal processes, dendrites and axons. Neurons receive signals over the dendrites and forward them by generating action potentials along the axons. Information is transmitted between neurons over chemical or electrical synapses. The human brain comprises about 10^{11} neurons, each of which can form up to 10^3 synaptic contacts to other neurons building a network of huge complexity.

Microglia constitute the immune cells in the CNS. They are distributed throughout the brain in a resting state, but scan the surrounding area with their highly motile processes. Upon brain damage, microglia become activated and function in the clearance of dead cells and cell debris and by the release of substances protecting (or sometimes harming) their vicinity (Kettenmann et al., 2011). Furthermore, microglia have been implicated in synaptic remodelling during development and they are activated in neurodegenerative diseases (Allen and Barres, 2009).

Astrocytes are the most abundant glial cells in the CNS. Many functions in brain homeostasis and development have been ascribed to astrocytes. They regulate the extracellular ion concentration, provide trophic support for neurons and oligodendrocytes and influence neuronal guidance, neurite outgrowth and synaptogenesis (reviewed by Araque and Navarrete, 2010). Moreover, astrocytes participate in assembly of the blood-brain-barrier (Ballabh et al., 2004) and control cerebral blood flow (Gordon et al., 2007). Intriguingly, during the last years rising evidence was collected that astrocytes are functional elements of synapses, regulating synaptic transmission and plasticity and thus playing a role in the processing of information (reviewed by Araque and Navarrete, 2010).

Ependymal cells are ciliated cells that line the ventricular surface in the CNS. They seem to be important for the development of the brain and for maintaining the cerebrospinal fluid (CSF) compartments (Del Bigio, 2010).

Oligodendrocytes are the myelin forming cells in the CNS. In contrast to its PNS counterpart (one Schwann cell forms one myelin segment) a single oligodendrocyte can build up to 40 myelin segments (internodes, Figure 2-3) around neuronal axons, thereby allowing fast signal conduction and supporting axonal integrity (Simons and Trotter, 2007; Nave, 2010a).

The importance of glial cells for brain function and maintenance immediately becomes apparent when studying evolution. The development of larger and more complex brains with growing cognitive capabilities led to increasing ratios of glial cells to neurons. While only small percentages of all neural cells are glia in primitive organisms, in

Drosophila they account for ~20-25%, in rodents for ~50-60% and in humans there are 10 times more glia than neurons (Verkhratsky, 2010).

2.3 Myelin in the CNS

Rapid conduction of nerve signals can easily be imagined as an evolutionary advantage allowing the prey as well as the predator to react fast. One solution to achieve this rapid conduction is to increase the diameter of the axon. Another solution is to insulate the axon with a myelin sheath. Both have been realised in several taxa, alone or in combination. The advantages of myelin are the much lower requirements for space and energy. For example, axon diameters and neuronal soma in the human brain would have to be 100-fold larger to achieve similar connection efficiencies in the absence of myelin (Hartline and Colman, 2007). The resulting "giant" brain would supposedly entail an evolutionary disadvantage.

In mammals, myelination largely occurs post-natally and carries on until early adulthood, in humans at least until the third decade of life. Intriguingly, some recent studies indicate that myelination plays a role in synaptic plasticity and thereby in learning and memory, particularly during adolescence. But even in adults, an increase in white matter structures was observed after learning complex tasks like piano playing or juggling (reviewed by Fields, 2010; Nave 2010a).

Formation and maintenance of the insulating myelin sheath does not seem to be the only function of oligodendrocytes. There is a strong interdependence between oligodendrocytes and the myelinated axon with mutual exchange of signals throughout their lifetime to preserve the so called axon-glial unit. Evidence from several studies suggests that the presence of oligodendrocytes is important for integrity and survival of axons (Griffiths et al., 1998; Lappe-Siefke et al., 2003). This seems to be particularly true for long axons that suffer from logistic problems of being supplied by their own cell soma which is located far away (Nave, 2010b).

2.3.1 Oligodendroglial development and myelination

During development of the CNS, oligodendrocyte precursor cells (OPCs) are generated in successive waves in ventral and dorsal regions of the subventricular zones in brain and spinal cord (Richardson et al., 2006). The OPCs proliferate and migrate throughout the CNS until they reach their target destination where they differentiate into myelination-competent mature oligodendrocytes. Migrating OPCs appear able to sense nearby OPCs by dynamically extending and retracting their processes, which is thought to ensure proper spacing of the cells needed for efficient myelination (Kirby et al.,

2006). Moreover, OPCs are produced in larger numbers than required for myelination. About half of the differentiating oligodendrocytes die during development, due to a lack of survival factors coming from astrocytes and neurons, thereby matching oligodendrocyte number to the number of axons requiring myelination (Barres and Raff, 1994). Differentiation of and myelination by oligodendrocytes are highly regulated processes to assure proper assembly and function of myelin. Several extrinsic (many of them axon-derived) and intrinsic factors and mechanisms involved here have been described so far (Figure 2-4; reviewed by Emery, 2010; Aggarwal et al., 2011a).

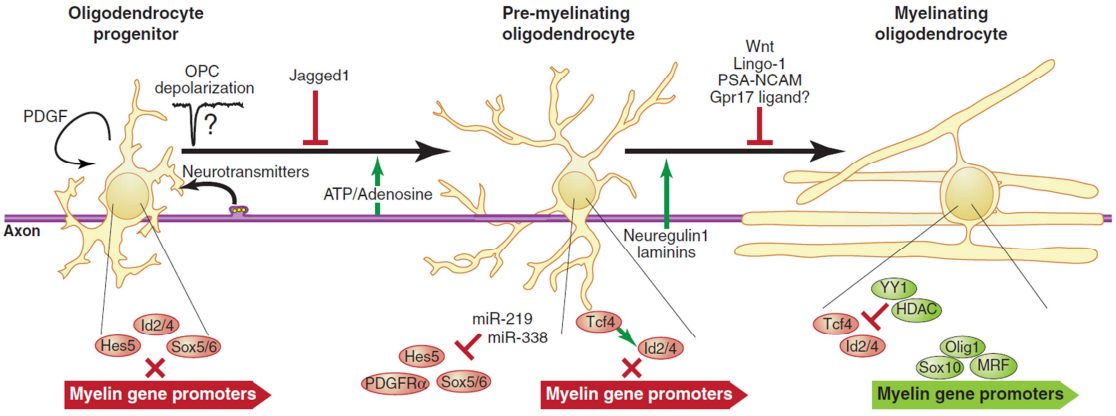


Figure 2-4 Control of oligodendroglial development and myelination

Oligodendrocyte differentiation is regulated by extrinsic signals (e.g. Wnt pathway activation or signalling from axonal Neuregulin-1) which are integrated through the action of intrinsic pathways (e.g. transcription factors like Tcf4 or micro-RNAs). Myelination is controlled by manipulating oligodendrocyte differentiation and by signalling from individual axons. Modified from (Emery, 2010).

An important concept is that electrical activity can drive myelination of the corresponding axon (Demerens et al., 1996), suggesting an involvement in synaptic plasticity by enhancing the transmitted signals (see also section 2.3; Fields, 2005). Moreover, a recent study identified control of local myelin protein synthesis as one mechanism for this kind of regulation (Wake et al., 2011). Not all axons become myelinated. Only those with diameters above 0.2 µm in the CNS and 1 µm in the PNS are myelinated. Furthermore, the thickness of the myelin sheath generally corresponds to axonal diameter. The g-ratio (axonal diameter divided by the diameter of axon plus myelin sheath) of most myelinated axons lies between 0.6 and 0.7 in any given species (Sherman and Brophy, 2005). In the PNS, axon size seems to be communicated to the myelinating Schwann cells by Neuregulin-1 (Nrg1) type III present on the axonal surface (Michailov, 2004) which moreover largely controls myelination in the PNS (Taveggia et al., 2005). In the CNS, Nrg1 type III promotes myelination but it is not such an instruc-

tive signal as in the PNS. Myelination in the CNS appears rather to be controlled by multiple signals which are at least partly redundant.

Loss of myelin, for example due to demyelinating diseases, can at least partially be compensated by remyelination, even in the adult CNS. Remyelination is thought to be carried out by resident OPCs which are activated upon myelin damage. Several studies indicate that the remyelination program recapitulates the mechanisms of developmental myelination. However, there is also evidence for variations between the 2 processes which could be explained by differences in the inflammatory environment and/or signalling from axons (reviewed by Fancy et al., 2011).

2.3.2 CNS Myelin structure

In the CNS, myelin is produced by oligodendrocytes. Following axon-glial contact, the oligodendrocyte extends its processes and spirally wraps around the axon with highly specialised membrane domains (Figure 2-3 and Figure 2-5). During active myelination, the membrane surface area has been estimated to grow at a rate of $5 – 50 \times 10^3$ µm^2 per cell and day while the cell body surface area comprises around 300 µm^2 (Baron and Hoekstra, 2009). Thus, a myelin forming cell requires highly efficient production and delivery systems in order to cope with the enormous amounts of myelin components needed by the expanding sheath. New membrane synthesis was suggested to occur at the tip of the processes as myelin protein messenger ribonucleic acids (mRNAs) and ribosomes were found there (Colman et al., 1982; Sherman and Brophy, 2005). Dependent on axon size (see section 2.3.1) up to 50 wraps around the axon are produced. In parallel, the cytoplasm is retracted from the processes resulting in a multilamellar compacted myelin sheath where the membrane surfaces are closely opposed to each other. Interconnected cytoplasmic pockets are embedded in the compact myelin and they are thought to serve the transport of molecules to and from the myelin sheath to ensure its physiological integrity, including maintenance and signalling events (Velumian et al., 2010).

Furthermore, the compact myelin (internode) is periodically interrupted by small unmyelinated gaps, the so called “nodes of Ranvier”. These gaps are densely occupied by voltage-gated sodium channels which enable the (fast) saltatory conduction of action potentials (rapid change of the membrane potential, nerve signal) along the axon, from gap to gap (Salzer, 1997). The nodes are flanked by the specialised ends of the internodes designated paranodes (or paranodal loops, membrane loops filled with cytoplasm, right hand micrograph in Figure 2-5) and juxtaparanodes, respectively (Figure 2-5). The paranodal loops are tightly connected to the axon surface via septate-

like junctions building a diffusion barrier for ions (and membrane components) and thereby enhancing the insulating properties of the internode. The juxtaparanode separates the paranode from the internode and contains fast potassium channels which are thought to promote repolarisation during action potential propagation. The small periaxonal space uniformly separates the compact myelin and the axon surface. The formation of these highly polarised domains and the clustering of ion channels and cell adhesion molecules are regulated by mutual signalling between the axon and the myelinating glial cell (Salzer et al., 2008; Susuki and Rasband, 2008).

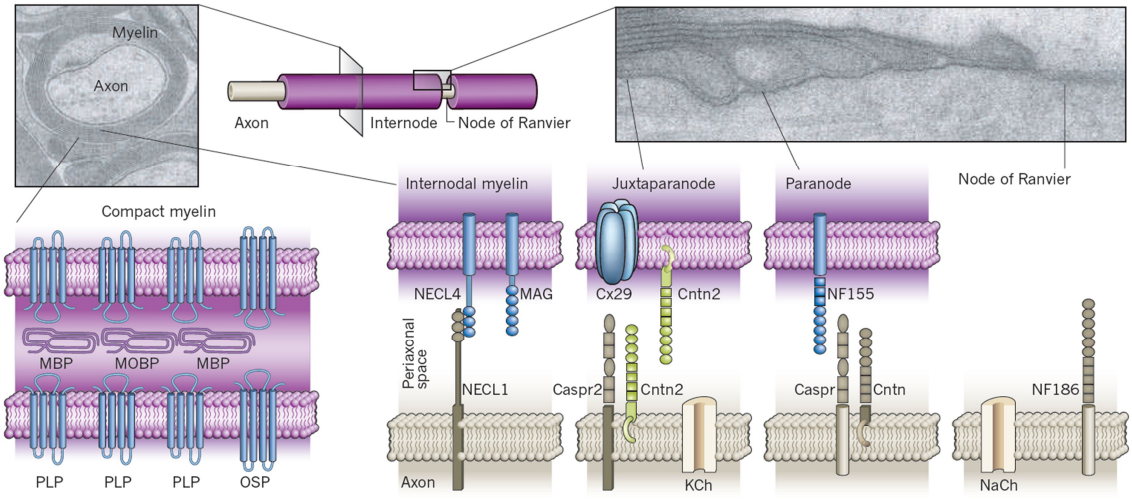


Figure 2-5 Structure of the axon-myelin unit in the CNS

Oligodendrocytes form a multilamellar compact myelin sheath around axons in the CNS (upper left electron micrograph, cross section of a myelinated axon from optic nerve). Several specific proteins (lower left part) contribute to the compact myelin structure (internode). The internodes are interrupted periodically by unmyelinated regions, the nodes of Ranvier, facilitating rapid saltatory conduction of action potentials by the presence of voltage-gated sodium channels (NaCh). Adjacent to the nodes of Ranvier one can distinguish two specialised axon-myelin contact regions containing specific cell adhesion proteins: the paranode and the juxtaparanode (right part). These regions are important for the functionality of the node of Ranvier and its separation from the internode. Caspr, Contactin-associated protein; Cntn, Contactin; Cx29, Connexin 29 kDa; KCh, fast potassium channels; MAG, Myelin-Associated Glycoprotein; MBP, Myelin Basic Protein; MOBP, Myelin Oligodendrocyte Basic Protein; NECL, Nectin-like Protein/SynCAM; NF155/186, Neurofascin 155 kDa/186 kDa; OSP, Oligodendrocyte-Specific Protein; PLP, Proteolipid Protein. Modified from (Nave, 2010a).

Compared to other cellular membranes, the myelin membrane has a higher lipid content, enhancing its insulating properties. About 70% of the dry weight of myelin is made up of Cholesterol, phospholipids (ethanolamine plasmalogen) and glycosphingolipids (sulfatide and galactosylceramide) (Figure 2-6; Simons and Trotter, 2007). The most abundant proteins in CNS myelin are Proteolipid Protein (PLP), its splice variant

DM20 and Myelin Basic Protein (MBP), together comprising approximately 80% of all CNS myelin proteins (Baron and Hoekstra, 2009). Amongst the less abundant myelin proteins are Myelin Oligodendrocyte Basic Protein (MOBP), Myelin-Associated Glycoprotein (MAG), Myelin Oligodendrocyte Glycoprotein (MOG), 2',3'-Cyclic Nucleotide 3'-Phosphodiesterase (CNP), Myelin And Lymphocyte Protein (MAL) and Oligodendrocyte Specific Protein (OSP) (Taylor et al., 2004; Roth et al., 2006; DeBruin and Harauz, 2007).

One study presented a slightly different view about the relative protein composition of CNS myelin. By mass spectrometry (quantification) analysis of a myelin enriched fraction, a large number of novel myelin-associated proteins was identified. Many of them were found to be present in low amounts and the presence of PLP and MBP was assessed as 17% and 8%, respectively, suggesting that previous investigations had overestimated their abundance (Jahn et al., 2009).

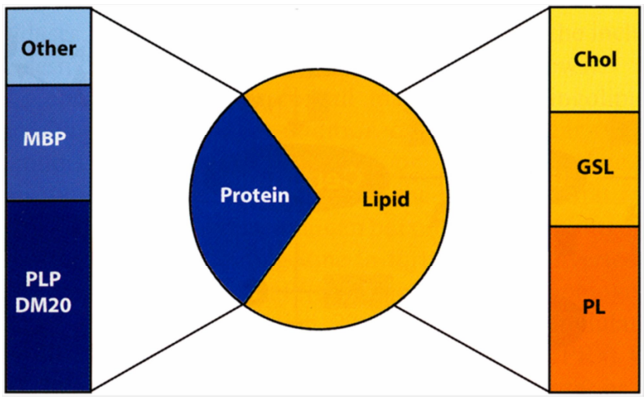


Figure 2-6 Protein and lipid content of CNS myelin

The myelin membrane is highly enriched in the lipid cholesterol (Chol), glycosphingolipids (GSL) and phospholipids (PL). The most abundant proteins in myelin are Proteolipid Protein (PLP), its splice variant DM20 and Myelin Basic Protein (MBP). Modified from (Lazzarini, 2004).

2.4 Myelin Basic Protein

2.4.1 Functions

MBP is so far the only known structural myelin protein that is essential for the formation of myelin in the CNS. Therefore, it was named the "executive molecule of myelin" (Boggs, 2006). The importance of MBP for CNS myelin is demonstrated by the naturally occurring mouse mutant "shiverer" where a large part of the mbp gene is deleted and which lacks almost all compact myelin in the CNS (Readhead and Hood, 1990). Furthermore, the "Long Evans shaker rat" contains a mutation in the mbp gene resulting in aberrant transcription and also in the absence of compact CNS myelin (Carre et al., 2002). MBP is also present in PNS myelin but does not seem to be as important for myelination here, probably because its function in the PNS can be compensated by

other myelin proteins such as PMP22, P0 and P2. However, also PNS myelin shows abnormalities in the shiverer mouse (Gould et al., 1995).

As its name indicates, MBP contains a high fraction of positively charged residues. The compaction of the myelin membrane by binding to the negatively charged lipids at the cytosolic membrane surface is considered as a main, and for myelination indispensable, function of MBP (Figure 2-5; Min et al., 2009). Nevertheless, many studies indicate that there is much more to its function. The above mentioned shiverer mutant is deficient in compact myelin but the oligodendrocytes still make contacts to axons and form some non-compacted wraps. However, axonal sodium-channel clustering and axon-glial junctions are abnormal (Rasband et al., 1999), alluding to a defect in axon-glial signalling (section 2.3.2). MBP appears to interact with a variety of cytosolic proteins, including cytoskeletal elements, acting as a linker or scaffolding protein. Moreover, MBP was connected to nuclear functions (section 2.4.2) and the regulation of other myelin proteins. There is growing evidence of multiple and diverse post-translational modifications of MBP such as phosphorylation or methylation, which very likely influence its adhesion to the membrane and to cytoskeletal elements as well as other functions (reviewed by Boggs, 2006; Harauz et al., 2009; Boggs et al., 2011). Interestingly, impulse conduction along myelinated axons can trigger reversible phosphorylation of MBP, suggesting an involvement of MBP in dynamic processes within the myelin compartment (Murray and Steck, 1984). A recent study implicates MBP in the establishment of the lipid enrichment in myelin by forming a size barrier, inhibiting the entry of proteins with large cytoplasmic domains (Aggarwal et al., 2011b).

2.4.2 MBP Isoforms

6 isoforms in mouse and 5 isoforms in human of myelin-associated MBP (also referred to as “classic” MBP isoforms) have been identified so far (Figure 2-7). They arise from alternative splicing of a single pre-mRNA which is transcribed from a large gene complex called “golli” (genes of oligodendrocyte lineage) (Campagnoni et al., 1993). The Golli (-MBP) proteins are the second family of proteins encoded by this gene complex and their pre-mRNA is generated from a different transcription start site. Golli proteins seem to have functions in nervous system development and in immune and hematopoietic cells (reviewed by Boggs, 2006; Fulton et al., 2010).

The classic MBP isoforms are differently expressed during development. Exon II-containing isoforms (Figure 2-7) are much more abundant during early development than in the adult CNS and, interestingly, during remyelination following nervous system injury. For isoforms lacking exon II the opposite expression pattern was observed.

In the adult human CNS, 18.5 kilodalton (kDa) is the predominant isoform while for mice it is the 14 kDa isoform (Boggs, 2006). Furthermore, the various isoforms also differ in their localisation. All are present in compact myelin but their distribution in myelin varies dependent on exon II (Karthigasan et al., 1996). In cultured cells, differential distribution of the isoforms was also observed. Isoforms lacking exon II are found at the plasma membrane while exon II-containing isoforms did not localise to the membrane but are present in the cytoplasm and the nucleus (Allinquant et al., 1991). Exon II-containing isoforms were shown to be actively transported into the nucleus suggesting a regulatory role early in myelination (Pedraza et al., 1997). Interestingly, the exon II-lacking 14 kDa but also the exon II-containing 17.22 kDa isoform on its own seems to be able to ensure the production of compact myelin and to rescue the shiverer phenotype. However, probably due to the lack of other isoforms, the rescue never was complete. Compared to wildtype mice, the number of myelinated fibres and the number of wraps per myelin segment were reduced, accompanied by slight deficits in myelin compaction (Kimura et al., 1998).

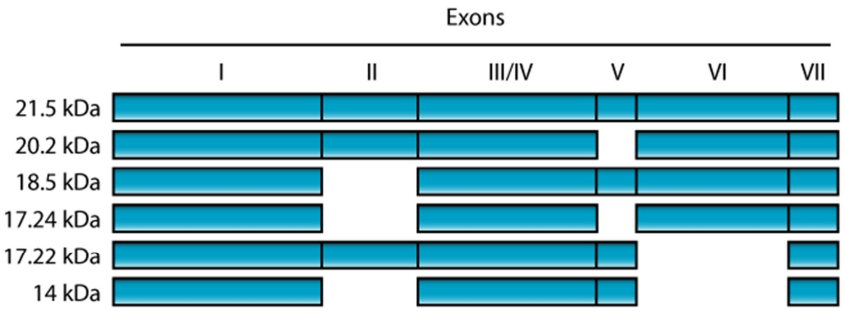


Figure 2-7 Organisation of the classic isoforms of murine MBP

There are 6 isoforms of classic MBP in mouse, generated by alternative splicing of exons II, V and VI. The molecular masses of the resulting proteins range from 14 to 21.5 kDa. Modified from (Boggs, 2006).

2.4.3 Localised synthesis of MBP

MBP mRNA has been found to be transported to the distal processes of oligodendrocytes and myelin where it becomes translated locally (see also sections 2.3.2, 2.6.1 and 2.7.1; Campagnoni et al., 1980; Colman et al., 1982; Barbarese et al., 1999). This process is assumed to ensure efficient and precise delivery of MBP to the site of myelin deposition and can be triggered by electrical activity or adhesion signalling from neighbouring axons and subsequent phosphorylation of an associated RNA-binding protein (see section 2.6.1; White, 2007; Wake et al., 2011). In zebrafish, transport of

MBP mRNA in oligodendrocytes was shown to be mediated by Kif1b. Disruption of this motor protein results in a hypomyelination phenotype, underscoring the importance of mRNA transport for myelination (Lyons et al., 2009). As for the corresponding proteins, the mRNAs of the classic MBP isoforms are also differentially distributed in space and time. Those coding for exon II-negative MBP are localised to oligodendroglial processes while exon II-positive forms remain in the cell body (de Vries et al., 1997). Furthermore, exon II-positive transcripts are more abundant during early developmental stages whereas the variants lacking exon II are predominantly expressed at later stages. Interestingly, these patterns also occur during CNS remyelination, supporting the significance of exon II-dependent expression for early and late phases of myelination (see also section 2.3.1; Jordan et al., 1990).

2.5 Fyn Kinase

Fyn belongs to the Src-family of non-receptor tyrosine kinases. 11 members of this family have been found in human and mice and they are named Blk, Brk, Fgr, Frk, Fyn, Hck, Lck, Lyn, Src, Srm and Yes (Manning et al., 2002; Caenepeel et al., 2004). In *Drosophila* only 2 members of the Src-family have been identified. Virus encoded Src (v-Src) was the first proto-oncogene to be identified (Martin, 2001).

So far, 2 isoforms of Fyn with a molecular weight of approximately 59 kDa, respectively, have been described in mice. They are generated by alternative splicing of exon 7. Not much is known about the differences between these isoforms although their activity is supposed to be quite similar (Resh, 1998). FynT was shown to be more effective in regulating cytoplasmic calcium levels (Davidson et al., 1994). The functional domains are almost the same and the sequences mainly vary in the linker region between the Src-homology (SH)2 and the kinase domain (section 2.5.2; Figure 2-8). FynT is predominantly expressed in T cells while the 3 amino acids longer FynB is present in the brain and other tissues.

2.5.1 Functions of Fyn in oligodendrocytes

Oligodendrocytes express the 3 Src-family members Fyn, Lyn and Src (Colognato et al., 2004). Fyn is the most prominent family member in oligodendroglial cells while Src is expressed only in low amounts.

A striking feature of mice deficient in Fyn is severe hypomyelination of the forebrain. Lack of the catalytic activity of Fyn is considered as cause for this phenotype as mice expressing a kinase inactive Fyn show the same alterations (Umemori et al., 1994; Sperber et al., 2001; Goto et al., 2008). As Fyn is also expressed in neurons, the ques-

tion arose if it was neuronal or oligodendroglial Fyn that is important for myelination. Over the years, several functions of Fyn have been discovered that support an involvement of oligodendroglial Fyn in myelinogenesis.

Fyn activity has been implicated in the regulation of OPC migration (Miyamoto et al., 2008), in oligodendrocyte differentiation (Relucio et al., 2009; Czopka et al., 2010) and in promoting target-dependent survival of oligodendrocytes (Colognato et al., 2004; Laursen et al., 2009).

Fyn expression and activity are upregulated during development of oligodendrocytes and the activity peaks with the most active part of myelination (Umemori et al., 1994; Krämer et al., 1999). Furthermore, inactivation of Fyn impairs process outgrowth of oligodendrocytes (Osterhout et al., 1999). This effect can to a large part be explained by Fyn-dependent regulation of Rho-family GTPases, which function in the remodelling of the actin cytoskeleton. On the one hand, Fyn activity inhibits RhoA (Wolf et al., 2001) and on the other hand, it activates Cdc42 and Rac1 (Liang et al., 2004), in both cases promoting process formation. Additionally, the actin-binding protein Mayven was shown to interact with Fyn and influence process outgrowth (Williams et al., 2005). Active Fyn also recruits the microtubule cytoskeleton by binding to the microtubule-associated protein Tau and to α-tubulin (Klein et al., 2002), thereby promoting cargo transport to the site of activation.

The above mentioned Fyn knockout mice exhibit reduced expression levels of MBP. Umemori and colleagues proposed in their work that Fyn stimulates MBP transcription by acting on the MBP promoter (Umemori et al., 1999). Moreover, our study suggests that Fyn is able to enhance translation of MBP mRNA by phosphorylation of the RNA-binding protein heterogeneous nuclear ribonucleoprotein (hnRNP) A2 (White et al., 2008), which is a central mediator in MBP mRNA metabolism (section 2.6.1). Fyn also was implicated in influencing MBP mRNA stability by acting on the RNA-binding protein QKI (Lu et al., 2005).

Several neuron- and extracellular matrix (ECM)-derived factors able to trigger Fyn activation have been identified. They act on a set of oligodendroglial surface receptors that build distinct membrane-associated signalling complexes with Fyn kinase manipulating the above described functions of Fyn (reviewed by Krämer-Albers and White, 2011).

2.5.2 Domain organisation and regulation of Fyn

All members of the Src-family share the same domain organisation (Figure 2-8). The N-terminus starts with an SH4 domain in which the most N-terminal glycine and cysteine

residue can be myristoylated and palmitoylated, respectively (Resh, 1998). These lipid modifications serve to anchor the kinase in the cytosolic leaflet of the plasma membrane. Palmitoylation was shown to be reversible (Palacios and Weiss, 2004) and is considered as a signalling event localising the kinase to lipid raft microdomains (Krämer-Albers and White, 2011). The next domain towards the C-terminus is unique for every member of the Src-family. It is followed by 2 domains instrumental in protein (substrate) binding. SH3 domains bind proline-rich ligands with the optimal (amino acid) target sequence RPLPPLP (class I) or ϕPPLPXR (class 2) where ϕ represents a hydrophobic residue and X any amino acid. Sequence specificity is low and variations lead to reduced binding affinities (Roskoski, 2004). SH2 domains bind to phosphorylated tyrosine residues (pY) and some downstream amino acids. The preferred target sequence of the Fyn SH2 domain is pYEEI (Songyang and Cantley, 1995) but varying sequences are also bound. Further parts of the ligand polypeptide can modify binding affinity (Roskoski, 2004).

The most C-terminal located domain is the kinase (or SH1) domain that functions in substrate phosphorylation. It is divided into a smaller N-terminal and a larger C-terminal lobe with the catalytic site in-between. The small lobe is primarily important for binding and orientation of Adenosine-5'-triphosphate (ATP) which serves as co-substrate for the kinase. The large lobe binds the protein substrate and supports ATP binding. Residues from the two lobes contribute to the catalytic site and kinase activity is affected by the spatial orientation of the lobes. An activation loop emerges from the large lobe. Autophosphorylation of tyrosine 420 (mouse FynB) inside that loop stabilises the active conformation of the kinase (Roskoski, 2004) and constitutes a broadly-used indicator for activated Src-family kinases. A short C-terminus follows the kinase domain and contains a further regulatory tyrosine residue (531 in mouse FynB). Phosphorylation of this tyrosine inhibits kinase activity (Brown and Cooper, 1996).

When Src-family kinases are inactive they exhibit a closed conformation (Figure 2-8) which is stabilised by intramolecular attachment of SH3 to the linker region between SH2 and the kinase domain and binding of SH2 to the phosphorylated regulatory tyrosine at the C-terminal tail (see above). Dephosphorylation of tyrosine 531 or binding of an external ligand to SH3 and/or SH2 can declamp the kinase leading to an open and active conformation. Autophosphorylation of tyrosine 420 can only occur when tyrosine 531 is dephosphorylated. In contrast tyrosine 531 can be phosphorylated in the presence of phospho-tyrosine 420. Double-phosphorylated kinase is active, thus tyrosine 420 overrides the inhibitory 531 (Roskoski, 2004).

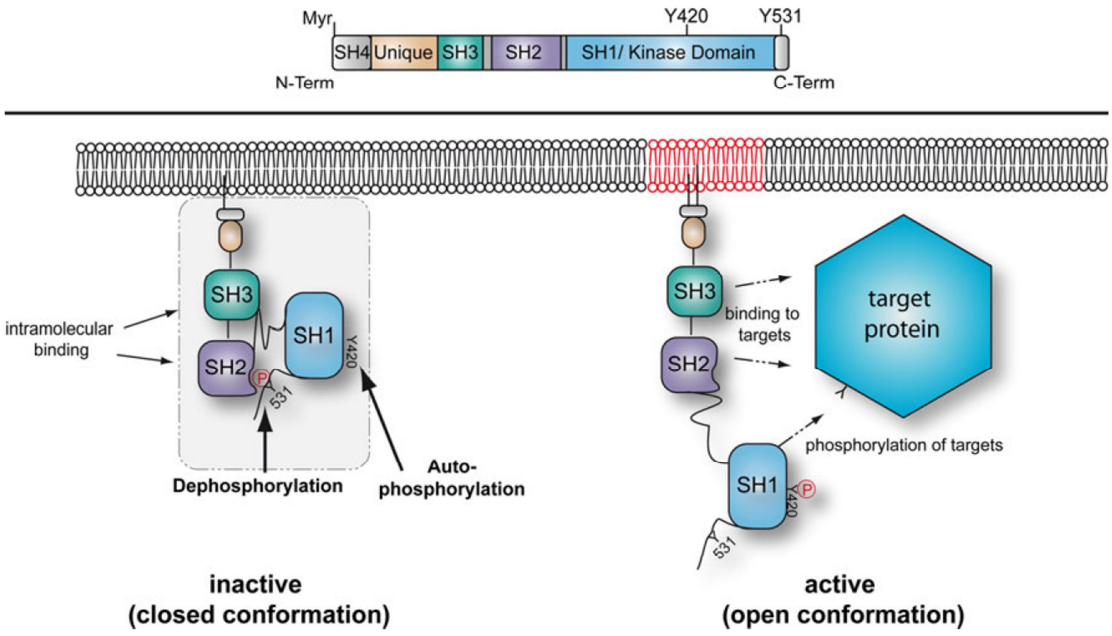


Figure 2-8 Domain organisation and activity regulation of Fyn kinase

Top: At the N-terminus Fyn kinase contains an SH4 domain which is myristoylated (Myr) and anchors the kinase in the plasma membrane, followed by a domain unique for each Src-family kinase and 2 protein binding domains (SH2 and SH3). Towards the C-terminus lies the SH1 or kinase domain including the regulatory tyrosine 420 and a short tail with another regulatory tyrosine (531) (residue numbers account for mouse FynB). Bottom: In its inactive state the kinase exhibits a closed conformation where the SH2 domain binds to the phosphorylated tyrosine 531 and the SH3 domain to the linker region between SH2 and the kinase domain. Upon dephosphorylation of tyrosine 531 or target binding to SH2 or SH3, the conformation is opened activating the kinase. Autophosphorylation of tyrosine 420 in the kinase domain stabilises the active conformation. Palmitoylation in the SH4 domain is thought to direct the kinase to lipid rafts (red). Modified from (Krämer-Albers and White, 2011).

Regulation of Fyn inhibition by manipulating the phosphorylation of tyrosine 531 can be catalysed by kinases such as C-terminal Src kinase (Csk) (Okada and Nakagawa, 1989) and Csk-homology kinase (Chk) (Zrihan-Licht et al., 1997). The opposite reaction, dephosphorylation of tyrosine 531 to activate Fyn was suggested for several phosphatases, such as Shp1 or 2 and CD45 (Roskoski, 2005) and has been shown to be carried out for receptor-like protein-tyrosine phosphatase alpha (PTPα) (Wang et al., 2009).

2.6 Heterogeneous nuclear ribonucleoproteins

The term “heterogeneous nuclear ribonucleoproteins” (hnRNPs) is used for a group of RNA-binding proteins that associate with RNA polymerase II transcripts (hnRNA or pre-mRNA) in the nucleus (Dreyfuss et al., 1993). Already during transcription, hnRNPs and other proteins form large complexes with pre-mRNAs (messenger ribonucleoprotein particles: mRNPs). They are involved in all steps of mRNA metabolism such as splicing,

mRNA transport and localisation and the regulation of mRNA translation and stability (Krecic and Swanson, 1999). Many hnRNPs individually have been shown to participate in several steps of the life of an mRNA. Hence, they can be primarily located in the nucleus and/or the cytoplasm and some of them undergo constant nuclear-cytoplasmic shuttling (Dreyfuss et al., 2002). The complex of mRNA and associated proteins is thought to be at least partially transcript-specific (Chaudhury et al., 2010). The complex is permanently remodelled on the way through the cell, changing the pattern of associated hnRNPs and other proteins and thus determining the current state and fate of the mRNA. This variable pattern of mRNA-bound proteins regulating mRNA metabolism was called the "mRNP code" (Singh and Valcárcel, 2005).

More than 20 hnRNPs, with additional isoforms in many cases, have been identified so far. Most hnRNPs are very abundant and ubiquitously expressed (Chaudhury et al., 2010). HnRNPs contain one or more RNA-binding domains and at least one auxiliary domain (Dreyfuss et al., 2002). 3 types of RNA-binding motifs in hnRNPs are known (Figure 2-9). The auxiliary domains are of diverse structure and can mediate for example protein-protein interactions or subcellular localisation (Chaudhury et al., 2010). The most frequent auxiliary domains known are the glycine-rich domains (Weighardt et al., 1996).

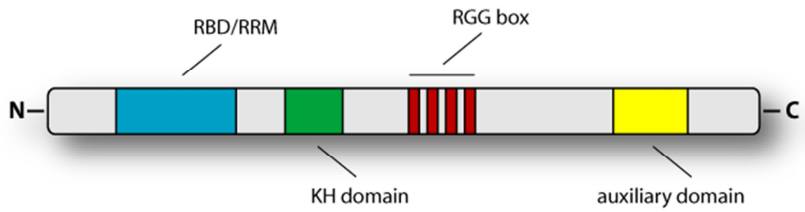


Figure 2-9 Common structural domains of hnRNP family members

HnRNPs contain at least one nucleic acid-binding domain and one auxiliary domain. The known RNA-binding motifs are the RNA-binding domain/RNA recognition motif (RBD/RRM), the hnRNP K-homology (KH) domain and the RGG box (contains closely spaced arginine-glycine-glycine repeats). Auxiliary domains are of variable structure and confer RNA- or protein-binding ability or influence the subcellular localisation of the hnRNP. Modified from (Carpenter et al., 2006).

HnRNPs influence the structure of and the interactions in mRNA-protein complexes. The function of hnRNPs depends on their binding activities to distinct mRNAs and proteins and can be regulated by their expression level as well as by post-translational modifications such as phosphorylation, methylation, ubiquitination and sumoylation (Chaudhury et al., 2010).

2.6.1 HnRNP A2

The 36 kDa ubiquitously expressed hnRNP A2 is one of 4 isoforms encoded by the hnRNP A2/B1 gene. The corresponding transcripts are generated by alternative splicing of exons 2 and 9 (Hatfield et al., 2002). HnRNP A2 has been implicated in nuclear and cytoplasmic functions where it seems to influence mRNA packaging, splicing, telomere regulation (He et al., 2009) and mRNA localisation (Barbarese et al., 1999), respectively. It was one of the first identified *trans*-acting factors involved in mRNA trafficking in neural cells. HnRNP A2 binds to a defined 11 nucleotide sequence called the hnRNP A2 response element (A2RE) (Munro et al., 1999) which has been found in the 3' untranslated regions (UTRs) of several localised mRNAs. For example in neurons, the mRNAs for α Calcium Calmodulin-dependent Protein Kinase II (αCaMKII), Activity-regulated Cytoskeleton-associated Protein (ARC) and Neurogranin (NG) seem to be targeted to dendrites by the so called A2-pathway (involving the *cis*-acting A2RE and the *trans*-acting hnRNP A2) (Gao et al., 2008).

The A2-pathway was initially described in oligodendrocytes and was shown to be responsible for the transport of MBP mRNA (section 2.4.3) to the myelin compartment (Ainger et al., 1997; Hoek et al., 1998). HnRNP A2 binds to MBP mRNA in the nucleus and both are exported to the cytoplasm where hnRNP A2-dependent MBP mRNA-transport granules (section 2.7.1) are assembled, apparently by the interaction of several hnRNP A2 proteins with the large microtubule-associated protein Tumor Overexpressed Gene (TOG) (Kosturko et al., 2005). In these granules, translation of MBP mRNA is suppressed and they are transported along the microtubule system to the periphery of the cell into the distal processes where the mRNA becomes translationally active to realise localised synthesis of MBP (Brumwell et al., 2002; Carson and Barbarese, 2005). Translational repression in the granule appears to be mediated by hnRNP E1 that binds to MBP mRNA over hnRNP A2 (Kosturko et al., 2006). Translational activation of MBP mRNA can be accomplished by the activity of the membrane-associated Fyn kinase (section 2.5.1) through tyrosine-phosphorylation of hnRNP A2 (White et al., 2008). The relative abundance of hnRNP A2 is approximately 20-fold higher in the nucleus compared to the cytoplasm in oligodendroglial cells (Kosturko et al., 2006). Compatible with the central role of hnRNP A2 in MBP mRNA metabolism, its expression and cytoplasmic localisation are increased during oligodendrocyte development (Maggipinto et al., 2004).

2.6.2 HnRNP F

Mouse hnRNP F has a relative molecular mass of about 48 kDa and is ubiquitously expressed (Honore et al., 1995). Typical for an hnRNP, it shows a strong localisation to the nucleus in most cells and a lower concentration in the cytoplasm (Matunis et al., 1994), although in some cell types the cytoplasmic localisation seems to prevail (Honore et al., 2004). HnRNP F contains 3 RNA recognition motifs (RRMs) that display some aberrations when compared to the classic RRMs and have therefore been termed "quasi-RRMs" (qRRMs). Nevertheless, they show specific RNA-binding activity that is unique for hnRNP F and its closely related family members from the hnRNP F/H subfamily. These subfamily members preferentially bind to poly(G) tracts with the optimal recognition sequence being "GGGA" (Caputi and Zahler, 2001; Alkan et al., 2006). 2 consecutive (G)-tracts in the RNA enhance the binding affinity (Dominguez and Allain, 2006) and it was demonstrated recently that the 3 qRRMs of human hnRNP F associate with RNA in a for RRMs very unusual manner, suggesting them to be a novel RRM class (Dominguez et al., 2010). Additionally to the qRRMs, hnRNP F comprises 2 auxiliary glycine-rich domains, one between the qRRMs 2 and 3 and the other at the C-terminus (Figure 2-10). The former seems to harbour a non-canonical nuclear localisation sequence and has been implicated in mediating nuclear-cytoplasmic shuttling of hnRNP F (Van Dusen et al., 2010).

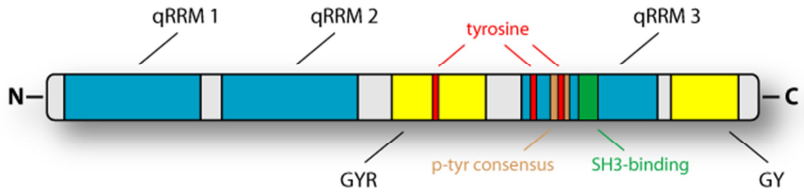


Figure 2-10 Domain structure of hnRNP F

HnRNP F comprises 3 quasi-RNA recognition motifs (qRRMs, blue) and 2 auxiliary domains (yellow) which are denoted according to their prevalent amino acid content (e.g. GYR = glycine-tyrosine-arginine-rich). The third qRRM contains a phospho-tyrosine consensus sequence (light brown) and a SH3-binding domain (green). Putative tyrosine-phosphorylation sites (Y246, Y298 and Y306) which are analysed in this study are marked in red. Modified from (Van Dusen et al., 2010).

Nuclear functions of hnRNP F seem to include splicing (Dominguez et al., 2010) and the regulation of polyadenylation (Veraldi et al., 2001). HnRNP F has been implicated in regulating proliferation downstream of mammalian target of rapamycin (mTOR) signalling (Goh et al., 2010). Furthermore, it seems to be able to influence apoptosis by controlling p53 mRNA levels (regulation of polyadenylation) (Decorsiere et al., 2011)

and/or by alternative splicing of Bcl-x pre-mRNA (Garneau et al., 2005). A cytoplasmic function of hnRNP F has been described only once so far. It participates in translation regulation of the *mu*-opioid receptor in mouse neuroblastoma cells (Song et al., 2011). Interestingly, a function in translation regulation was also shown for the *Drosophila* homologue of hnRNP F, Glorund, which controls Nanos synthesis in oocytes (Kalifa et al., 2006).

In oligodendrocytes, the only published function to date of hnRNP F is a role in the alternative splicing of PLP/DM20 pre-mRNA (Wang et al., 2007).

2.7 Cytoplasmic RNA granules

As soon as transcription has started, the nascent transcript is bound by RNA-binding proteins. The composition of individual mRNPs determines the fate of the containing mRNA at every step of its life. Constant remodelling of the mRNPs ensures and controls their transition between the different stations like splicing, transport, translation and eventually degradation. Remodelling is influenced by abundance of mRNA and bound proteins and the latter's post-translational modifications (Moore, 2005). After processing in and export from the nucleus, many mRNAs are immediately translated on polysomes (several ribosomes associated with the mRNP). Other mRNAs are stored until a signal triggers their translation and again others are transported to a certain destination in the cell where they become locally translated. Both storage and transport involve the package of the mRNAs into so called cytoplasmic "RNA granules", large RNA-protein complexes devoid of a limiting membrane, in which the mRNAs reside in a translationally inactive state. There are distinct types of cytoplasmic RNA granules, serving different functions (Figure 2-11; sections 2.7.1, 2.7.2 and 2.7.3). Cytoplasmic RNA granules are considered as highly dynamic structures which can be closely associated and undergo constant exchange of their components, allowing mRNAs to rapidly shift between translation, storage or decay (reviewed by Anderson and Kedersha, 2009; Erickson and Lykke-Andersen, 2011).

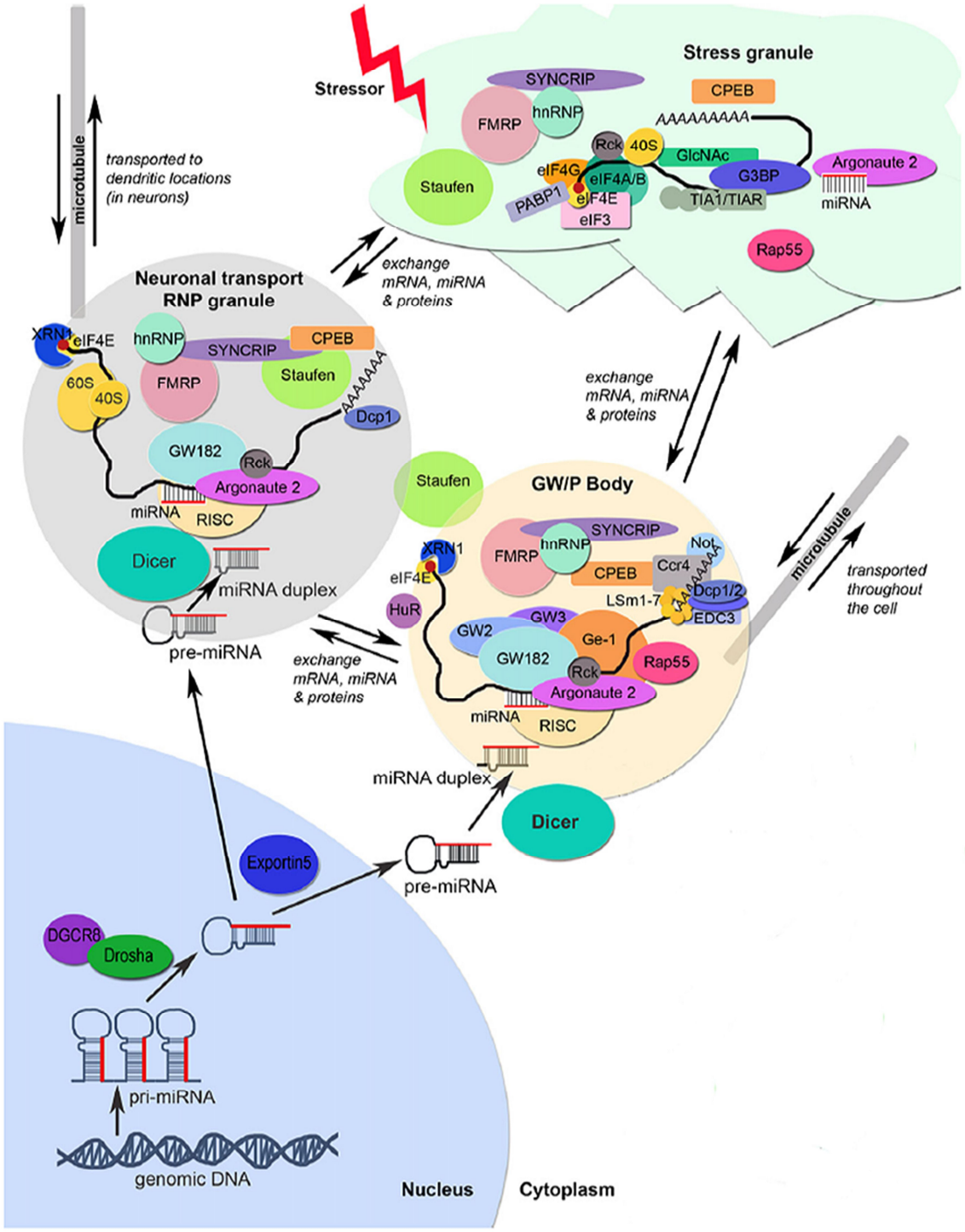


Figure 2-11 Cytoplasmic RNA granules

Schematic representation of the interactions between cytoplasmic transport granules, processing (P-) bodies and stress granules and some of the major components of the corresponding granule type. Note that some components can be found in more than one single type of granule (e.g. hnRNPs) while others are specific for a certain type (e.g. TIA1). Modified from (Moser and Fritzler, 2009).

2.7.1 Transport granules

Differential spatial distribution of proteins allows a cell to build up functionally distinct compartments and establish polarity, crucial processes for a cell to acquire its specific physiology. A powerful means to achieve local concentrations of a certain protein is to first localise its mRNA (translationally silent) to the desired place and then initiate protein synthesis. It has become clear during the last years that mRNA localisation is a widespread mechanism instrumental in many cellular functions. It enables a cell to quickly trigger local protein synthesis independent of transcription in the nucleus which lies potentially quite far away (Rodriguez et al., 2008). Well-known examples are the localisation of mRNAs to neuronal synapses which is thought to contribute to synaptic plasticity (Moser and Fritzler, 2009) and the localisation of MBP mRNA to the distal processes of oligodendrocytes to ensure efficient production of this major myelin protein at the site of myelin synthesis (Figure 2-12, see also sections 2.4.3 and 2.6.1).

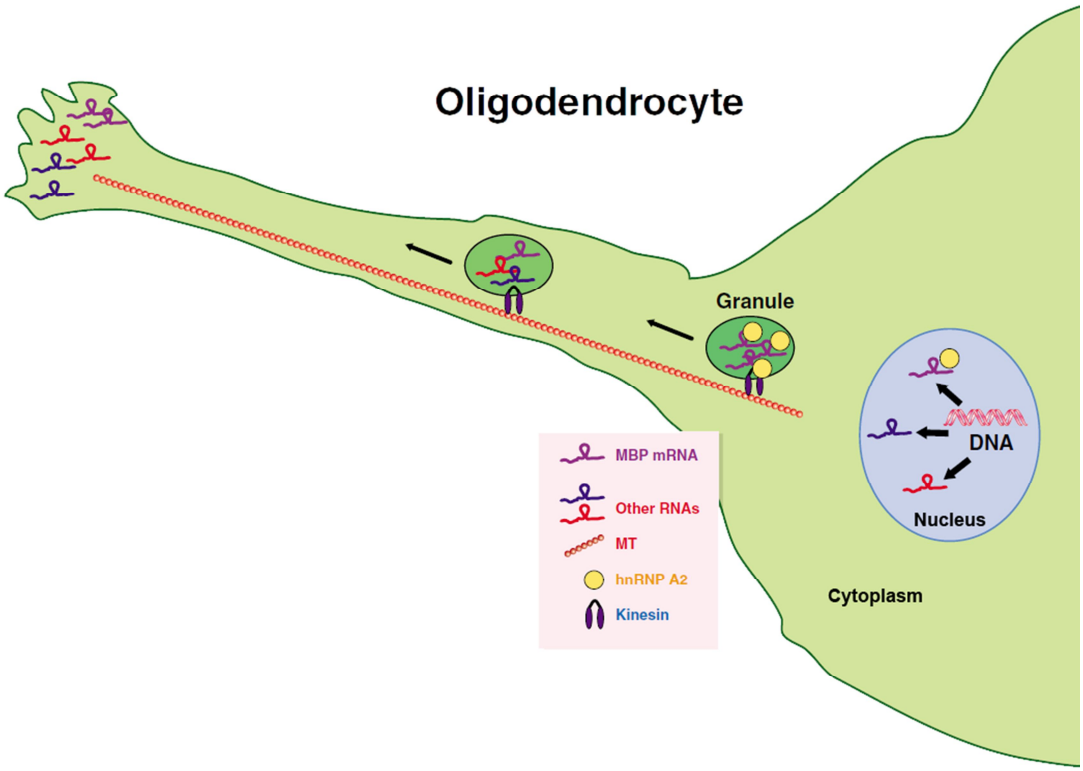


Figure 2-12 MBP mRNA transport in oligodendrocytes

After export from the nucleus, MBP mRNA is assembled into cytoplasmic RNA transport granules and transported in a translationally silenced state along the microtubule (MT) cytoskeleton to the periphery of the cell where translation can be triggered. This process is mediated by the *trans*-acting RNA-binding protein hnRNP A2 that binds to a *cis*-acting localisation element (A2RE) in the 3'UTR of MBP mRNA. Modified from (Shav-Tal and Singer, 2005).

Cytoplasmic transport of mRNA typically involves the formation of RNA transport granules, often referred to as neuronal transport granules. Sorting of mRNAs to transport granules is mediated by *cis*-acting signals (localisation elements; often parts of 3'UTRs; A2RE in MBP mRNA) in the mRNA which are recognised by specific *trans*-acting factors (RNA-binding proteins; hnRNP A2 in MBP mRNA). Several mRNAs (also coding for different proteins) can be assembled into one granule. Further components of the 150 – 1000 nm large transport granules include various RNA-binding proteins, ribosomal subunits, members of the translation initiation machinery, miRNAs and motor proteins in order to facilitate movement along the (mostly microtubule) cytoskeleton (reviewed by Moser and Fritzler, 2009).

2.7.2 Stress granules

Stress granules (SGs) are about 100 – 2000 nm in diameter and of heterogeneous shape. They are formed upon exposure of a cell to different kinds of environmental stressors including heat, hypoxia, glucose deprivation, oxidative conditions or UV irradiation. This type of cytoplasmic granule is considered as part of the cell's adaptation to the stress situation. During stress most of the normally translated mRNAs are localised to SGs and kept translationally silent and stable. Concomitant with this, translation of specific mRNAs, whose products help to cope with the stress, is increased. When the stress disappears, mRNA metabolism is reprogrammed and the stored mRNAs can re-enter a translationally active status. Thereby, energy can be saved during stress for necessary repairs and a fast and efficient return to the "normal" metabolism is ensured (Anderson and Kedersha, 2006; Erickson and Lykke-Andersen, 2011).

A major constituent of SGs are stalled 48S (translation) pre-initiation complexes including small ribosomal subunits and several eukaryotic translation initiation factors (eIFs). Furthermore, many RNA-binding proteins and miRNAs can be found there. SG assembly is dependent on the phosphorylation of eIF2α. Downstream of eIF2α, formation of SGs is promoted by self-aggregation of T-cell intracellular antigen (Tia)-1 or Ras-GAP SH3 binding protein (G3BP), further characteristic components of SGs (Moser and Fritzler, 2009).

2.7.3 Processing bodies

Processing (P-) bodies are thought to constitute discrete cytoplasmic foci where mRNA decay occurs, a model first suggested by Bashkirov and colleagues in 1997 (Bashkirov et al., 1997). In contrast to SGs, P-bodies are uniformly shaped, spheroid particles with a size of about 100 – 300 nm (Moser and Fritzler, 2009). Moreover, they are present

under “normal” conditions, but their number and size increase due to stress. P-bodies can move along microtubules and they seem to be able to “dock” to SGs and exchange components (Figure 2-11). It was speculated that SGs are a kind of mediator between polysomes and P-bodies evolving during stress to cope with the rapidly increasing amounts of non-translating mRNAs, sorting some to degradation and others to storage (Anderson and Kedersha, 2006).

Major elements in P-bodies are components of the 5’-3’ mRNA decay machinery, the nonsense-mediated decay pathway and the RNA-induced silencing complex (RISC), but no ribosomal subunits or translation initiation factors. Typical molecules enriched in P-bodies and contributing to these pathways are the 5’-3’ exoribonuclease 1 (XRN1) and the mRNA decapping enzymes 1 and 2 (Dcp1 and 2) (Moser and Fritzler, 2009).

2.8 Aim of the study

The non-receptor tyrosine kinase Fyn had been shown to play a major part in CNS myelination and evidence had been collected supporting the model that Fyn activity in oligodendrocytes significantly contributes to this phenotype. Potential oligodendroglial targets of Fyn had been identified that required validation. Moreover, it was planned to investigate the functions of these putative Fyn targets in oligodendrocytes in order to gain further insight into the important role of Fyn kinase in oligodendroglial physiology and myelination.

3 MATERIALS AND METHODS

3.1 Resources

3.1.1 Equipment

Transfection devices	
Gene Pulser®	Bio-Rad
Amaxa™ Nucleofector II	Lonza
Centrifuges	
Biofuge® *fresco*	Heraeus
Megafuge® 1.0 R	Heraeus
3K20	Sigma-Aldrich
Optima™ MAX-E (ultra-centrifuge)	Beckman Coulter
Microscopes	
DM LB Digital camera: DFC 350 F	Leica
DM 6000 B Digital camera: DFC 360	Leica
Other equipment	
Biotrak II Plate Reader (ELISA)	Amersham/GE Healthcare
Photospectrometer Ultrospec™ 2100 pro	Amersham/GE Healthcare
Nanodrop™ 1000 Photospectrometer	Thermo Scientific
OptiMax X-Ray (film processor)	MS Laborgeräte
T3 Thermocycler (PCR-Machine)	Biometra
Infinite® 200 (luminometer)	Tecan
StepOne™ (Real-Time-PCR system)	Applied Biosystems
LightCycler® 1.5 (Real-Time-PCR system)	Roche Applied Science

3.1.2 Materials

Chemicals	Sigma-Aldrich Roth
Plastic ware	Sarstedt BD Falcon
Glass ware	VWR
Miscellaneous	See text

3.1.3 Buffers and Media

General Buffers	
PBS	150 mM NaCl; 8 mM Na_2HPO_4; 1.7 mM NaH_2PO_4; adjust pH to 7.2
TBS	150 mM NaCl ; 50 mM Tris; adjust pH to 7.2
TBST	TBS + 0.1% (v/v) Tween 20
Cell culture	
10x PLL	0.1 % PLL in ddH_2O
Sato 1% HS (Oli-*neu*)	13.4 g/l DMEM; 2 g/l $NaHCO_3$; 0.01 g/l transferrin; 100 µg/l insulin (stock 10 µg/ml); 100 µM putrescine; 200 nM progesterone; 500 nM TIT; 220 nM Na_2SeO_3; 520 mM L-thyroxine; 0.05% gentamycine; 1% (v/v) horse serum; filtrate sterile
Differentiation medium (Oli-*neu*)	Sato 1% HS + daily addition of 1 mM dbcAMP
Sato/B27 1% HS (primary oligodendrocytes)	13.4 g/l DMEM; 2 g/l $NaHCO_3$; 20 ml/l B27 supplement; 0.011% pyruvate; 500 nM TIT; 520 mM L-thyroxine; 0.05% gentamycine; 1% (v/v) horse serum; filtrate sterile
$HBSS^+$	500 ml HBSS (1x)+ 7.5 ml $MgSO_4$ (stock: 10% (w/v))
COS7 medium	DMEM + L-Glutamine containing 10% FCS

COS7 transfection medium	DMEM + L-Glutamine containing 1% FCS (5ml FCS were pre-treated with 250 µl Protein A Sepharose to remove IgGs from the serum)
1% trypsin	100 ml 2,5% (v/v) trypsin; 15 ml HBSS (10x); 135 ml H_2O; 125 mg DNase I; adjust pH to 7.8; filtrate sterile; store aliquots at -20°C
Trypsin/EDTA „low“	0.2 ml 1% trypsin; 0.02% EDTA (stock: 0.2 % in HBSS) in HBSS
Freezing medium	70% (v/v) RPMI 1640; 20% (v/v) FCS; 10% (v/v) DMSO; filtrate sterile
Molecular biology	
50 x TAE buffer	242 g Tris (2 M); 100 ml 0.5 M EDTA pH 8.0 (50 mM); 57.1 ml acetic acid; fill up to 1 l with ddH_2O
LB medium	10 g trypton; yeast extract; 10 g NaCl; fill up to 1000 ml with ddH_2O and adjust pH to 7.4; autoclave
LB agar	4.5 g agar-agar in 300 ml LB medium; autoclave
SOC medium	20 g trypton; 5 g yeast extract; 0.5 g NaCl; 2.5 mM KCl; fill up to 1 l with ddH_2O and adjust pH to 7; autoclave; add 20 mM sterile glucose prior to use
Protein Biochemistry	
Lysis buffer	50 mM Tris-HCl, pH 7.4; 1% (v/v) NP-40; 0.25% (w/v) sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; Halt® Protease and Phosphatase Inhibitor Cocktails (Thermo Fisher) were added if appropriate
Kinase buffer	50 mM PIPES, pH 7.02; 10 mM $MgCl_2$
4x sample buffer	200 mM Tris-HCL, pH 6.8; 10% (w/v) SDS; 0.4% (w/v) bromphenol blue, 40% (v/v) glycerol, 400 mM DTT (if reducing conditions are desired)
SDS running buffer for electrophoresis (5x)	125 mM Tris; 1.25 M glycine; 0.5 % (w/v) SDS; adjust pH to 8.3
Stacking/Separation gel for SDS PAGE	See tables A8-9 and A8-10, section A8.43 in „Molecular Cloning – A Laboratory Manual“ (Sambrook and Russell, 2001)
Western Blot transfer buffer	24 mM Tris; 192 mM glycine; 20% ethanol

10 x Ponceau S	2% (w/v) Ponceau S; 30% (w/v) trichloric acid; 30% (w/v) sulfosalicylic acid
Blocking buffer	4% (w/v) dry milk powder in TBST or 3% (w/v) BSA in TBST (for phospho-protein analysis)
Stripping buffer	Add 16.7 ml 37% HCl to 700 ml ddH_2O; adjust pH to 2.0 with approximately 230 ml of 1 M glycine
ECL solution	Solution A: 200 ml 0.1 M Tris-HCl, pH 8.6; 50 mg luminol; store at 4°C Solution B: dissolve 11 mg para-hydroxy coumaric acid in 10 ml DMSO; keep dark Development: combine 1 ml solution A + 100 µl solution B + 0.3 µl H_2O_2
Immunocytochemistry	
Fixation solution	4% (w/v) paraformaldehyde in PBS
Blocking solution	PBS 10% horse serum
Mounting medium	2.4 g moviol 4-88; 6 g glycerol; 6 ml ddH_2O; 12 ml 0.2 M Tris, pH 8.5

3.1.4 Software

AIDA Image Analyzer 3.28	Raytest
CLC Sequence Viewer 6.4	CLC bio
Clone Manager 9.1	Scientific & Educational Software
Illustrator CS2	Adobe
ImageJ 1.38n	Wayne Rasband, NIH
Nanodrop Operating Software V3.7.1	Thermo Scientific
Photoshop CS2	Adobe

3.2 Antibodies

3.2.1 Primary antibodies

3.2.1.1 Monoclonal antibodies

Antigen	Name/Clone	Species	Application	Source
CNP	11-5B	mouse	1:500 (WB)	Sigma-Aldrich
F3	11-111	mouse	1:1000 (WB); 1:2 (IXL)	Dr. Rathjen, Berlin
Fyn	25	mouse	1:250 (WB); 1:50 (ICC)	BD Biosciences
hnRNP A2	EF67	mouse	1:500 (WB); 1:200 (ICC); 1:100 (IP)	Dr. Rigby, Dartmouth Medical School, USA
L1	555	rat	1:100 (WB)	Dr. Rathjen, Berlin
MBP	MCA409S	rat	1:1000 (WB); 1:500 (ICC)	Serotec
MOG	8-18C5	mouse	1:100 (WB)	Dr. Linington, University of Glasgow
Myc	9E10	mouse	1:1000 (WB); 1:200 (ICC); 1:100 (IP)	Sigma-Aldrich
NG2	-	rat	1:20 (ICC)	Dr. Trotter, University of Mainz
Phospho-tyrosine	4G10	mouse	1:500 (WB)	Millipore
PLP	AA3	rat	1:10 (ICC)	M. B. Lees, Waltham, MA
Sulfatide	O4	mouse	1:10 (IXL)	Sommer and Schachner, 1981

WB: Western Blot; ICC: Immunocytochemistry; IP: Immunoprecipitation; IXL: Immunocrosslinking

3.2.1.2 Polyclonal antibodies

Antigen	Name	Species	Application	Source
Cleaved Caspase 3	Cleaved Caspase-3 (Asp175)	rabbit	1:300 (WB)	Cell Signaling
DDX5	DDX5 (A300-523A)	rabbit	1:1000 (WB); 1:200 (ICC)	Bethyl Laboratories
Fyn	Fyn3 (SC-16)	rabbit	1:500 (WB); 1:50 (IP)	Santa Cruz
GAPDH	GAPDH (A300-641A)	rabbit	1:3000 (WB)	Bethyl Laboratories
hnRNP E1	T-18	goat	1:100 (WB)	Santa Cruz
hnRNP F	Ab50982	rabbit	1:1500 (WB); 1:250 (ICC)	abcam
Myc	Myc-Tag	rabbit	1:1000 (WB)	Cell signaling
Olig 2	Anti-Olig-2	rabbit	1:400 (ICC)	Millipore
p130Cas	C-20	rabbit	1:200 (WB); 1:50 (ICC)	Santa Cruz
Sox10	-	guinea pig	1:2000 (ICC)	Dr. Wegner, University of Erlangen
Src-pY^{418}	Src-pY^{418}	rabbit	1:1000 (WB); 1:100 (ICC)	Invitrogen
Tia-1	(C-20)	goat	1:1000 (WB); 1:400 (ICC)	Santa Cruz

WB: Western Blot; ICC: Immunocytochemistry; IP: Immunoprecipitation

3.2.2 Secondary antibodies

Target species	Host species	Conjugation	Application	Source
goat (specific)	bovine	HRP	1:5000 (WB)	Dianova
goat	donkey	Cy5	1:100 (ICC)	Dianova
guinea pig	goat	Cy5	1:100 (ICC)	Dianova
human	goat	HRP	1:2000 (WB)	Thermo Fisher Scientific
human	goat	-	1:100 (IXL)	Dianova
mouse	goat	HRP	1:10000 (WB)	Dianova
mouse (specific)	goat	HRP	1:5000 (WB)	Dianova
mouse	goat	Cy2	1:200 (ICC)	Dianova
mouse (specific)	goat	Cy3	1:100 (ICC)	Dianova
mouse	donkey	Alexa 546	1:400 (ICC)	Invitrogen
rabbit	goat	HRP	1:10000 (WB)	Dianova
rabbit	donkey	Cy2	1:200 (ICC)	Dianova
rabbit	goat	Cy3	1:1000 (ICC)	Dianova
rat	goat	HRP	1:10000 (WB)	Dianova
rat	goat	Alexa 488	1:400 (ICC)	Invitrogen
rat	goat	Cy5	1:100 (ICC)	Dianova

WB: Western Blot; ICC: Immunocytochemistry; IXL: Immunocrosslinking

3.3 DNA analysis and manipulation

3.3.1 Isolation of total RNA from cells

Total RNA was isolated from primary oligodendrocytes or Oli-*neu* cells by using the "RNeasy® Plus Mini Kit" from Qiagen according to the manufacturer's instructions.

3.3.2 Polymerase Chain Reaction (PCR)

To amplify deoxyribonucleic acid (DNA) from plasmids or complementary DNA (cDNA), a polymerase chain reaction (PCR) was carried out with the "Pfu Turbo" polymerase from Stratagene. Reactions were prepared as follows:

Pfu Turbo reaction buffer (10x)	10 µl
Forward primer [10 µM]	3 µl
Reverse primer [10 µM]	3 µl
dNTPs [2.5 nM each]	4 µl
Template DNA	~ 50 ng
Pfu Turbo polymerase	1 µl
Nuclease-free dH_2O	Fill up to 100 µl

Thermo-cycling conditions can be exemplified as follows:

	Step	Temperature	Time	
1	Initial denaturation	95°C	2 min	
2	Denaturation	95°C	45 sec	Repeat steps 2-4 between 25 and 40 times
3	Primer annealing	55-65°C (~ primer T_m -5°C)	45 sec	
4	Extension	72°C	1 min per kb	
5	Final extension	72°C	10 min	

Reactions were analysed by agarose gel electrophoresis (section 3.3.12).

3.3.3 Reverse transcription-PCR (RT-PCR)

For plasmid constructions, total RNA was reverse transcribed with the "Transcriptor High Fidelity cDNA Synthesis Kit" (Roche Applied Science) using the protocol below:

12.85 µl of RNA (maximal volume) were mixed with 0.4 µl random hexameric primers, incubated for 10 min at 65°C and then kept at 4°C. A master-mix was prepared using the following amounts:

Transcriptor High Fidelity Reaction buffer	4 µl
Protector RNase Inhibitor	0.5 µl
dNTP Mix, PCR-grade	2 µl
Transcriptor High Fidelity Reverse Trancriptase	0.25 µl

To each mixture of RNA and primers 6.75 ml master-mix were added and the below stated incubations were carried out in a thermo-cycler:

25°C	20 min
55°C	30 min
85°C	5 min

Desired segments of the obtained cDNA were amplified by PCR with gene-specific primers (see section 3.3.2).

Alternatively, reverse transcription and PCR were performed in the same tube using the "One Step RT-PCR Kit" from Qiagen or the "Super Script™ III One-Step RT-PCR System with Platinum® *Taq* High Fidelity" from Invitrogen according to the manuals' instructions.

To prepare cDNA as template for a quantitative PCR (section 3.3.4), RNA was reverse transcribed with the "Transcriptor High Fidelity cDNA Synthesis Kit" (Roche Applied Science) as described above in this section or with the "Quantitect Reverse Transcription Kit" from Qiagen following the instructions in the manual.

3.3.4 Quantitative PCR (qPCR)

Quantitative PCR was performed with the Real-Time PCR-System "StepOne™" from Applied Biosciences. 1 – 2 µl of a generated cDNA (5 – 50 ng/µl) (section 3.3.3) were utilised for a "TaqMan® gene expression assay" according to the manufacturer's instructions for 10 µl reaction volumes. The following primer/probe pairs (all from Applied Biosciences) were used:

Gene	Product number	Amplicon site	Specificity
β-actin	Mm00607939_s1	Exon 6	-
MBP	Mm01266402_m1	Spans "classic" exons 3 and 4	All "classic" isoforms
PgK1	Mm01225301_m1	Spans exons 6 and 7	-

Alternatively, qPCR was carried out using the "LightCycler® TaqMan Master Kit" and a "LightCycler® 1.5" capillary-based system (both from Roche Applied Science). The primers and probes for detection of hnRNP F, hnRNP A2, MBP, *Firefly* and *Renilla* luciferase were designed using the web-based "Universal ProbeLibrary Assay Design Center" from Roche Applied Science:

http://qpcr.probefinder.com/organism.jsp

3.3.5 Site-directed mutagenesis

Mutant constructs were generated with the "QuickChange® II Site Directed Mutagenesis Kit" from Agilent Technologies according to the manual's instructions. The used mutagenic primers are listed in section 3.3.13. They were designed using the "QuickChange® Primer Design" program which is available at:

https://www.genomics.agilent.com/CollectionSubpage.aspx?PageType=Tool&SubPageType=ToolQCPD&PageID=15

3.3.6 DNA restriction

Restriction of DNA (plasmids, PCR-products) for analytical or preparative purposes was performed as follows:

	Analytical	Preparative
DNA	~ 1 µg	10 µg
Enzyme buffer (10x)	2 µl	10 µl
Enzyme [20 U/µl]	0.5 µl	1 µl
Nuclease-free dH_2O	Fill up to 20 µl	Fill up to 100 µl
Digestion time	60 min	90 min

Restriction enzymes were purchased from New England BioLabs. Double digests were carried out according to the company's instructions at:

http://www.neb.com/nebecomm/DoubleDigestCalculator.asp

Reactions were analysed by agarose gel electrophoresis.

3.3.7 DNA ligation

Vector backbone and DNA insert were ligated using the "T4 DNA Ligase" from Fermentas following this reaction setup:

Ligase buffer (10x)	2 µl
T4 DNA Ligase [5 U/µl]	1 µl
Vector	~50 ng
Insert	5:1 molar ratio insert:vector
Nuclease-free dH_2O	Fill up to 20 µl

The reaction mixture was incubated for 1 hour at 22°C.

3.3.8 Transformation of bacteria

Standard cloning was carried out with chemically competent "Top 10 F' *E. Coli*" bacteria from Invitrogen.

Genotype Top 10 F':

F' {*lac*Iq Tn*10* (TetR)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1*ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

50 µl of bacteria suspension were thawed on ice. 14 µl of a ligation reaction (see section 3.3.7) were added and the mixture was incubated on ice for 30 min. After the cells were heat-pulsed for 1 min at 37°C, 1 ml of warm (37°C) lysogeny broth (LB) medium without antibiotics was added and the bacteria were incubated for 30 min at 37°C. Finally, the bacteria were plated on LB agar plates containing the appropriate antibiotic.

3.3.9 Plasmid preparation from bacteria

3.3.9.1 Minicultures

Clones of transformed bacteria (see section 3.3.8) were picked and grown over night at 37°C in 4 ml LB medium containing selective antibiotics, respectively. Plasmids were purified from 2 ml of the resulting bacteria suspension with the "PureLink™ Quick Plasmid Miniprep Kit" from Invitrogen according to the suggestions in the manual.

3.3.9.2 Maxicultures

100 µl from a miniculture (see section 3.3.9.1) or from a glycerol stock suspension (200 µl sterile glycerol + 800 µl bacteria suspension; storage at -80°C) were added to 100 – 400 ml LB medium and grown over night at 37°C. Plasmids were purified from the whole suspension using the "PureLink™ HiPure Plasmid Maxiprep Kit" from Invitrogen according to the manufacturer's instructions.

3.3.10 DNA purification from reactions

PCR or restriction products (see sections 3.3.2, 3.3.3 and 3.3.6) were purified from buffer components etc. in order to facilitate further processing. The purification from solutions was carried out using the "QIAquick® PCR Purification Kit" from Qiagen while DNA fragments were purified from agarose gels with the "QIAquick® Gel Extraction Kit" from Qiagen, in both cases following the directions in the manual.

3.3.11 Determination of nucleic acid concentrations

Concentrations of nucleic acid solutions were determined with a "Nanodrop™ 1000 Spectrophotometer" from Thermo Scientific using the "Nanodrop™ Operating Software V3.7.1".

Alternatively, nucleic acid concentrations were assessed by measuring the absorption of a solution at 280 nm with the photospectrometer "Ultrospec 2100 pro" from Amersham/GE Healthcare.

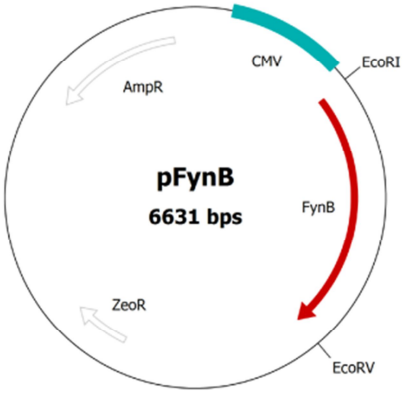
3.3.12 Agarose gel electrophoresis

For analysis of DNA fragments, they were separated by size by performing agarose gel electrophoresis. 1% (weight per volume (w/v)) agarose was dissolved in boiling Tris-acetate-EDTA (TAE) buffer. The solution was cooled down until no vapour was visible any more. Then, 6 µg/ml ethidium bromide were added and the solution was poured into a horizontal gel chamber. The solid gel was covered with TAE buffer and the samples were loaded on the gel after mixing them with a 6x loading dye solution (Fermentas). Electrophoresis was carried out by applying 5V/cm of distance between the electrodes. The "GeneRuler™ 1 kb DNA ladder" from Fermentas was used as marker to assess the size of the separated DNA fragments.

3.3.13 Generated expression vectors and used primers

To obtain expression vectors coding for wildtype mouse FynB, the coding sequence (CDS) of FynB was amplified by RT-PCR on total RNA from primary mouse oligodendrocytes (section 3.4.1) followed by cloning into the EcoRI/EcoRV sites of the pcDNA 3.1 Zeo + vector from Invitrogen (Figure 3-1). In the course of the amplification step, a Kozak sequence was added before the "Start" codon ATG to determine the site of translation initiation. Constitutive active Fyn (Y531F; Fyn+) was generated by exchanging tyrosine (Y) 531 in the C-terminal regulatory tail of the kinase for phenylalanine (F) by site-directed mutagenesis (section 3.3.5), preventing the possibility to phosphorylate this residue and thereby the kinase to change to its inactive conformation (section 2.5.2; Brown and Cooper, 1996).

Kinase inactive Fyn (K299M; Fyn-) was also obtained by site-directed mutagenesis, this time by mutation of lysine (K) 299 to methionine (M). Lysine 299 is part of the ATP-binding domain of the kinase. The mutation inhibits the binding of ATP, rendering the kinase inactive (section 2.5.2; Osterhout et al., 1999).



Primers:

EcoRI Kozak-sequence

forward: 5' - CGAATTCGCCACCATGGGCTGTGTGCAATG - 3'
reverse: 5' - CAGATATCTCACAGGTTTTCACCGGGCTG - 3'

EcoRV

Template for insert:

Total RNA from primary mouse oligodendrocytes

Vector backbone:

pcDNA 3.1 Zeo +

Variants:

Constitutive active Fyn (Y531F; Fyn +)

Mutagenic primers:

Forward: 5' - CCACAGAGCCCCAGTTCCAGCCCGGTGAAAA - 3'
Reverse: 5' - TTTTCACCGGGCTGGAACTGGGGCTCTGTGG - 3'

Kinase dead Fyn (K299M; Fyn -)

Mutagenic primers:

Forward primer: 5' - GAATGGAAATACAAAAGTAGCCATAATGACCCTTAAGCCAG - 3'
Reverse primer: 5' - CTGGCTTAAGGGTCATTATGGCTACTTTTGTATTTCCATTC - 3'

Figure 3-1 Expression vectors for FynB from mouse

The coding sequence of mouse FynB was amplified by RT-PCR and cloned into the EcoRI/EcoRV sites of the pcDNA 3.1 Zeo + vector (Invitrogen). Constitutive active (Fyn+) and kinase dead (Fyn-) mutants were obtained by site-directed mutagenesis (mutated sites marked in red) exchanging tyrosine 531 for phenylalanine (Y531F) and lysine 299 for methionine (K299M), respectively.

Expression vectors for myc-His- and Yellow Fluorescent Protein- (YFP-)tagged FynB constructs were generated by PCR on the above described FynB plasmids and subsequent cloning into the EcoRI/XbaI sites of the pcDNA 4/TO/myc-His vector (Invitrogen; Figure 3-2) or the EcoRI/SalI sites of the peYFP-N1 vector (Clontech; Figure 3-3), respectively, resulting in C-terminally tagged FynB variants. Again, Kozak sequences were added with the primers.

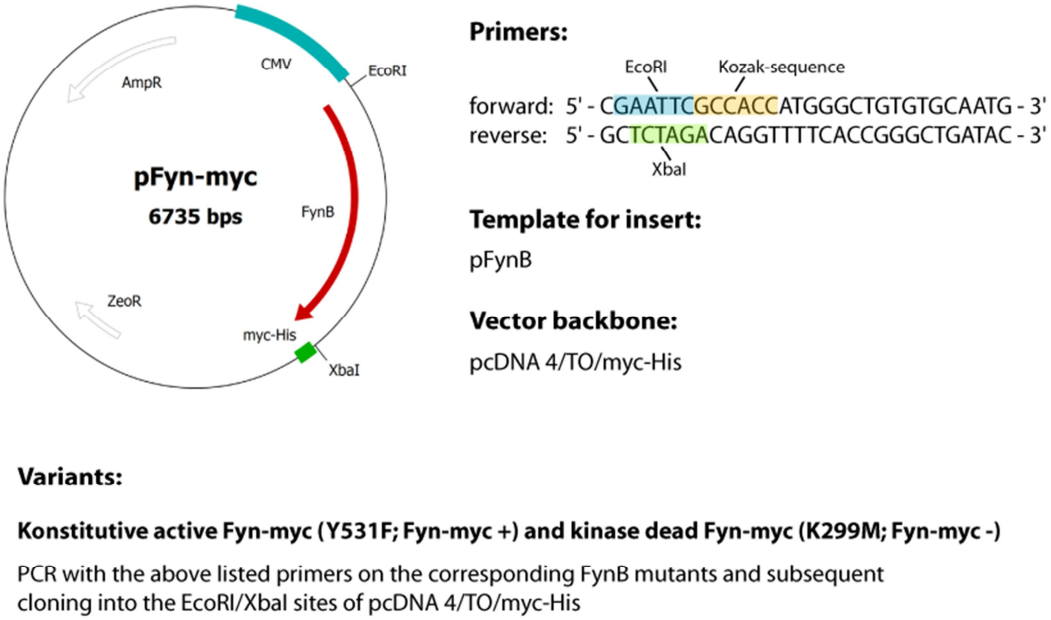


Figure 3-2 Expression vectors for myc-tagged FynB

The coding sequences of FynB and its constitutive active (short: Fyn+) and inactive (short: Fyn-) variants were amplified by PCR and cloned into the EcoRI/XbaI sites of the pcDNA 4/TO/myc-His vector (Invitrogen), respectively. From these plasmids C-terminal myc-His-tagged FynB fusion proteins (short: Fyn-myc) can be expressed.

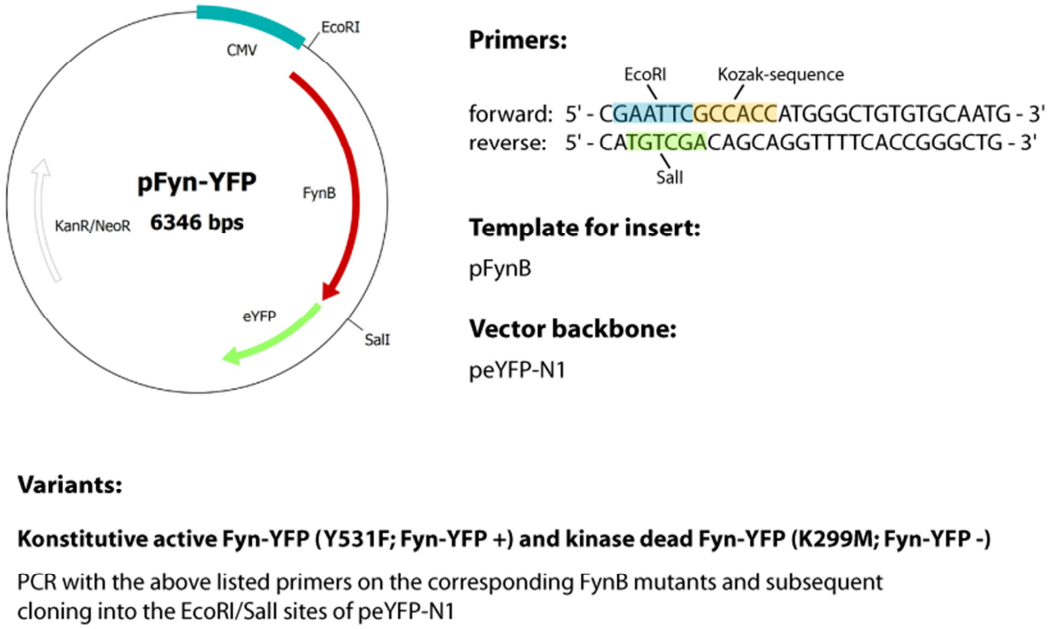


Figure 3-3 Expression vectors for YFP-tagged FynB

The coding sequences of FynB and its constitutive active (short: Fyn+) and inactive (short: Fyn-) variants were amplified by PCR and cloned into the EcoRI/SalI sites of the peYFP-N1 vector (Clontech), respectively. From these plasmids C-terminal YFP-tagged FynB fusion proteins (short: Fyn-YFP) can be expressed.

A construct for wildtype mouse hnRNP F was obtained by amplifying its CDS by PCR on the peGFP-hnRNP F vector (Gonsior, 2007). The resulting fragment was introduced into the EcoRV/XhoI sites of the pcDNA 3.1 Zeo + vector (Invitrogen; Figure 3-4). With the primers a Kozak sequence was added before the "Start" codon ATG to determine the site of translation initiation.

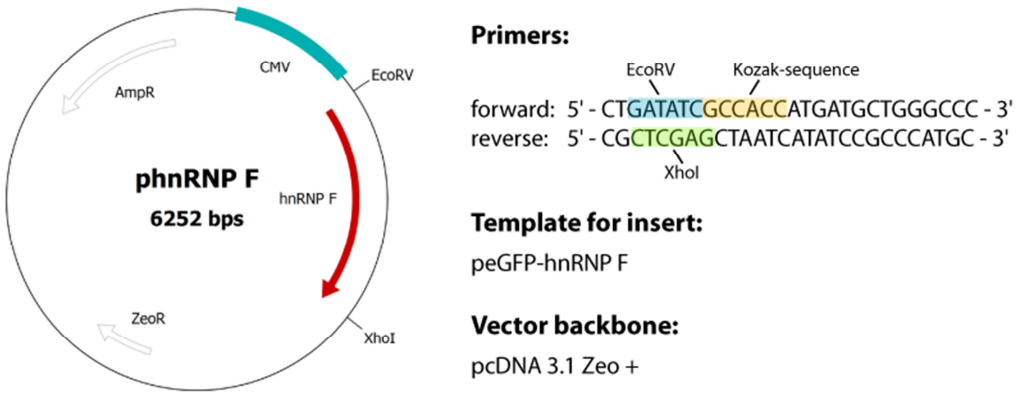


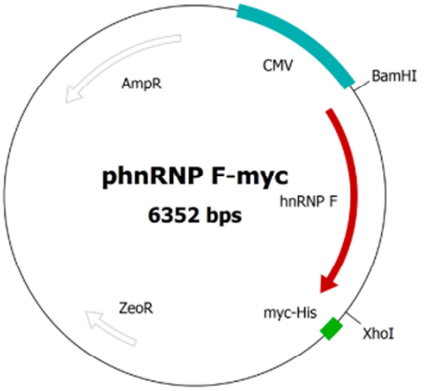
Figure 3-4 Expression vector for hnRNP F

The coding sequence of mouse hnRNP F was amplified by PCR and introduced into the EcoRV/XhoI sites of the pcDNA 3.1 Zeo + vector (Invitrogen).

Similarly, a PCR on peGFP-hnRNP F with subsequent cloning into the BamHI/XhoI sites of the pcDNA 4/TO/myc-His vector (Invitrogen) yielded in a plasmid coding for C-terminally myc-His-tagged hnRNP F (short: F-myc; Figure 3-5). Also here, a Kozak sequence was added with the primers.

For analysis of putative tyrosine phosphorylation sites, mutants of F-myc were generated in which a certain tyrosine residue (Y) was exchanged for phenylalanine (F), respectively. This was achieved by site-directed mutagenesis of wildtype F-myc (Y246F) or by performing a PCR on previously made mutants of peGFP-hnRNP F (Gonsior, 2007). The amplified mutant CDSs of hnRNP F were then cloned into the BamHI/XhoI sites of the pcDNA 4/TO/myc-His vector from Invitrogen (Figure 3-5).

In order to generate a tool for investigations on cellular events post-transcriptionally influencing the expression of MBP dependent on its 3'UTR, this region was amplified by PCR on a plasmid containing the 14 kDa isoform of MBP and its 3'UTR (provided by Dr. M Simons, University of Göttingen) The 3'UTR of MBP was then introduced into the EcoRI/XhoI sites of a pcDNA 3.1 + vector (Invitrogen), downstream of the CDS of *Firefly* luciferase (Figure 3-6).



Primers:

BamHI Kozak-sequence

forward: 5' - CGGATCCGCCACCATGATGCTGGGCCC - 3'
reverse: 5' - CGCTCGAGATCATATCCGCCCATGCTG - 3'

XhoI

Template for insert:

peGFP-hnRNP F

Vector backbone:

pcDNA 4/TO/myc-His

Variants:

hnRNP F-myc Y246F

Mutagenic primers:

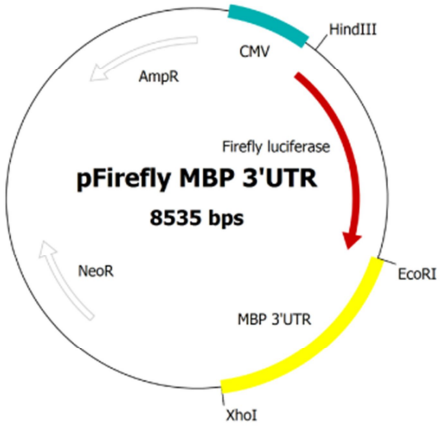
Forward: 5' - CTATGGGGGCTATGAAGAATTCAGTGGCCTCAG - 3'
Reverse: 5' - CTGAGGCCACTGAATTCTTCATAGCCCCCATAG - 3'

hnRNP F-myc Y298F and Y306F

PCR on the corresponding eGFP-hnRNP F mutants with the above listed primers and subsequent cloning into the BamHI/XhoI sites of pcDNA 4/TO/myc-His

Figure 3-5 Expression vector for myc-tagged hnRNP F

The coding sequence of mouse hnRNP F was amplified by PCR and introduced into the BamHI/XhoI sites of the pcDNA 4/TO/myc-His vector (Invitrogen), resulting in a plasmid coding for C-terminal myc-His-tagged hnRNP F (short: hnRNP F-myc or F-myc). Mutant constructs were either generated by site-directed mutagenesis of F-myc (Y246F, mutated site marked in red) or by PCR on the corresponding peGFP-hnRNP F mutants (Y298F and Y306F) followed by the previously described cloning step. Thereby, F-myc mutants were obtained where a single tyrosine is changed to phenylalanine, respectively.



Primers (MBP 3' UTR):

EcoRI

forward: 5' - AGAATTCGAGCCCTCCCCGCTCAGC - 3'
reverse: 5' - CACTCGAGTACCGTCGACTGCACAGTTCCG - 3'

XhoI

Template for insert:

pIRES2-EGFP containing the CDS of MBP14 and its complete 3'UTR

Vector backbone:

pcDNA 3.1 + containing *Firefly* luciferase in the HindIII/EcoRI sites

Figure 3-6 MBP 3'UTR reporter construct

The complete 3'UTR of classic MBP was amplified by PCR on a plasmid and cloned downstream of the coding sequence of *Firefly* luciferase, yielding a reporter construct for the analysis of MBP 3' UTR-dependent cellular processes.

3.3.14 RNA interference

In this study, RNA interference (RNAi) was performed by using small synthetic double stranded RNA molecules, called small interfering RNA (siRNA). Transfection of these oligonucleotides into cells leads to reduced expression (knockdown) of a certain targeted protein at the mRNA level, mediated by the cytoplasmic RISC complex (Agrawal et al., 2003). This allows for analysis of cellular processes dependent on the selected protein. The following siRNAs were used:

Target	Target sequence	siRNA data
Non-silencing control	AATTCTCCGAACGTGTCACGT	Source: Qiagen; RNA sequence: r(UUCUCCGAACGUGUCACGU)dTdT (sense) r(ACGUGACACGUUCGGAGAA)dTdT (antisense)
Fyn	CTCGTTGTTTCTGGAGAAGAA	Source: Qiagen; RNA sequence: r(CGUUGUUUCUGGAGAAGAA)dTdT (sense) r(UUCUUCUCCAGAAACAACG)dAdG (antisense)
hnRNP F	GCAUGGGCGGAUAUGAUUA AGGAGGAAGUUAGAUCAUA GUACAUUGGCAUUGUGAAA CAACGGAGAACGACAUUUA	Source: Dharmacon (Thermo Scientific) siGENOME SMARTpool M-051363-00-0005 (Mouse 4833420I20RIK)

3.4 Cell culture

3.4.1 Preparation and culture of primary oligodendrocytes

Primary mouse oligodendrocytes were prepared according to (Trotter et al., 1989). The animals came from the animal facility of the Johannes Gutenberg University in Mainz.

Brains from embryonic day 14 – 16 C57/BL6 mice were freed from meninges, incubated shortly in 1% trypsin and washed with Hank's balanced salt solution + (HBSS+). To obtain a single cell suspension, the brains were put in a 0.05% DNase solution and passed several times through Pasteur pipettes with decreasing diameters. The dissociated cells were spun down for 10 min at 190g and 4°C and resuspended in HBSS+. After they were pelleted again, the cells were resuspended in Dulbecco's Modified Eagle Medium (DMEM) with 10% horse serum (HS) and plated on poly-L-lysine (PLL)-coated dishes (whole brain culture) at a density of 3.7 – 4.4 x 10^5 cells per cm^2.

5 days later, removal of neurons from the culture was achieved by immune cytolysis using monoclonal M5 antibody (1:10, from 358-hybridoma supernatant) and complement from guinea pig (1:15). Further 4 days later, microglia were removed by moderate tapping of the culture vessel. On day 14 in culture, microglia were detached again and finally the oligodendrocytes were taken off the astrocyte layer (on the ground of the culture vessel) by intense shaking ("oligo shake"). The oligodendrocytes were plated in B27 1% HS medium ((Bottenstein and Sato, 1979) modified Sato medium according to (Trotter et al., 1989)) on PLL-coated culture dishes at a density of 1.4 – 2.1 x 10^5 cells per cm^2. Besides stated elsewhere, the cells were kept in the presence of 10 ng/ml Platelet Derived Growth Factor (PDGF) and 5 ng/ml basic Fibroblast Growth Factor (bFGF) for 2 days, to promote proliferation and survival (Miller, 2002; Colognato and ffrench-Constant, 2004).

Primary oligodendrocytes were cultured in B27 1% HS medium at 37°C and 8% CO_2.

3.4.2 Oli-*neu* cells

The oligodendroglial cell line Oli-*neu* was generated by gene transfer using replication-deficient retroviruses to infect primary oligodendrocytes. The transferred t-*neu* oncogene is a constitutive active variant of the c-*neu* proto-oncogene and it is expressed in Oli-*neu* cells under the control of a thymidine kinase promoter (Jung et al., 1995). The cells have the character of oligodendrocyte precursor cells but can be induced to differentiate by daily addition of 1 mM dibutyryl cyclic AMP (dbcAMP).

Oli-*neu* cells were cultured on PLL-coated dishes with Sato 1% HS medium at 37°C and 8% CO_2. For passaging, the cells were detached from the surface by incubating them for 1 – 2 min with warm (37°C) Trypsin/EDTA "low". The reaction was stopped by the addition of cold (4°C) phosphate buffered saline (PBS) 10% HS. For long time storage, Oli-*neu* cells were frozen in freezing medium at -80°C and kept in liquid nitrogen afterwards.

3.4.3 Cos7 cells

Cos7 cells were generated by transformation of a monkey kidney fibroblast cell line (CV-1 cells). They express the large T-antigen from Simian Virus 40 (SV40) which promotes the replication of plasmids containing an SV40 origin of replication. This leads to high expression rates of proteins encoded by such vectors, making these cells ideal for the production of recombinant proteins.

Cos7 cells were cultured in DMEM 10% foetal calf serum (FCS) at 37°C and 10% CO_2. For transfection, serum concentration was lowered to 1% to improve transfection effi-

ciencies and expression. Passaging and storage was carried out as described for Oli-*neu* cells (section 3.4.2).

3.4.4 Transfection

3.4.4.1 Plasmids

Fugene® HD (Promega; previously Roche Applied Science):

This method was used to transfect Oli-*neu* cells on 6-well plates or 6 cm culture dishes. The following protocol (in principle matching the instructions in the manual) applies to the 6-well format (2 ml of medium per well) and was scaled up for other formats according to the amount of culture medium (4 ml for 6 cm dishes). 10^5 cells were seeded per well. On the next day the complete culture medium was replaced with fresh medium prior to transfection. 2 µg plasmid DNA were diluted in 100 µl DMEM, 4 µl Fugene® HD were added and the mixture was vortexed briefly. After 15 min incubation at room temperature (RT) the mixture was applied dropwise to the culture medium.

Polyethylenimine (PEI):

PEI was used to transfect Oli-*neu* cells on 6-well plates as well as 6 or 10 cm culture dishes. The following protocol applies to the 6-well format (2 ml of medium per well) and was scaled up for other formats according to the amount of culture medium (4 ml for 6 cm and 8 ml for 10 cm dishes). 10^5 cells were seeded per well 12 hours prior to transfection. DMEM and PEI were pre-warmed to RT. 1 µg of plasmid DNA and 4 µl PEI were diluted in 30 µl DMEM, respectively. After 10 min incubation at RT, the solutions were combined, briefly vortexed and incubated for another 15 min at RT. Finally, the transfection mixture was added dropwise to the culture medium. 4 – 6 hours after transfection, the complete medium was replaced by fresh medium.

JetPEI™ (Polyplus-transfection):

With this method plasmids coding for the L1-Fc fusion protein (Oleszewski et al., 1999) were introduced into Cos7 cells for the production of recombinant L1-Fc. 1.5 x 10^6 Cos7 cells were plated into 15 cm culture dishes in 18 ml transfection medium (section 3.4.3). Transfection was carried out on the following day. 12 µg of plasmid DNA and 24 µl JetPEI™ were mixed with 1 ml of a sterile 150 mM NaCl solution, respectively. The JetPEI™ containing solution was added to the DNA containing solution and incubated at RT for 30 min. Finally, the mixture was administered to the culture dish.

Electroporation (Gene Pulser® Xcell™, Bio-Rad):

10 – 15 µg of plasmid DNA were mixed with 1.8 x 10^6 Oli-*neu* cells in 600 µl warm (37°C) Sato 1% HS medium in a 4 mm electroporation cuvette. The cell suspension was incubated for 5 min at RT, pulsed (220 V, 950 µF; exponential decay program) with a Gene Pulser® Xcell™ device, incubated another 5 min at RT and plated on PLL-coated 10 cm culture dishes. Alternatively, 2 transfections were combined on a 15 cm dish. The complete culture medium was replaced with fresh medium 4 – 16 hours after transfection.

3.4.4.2 siRNA

Fugene® HD (Promega; previously Roche Applied Science):

Transfection was performed in principle following the manufacturer's suggestions. 10^5 cells were seeded per well. On the next day the complete culture medium was replaced with fresh medium prior to transfection. 100 pmol siRNA were diluted in 100 µl DMEM, 4 µl Fugene® HD were added and the mixture was vortexed briefly. After 15 min incubation at RT, the mixture was applied dropwise to the culture medium.

Basic Primary Neurons Nucleofector® Kit (Amaxa™ Nucleofector II, Lonza):

10^6 Oli-*neu* cells or 4 x 10^6 primary oligodendrocytes (suspension after "oligo shake" (section 3.4.1) were pelleted, resuspended in 100 µl nucleofector solution and combined with 160 pmol siRNA. The mixture was transferred to an electroporation cuvette and nucleofected by executing program "O-005" of the Nucleofector® II device. Carefully, 500 µl warm (37°C), CO_2-equilibrated RPMI 1640 medium were added, the whole solution was taken up in a plastic pipette and carefully plated on PLL-coated culture dishes (3.5 or 10 cm for primary oligodendrocytes or Oli-*neu* cells, respectively) containing CO_2-equilibrated Sato 1% HS or B27 1% HS (with growth factors, see section 3.4.1) medium. 4 – 6 hours after nucleofection, the complete medium was exchanged with fresh medium containing growth factors.

3.5 Protein analysis

3.5.1 Cell lysis

Cells were scraped off in cold lysis buffer (50 mM Tris pH 7.4; 150 mM NaCl; 1 mM Ethylen-diamine-tetraacetic acid (EDTA) pH 7.4; 1% (volume per volume (v/v)) NP-40; 0.25% (v/v) sodium deoxycholate if applicable (enhances stringency in immunoprecipitations) containing "HALT™" protease and phosphatase inhibitor cocktails (Thermo

Scientific) and incubated on a rotating wheel for 45 min. Post-nuclear supernatants were obtained by pelleting the nuclei for 10 min at 300g and 4°C.

For simultaneous analysis of nuclear and cytoplasmic fractions, cells were lysed using the "NE-PER™ nuclear and cytoplasmic fractionation kit" (Pierce) according to the manufacturer's instructions.

To deplete from RNA, lysates were treated with 50 µg/ml RNase A (Invitrogen) or with 50 U/ml "RNasin®" (Promega; control condition) for 12 min at 37°C.

Granule-free lysates were obtained by ultra-centrifugation of post-nuclear supernatants for 30 min at 136000g and 4°C.

3.5.2 Determination of protein concentrations

Protein concentrations of lysates were determined using the "BCA Protein Assay Kit" from Novagen following the manufacturer's instructions for the "enhanced assay".

3.5.3 Purification of proteins

3.5.3.1 L1-Fc purification

3 – 5 days after transfection with L1-Fc plasmids (see section 3.4.4.1), the complete medium of the Cos7 cells was collected (L1-Fc protein is secreted to the medium). Purification was carried out at 4°C using a "HiTrap™ Protein A Column" (1 ml, Amersham Biosciences) connected to a peristaltic pump with the flow rate adjusted to 1.5 ml/min. Initially, the column was washed with ~50 ml PBS. The collected medium was centrifuged for 10 min at 1000g and 4°C to remove dead cells and cell fragments that could clog the column. The entire medium was then applied to the column followed by a washing step with ~50 ml PBS. Washing was performed until the optical density (OD) of the flow through at a wavelength of 280 nm reached the value of 0, to check for unspecifically bound proteins. In order to elute L1-Fc, the column was connected to a syringe and 2 ml of elution buffer (0.1 M glycine, pH 2.7) were applied. The eluate was collected in tubes containing 120 µl 1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) to immediately raise the pH to a neutral level. After repeating the elution step, the eluate was dialysed twice using a "Slide-A-Lyzer® Dialysis Cassette" (0.5 – 3 ml, Pierce) in 2 l PBS for 2 hours, respectively. Finally, the dialysed solution was concentrated with "Centricon®" centrifugal filter devices (YM-100, Millipore) to about 500 µl and the protein concentration was measured as described in section 3.5.2. The presence and purity of L1-Fc were assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent Coomassie staining and Western anal-

ysis, respectively (sections 3.5.5 to 3.5.7). Purified L1-Fc was stored in 10 µg aliquots at -20°C.

3.5.3.2 Immunoprecipitation

All incubation steps were carried out at 4°C on a rotating wheel.

Antibody-bead complexes were built by incubation of Protein A or G Sepharose with the appropriate amount of antibody in 500 µl PBS for 2 hours (the Sepharose with the highest binding affinity was chosen according to (Bonifacino et al., 2001)). Cell lysates were incubated with 20 µl packed Sepharose for 45 min (pre-clear). For immunoprecipitation (IP), pre-cleared lysates were incubated with the prepared antibody-bead complexes for 4 hours or overnight. Beads were washed 4 times with 1 ml lysis buffer and once with 1 ml PBS. Between the washing steps, the beads were spun down with 1000g for 10 sec at 4°C. To elute the immunoprecipitates from the beads, they were incubated in each case with 45 µl 2x sample buffer (see section 3.1.3) for 10 min at 90°C and 800 rounds per minute (rpm) on a shaking device. Beads were spun down briefly and 40 µl of the supernatant were collected.

Tyrosine-phosphorylated proteins were purified by IP using 4G10 antibody-coupled agarose beads according to the manufacturer's instructions (Millipore) using 50 µl slurry per IP. Elution from the beads was performed as described above.

3.5.3.3 Co-immunoprecipitation of protein/RNA complexes

In principle, protein-RNA co-IPs were carried out as stated in section 3.5.3.2 (with 4 hours incubation of the lysate with the antibody-bead complex), but buffers were prepared with DEPC-treated H_2O and 50 U/ml "RNasin®" (Promega) were added. Proteins and RNA were eluted from the beads by incubation in each case with 0.2% (w/v) SDS and 2% (v/v) β-mercaptoethanol in 100 µl nuclease-free H_2O for 5 min at 70°C, of which 10 µl were used for Protein analysis (SDS-PAGE and Western Blotting, sections 3.5.5 and 3.5.7). For RNA analysis, the remaining 90 µl were diluted in 700 µl "QIAzol® Lysis Reagent" and RNA purification was performed with the "miRNeasy Plus Mini Kit" following the manual's directions. Synthesis of cDNA and qPCR were carried out according to sections 3.3.3 and 3.3.4.

3.5.4 Fyn kinase assay

Proteins were immunoprecipitated as described in section 3.5.3.2. The first two washing steps of the IP were performed with lysis buffer. The third washing step was performed with kinase buffer (50 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES),

pH 7.02; 10 mM $MgCl_2$) and the last one with kinase buffer containing 50 µM ATP. In each case, 30 ng active recombinant human FynB (Fyn A, active; SignalChem) were added to the beads in 40 µl kinase buffer with 50 µM ATP. As control condition, the kinase was replaced with an equal amount of distilled H_2O (dH_2O). The assay was carried out incubating the beads for 20 min at 30°C and 800 rpm on a shaking device. The reaction was stopped and the proteins were eluted by the addition of 15 µl 4x sample buffer (see section 3.1.3) and heating of the beads for 5 min at 90°C at 800 rpm on a shaking device. Phosphorylated proteins were analysed by SDS-PAGE and Western Blotting.

3.5.5 SDS-PAGE

For analysis, proteins were separated by size performing a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Gels were prepared following the suggestions in "Molecular Cloning – A Laboratory Manual" (Sambrook and Russell, 2001), tables A8-9 and A8-10 in section A8.43, respectively. Depending on the sizes of the proteins to be investigated, different acrylamide concentrations were used for the separation gels. Pouring and running of the gels were carried out utilising the "Mini PROTEAN® 3 System" from Bio-Rad.

Alternatively, proteins were separated with the "Novex® NuPAGE® SDS-PAGE Gel system" (pre-cast gels) from Invitrogen using 4 – 12% Bis-Tris gradient gels in MOPS or MES buffer, dependent on the desired separation pattern.

Before loading on the gel, protein samples were mixed with an appropriate amount of 4x sample buffer and heated for 5 min at 90°C to denature the proteins. To assess the size of the separated proteins, the "SigmaMarker™ Wide Range" from Sigma-Aldrich or the prestained marker "Precision Plus" from Bio-Rad was run on the same gels.

3.5.6 Coomassie staining

To visualise proteins in the gel after SDS-PAGE, the colloidal Coomassie staining solution "Roti®-Blue" from Roth was used as described in the manual.

3.5.7 Western Blotting

For Immunodetection of proteins following SDS-PAGE, they were blotted from the gels on polyvinylidene fluoride (PVDF) membranes ("Immobilon™-P Transfer Membrane", Millipore) using the "Mini Trans-Blot® Electrophoretic Transfer Cell" system (Bio-Rad) for 3 hours at 200 mA or for 12 hours at 50 mA.

Following the blotting procedure, the membrane was washed briefly in dH_2O and proteins (in particular the marker bands) were stained with Ponceau S (see section 3.1.3). The membrane was washed again in dH_2O to remove the staining solution.

In order to block unspecific binding to the membranes, they were incubated for at least 30 min in blocking buffer (see section 3.1.3). First antibodies were applied in blocking buffer for 1 – 3 hours at RT or overnight at 4°C followed by three times washing of the membrane for at least 10 min each in Tris buffered saline 0.1% (v/v) Tween 20 (TBST). Secondary antibodies coupled with horseradish peroxidase (HRP) also were incubated in blocking buffer for 30 – 60 min at RT. Hereafter, two washing steps were carried out with TBST again but the last wash was performed with TBS only.

HRP activity was utilised for an enhanced chemiluminescence reaction (ECL, see section 3.1.3) and the proteins were subsequently visualised on X-ray films ("Hyperfilm™ ECL", Amersham/GE Healthcare).

Membranes were stored at -20°C and could be used for new detections later.

To remove disturbing primary and/or secondary antibodies from the membrane, it was incubated with stripping buffer (see section 3.1.3) for 1 – 4 hours (replacing the buffer every hour) or twice for 5 min at RT, respectively.

3.6 Immunocytochemistry

Coverslips on ice were washed two times with cold PBS. The subsequent steps were performed at RT, besides stated otherwise. The cells were fixed for 15 min in 4% (w/v) paraformaldehyde and permeabilised with 0.1% Triton X-100 in PBS for 2 min. After blocking with 10% HS in PBS for at least 1 hour, primary antibodies were applied for 1 hour at RT or overnight at 4°C in blocking medium. After three washing steps with PBS for 2 min each, detection was performed with secondary antibodies conjugated with a fluorescent dye in blocking medium for 20 min. In some cases nuclei were stained with 4'6-Diamidino-2-phenylindole (DAPI) for 2 min. The three washing steps were repeated after secondary antibody incubation and/or DAPI staining, respectively. Coverslips were shortly dipped in double-distilled H_2O (ddH_2O) before they were mounted in Moviol on object slides.

Images were acquired using a microscope (DM LB) with a 40×/0.7 numerical aperture (NA) objective lens or a 100×/1.3 NA oil objective lens connected to a digital camera (DFC 350F) using Application Suite 2.5.0 software or alternatively using a DM 6000 B microscope with a 20x/0.7 NA objective lens, a 40x/0.75 objective lens or a 63×/1.32 NA oil objective lens connected to a digital camera (DFC 360) using LASAF software (all

from Leica). Stacked images were processed by blind deconvolution with 5 iterations and single planes were shown. Images were adjusted using Image J (Wayne Rasband, NIH) and Photoshop (Adobe).

3.7 L1-Fc binding

Oli-*neu* cells or primary oligodendrocytes were allowed to differentiate for 3 days. The cells were put on ice and washed twice with cold PBS. 15 nM L1-Fc (see section 3.5.3.1) or human Immunoglobulin G (IgG, control-Fc) were applied to the cells for 1 hour in 3% (w/v) bovine serum albumin (BSA) in PBS at 4°C. The cells were washed two times on ice with cold PBS and incubated with a goat-anti-human IgG antibody (1:100 in 3% BSA in PBS) for 2 min at 4°C followed by 5 min incubation at 37°C. Again, the cells were put on ice and washed twice with cold PBS, before they were lysed in the presence of protease and phosphatase inhibitors.

3.8 Cell ELISA (L1-Fc)

1.5 x 10^4 Oli-*neu* cells per well were seeded into a 24-well plate where they were allowed to differentiate for 4 days. The cells were either treated with 25 nM L1-Fc, 25 nM human IgG (control-Fc) 25 nM L1-Fc in the presence of antibody against F3 (1:2) or with 25 nM L1-Fc in the presence of O4 antibody (1:10) for 1 hour at 4°C. After 2 washing steps with PBS, the cells were fixed for 10 min with 4% paraformaldehyde. A blocking step was performed with PBS 10% FCS for 30 min at room temperature before the cells were incubated for 60 min with HRP-coupled goat-anti-human Fc antibody (1:2000 in PBS, Pierce). The cells again were washed twice with PBS followed by incubation with tetramethylbenzidine (Pierce) according to the manufacturer's suggestions to detect bound HRP. From each well 100 µl were transferred to a 96-well plate and colour development was measured in a "Biotrak II Plate Reader" from Amersham/GE Healthcare.

3.9 Luciferase assay (MBP 3'UTR-dependent translation)

The luciferase assays were carried out with Oli-*neu* cells on 6-well culture plates.

1 day after siRNA treatment (Amaxa or Fugene HD, section 3.4.4.2), Oli-*neu* cells were transfected (Fugene HD, section 3.4.4.1) with 250 ng A2RE *Firefly* luciferase reporter (White, 2007) or 250 ng full length-MBP 3'UTR *Firefly* luciferase reporter (see Figure 3-6 and Figure 3-7), 100 ng *Renilla* luciferase and 1650 ng peGFP C3 (Clontech) plasmids. Both luciferase constructs (*Firefly* and *Renilla*) are driven by the same viral promoter

but the *Firefly* luciferase construct contains a part of the 3'UTR of MBP (or the whole 3'UTR) containing the A2RE. On the following day, the cells were scraped off in 350 µl PBS of which 150 µl (divided in three replicates with 50 µl each) were used in a DualGlo® luciferase assay (Promega) according the manufacturer's instructions. *Firefly* luciferase activities were normalized with *Renilla* luciferase activities. In each case, half of the 200 µl of the remaining cell suspension in PBS were used for protein analysis by SDS-PAGE and Western Blotting (sections 3.5.5 and 3.5.7) or total RNA analysis by quantitative RT-PCR (qRT-PCR) (sections 3.3.3 and 3.3.4) for *Firefly* and *Renilla* luciferase mRNAs.

For the experiments with overexpression of hnRNP F, Oli-*neu* cells were transfected with the above stated amounts of luciferase constructs together with 1650 ng peGFP C3 or 1650 ng hnRNP F in pcDNA 3.1 Zeo + (Invitrogen). On the next day, analysis was carried out as described above.

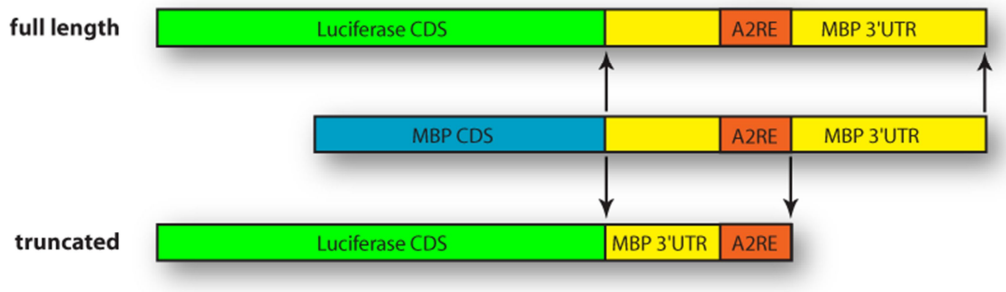


Figure 3-7 Schematic representation of the MBP 3'UTR luciferase reporter constructs

Either a part or the whole 3'UTR of MBP was fused to the coding sequence (CDS) of *Firefly* luciferase yielding in a truncated or a full length reporter construct, respectively, suitable for the investigation of MBP 3'UTR-dependent effects. Note that the recognition sequence of hnRNP A2, the A2 response element (A2RE), is included in both constructs. Adapted from (White, 2007).

4 RESULTS

The Src-family non-receptor tyrosine kinase Fyn was found to play a critical role in myelination in the CNS when mice deficient for Fyn were investigated (Sperber et al., 2001). As the Fyn knockout was generated in all cell types and not conditionally, one could assume that the myelination phenotype could be due to the missing action of Fyn in (for example) neurons. However, there is strong evidence that the lack of Fyn in oligodendrocytes plays a major role in the observed phenotype. Fyn activity peaks during the most active phase of myelination (Krämer et al., 1999). Fyn has been shown to be involved in differentiation (Osterhout et al., 1999; Klein et al., 2002; Relucio et al., 2009) and target dependent survival (Colognato et al., 2004; Laursen et al., 2009) of oligodendrocytes. Furthermore, it has been implicated in regulating the expression of MBP which is indispensable for the formation of the myelin sheath (Readhead and Hood, 1990). Fyn promotes transcription of MBP (Umemori et al., 1999) and it is able to enhance MBP mRNA translation at the axon-glial contact site by phosphorylation of a central mediator of MBP mRNA metabolism, hnRNP A2 (White, 2007).
Previously (White, 2007), further potential targets of Fyn in oligodendrocytes were identified. In order to verify these candidates and to enable investigation of the consequences of Fyn activity in oligodendrocytes, several wildtype and mutant Fyn constructs were generated (section 4.1). Furthermore, additional work on the oligodendroglial role of the known Fyn target hnRNP A2 (section 4.2) and a putative co-operator of A2 (DDX5, section 4.3) was carried out. The present study also addresses analysis of p130Cas (section 4.4) and hnRNP F (section 4.5), newly identified (see above) potential targets of Fyn in oligodendrocytes.

4.1 Fyn constructs

4.1.1 Generation and testing of wildtype and mutant FynB constructs

There are two known isoforms of Fyn in mice. FynT is present in T-lymphocytes whereas FynB is mainly expressed in the brain (Resh, 1998). In the first step of the construct generation, a reverse transcription-PCR was performed on total RNA isolated from primary mouse oligodendrocytes. The goal was to obtain oligodendrocyte-specific cDNA for Fyn. The used primers flank the coding sequence of Fyn and are able to generate cDNAs for both isoforms. However, only the cDNA for FynB was amplified (data not shown) confirming that this is the resident isoform in oligodendrocytes. The FynB coding sequence was cloned into the pcDNA 3.1 Zeo + vector (Figure 3-1). In order to

be able to investigate cellular processes dependent on Fyn activity, a constitutive active (Fyn+) and a kinase dead (Fyn-) version of the newly formed wildtype FynB construct (Fyn wt) were created by site-directed mutagenesis. To obtain Fyn+, the negative regulatory tyrosine residue (Y531 in mouse FynB, section 2.5.2) at the C-terminus of Fyn was exchanged for phenylalanine, rendering the kinase unable to adopt its inactive conformation. For Fyn-, a lysine residue (K299 in mouse FynB, section 2.5.2) in the ATP-binding site of the kinase domain was mutated to methionine leading to an inability to bind ATP and hence to phosphorylate target proteins.
First, the pattern of target proteins of FynB in oligodendrocytes was investigated. Oli-*neu* cells were transfected with the active or inactive constructs of FynB. The cells were lysed 2 days following transfection, the proteins were separated by SDS-PAGE and analysed by Western Blotting with antibodies specific for phosphorylated tyrosine residues and activated Fyn kinase. As shown in Figure 4-1 A, FynB activity leads to the phosphorylation of many proteins in oligodendroglial cells.

Prior to this, the Fyn-dependent tyrosine-phosphorylation of the RNA-binding protein hnRNP F was examined in oligodendrocytes (Gonsior, 2007). This was done with constitutive active and inactive FynT constructs and a Green Fluorescent Protein (GFP) fusion protein of hnRNP F (GFP-F) with both proteins derived from mouse. FynB and T are structurally highly identical and evidence suggests that they exhibit comparable activity (Resh, 1998). Anyhow, it seems more logical to investigate Fyn-dependent actions in oligodendrocytes using the inherent isoform. Therefore, the analysis of the phosphorylation of GFP-F was repeated dependent on FynB. Oli-*neu* cells were co-transfected with FynB+ or FynB- and GFP-F, respectively. 2 days after transfection, GFP-F was immunoprecipitated with antibodies against GFP and its tyrosine-phosphorylation was checked on Western Blots. Fitting to the previous studies, GFP-F also is phosphorylated in response to FynB activation (Figure 4-1 B).

All analyses of Fyn-dependent events in this thesis were thus carried out with the FynB constructs and with the exception of Figure 4-1 it will be referred to them simply as Fyn+, Fyn wt or Fyn-.

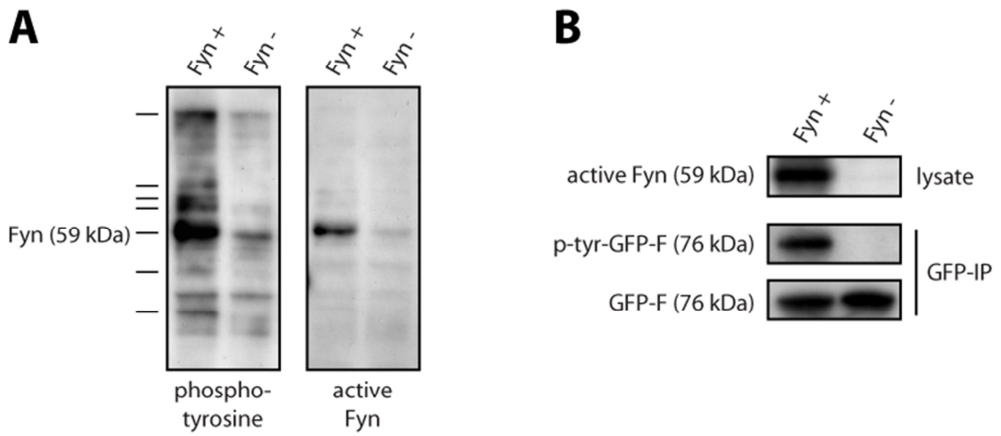


Figure 4-1 Pattern of phosphorylation-targets of active FynB in oligodendroglial cells

A) Oli-*neu* cells were transfected with plasmids coding for constitutive active (+) or kinase inactive (-) FynB, respectively. 2 days later, cell lysates were analysed on Western Blots with antibodies against phosphorylated tyrosine and activated Fyn kinase. Horizontal lines mark proteins that appear phosphorylated in response to Fyn activation. B) GFP-hnRNP F (GFP-F) is a downstream target of FynB in oligodendrocytes. Oli-*neu* cells were co-transfected with active or inactive FynB and GFP-F. After 2 days, lysates and GFP-F immunoprecipitations (GFP-IP) were analysed on Western Blots for phosphorylation of GFP-F.

Having investigated the general target pattern of Fyn in oligodendrocytes, the subcellular distribution of the mutant Fyn proteins was assessed. Oli-*neu* cells were transfected with Fyn+ or Fyn- and analysed by immunostaining for total and activated Fyn kinase after they were allowed to differentiate for 2 days. As previously shown for endogenous oligodendroglial Fyn (Osterhout et al., 1999; Colognato et al., 2004) and as expected for the plasma membrane-tethered molecule, both Fyn mutants localise to the cytoplasm of oligodendroglial cells occupying also the distal parts of the cells' processes. Moreover and also as expected, Fyn+ displays a much higher degree of activation (determined by detection of the phosphorylated regulatory tyrosine 420, section 2.5.2) than Fyn- (Figure 4-1 and Figure 4-2). Therefore, the mutant Fyn proteins appear to show a normal subcellular distribution and the mutations have the expected effects on the kinase activity.

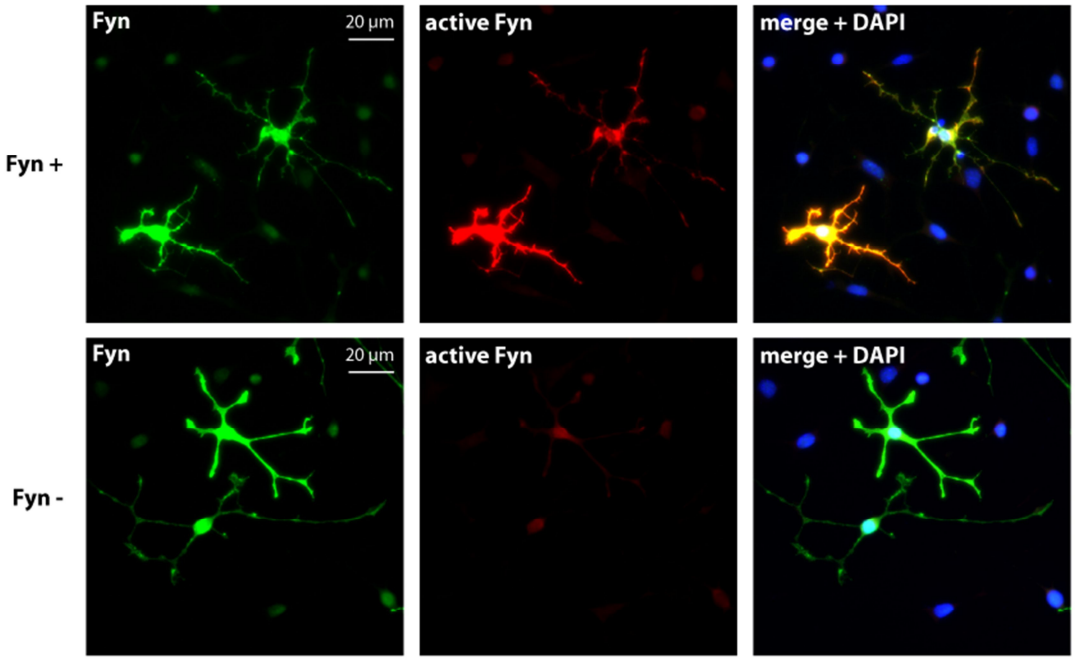


Figure 4-2 Subcellular distribution of the mutant Fyn proteins in oligodendroglial cells

Oli-*neu* cells were transfected with plasmids coding for constitutive active (+) or kinase inactive (-) Fyn, respectively. After 2 days, the cells were fixed and immunostained for total and activated Fyn kinase. Nuclei were stained with DAPI. Note the strong expression of Fyn in transfected cells and the differences in active Fyn staining.

4.1.2 Generation and testing of myc- and YFP-tagged Fyn constructs

To broaden the technical possibilities of experiments using Fyn kinase, further constructs were generated in which the 3 Fyn variants (+, wt and -) were fused to a myc- or an YFP-tag, respectively. This allows for a wider choice of purification methods as the myc- or the YFP-tag can be additionally used. Moreover, the transfected Fyn proteins can be distinguished more easily from the endogenous protein, for example by size on Western Blots or by immunostaining of the tag. The coding sequences for Fyn+, wt and - were cloned into expression vectors where they were fused to a myc- or YFP-tag, respectively, with the tag attached to the C-terminus of the molecule. This orientation was chosen taking in account the structure of Fyn. The N-terminus can be post-translationally modified by fatty acids in order to tether the kinase to the plasma membrane (section 2.5.2). It is likely, that space would be limited for a tag with the N-terminus in close proximity to the membrane. To avoid sterical problems or the risk that such tagged Fyn would not be located to the plasma membrane, the positioning of the tags described above was selected.

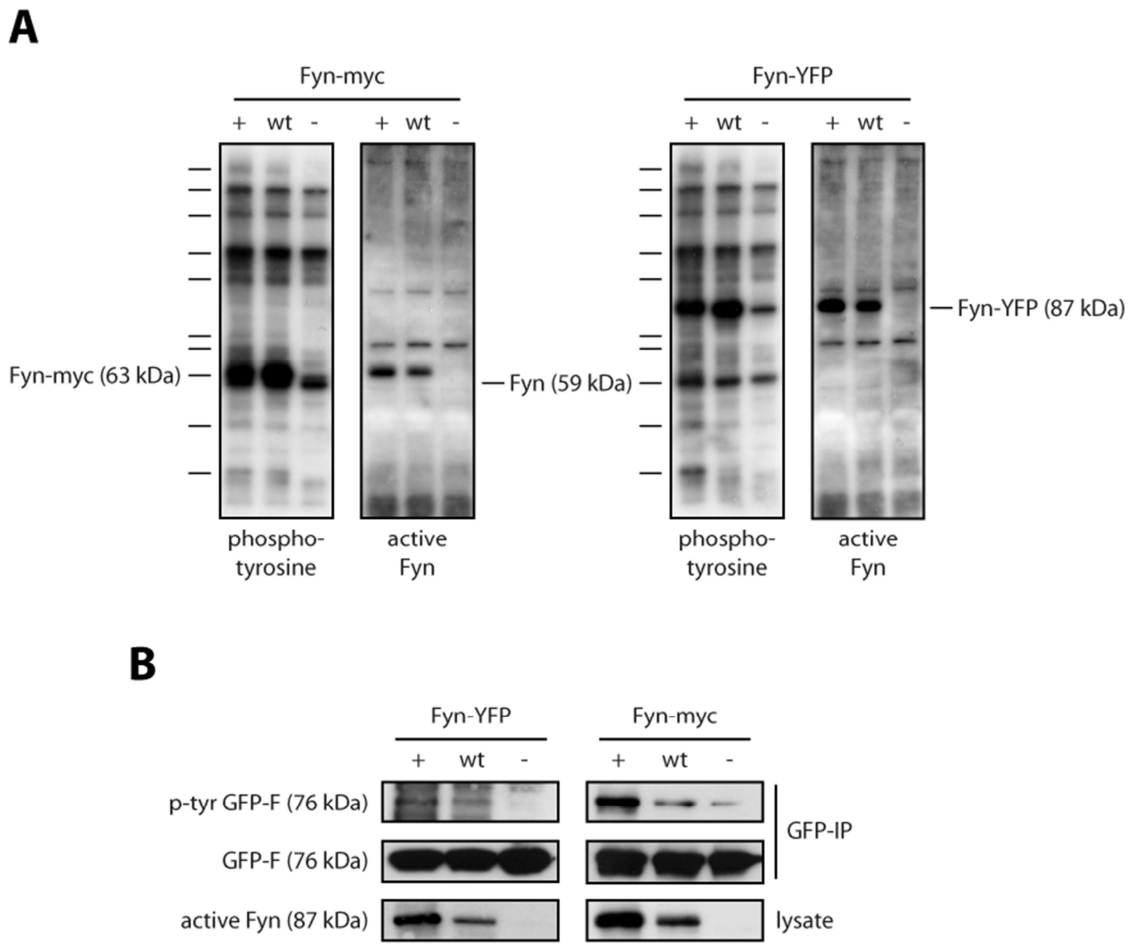


Figure 4-3 Myc- and YFP-tagged Fyn variants show kinase activity

A) Tyrosine-phosphorylation patterns of myc- and YFP-tagged Fyn. Oli-*neu* cells were transfected with active (+) wildtype (wt) or kinase inactive (-) Fyn-myc or -YFP. 2 days later, cell lysates were analysed on Western Blots with antibodies against phosphorylated tyrosine and activated Fyn kinase. Horizontal lines mark proteins that appear phosphorylated due to Fyn activation. Note the differing sizes of endogenous (59 kDa), myc- (63 kDa) and YFP-tagged (87 kDa) Fyn and the bands representing active Fyn in the presence of Fyn+ and Fyn wt. B) GFP-F is phosphorylated by the tagged Fyn mutants. Oli-*neu* cells were co-transfected with GFP-hnRNP F (GFP-F) and myc- or YFP-tagged variants of the mutant Fyn constructs, respectively. Following 2 days in culture, lysates and GFP-F immunoprecipitations (GFP-IP) were analysed on Western Blots for phosphorylation of GFP-F.

In order to assess their phosphorylation capabilities, the tagged recombinant Fyn constructs (+, wt and -) were transfected into Oli-*neu* cells and similar to the original constructs, Western Blot analysis for tyrosine-phosphorylated proteins and activated Fyn kinase was performed. As observed in the presence of the untagged constructs, many proteins appear to be phosphorylated in response to Fyn activation using the tagged constructs and the pattern of target proteins seems to be comparable between the Fyn-myc and -YFP variants (Figure 4-3 A).

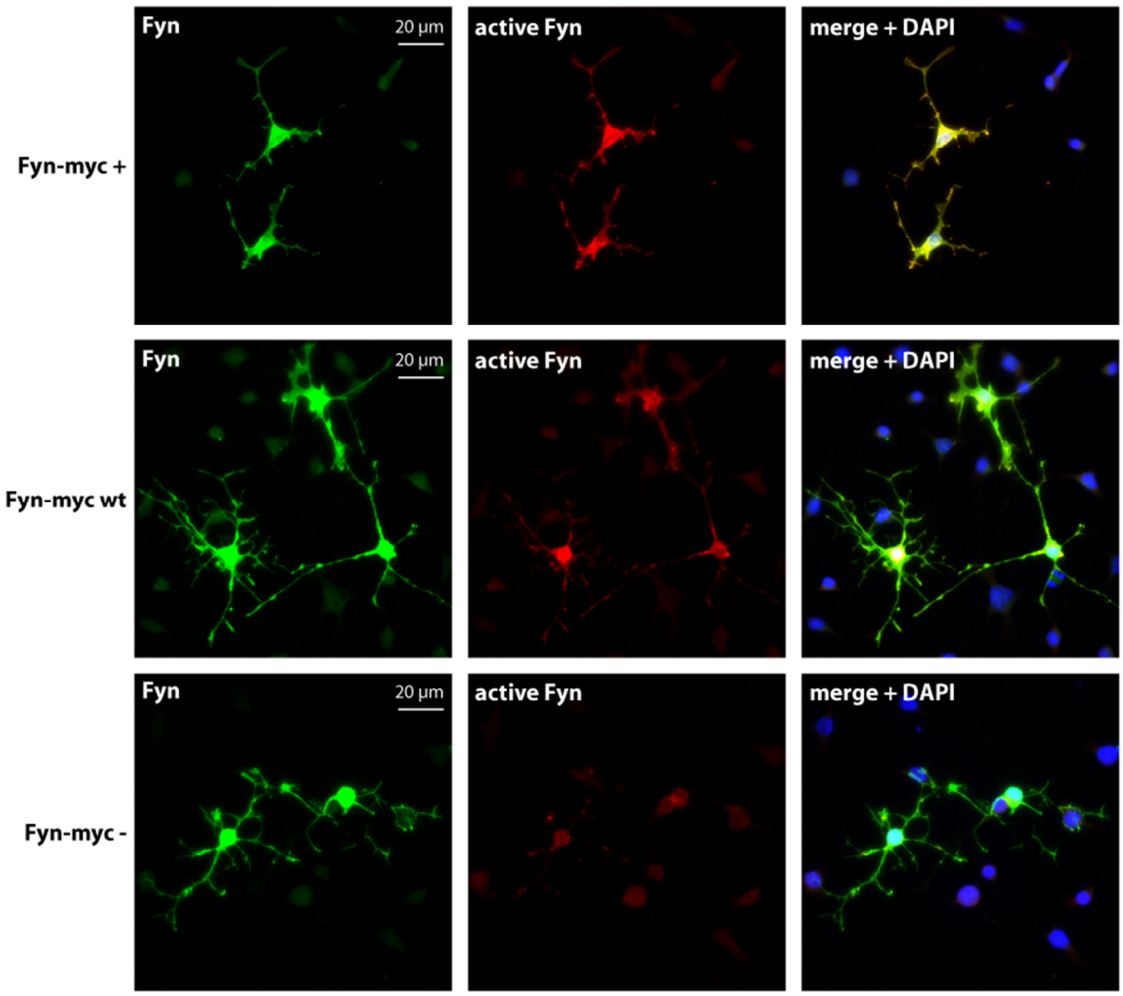


Figure 4-4 Subcellular distribution of Fyn-myc in oligodendroglial cells

Oli-*neu* cells were transfected with plasmids coding for constitutive active (+), wildtype (wt) or kinase inactive (-) Fyn-myc, respectively. After 2 days, the cells were fixed and immunostained for total and activated Fyn kinase. Nuclei were stained with DAPI. Note the strong expression of Fyn in transfected cells and the differences in active Fyn staining.

Additionally, the kinase activity was tested on a previously described target, GFP-F (Gonsior, 2007; Figure 4-1 B). The tagged constructs were co-transfected with GFP-F in Oli-*neu* cells. Proteins were extracted, GFP-F was immunoprecipitated and the tyrosine-phosphorylation of GFP-F as well as the activity of Fyn was tested on Western Blots. GFP-F is phosphorylated in the presence of both tagged variants of Fyn, even though Fyn-YFP seems to be less effective than Fyn-myc (Figure 4-3 B) which could result from sterical hindrance between the YFP of Fyn-YFP and the GFP of GFP-F. This indicates that the generated constructs can specifically phosphorylate the targets of Fyn, but one cannot exclude that a tag, in particular the relatively large tag in Fyn-YFP, leads to sterical problems and unphysiological results in some instances.

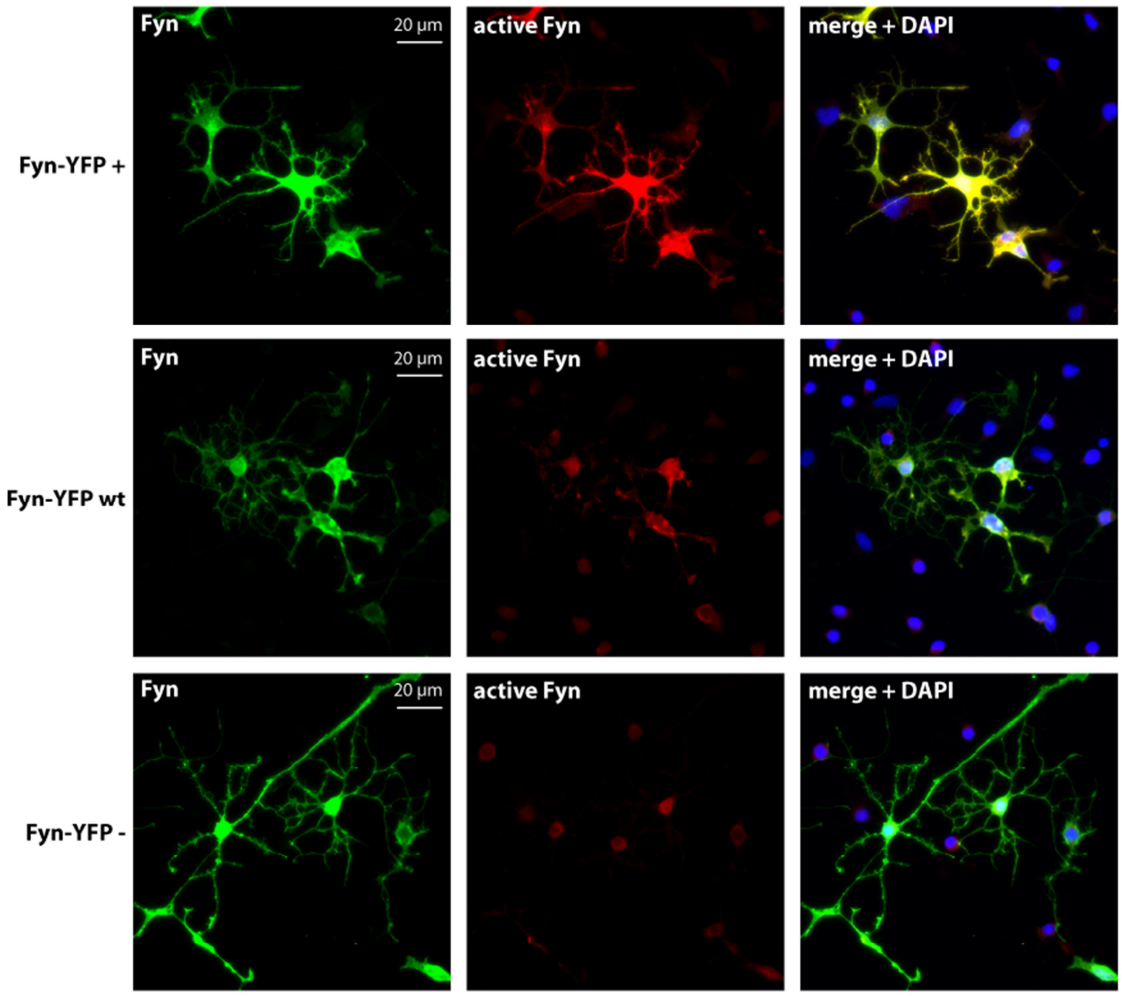


Figure 4-5 Subcellular distribution of Fyn-YFP in oligodendroglial cells

Oli-*neu* cells were transfected with plasmids coding for constitutive active (+), wildtype (wt) or kinase inactive (-) Fyn-YFP, respectively. After 2 days, the cells were fixed and immunostained for total and activated Fyn kinase. Nuclei were stained with DAPI. Note the strong expression of Fyn in transfected cells and the differences in active Fyn staining.

Furthermore, to examine if the subcellular localisation of the tagged Fyn-constructs would be normal in oligodendrocytes, a fraction of the cells used in Figure 4-3 A was grown on coverslips for 2 days, fixed and immunostained with antibodies directed against total or active Fyn. The subcellular distribution of both the myc- (Figure 4-4) and the YFP-tagged (Figure 4-5) Fyn proteins resembles the distribution of untagged and endogenous Fyn. Also, the activity of the tagged Fyn-mutants seems to be as expected as the Fyn+ variants show the highest and the Fyn- variants the lowest activation on the Blots (Figure 4-3 A) as well as in immunostainings (Figure 4-4 and Figure 4-5). Taken together, the data suggests that these constructs are valid tools to investigate Fyn-dependent processes in oligodendrocytes.

4.2 The Fyn target hnRNP A2 links neuron-glial signalling to RNA granule remodelling

MBP is a major structural protein of CNS myelin and crucial for compaction of the myelin sheath. The mRNA of MBP is sorted into mRNA transport granules following its export from the nucleus. In the granules, the mRNA is maintained in a translationally silent state and it is transported to the processes of oligodendrocytes (Barbarese et al., 1999). A main mediator of formation and transport of these granules is the RNA-binding protein hnRNP A2 which binds to a defined region in the 3'UTR of MBP mRNA, called the A2RE (Ainger et al., 1997; Hoek et al., 1998). A signalling cascade between neurons and oligodendrocytes was proposed that leads to activation of the oligodendroglial Fyn kinase. Furthermore, it was found that Fyn is able to phosphorylate hnRNP A2 directly and to enhance translation of MBP mRNA, very likely initiated by phosphorylation of hnRNP A2 (White, 2007).

4.2.1 L1-CAM binds to oligodendroglial cells via F3

Binding of the neuronal cell adhesion molecule (CAM) L1 to the oligodendroglial cell adhesion molecule F3 (Koch et al., 1997) was shown to initiate Fyn activation and the signalling cascade described above. F3 was reported before to be able to form a membrane-associated complex with Fyn (Krämer et al., 1999). A cell ELISA was used to demonstrate L1 binding to F3. A recombinant L1-Fc fusion protein was found to bind to oligodendroglial cells and this binding was reduced by the addition of antibodies against F3, by competition of the antibody with L1-Fc. To exclude that L1-Fc bound to the cells via its Fc-part, the experiment was repeated adding a control where the cells were incubated with human IgG, as the Fc-part of L1-Fc is from human origin. Furthermore, the antibody competition was also performed with an antibody (α-O4) specific for an oligodendroglial surface epitope other than F3, thereby controlling for unspecific competition. Figure 4-6 shows that the binding of L1-Fc to oligodendroglial cells is independent of the Fc-region but dependent on free F3 surface epitopes.

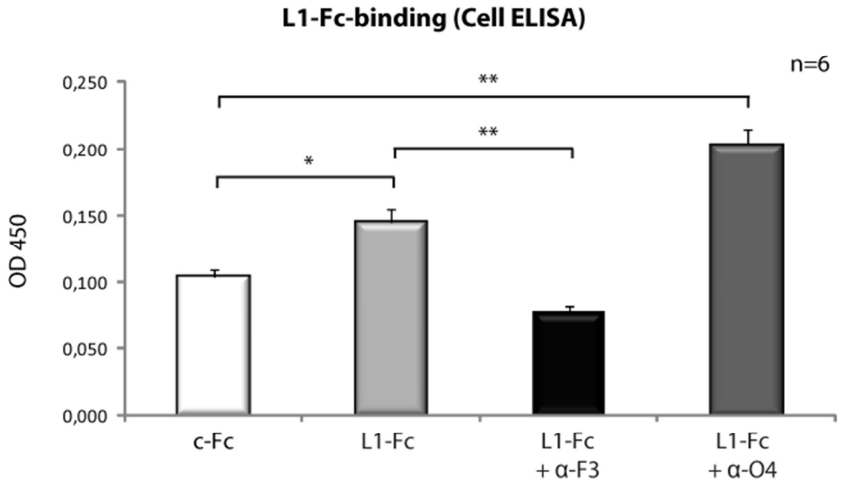


Figure 4-6 L1-Fc binds to oligodendroglial cells in an F3-dependent manner

Oli-*neu* cells were allowed to differentiate for 2 days and then were incubated with 75 nM control-Fc (human IgG, c-Fc) or 75 nM L1-Fc with and without the presence of antibodies against F3 or O4 (control) to compete for potential binding sites of L1-Fc. The binding was assessed by cell ELISA measuring the optical density at a wavelength of 450 nm (OD 450) (see section 3.8 for details). Error bars indicate SEM; * $p < 0.05$, ** $p < 0.01$ (Student's ttest).

4.2.2 L1-CAM triggers tyrosine-phosphorylation of hnRNP A2 in oligodendrocytes mediated by Fyn kinase

It was further demonstrated in the thesis of Robin White (White, 2007) that the incubation of oligodendroglial cells with L1-Fc triggers the activation of Fyn kinase (via F3). This experiment also was repeated with human IgG as control to confirm the specificity of Fyn activation by L1, as can be seen in the Western analysis of L1-Fc-treated Oli-*neu* cells (Figure 4-7 A). Then, as hnRNP A2 was identified as a target of Fyn in oligodendrocytes, the L1-dependent tyrosine-phosphorylation of A2 was investigated. Primary oligodendrocytes were incubated with L1-Fc or human IgG as control. The cells were lysed subsequently, tyrosine-phosphorylated proteins were purified by immunoprecipitation and analysed for the presence of hnRNP A2 by Western Blotting (Figure 4-7 B). To determine whether the observed L1-induced phosphorylation of A2 was mediated by Fyn, the experiment was recapitulated in Oli-*neu* cells that had been treated with control or Fyn siRNA, respectively (Figure 4-7 C). The results confirm the concept that L1-binding to oligodendrocytes activates Fyn which in turn phosphorylates hnRNP A2.

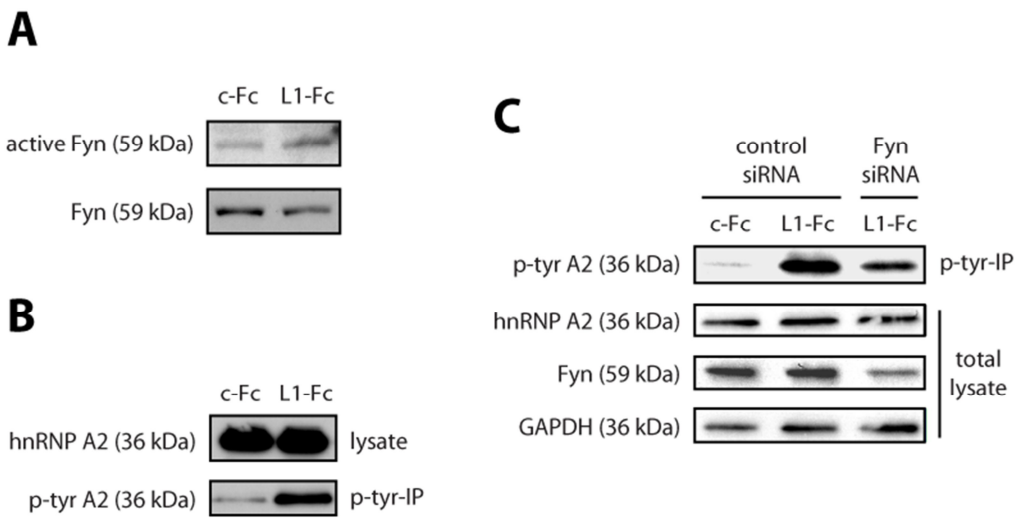


Figure 4-7 L1-Fc triggers tyrosine-phosphorylation of hnRNP A2 through Fyn kinase

A) Fyn is activated by L1-Fc binding. Oli-*neu* cells were incubated with 75 nM control-Fc (human IgG, c-Fc) or 75 nM L1-Fc. Cell lysates were analysed on Western Blots for total and active Fyn. B) L1-Fc binding triggers tyrosine-phosphorylation of hnRNP A2. Primary oligodendrocytes were incubated with 75 nM control-Fc or 75 nM L1-Fc. Tyrosine-phosphorylated proteins were immunoprecipitated (p-tyr-IP) and analysed on Western Blots (for hnRNP A2) together with the corresponding cell lysates. C) HnRNP A2 phosphorylation is reduced by knockdown of Fyn. Oli-*neu* cells were incubated with 75 nM control-Fc or 75 nM L1-Fc. Immunoprecipitations and analysis were performed as in B, here in the presence of control or Fyn siRNA. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) was blotted as loading control here.

4.2.3 L1-binding to oligodendroglial cells leads to increased levels of soluble cytosolic hnRNP A2 and hnRNP E1

Another RNA-binding protein, hnRNP E1, was also shown to be a component of hnRNP A2-containing transport granules, binding to MBP mRNA via A2 and to be responsible in part for translational repression of MBP during cytoplasmic transport. Release of hnRNP E1 from the granule was suggested as mechanism to initiate translation of MBP mRNA (Kosturko et al., 2006). As Fyn activity enhances MBP mRNA translation, it was investigated if the L1-triggered events in oligodendrocytes could modify the structure of MBP mRNA granules: in particular, if the association of E1 and/or A2 with the granule was altered by L1 binding. First, granule-containing post-nuclear lysates from Oli-*neu* cells were treated with RNase A to destroy the RNA and thus disrupt RNA granules. Subsequently, the lysates were cleared from the remaining granules by ultracentrifugation. The resulting granule-free lysates were analysed by SDS-PAGE and Western Blotting for the presence of the hnRNPs A2 and E1. The non-granular amounts of A2 and E1 were densitometrically quantified on Blots from several experiments and compared to untreated granule-free lysates (control), followed by statistic

evaluation. Figure 4-8 A illustrates that disruption of RNA granules leads to increased levels of free cytosolic hnRNP A2 and E1. A similar increase was observed when Oli-*neu* cells were incubated with L1-Fc followed by ultra-centrifugation of the lysates and statistical analysis (Figure 4-8 B). Taken together, the findings suggest that L1-signalling to oligodendrocytes can manipulate hnRNP A2-dependent RNA granule structures.

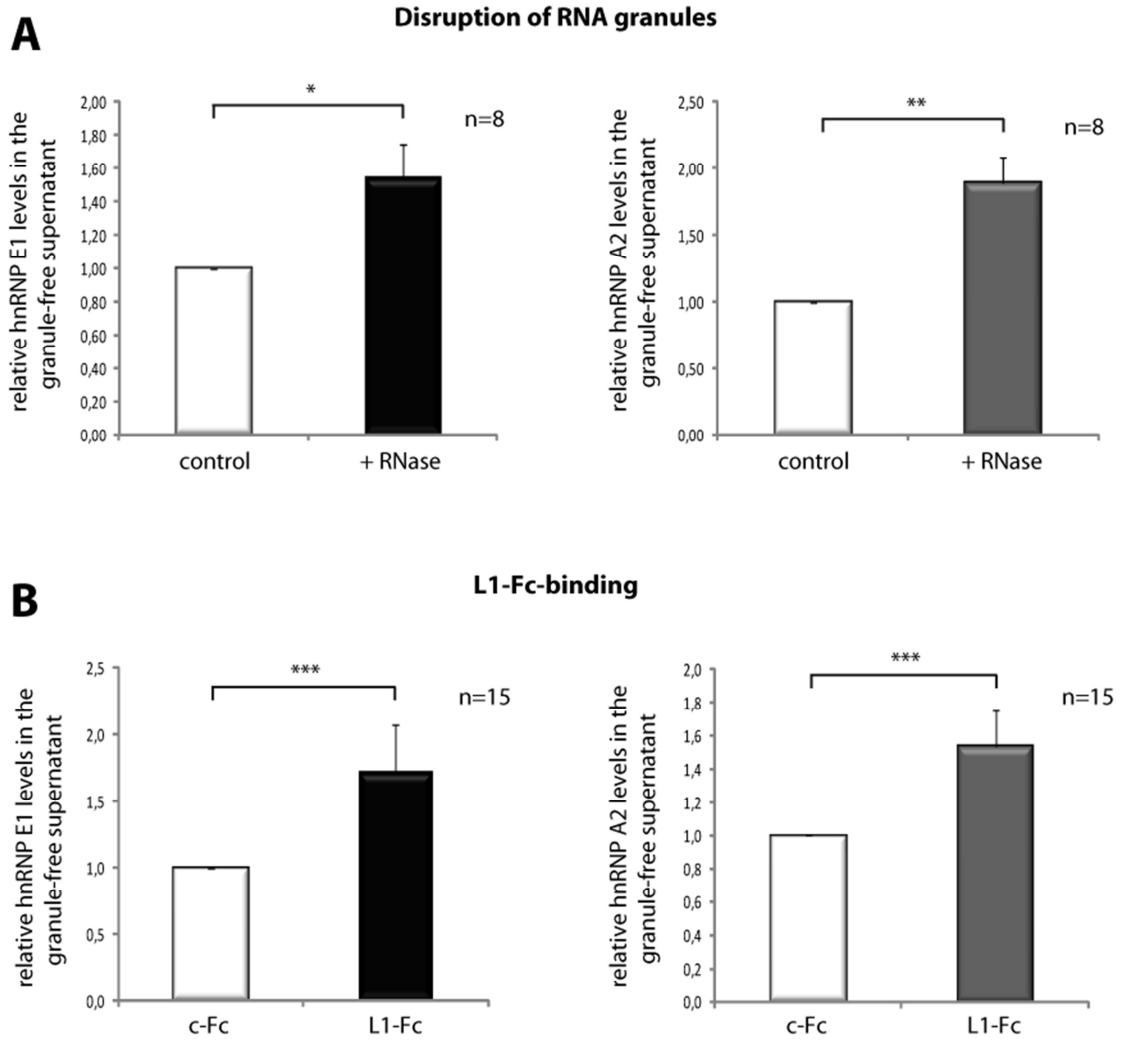


Figure 4-8 HnRNPs A2 and E1 are released from RNA granules by L1-Fc binding

A) Disruption of RNA granules leads to increased levels of hnRNP A2 and E1 in the soluble cytoplasmic fraction. Post-nuclear lysates from Oli-*neu* cells were treated with RNase A. Subsequently, the lysates were depleted from granules by ultra-centrifugation and analysed on Western Blots. For statistical evaluation, hnRNP levels were quantified densitometrically and related to GAPDH. B) L1-Fc-binding leads to increased levels of hnRNP A2 and E1 in the soluble cytoplasmic fraction. Oli-*neu* cells were incubated with 75 nM control-Fc (human IgG, c-Fc) or 75 nM L1-Fc. Postnuclear lysates were depleted from granules by ultra-centrifugation followed by analysis as in A. Error bars indicate SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Wilcoxon signed-rank test).

4.3 DDX5, a co-worker with hnRNP A2?

The DEAD box-family RNA helicase DDX5 was identified as one of several potential cytoplasmic binding partners of hnRNP A2 (White, 2007). Members of the DEAD box-family are ATP-dependent RNA-binding proteins which are able to modify secondary RNA structures and RNA-RNA as well as RNA-protein interactions. They are involved in various aspects of RNA metabolism including translation initiation (reviewed by Rocak and Linder, 2004; Abdelhaleem, 2005). Moreover, DDX5 was found in RNA granules isolated from total brain (Elvira et al., 2006) or neuronal cell cultures (Kanai et al., 2004). As DDX5 was found co-immunoprecipitating with cytoplasmic hnRNP A2 in oligodendroglial cells it could play a role in hnRNP A2-dependent (and MBP mRNA-containing) transport granules. Therefore, DDX5 was investigated in oligodendrocytes.

4.3.1 DDX5 is present in the nucleus and the cytoplasm of oligodendroglial cells

First, the subcellular localisation of DDX5 in oligodendroglial cells was assessed. Oli-*neu* cells were fixed after 2 days in culture and immunostained for DDX5. Figure 4-9 illustrates that the helicase is heavily enriched in the nucleus of oligodendroglial cells but it is also present in lower amounts in the cytoplasm which is a prerequisite for a possible function in oligodendroglial RNA transport granules.

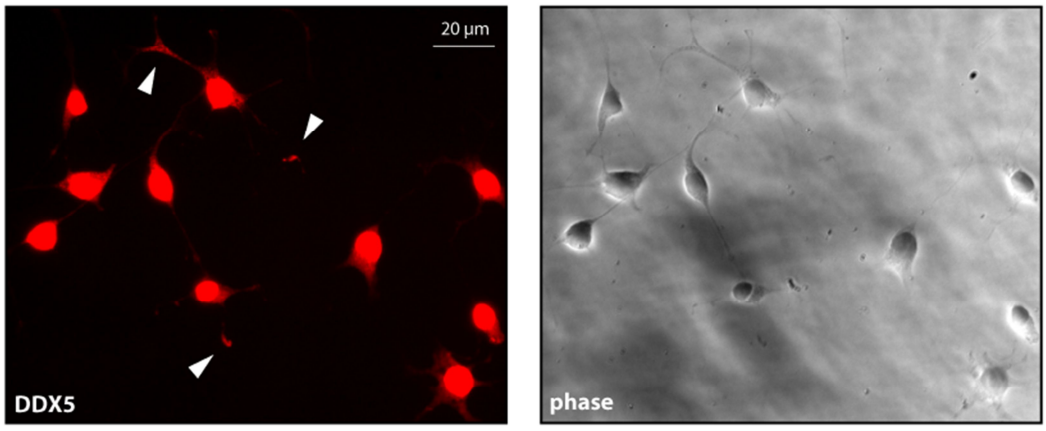


Figure 4-9 Subcellular localisation of DDX5 in oligodendroglial cells

DDX5 is highly expressed in the nucleus and can be found in lower amounts in several cytoplasmic foci (white arrowheads), additionally. Oli-*neu* cells were fixed and immunostained for DDX5.

4.3.2 HnRNP A2 co-immunoprecipitates with DDX5

DDX5 was initially found co-immunoprecipitating with hnRNP A2. Here, the reciprocal immunoprecipitation (IP) was performed to confirm the potential interaction between the two RNA-binding proteins. DDX5 was immunoprecipitated from post-nuclear lysates of differentiated Oli-*neu* cells. The precipitates were analysed on Western Blots for DDX5 and co-purifying proteins. Figure 4-10 shows that hnRNP A2 is enriched in the IP of DDX5. In contrast, a control protein (F3) was not enriched in the DDX5-IP, indicating the specificity of the results and that hnRNP A2 and DDX5 interact in the cytoplasm of oligodendrocytes. This confirms a possible role of DDX5 in hnRNP A2-dependent RNA transport granules in oligodendrocytes.

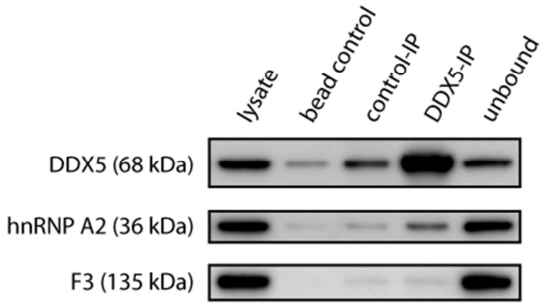


Figure 4-10 HnRNP A2 co-immunoprecipitates with cytoplasmic DDX5

Immunoprecipitations with antibodies against DDX5 (DDX5-IP) or isotype-matched control antibodies (control-IP) were performed from Oli-*neu* post-nuclear lysates and analysed on Western Blots.

4.3.3 DDX5 is not a direct target of oligodendroglial Fyn kinase

DDX5 was shown to be tyrosine-phosphorylated in HeLa cells and phosphorylation appears to affect its RNA helicase activity (Yang et al., 2005). As DDX5 could play a role in MBP mRNA-containing transport granules, for example in translation initiation, and as Fyn activity enhances translation of MBP mRNA, it was examined if DDX5 was a target of Fyn in oligodendrocytes. Oli-*neu* cells were transfected with Fyn+ or Fyn-. After 2 days in culture, the cells were lysed, tyrosine-phosphorylated proteins were immunoprecipitated and analysed by Western Blotting together with the initial lysates for the presence of DDX5. As seen in Figure 4-11 A, tyrosine-phosphorylated DDX5 appears to be present in similar amounts, regardless if purified from active or inactive Fyn containing cells.

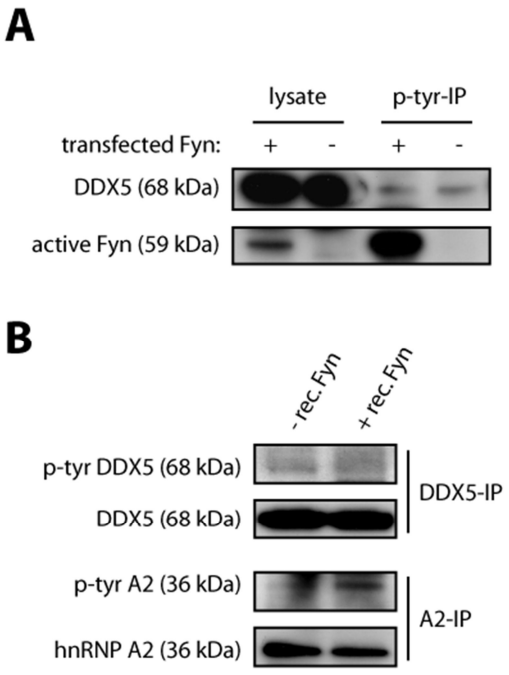


Figure 4-11 DDX5 is not a target of Fyn kinase in oligodendroglial cells

A) Fyn activation does not lead to tyrosine-phosphorylation of DDX5. Oli-*neu* cells were transfected with constitutive active (+) or inactive (-) Fyn kinase constructs. Tyrosine-phosphorylated proteins were immunoprecipitated (p-tyr-IP) and analysed on Western Blots with antibodies recognising DDX5 and activated Fyn kinase. B) Fyn kinase assay. DDX5 or hnRNP A2 (as positive control) were immunoprecipitated (IP) from Oli-*neu* cells and incubated with recombinant active Fyn kinase. Tyrosine-phosphorylation was investigated on Western Blots. Note the higher degree of phosphorylation of hnRNP A2 in the presence of recombinant Fyn in contrast to DDX5.

Subtle differences in the amount of phosphorylation can be difficult to detect, as the results obtained (Figure 4-11 A) are highly dependent on the efficiencies of the transfection as well as the immunoprecipitation. To check for a possible phosphorylation of DDX5 by Fyn in a more direct approach, a Fyn kinase assay was performed. DDX5 was immunoprecipitated from Oli-*neu* cells and incubated with recombinant active Fyn. Again, Western analysis showed no effect of the presence of active Fyn compared to its absence on the levels of tyrosine-phosphorylation of DDX5 (Figure 4-11 B). The experiment also was carried out with hnRNP A2 as control. A2 is directly phosphorylated by Fyn (White, 2007) which was confirmed here, and serves as a positive control. Taken together, DDX5 does not seem to be a target of Fyn in oligodendrocytes.

Further analysis of the functions DDX5 in oligodendrocytes was pursued by Peter Hoch-Kraft (Hoch-Kraft, 2010).

4.4 p130Cas, multiadaptor and Fyn target

P130Cas (Crk-associated substrate) is a cytoplasmic multiadaptor protein which can integrate various signals and subsequently affect many downstream pathways. The protein does not exhibit enzymatic or transcriptional activity. It controls signalling pathways by binding to distinct signalling donors and acceptors, thereby building diverse multiprotein complexes. P130Cas is heavily phosphorylated on multiple serine and tyrosine residues. This is thought to control its association to different binding

partners, thereby controlling p130Cas-dependent signalling (Defilippi et al., 2006). Several functions of p130Cas have already been described in different cell types. It is important for remodelling of the actin cytoskeleton, process elongation and migration (Honda et al., 1998; Huang et al., 2002). Furthermore, it plays a role in cell survival, apoptosis, cell transformation and cancer (reviewed by Defilippi et al., 2006). P130Cas is a known target of Src-family kinases including Fyn and contains many putative and proven Src-family-dependent phospho-sites (Manie et al., 1997; Defilippi et al., 2006; Huang et al., 2006). In oligodendrocytes, p130Cas has not been studied to date. But it was identified as a potential target of Fyn in oligodendrocytes (White, 2007). The Fyn-dependent tyrosine-phosphorylation of p130Cas was investigated. A role in actin cytoskeleton rearrangement and (target dependent) survival signalling could be important in the context of myelination.

4.4.1 Subcellular localisation of p130Cas in oligodendrocytes

The subcellular localisation of p130Cas was examined. Primary oligodendrocytes were allowed to differentiate for 3 days, followed by fixation and immunostaining of the cells with antibodies against p130Cas and Sox10 (oligodendroglial marker). Figure 4-12 displays that p130Cas is expressed in the cytoplasm of oligodendrocytes with a higher occurrence in the cell soma than in the processes.

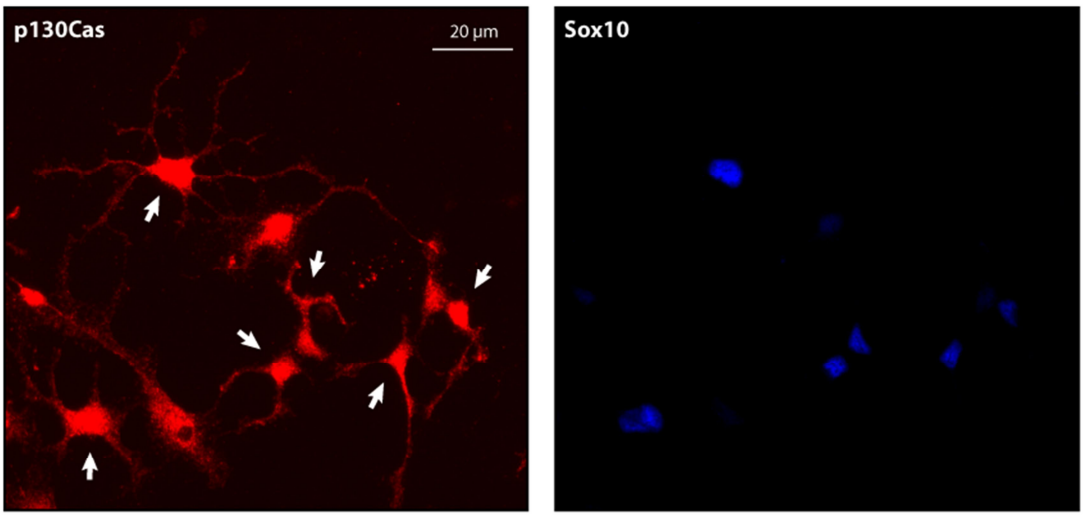


Figure 4-12 Subcellular localisation of p130Cas in oligodendrocytes

P130Cas appears to be present in the cytoplasm of oligodendrocytes showing a stronger staining in the soma than in the processes. Primary oligodendrocytes were fixed after 3 days in culture and immunostained for p130Cas. The transcription factor Sox10 is used as marker for oligodendrocytes here (white arrows).

4.4.2 P130Cas is a target of Fyn in oligodendroglial cells

To verify that p130 is a target of Fyn in oligodendroglial cells, Oli-*neu* cells were transfected with expression constructs coding for Fyn+ or Fyn-. Tyrosine-phosphorylated proteins were isolated by immunoprecipitation and analysed together with total lysates for the presence of p130Cas by Western Blotting. Figure 4-13 A depicts that p130Cas is tyrosine-phosphorylated in response to Fyn activation. In order to strengthen this finding, a possible interaction between Fyn and p130Cas was investigated. Western analysis of Fyn kinase immunoprecipitated from Oli-*neu* cells shows that p130Cas co-immunoprecipitates with Fyn (Figure 4-13 B).

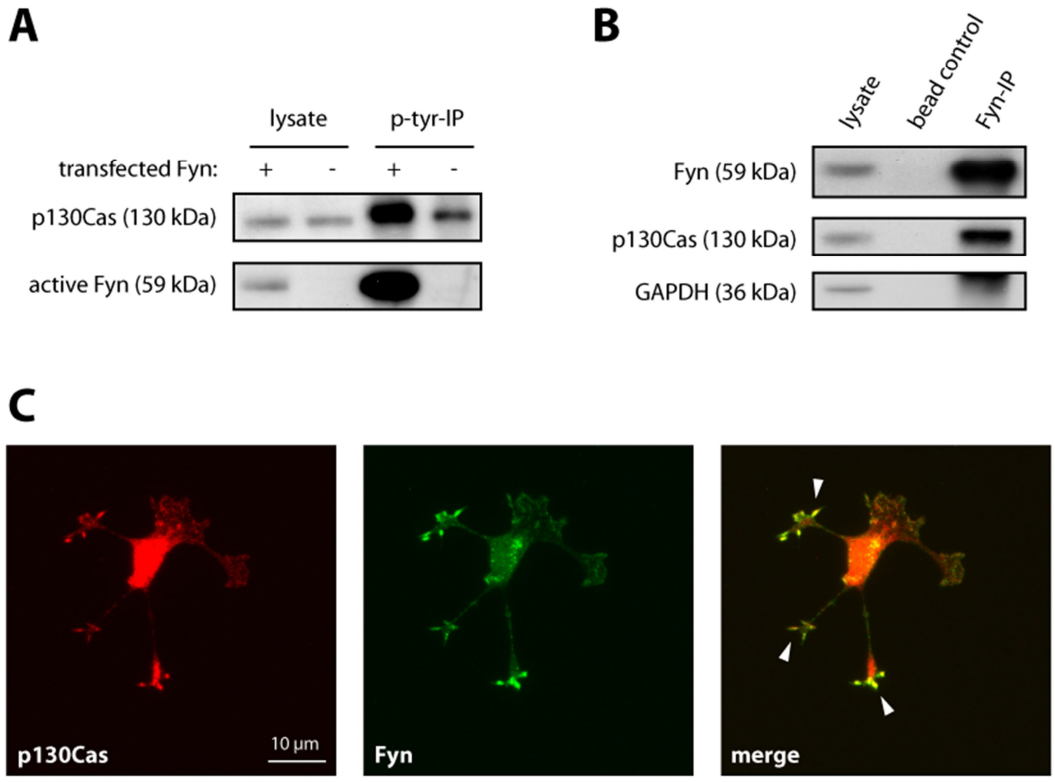


Figure 4-13 P130Cas is a target of Fyn in oligodendroglial cells

A) Fyn activation leads to tyrosine-phosphorylation of p130Cas. Oli-*neu* cells were transfected with constitutive active (+) or inactive (-) Fyn kinase constructs. Tyrosine-phosphorylated proteins were immunoprecipitated (p-tyr-IP) and analysed on Western Blots with antibodies against p130Cas and activated Fyn kinase. B) P130Cas co-immunoprecipitates with Fyn from an Oli-*neu* cell lysate. The immunoprecipitation (Fyn-IP) was analysed on Western Blots. The absence of GAPDH in the IP-lane confirms the specificity of the interaction between Fyn and p130Cas. C) Fyn and p130Cas co-localise in oligodendroglial cells. Oli-*neu* cells were allowed to differentiate for 1 day, fixed and immunostained for Fyn and p130Cas. Selected areas of co-localisation are highlighted by white arrowheads.

The absence of the cytosolic protein Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the IP-lane confirms the specificity of the experiment. Additionally, differentiated Oli-*neu* cells were immunostained for Fyn and p130Cas. Figure 4-13 C illustrates co-localisation of the enzyme and its putative substrate in several areas. The overlap is particularly prominent in the growth cones of the processes indicating that p130Cas could exhibit its function in cytoskeletal rearrangement (white arrowheads).

One can conclude from these findings that p130Cas is a direct target of Fyn in oligodendrocytes.

4.4.3 P130Cas plays a role in oligodendroglial apoptosis

A connection between p130Cas and apoptosis has been reported in the literature (Defilippi et al., 2006). To investigate a similar function in oligodendrocytes, primary oligodendrocytes were treated with p130Cas or control siRNA which led to a good knockdown of p130Cas, as can be seen in Figure 4-14. The Western analysis suggests a higher rate of apoptosis in cells lacking p130Cas compared to control cells, as the amount of cleaved caspase 3, an early marker of apoptosis, is increased. Levels of GAPDH were checked as a loading control and are slightly decreased in the presence of p130Cas siRNA indicating here that the number of cells was reduced.

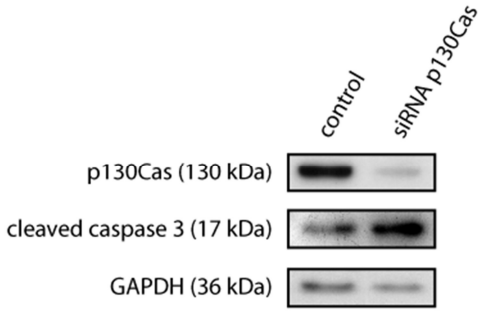


Figure 4-14 Depletion of p130Cas increases apoptosis in oligodendrocytes

2 days following the treatment of primary oligodendrocytes with siRNA against p130Cas, the cells were lysed and protein levels were examined on Western Blots. Note the increased amounts of active caspase 3 after reduction in p130Cas levels. GAPDH serves as a loading control.

4.5 Post-transcriptional regulation of MBP by the Fyn target hnRNP F

Like p130Cas (section 4.4) the RNA-binding protein hnRNP F was identified as potential target of Fyn kinase in oligodendroglial cells (White, 2007; Gonsior, 2007). HnRNP F is ubiquitously expressed and has been related to several cellular functions, most of all splicing (Dominguez et al., 2010). In oligodendrocytes, hnRNP F was shown to be involved in the alternative splicing of PLP/DM20, the major structural protein of CNS myelin (Wang et al., 2007).

4.5.1 Expression of hnRNP F in oligodendrocytes

The subcellular localisation of hnRNP F in oligodendrocytes was investigated. Primary oligodendrocytes were fixed at different stages of differentiation and immunostained for hnRNP F and oligodendrocyte-specific proteins. Figure 4-15 depicts the presence of hnRNP F in Nerve-Glia antigen 2- (NG2-)positive oligodendrocyte precursor cells as well as in more mature PLP- and MBP-positive cells. As one would expect due to its role in the splicing of PLP/DM20 it shows a clear nuclear localisation in oligodendrocytes (yellow arrowheads in Figure 4-15). Moreover, it is also present in the cytoplasm at all differentiation stages where its distribution reaches into the cells' processes and appears granular (inserts and white arrows in Figure 4-15). This relation of a very strong expression in the nucleus to much lower amounts of protein in the cytoplasm is typical for hnRNPs (Chaudhury et al., 2010).

The oligodendroglial expression of hnRNP F was confirmed by Western analysis of lysates from primary oligodendrocytes, prepared at days 2, 4 and 6 of differentiation in culture. The Blots and the corresponding graph in Figure 4-16 illustrate that, in agreement with previous findings (Wang et al., 2007), the protein levels of hnRNP F decrease to about 50% during differentiation of oligodendrocytes. The myelin proteins MBP, MOG and CNP were examined by Western Blotting to reveal the increasing differentiation of the cells.

The cytoplasmic staining of hnRNP F suggests a novel function in oligodendrocytes additional to its known nuclear function as splicing factor. The granular pattern of hnRNP F in the cytoplasm is comparable to the appearance of the RNA-binding proteins hnRNP A2 and E1 which are known to be part of RNA-transport granules containing MBP mRNA and to function in the regulation of its transport and translation. Interestingly, for the homologue of hnRNP F in *Drosophila*, named Glorund, a cytoplasmic function was identified: it participates in the regulation of the translation of defined mRNAs in the oocytes of the fly (Kalifa et al., 2006; Kalifa et al., 2009). Moreover,

hnRNP F was proposed to play a regulatory role in translation regulation in mammalian cells (Song et al., 2011). These findings point towards a participation of hnRNP F in oligodendroglial RNA transport granules.

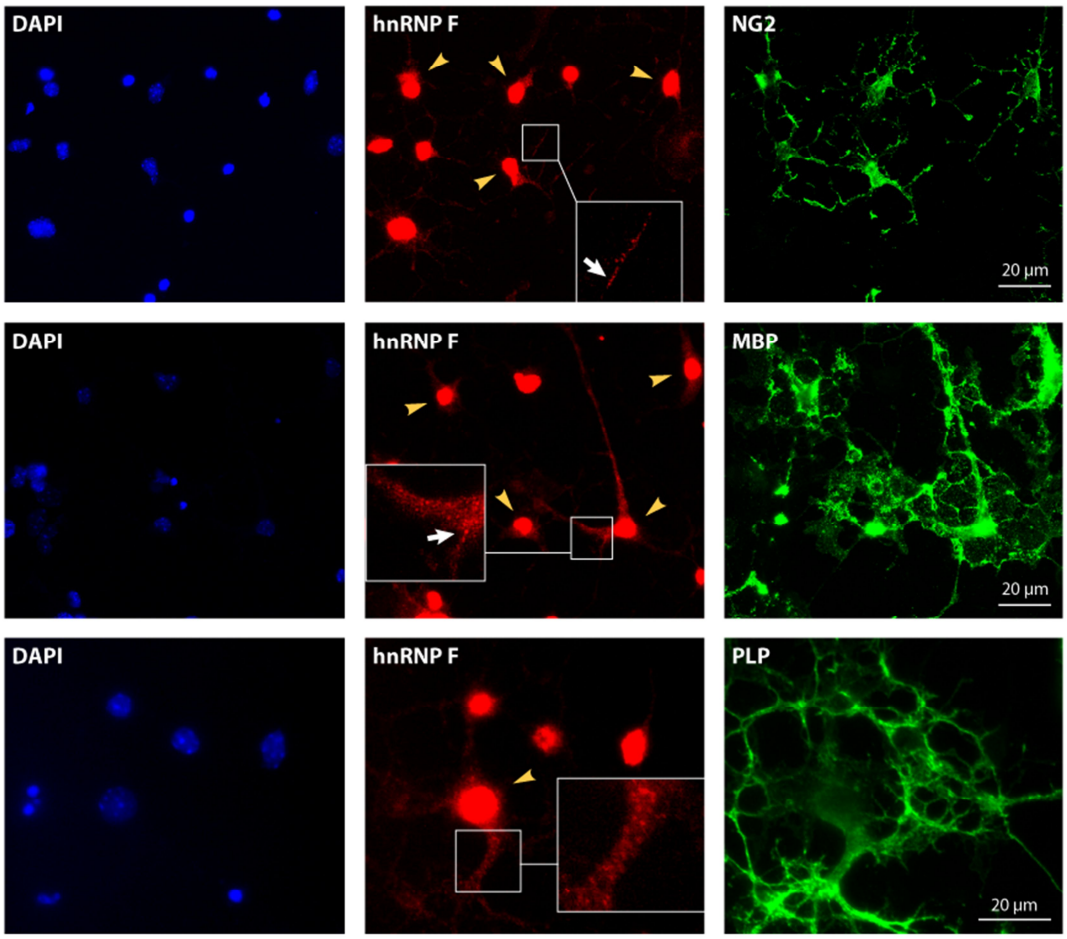


Figure 4-15 HnRNP F is localised in the nucleus and the cytoplasm of oligodendrocytes

In addition to its prominent nuclear localisation in oligodendrocytes (yellow arrowheads), hnRNP F is present in granular structures in the cytoplasm (white arrows) including the processes. Primary mouse oligodendrocytes after 2 or 4 days in culture were stained with antibodies to hnRNP F and markers for oligodendrocyte precursor cells (NG2; 2 days *in vitro*) or more mature oligodendrocytes (MBP, PLP; 4 days *in vitro*). Cell nuclei were stained with DAPI. Inserts show enlarged areas.

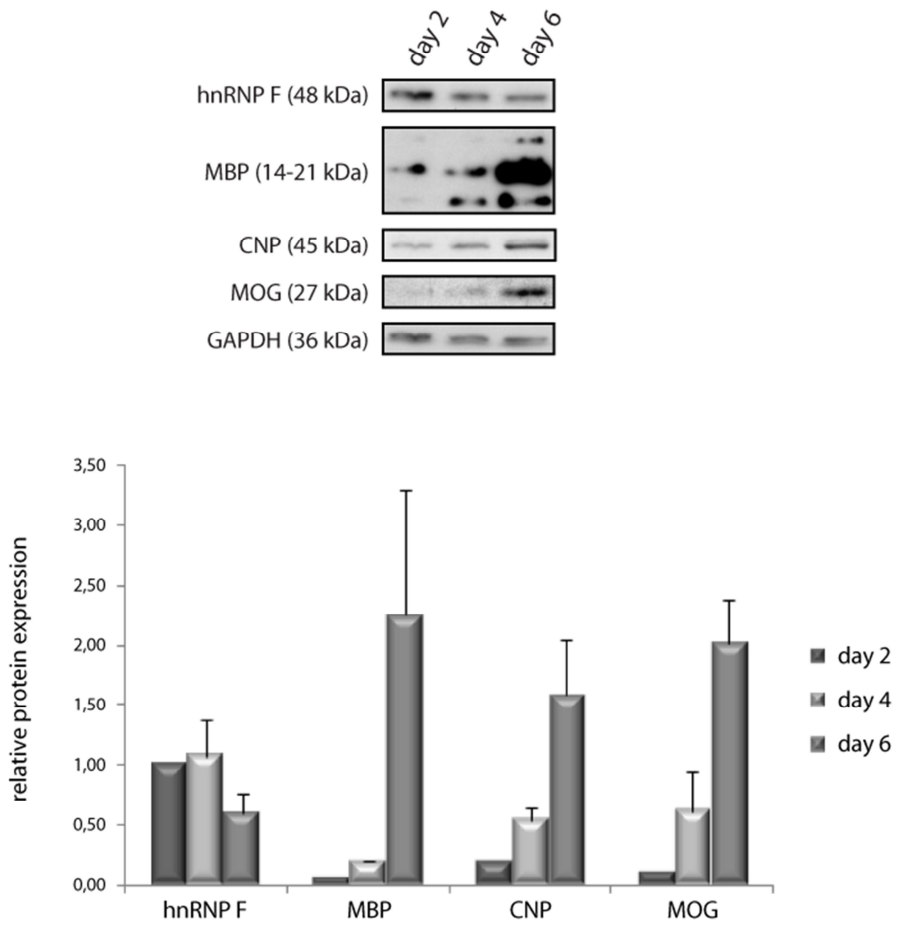


Figure 4-16 Expression of hnRNP F during oligodendrocyte differentiation

Western Blots of lysed primary oligodendrocytes showing the expression of hnRNP F in immature and mature oligodendrocytes (2, 4 and 6 days *in vitro*). MBP, CNP and MOG serve as markers for the ongoing differentiation of the cells and GAPDH is shown as loading control. The graph displays the densitometrical evaluation of Western Blots from 4 independent experiments. Protein levels were normalised to GAPDH. Error bars indicate SEM.

4.5.2 Cytoplasmic association of hnRNP F and hnRNP A2 in oligodendrocytes

The most thoroughly characterised RNA transport granules in oligodendrocytes are those containing mRNA for MBP. As mentioned above, a main mediator of the transport of these granules and the regulation of translation of their mRNAs is the RNA-binding protein hnRNP A2, which binds to MBP mRNA over an 11 nucleotide sequence in the 3'UTR called the A2RE. To determine whether hnRNP F contributes to MBP mRNA-containing granules, a cytoplasmic association of F with hnRNP A2 was analysed.

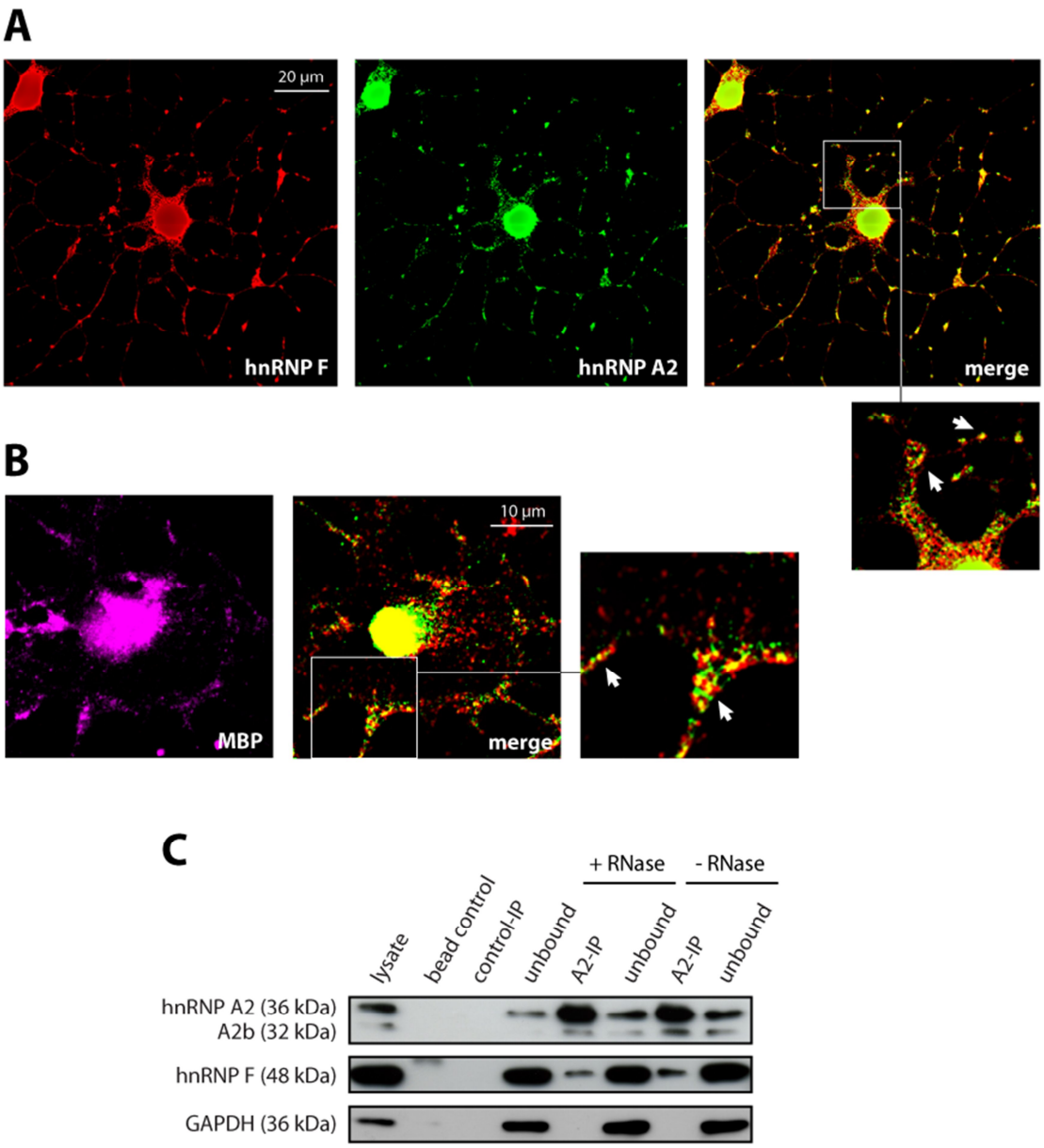


Figure 4-17 HnRNP F and hnRNP A2 associate in cytoplasmic granular structures in oligodendrocytes

A) Partial co-localization of hnRNP F and hnRNP A2 in granular structures (white arrows) in the cytoplasm of Oli-*neu* cells that were allowed to differentiate for 3 days and immunostained for hnRNP F and hnRNP A2. Single deconvoluted planes are depicted; the insert shows an enlarged area. B) Partial co-localization of hnRNP F and hnRNP A2 in granular structures (white arrows) in the cytoplasm of primary mouse oligodendrocytes that were immunostained for hnRNP F and hnRNP A2 after 3 days in culture. MBP is shown as oligodendroglial marker. Single deconvoluted planes are depicted; the insert shows an enlarged area. C) HnRNP F co-immunoprecipitates with hnRNP A2. Immunoprecipitations with hnRNP A2 (A2-IP) or isotype-matched control antibodies (control-IP) were performed from RNase- or untreated Oli-*neu* post-nuclear lysates and analysed on Western Blots. The absence of GAPDH in the A2-IP confirms the specificity of hnRNP F binding. Note that the hnRNP A2 Western Blots show an additional lower band at approximately 32 kDa which is likely to be the hnRNP A2/B1 splice variant hnRNP A2b.

Oli-*neu* cells or primary oligodendrocytes were allowed to differentiate for 3 days. The cells were fixed and immunostained with antibodies to hnRNP F and hnRNP A2. Primary oligodendrocytes were additionally stained for MBP protein. A partial overlap of the hnRNPs can be seen in the cytoplasm of both Oli-*neu* cells and primary oligodendrocytes (Figure 4-17 A + B) with the appearance of granular structures (see also white arrows in the inserts of (Figure 4-17 A + B). Interestingly, MBP protein seems to be enriched in cytoplasmic areas where there is a high abundance of granular structures positive for hnRNP F and/or A2.
To demonstrate the interaction of the two hnRNPs with a different approach, hnRNP A2 was immunoprecipitated from RNase- or untreated Oli-*neu* post-nuclear lysates and co-purifying proteins were examined by Western Blotting. Figure 4-17 C shows that hnRNP F co-immunoprecipitates with hnRNP A2 and that this interaction does not depend on the presence of RNA. GAPDH was probed as control and does not co-immunoprecipitate here.
Taken together, these observations suggest that hnRNP F and hnRNP A2 interact in a subset of cytoplasmic granules in oligodendrocytes.

4.5.3 HnRNP **F** is associated with oligodendroglial RNA granules and binds to MBP mRNA

In order to further investigate an association of hnRNP F with RNA granules, post-nuclear supernatants from Oli-*neu* cells were treated with RNase A to disrupt RNA granules. These lysates were subsequently subjected to an ultra-centrifugation step to remove the remaining granules from the solution. The granule-free lysates were analysed on Western Blots for the presence of hnRNP F. The amount of free soluble hnRNP F is increased in the cytoplasm of oligodendroglial cells when RNA granules are destroyed (Figure 4-18 A). The statistics are derived from densitometrical analysis of the hnRNP F-signals on Western Blots which were normalised to the amounts of GAPDH, respectively.

HnRNP F is thus present in RNA granules: does it also bind to MBP mRNA? Due to the inability to immunoprecipitate endogenous hnRNP F with the available antibodies, a recombinant myc-tagged hnRNP F (F-myc) was co-transfected with a plasmid coding for the 14 kDa isoform of MBP including the MBP 3'UTR into Oli-*neu* cells. After 3 days in culture, F-myc was immunoprecipitated with antibodies specific for the myc-tag and associated mRNAs were purified and analysed by qRT-PCR. The amounts of MBP (and β-actin) mRNA in the IPs were normalised to the presence of the non-granule associated phosphoglycerate kinase 1 mRNA. The Western Blot in Figure 4-18 B illustrates that F-myc was successfully immunoprecipitated in the myc-IP whereas no F-myc could be detected in the control-IP that was carried out with isotype-matched antibodies. The

graph in Figure 4-18 B shows a significant enrichment of MBP mRNA in the myc-IP compared to the control-IP and to β-actin mRNA, another transport granule-associated mRNA which is not significantly enriched here.

These findings support the assumption that hnRNP F is a component of oligodendroglial transport granules containing MBP mRNA.

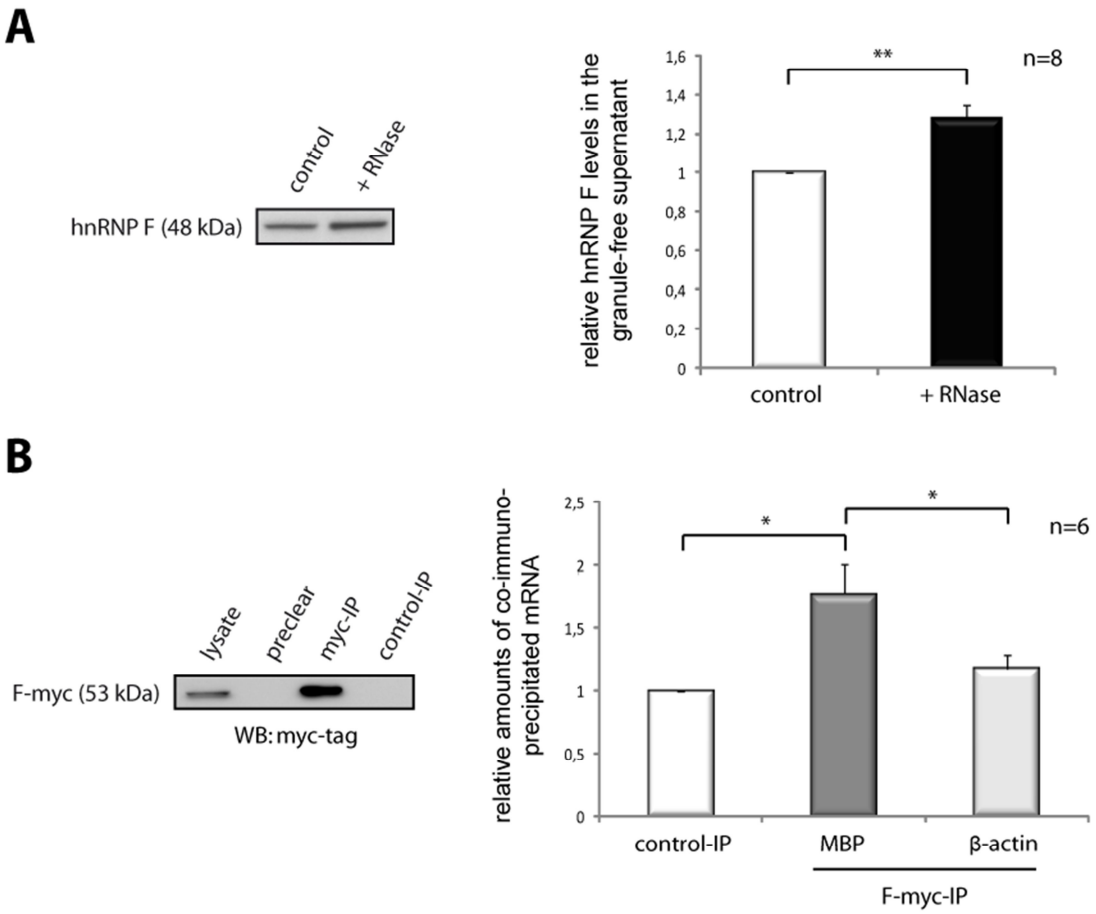


Figure 4-18 HnRNP F is a component of RNA granules and binds to MBP mRNA

A) Disruption of RNA granules increases the amount of soluble cytoplasmic hnRNP F. Post-nuclear lysates of Oli-*neu* cells were treated with RNase A and after depletion of RNA granules by ultracentrifugation, soluble cytoplasmic proteins were analysed on Western Blots. The change in soluble hnRNP F protein was densitometrically quantified and normalised to GAPDH. B) MBP mRNA co-immunoprecipitates with hnRNP F-myc. Oli-*neu* cells were co-transfected with myc-tagged hnRNP F (F-myc) and MBP14 including the 3'UTR. After the cells were allowed to differentiate for 3 days, immunoprecipitations with antibodies against the myc-tag (F-myc-IP) or with isotype-matched control antibodies (control-IP) were performed from post-nuclear lysates and associated mRNAs were analysed by qRT-PCR. MBP and β-actin mRNA levels were normalised to phosphoglycerate kinase 1 mRNA. Error bars indicate SEM; * $p < 0.05$, ** $p < 0.01$ (Wilcoxon signed-rank test).

4.5.4 HnRNP F regulates expression of MBP by a post-transcriptional mechanism

The data suggest an involvement of hnRNP F in the processing of MBP mRNA containing transport granules in oligodendrocytes. To investigate the influence of hnRNP F on MBP expression, primary oligodendrocytes were treated with hnRNP F-directed or control siRNA. The cells were lysed after 3 days in culture and protein levels were compared on Western Blots. Figure 4-19 A displays the high efficiency of hnRNP F knockdown which leads to decreased amounts of MBP protein (all classic isoforms). GAPDH was probed as a loading control. Densitometrical analysis of Western Blots from several experiments (graph in Figure 4-19 A) shows that MBP protein is reduced to 60% in the absence of hnRNP F, whereas other myelin proteins such as CNP and MOG seem to be unaffected.

As depicted in Figure 4-19 B, which is derived from a quantification of mRNAs from the above hnRNP F siRNA experiments, reduction of hnRNP F has no significant impact on the level of MBP mRNA, leading to the conclusion that hnRNP F affects MBP expression by a post-transcriptional mechanism. The expression of hnRNP A2 was unaltered demonstrating the specificity of the effects.

The observed effect of hnRNP F on MBP expression raises the question if MBP protein is moderately reduced in more or less all cells or if there is a certain population of oligodendrocytes that fail to produce MBP at all. Primary oligodendrocytes were treated with hnRNP F siRNA and the cells were immunostained for MBP. In addition, the cells were stained for Olig2 as marker for both immature and mature oligodendrocytes. MBP- and Olig2-positive cells were counted in each condition and normalised to DAPI-staining which highlights the cell nuclei. Figure 4-20, in which representative images and the statistical evaluation of 4 experiments can be seen, shows that oligodendrocyte numbers (Olig2-positive cells) are unaltered in the absence of hnRNP F but that there is a reduction in the number of cells positive for MBP to about 80%.

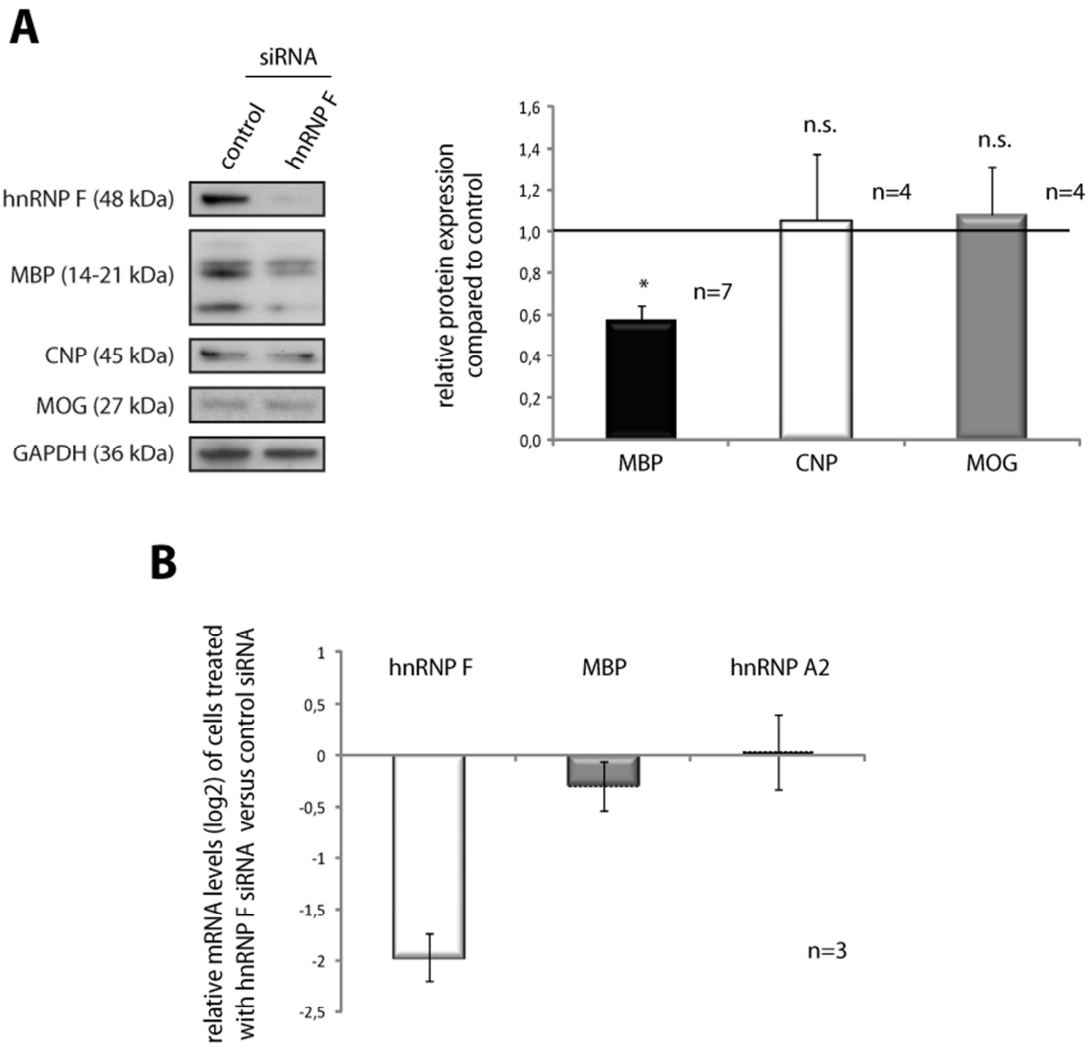


Figure 4-19 Reduction in hnRNP F levels impairs MBP expression by post-transcriptional mechanisms

A) Knockdown of hnRNP F specifically affects MBP protein levels. Primary mouse oligodendrocytes were treated with hnRNP F or control siRNA and allowed to differentiate for 3 days. Protein levels were quantified by densitometric analysis of Western Blots and the expression of MBP, CNP and MOG was normalised to GAPDH. B) Knockdown of hnRNP F has no impact on levels of MBP mRNA. Primary mouse oligodendrocytes were treated as in A, but here mRNA levels were analysed by qRT-PCR and normalised to β-actin mRNA. Error bars indicate SEM; * $p < 0.05$ (Wilcoxon signed-rank test).

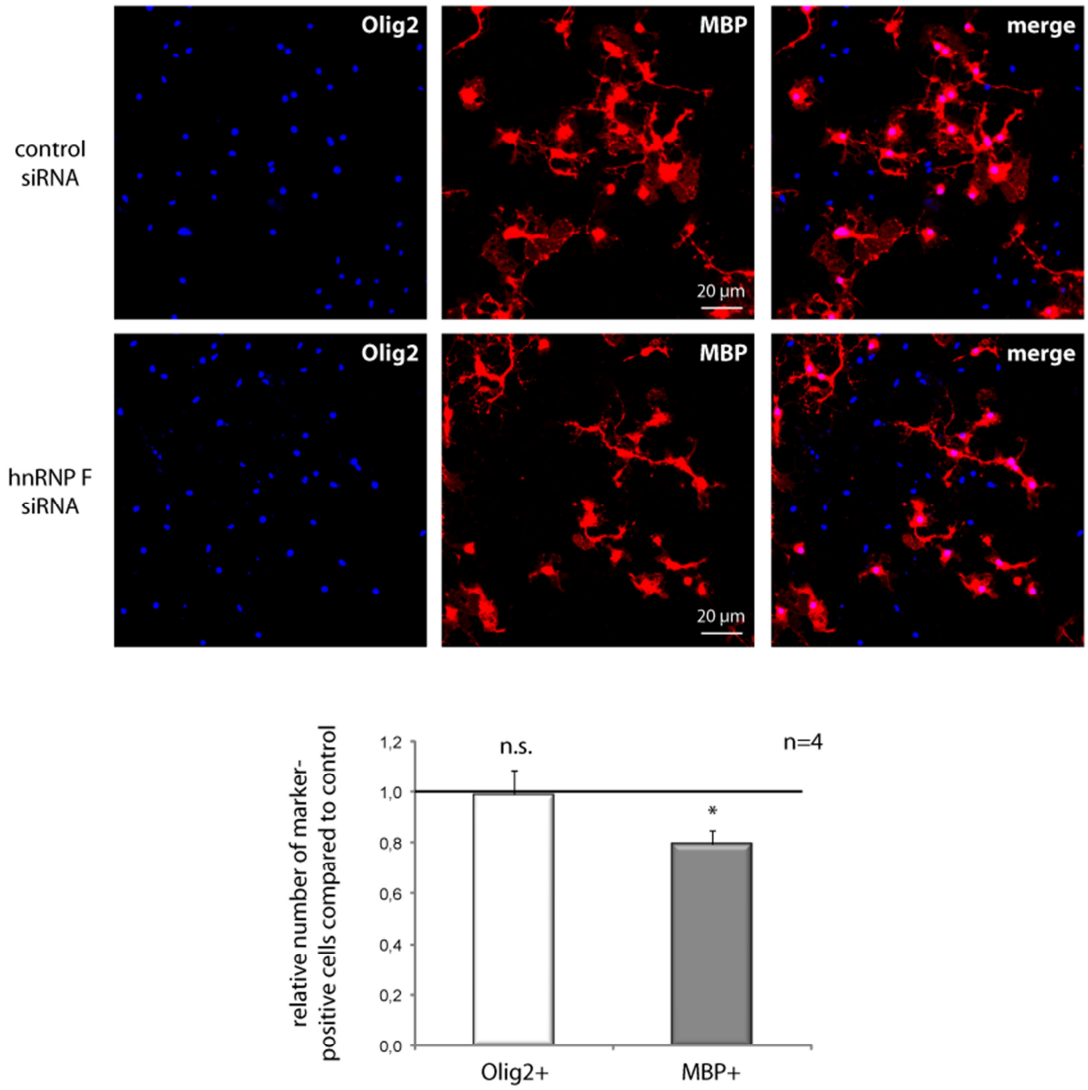


Figure 4-20 Reduced number of MBP-positive oligodendrocytes in the absence of hnRNP F

Primary mouse oligodendrocytes were treated with hnRNP F or control siRNA, allowed to differentiate for 3 days and immunostained for MBP and Olig2 (a marker for immature and mature oligodendrocytes). The graph shows the statistical evaluation of hnRNP F siRNA compared to control siRNA treated cells of 4 independent experiments. In each experiment marker-positive cells were counted in 15 different fields of view and related to DAPI which stains all nuclei (not shown). Error bars indicate SEM; * $p < 0.05$ (Student's ttest).

4.5.5 Levels of hnRNP F are critical for post-transcriptional regulation of MBP mRNA

To further analyse hnRNP F-dependent post-transcriptional regulation of MBP mRNA, a luciferase-based MBP reporter assay was performed. Two luciferase constructs were used in this assay. One contained the CDS of *Firefly* luciferase attached to a 378 nucleotide region of the 3'UTR of the classic MBP isoforms including the A2RE, and the other containing the CDS of *Renilla* luciferase without an attached element as control. This assay is independent of transcriptional effects as both luciferase plasmids share the same viral promoter. Furthermore, general translational effects and varying transfection efficiencies can be corrected for by normalising the *Firefly* activity versus *Renilla* activity. With the help of these constructs, a positive effect of Fyn activity on MBP mRNA translation was shown before (White, 2007).

Oli-*neu* cells that had either been treated with siRNA or transfected with plasmids to achieve knockdown or overexpression of hnRNP F were co-transfected with the luciferase constructs. On the following day the cells were lysed and luciferase activities were measured in a DualGlo® assay. Both knockdown (Figure 4-21 A) and overexpression (Figure 4-21 B) of hnRNP F significantly impair reporter translation. Interestingly, similar results were obtained using reporter constructs comprising the whole 3'UTR of MBP (data not shown). This indicates that the interaction of hnRNP F and MBP mRNA occurs within the first 378 nucleotides of its 3'UTR.

Compared to the reduction of MBP protein when primary oligodendrocytes were treated with hnRNP F siRNA (Figure 4-19 A), the reduction of reporter expression after the same treatment here is rather modest. A possible explanation is the use of viral promoters in the luciferase reporter constructs. The reporter mRNA is produced in high amounts, thereby very likely titrating out the factors that control transport and translational repression of MBP mRNA. The resulting "leaky" translation would weaken an observed reduction in reporter expression.

To control for possible effects of hnRNP F manipulation on the stability of the luciferase mRNAs, their levels were quantified by reverse transcription and subsequent qPCR. No significant alteration of luciferase mRNA amounts could be detected (right graphs in Figure 4-21).

These results confirm a post-transcriptional involvement of hnRNP F in MBP mRNA metabolism and suggest that a distinct level of hnRNP F protein is required to ensure efficient translation of MBP.

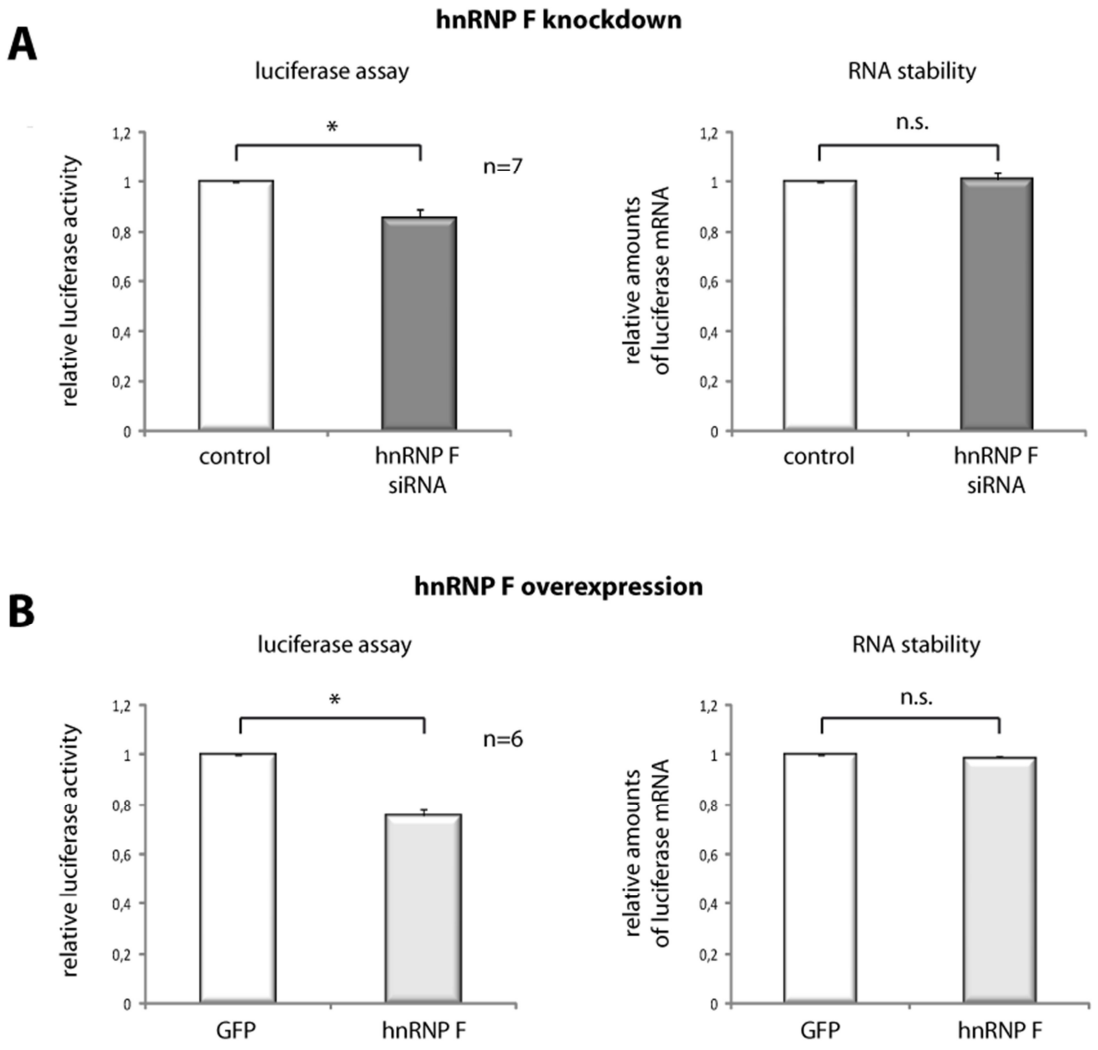


Figure 4-21 HnRNP F levels are critical for post-transcriptional expression of an MBP reporter gene

A) Reduction of hnRNP F impairs post-transcriptional MBP reporter expression. Oli-*neu* cells were treated with hnRNP F-directed or control siRNA. Subsequently, luciferase-based reporters containing parts of the 3'UTR of MBP (including the A2RE) as regulatory elements were used to measure post-transcriptional activity in DualGlo® assays. Relative amounts of luciferase mRNA were quantified using qRT-PCR to exclude effects on mRNA stability. B) Overexpression of hnRNP F impairs post-transcriptional MBP reporter expression. Oli-*neu* cells were transfected with hnRNP F or GFP (control) expression vectors. Luciferase assays were performed according to A. Error bars indicate SEM; * $p < 0.05$ (Wilcoxon signed-rank test).

4.5.6 Knockdown of hnRNP F does not affect proteasomal degradation of MBP in oligodendrocytes

The finding that knockdown of hnRNP F leads to a decrease of MBP protein levels can be explained in two ways. Either there is less MBP produced or there is more MBP degraded. As other myelin proteins and the cytosolic protein GAPDH were not affected by hnRNP F reduction (Figure 4-19 A), an influence on degradation would have to be

specific for MBP. To check for a degradation-dependent effect, the impact of hnRNP F knockdown on MBP was tested in primary oligodendrocytes (according to section 4.5.4) in the presence of the proteasomal inhibitor N-acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN) as previously described (Krämer-Albers et al., 2006). In the control condition the cells were treated with dimethyl sulfoxide (DMSO), which was used as a solvent for ALLN. Figure 4-22 indicates that there is an effect of the inhibition of the proteasome, because the Western Blots and the graph, where amounts of MBP were normalised to GAPDH, tend to show increased levels of MBP upon ALLN treatment. But there is no obvious change in the effect on MBP triggered by hnRNP F depletion, suggesting that it is independent of proteasomal degradation.

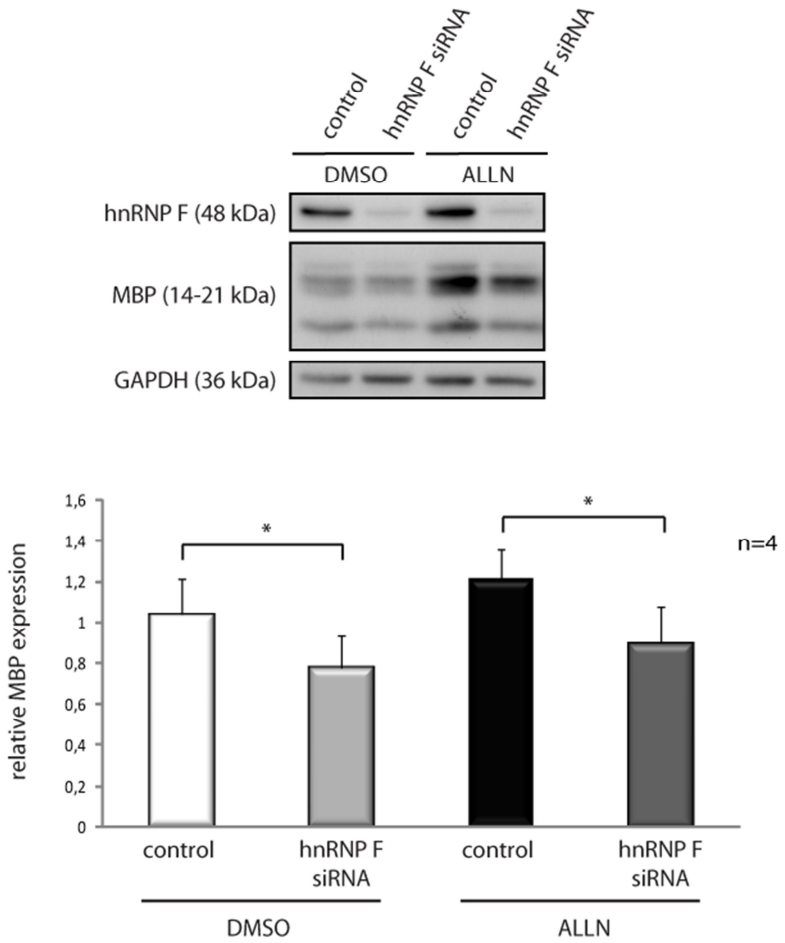


Figure 4-22 The effect of hnRNP F on MBP expression is independent of proteasomal degradation

Primary mouse oligodendrocytes were treated with hnRNP F or control siRNA and allowed to differentiate for 3 days. Before lysis, the siRNA-treated cells were incubated with the proteasomal inhibitor ALLN or as control with DMSO. Lysates were examined on Western Blots with antibodies to hnRNP F, MBP and GAPDH (loading control). The graph displays statistical evaluation of 4 independent experiments. Note the persistence in MBP reduction despite inhibition of the proteasome. Error bars indicate SEM; * $p < 0.05$ (Student's ttest).

4.5.7 Transport of hnRNP A2-dependent RNA granules appears normal in oligodendrocytes deficient for hnRNP **F**

The presence and functional effect of hnRNP F in MBP mRNA-containing cytoplasmic transport granules raises the question as to which point in the granule's lifetime the RNA-binding protein acts. In order to investigate a possible function of hnRNP F in granule dynamics, the well-accepted binding partner of MBP mRNA, hnRNP A2, was used as marker for MBP mRNA granules. As before, primary oligodendrocytes were treated with siRNA against hnRNP F. 3 days later the cells were fixed and immunostained for hnRNP A2 and MBP. Figure 4-23 shows no obvious difference in the localisation of hnRNP A2 or MBP in the absence of hnRNP F compared to control conditions. Moreover, in both conditions hnRNP A2 is enriched in the cytoplasm and processes of oligodendrocytes in areas that are also highly positive for MBP. These results suggest that hnRNP F does not have an influence on the localisation of hnRNP A2- and MBP mRNA-containing transport granules.

4.5.8 HnRNP **F** is tyrosine-phosphorylated in the cytoplasm of oligodendroglial cells upon Fyn kinase activation

HnRNP F was identified as a potential target of Fyn kinase in oligodendrocytes and this was confirmed by the Fyn-dependent phosphorylation of a GFP-hnRNP F fusion protein (White, 2007; Gonsior, 2007). To test this with a more physiological approach, the tyrosine-phosphorylation of endogenous hnRNP F was examined in response to Fyn activation. Oli-*neu* cells were transfected with Fyn+, Fyn wt or Fyn-. Subsequently, tyrosine-phosphorylated proteins were isolated by immunoprecipitation and analysed by Western Blotting for the presence of hnRNP F. As Figure 4-24 A illustrates, there is considerably more tyrosine-phosphorylated hnRNP F in the presence of active Fyn than in its absence. In agreement with this finding, there is less unphosphorylated hnRNP F in the lysate after the IP (unbound in Figure 4-24 A) when Fyn is active compared to the presence of inactive Fyn. This confirms endogenous hnRNP F as a downstream target of Fyn kinase in oligodendroglial cells.

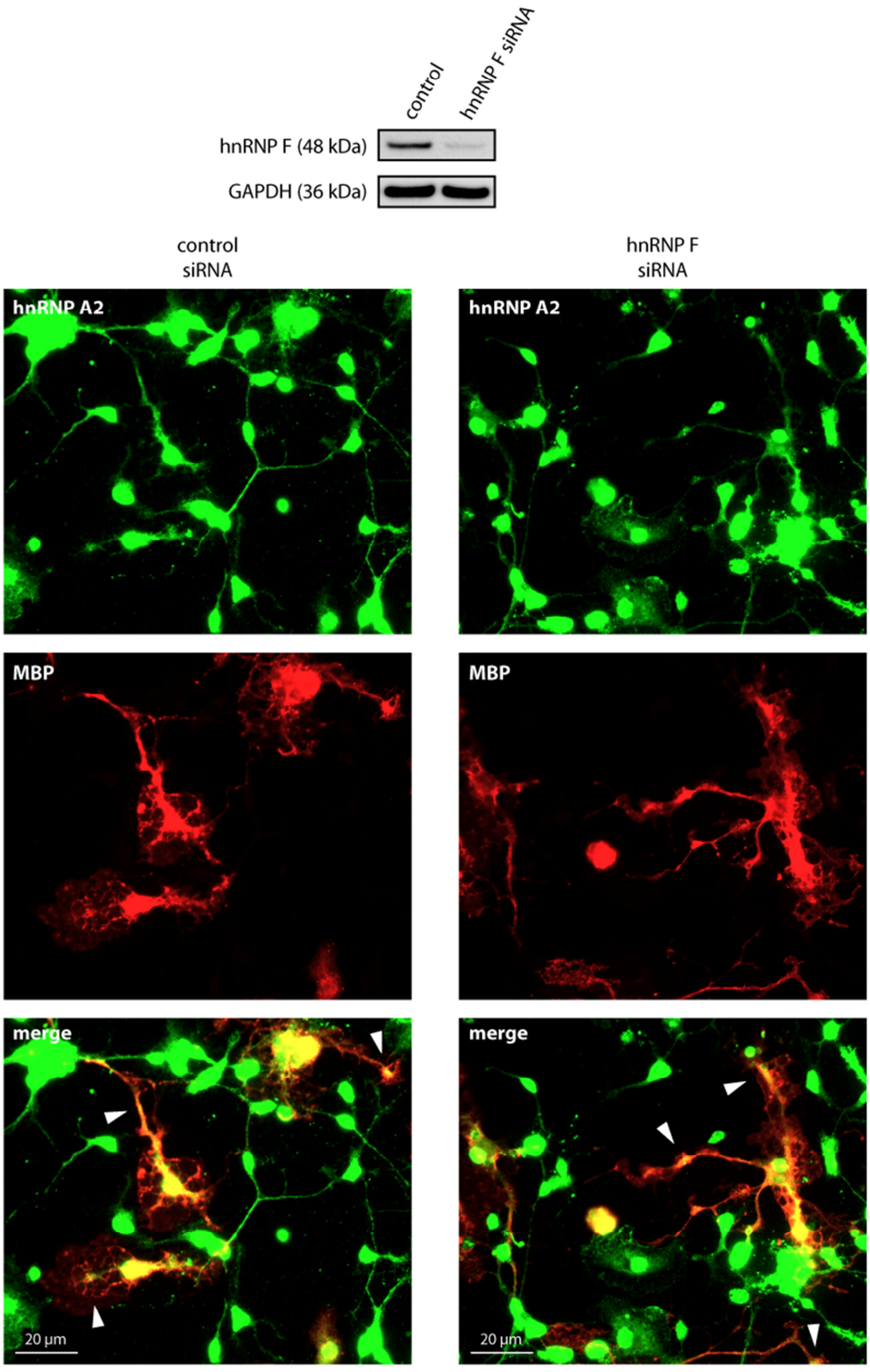


Figure 4-23 MBP and hnRNP A2 localisation are not affected by hnRNP F knockdown

Primary mouse oligodendrocytes were treated with hnRNP F or control siRNA and allowed to differentiate for 3 days. The knockdown of hnRNP F was assessed by Western analysis. GAPDH serves as loading control. Cells were immunostained for hnRNP A2 and MBP. Note the characteristic distribution of MBP and the cytoplasmic localisation of hnRNP A2, in particular its concentration at foci where MBP is highly abundant (white arrowheads).

HnRNP F is localised in the nucleus and the cytoplasm of oligodendroglial cells (see section 4.5.1). Thus, it could be phosphorylated in either compartment, indirectly in the nucleus by another kinase which is activated downstream of Fyn or directly in the cytoplasm where it would be accessible to Fyn. To answer this, the active and inactive Fyn mutants were co-transfected with F-myc into Oli-*neu* cells. After 2 days in culture, cytoplasmic fractions of the cells were separated from nuclear fractions and immunoprecipitations with myc-directed antibodies were carried out. Western analysis of the immunoprecipitated F-myc (Figure 4-24 B) depicts that tyrosine-phosphorylated F-myc is only present in the cytoplasm of oligodendroglial cells. Moreover, activated Fyn co-immunoprecipitates with F-myc from cytoplasmic extracts. To test the phosphorylation of F-myc by Fyn in a more direct way, an *in* vitro assay was performed. F-myc was immunoprecipitated from previously transfected Oli-*neu* cells and incubated with recombinant active Fyn kinase. As Western analysis in Figure 4-24 C shows, F-myc is strongly tyrosine-phosphorylated in the presence of recombinant Fyn compared to control conditions where no Fyn was added.

These results strongly suggest that the Fyn-dependent phosphorylation of hnRNP F takes place in the cytoplasm of oligodendrocytes.

The specific effects that Fyn kinase exerts on hnRNP F by phosphorylation could be best assessed when the phosphorylation site in hnRNP F is defined. Therefore, an attempt was made to identify this site (or several sites) in a previous study (Gonsior, 2007). By bioinformatical prediction analysis, two putative tyrosines (Y298 and Y306) were mutated to phenylalanine (F) in GFP-F-constructs and examined for their Fyn-dependent tyrosine-phosphorylation: this was still detectable. Then, a third promising tyrosine residue (Y246), which was found to be phosphorylated in human hnRNP F (UniProtKB/Swiss-Prot, 2011) was mutated. Furthermore, in this analysis the GFP-tagged constructs have the disadvantage of containing several tyrosines in the GFP region. Thus, to rule out that hnRNP F is phosphorylated in this experiment solely due to phosphorylation of the GFP portion, the mutants were cloned into myc-expression vectors resulting in tyrosine-mutant variants of F-myc.

These variants were co-transfected in Oli-*neu* cells with Fyn+ or Fyn-. Myc-specific immunoprecipitations were performed and the tyrosine-phosphorylation of the mutants was analysed on Western Blots. All F-myc variants generated still exhibit tyrosine-phosphorylation, even though the Y306F mutant seems to be less phosphorylated than the others (Figure 4-24 D), indicating that the mutated tyrosine residues are not exclusively phosphorylated in response to Fyn activation, if at all.

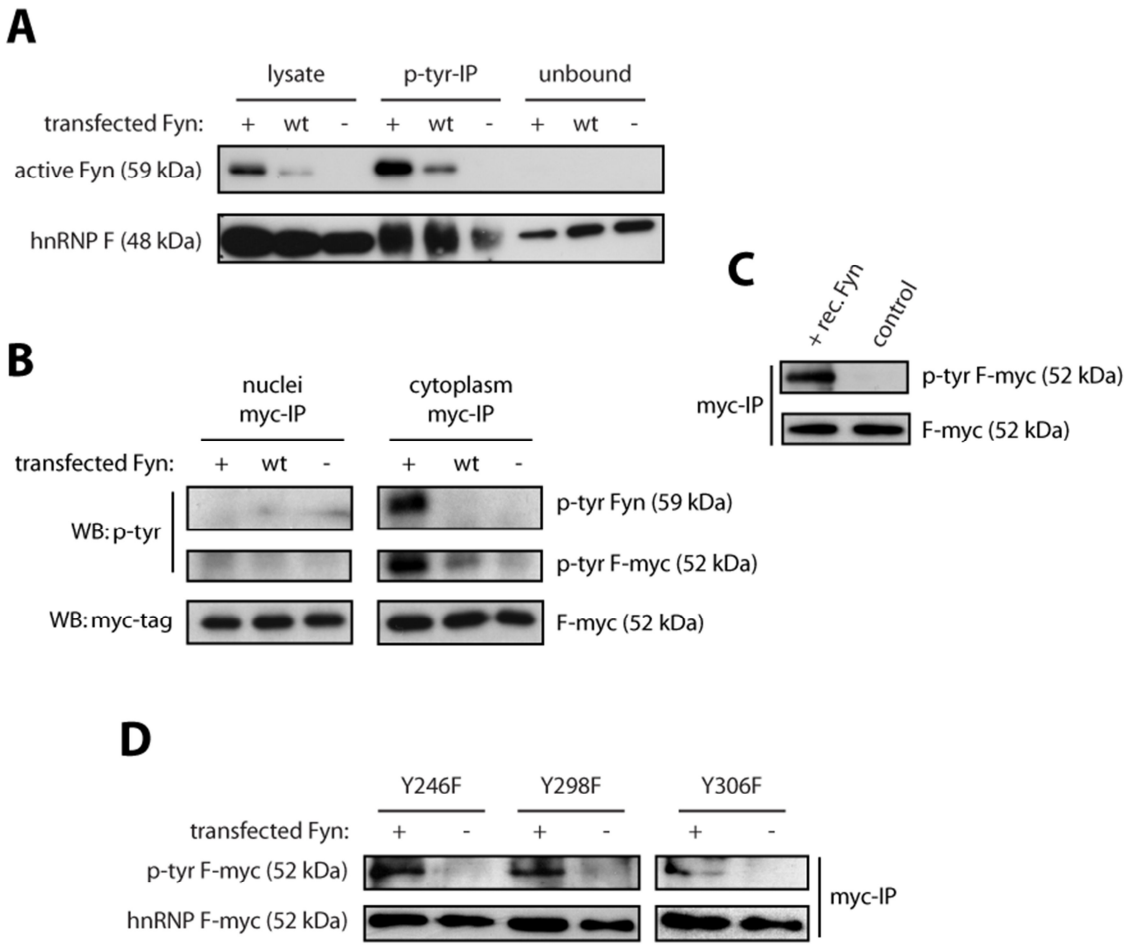


Figure 4-24 Cytoplasmic hnRNP F is a target of Fyn kinase

A) HnRNP F is tyrosine-phosphorylated in response to Fyn activity. Oli-*neu* cells were transfected with constitutive active (+), wildtype (wt) or kinase inactive (-) Fyn constructs. After 2 days, tyrosine-phosphorylated proteins were immunoprecipitated (p-tyr-IP) and analysed on Western Blots together with total lysates and proteins that were not precipitated (unbound). B) Fyn-dependent tyrosine-phosphorylation of hnRNP F occurs in the cytoplasm of oligodendrocytes. Oli-*neu* cells were co-transfected with Fyn+, Fyn wt or Fyn- together with myc-tagged hnRNP F (F-myc). 2 days later, separate nuclear and cytoplasmic fractions were prepared, immunoprecipitations with antibodies against the myc-tag (myc-IP) were performed and subsequently analysed on Western Blots (WB) for tyrosine-phosphorylated proteins and total amounts of F-myc. C) HnRNP F can be directly phosphorylated by Fyn. F-myc was immunoprecipitated from transfected Oli-*neu* cells and incubated with recombinant active Fyn. Tyrosine-phosphorylation and total F-myc levels were assessed by Western Blotting. D) Investigation of Fyn-dependent tyrosine-phosphorylation sites in hnRNP F. Oli-*neu* cells were co-transfected with Fyn+ or Fyn- and mutants of F-myc where the indicated tyrosine residues were changed to phenylalanine (Y->F), respectively. Mutant proteins were immunoprecipitated with antibodies to the myc-tag and investigated for tyrosine phosphorylation by Western analysis.

4.5.9 Fyn activity leads to a release of hnRNP F from granules and MBP mRNA

Fyn activity leads to cytoplasmic phosphorylation of hnRNP F and enhanced translation of MBP mRNA. The latter is thought to be mediated by phosphorylation of hnRNP A2 and subsequent release of hnRNP A2 and E1 from the granule (section 4.2.3). Therefore, an impact of Fyn on the association of hnRNP F with cytoplasmic RNA granules and MBP mRNA was investigated.

Oli-*neu* cells were transfected with Fyn wt or Fyn- and allowed to differentiate for 2 days. Post-nuclear lysates were prepared and subjected to ultra-centrifugation to deplete the lysate from RNA granules. The resulting granule-free lysates and total lysates were tested by Western Blotting. Densitometrical analysis of 4 such experiments, where granule-free levels of hnRNP F were related to total levels, shows a significant increase of granule-free, soluble hnRNP F in the presence of active compared to inactive Fyn (Western Blots and graph in Figure 4-25 A).

To reveal if Fyn activity also influences the binding of hnRNP F to MBP mRNA, Oli-*neu* cells were co-transfected with Fyn wt or Fyn- and F-myc. F-myc was immunoprecipitated from these cells and the associated mRNAs were purified and analysed by qRT-PCR. As illustrated by the evaluation of several independent experiments where MBP mRNA levels were normalised to levels of phosphoglycerate kinase 1 mRNA (Figure 4-25 B), activation of Fyn kinase decreases the amount of MBP mRNA co-purifying with hnRNP F.

Taken together, these results provide evidence that Fyn activity releases hnRNP F from RNA granules and MBP mRNA.

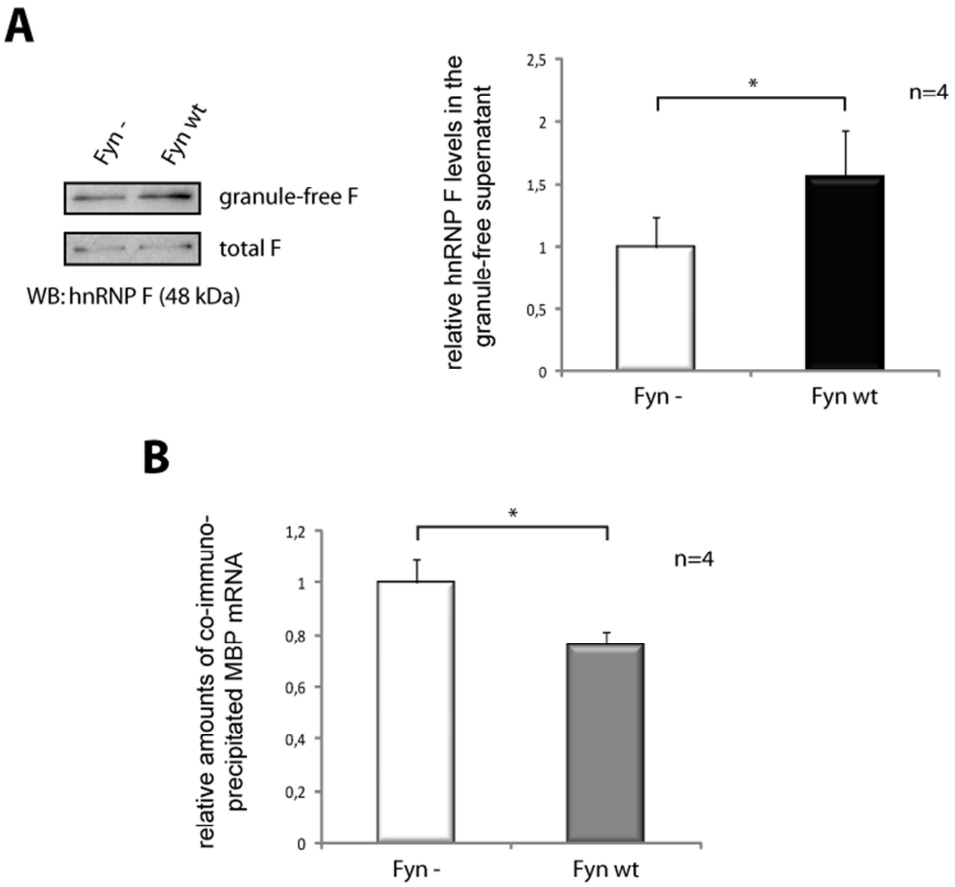


Figure 4-25 Fyn activity releases hnRNP F from granules and MBP mRNA

A) Fyn activity leads to a release of hnRNP F from granules into the cytosolic fraction. Oli-*neu* cells were transfected with Fyn wt or Fyn- constructs. After 2 days, post-nuclear total lysates and granule-free lysates, obtained by ultra-centrifugation, were analysed on Western Blots. The levels of hnRNP F in the non-granule fraction were quantified densitometrically and normalised to total hnRNP F levels. B) Fyn activity releases hnRNP F from MBP mRNA. Oli-*neu* cells were co-transfected with F-myc and Fyn wt or Fyn-, respectively. After they were allowed to differentiate for 3 days, immunoprecipitations with antibodies against the myc-tag or control antibodies were performed from post-nuclear lysates and associated mRNAs were analysed by qRT-PCR. MBP mRNA levels were normalised to levels of phosphoglycerate kinase 1 mRNA. Error bars indicate SEM; * $p < 0.05$ (Student's t-test).

4.6 HnRNP A2 localises to stress granules in oligodendroglial cells

Recently, it was published that MBP mRNA can be localised in SGs in oligodendrocytes (Wang et al., 2010). SGs are large cytoplasmic complexes consisting of mRNAs and associated proteins. They form reversibly upon exposure of a cell to a variety of stresses such as heat, oxidative stress or nutrient deprivation. SGs are believed to store and protect translationally inactive mRNAs, constituting part of the cellular metabolic adaptation during stress situations (Anderson and Kedersha, 2006).

As hnRNP F and hnRNP A2 are associated with MBP mRNA, a localisation of these RNA-binding proteins to oligodendroglial SGs was investigated. SGs were induced in Oli-*neu* cells by applying a heat shock for 30 min at 44°C. The cells were fixed and immunostained for the two hnRNPs and for Tia-1, a marker and necessary component of SGs. Figure 4-26 displays Oli-*neu* cells with Tia-1-positive SGs that are mainly concentrated around the nucleus. Furthermore, some of these SGs stain positive for hnRNP A2 (white arrows in Figure 4-26) while others do not (yellow arrowheads in Figure 4-26). Interestingly, no co-localisation of hnRNP F with stress granules was observed (data not shown).

Thus, hnRNP A2 seems to be sorted to SGs in oligodendrocytes.

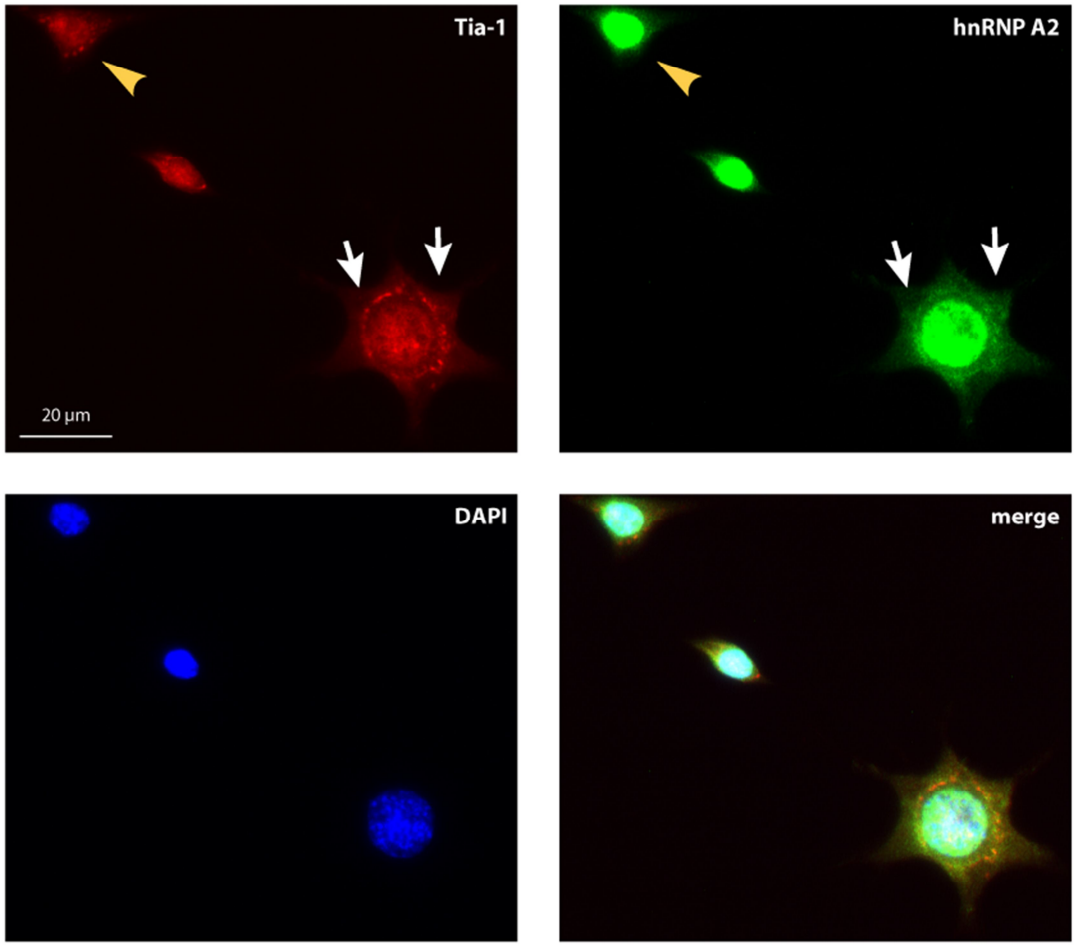


Figure 4-26 HnRNP A2 associates with oligodendroglial stress granules

Oli-*neu* cells were exposed to a 30 min heat shock at 44°C, fixed and immunostained. Tia-1 represents a marker for stress granules. Nuclei were stained with DAPI. Note the presence of stress granules positive (white arrows) or negative (yellow arrowheads) for hnRNP A2.

5 DISCUSSION

Myelination requires precisely regulated and efficient delivery in time and space of vast amounts of myelin components to the axon glial contact site (Simons and Trotter, 2007). MBP is the second-most abundant protein in myelin and it is essential to form this compacted multilamellar sheath (Readhead and Hood, 1990). The discovery of MBP mRNA and ribosomes in purified myelin fractions (Colman et al., 1982) was a first hint pointing at transport of the mRNA to the myelin compartment with localised translation to ensure the deposition of MBP in the specific myelin domain. Since then, transport of mRNAs has been described in multiple cell types, for example β-actin mRNA in fibroblasts and neurons (Lawrence and Singer, 1986; Hüttelmaier et al., 2005), or mRNAs coding for synaptic proteins in neurons (Gao et al., 2008). Localisation of mRNAs helps to establish specialised domains in polarised cells and allows efficient protein synthesis which can be triggered quickly in response to local cues, independently of transcription. Thus, mRNA localisation is a mechanism that appears to be ideally suited for the demanding task to precisely and effectively deliver myelin components and has in fact been realised for the "executive" myelin protein MBP.

5.1 The hnRNP A2 pathway is a central mediator of MBP mRNA metabolism and is regulated by Fyn kinase

It was shown in the last century that the mRNA of MBP is sorted to RNA transport granules after export from the nucleus in which it is transported in a translationally inactive state to the distal processes of oligodendrocytes where it is eventually locally translated (Ainger et al., 1993; Barbarese et al., 1999). The RNA-binding protein hnRNP A2 was identified as a main mediator of this transport process (called the "A2 pathway"), binding already in the nucleus as a *trans*-acting factor to the *cis*-acting A2RE in the 3'UTR of MBP mRNA (Ainger et al., 1997; Hoek et al., 1998). Furthermore, the RNA-binding protein hnRNP E1 is recruited to MBP mRNA by hnRNP A2 and was proposed to be responsible for translational repression during transport (Kosturko et al., 2006). Intriguingly, a signalling cascade between the neuronal CAM L1 and the oligodendroglial CAM F3 was later discovered which leads to the activation of the plasma membrane-tethered Fyn kinase in oligodendrocytes. Furthermore, active Fyn was shown to directly phosphorylate hnRNP A2 and to enhance translation of an MBP mRNA reporter containing the A2RE, supposedly by modification of the transport

granule structure. This was the first demonstration that axon-glial contact can lead to local synthesis of a myelin protein (White, 2007).

Here, the F3-dependent binding of L1 to oligodendroglial cells was confirmed in a cell ELISA approach (section 4.2.1). Moreover, L1 binding to primary oligodendrocytes as well as to the oligodendroglial cell line Oli-*neu* was shown to lead to tyrosine-phosphorylation of hnRNP A2 which could be blocked by depleting the cells of Fyn kinase (section 4.2.2). These findings further endorse the previously proposed model that hnRNP A2 is phosphorylated upon axon-glial contact involving signalling via L1 on the neuronal side, F3 on the oligodendroglial side and finally Fyn kinase at the inner leaflet of the oligodendroglial plasma membrane. As a last step of this signalling cascade, hnRNP A2 phosphorylation by the Src-family kinase Fyn was suggested to initiate translation of associated MBP mRNA (White, 2007), a mechanism of Src kinase control that had been described in other systems (Hüttelmaier et al., 2005; Ostareck-Lederer et al., 2002). It was proposed before that dissociation of hnRNP E1 from MBP mRNA-containing granules had to occur in order to relieve the mRNA from translational repression (Kosturko et al., 2006). Here, it was shown that L1-binding to Oli-*neu* cells triggers the release of both hnRNP A2 and E1 from the cytoplasmic granule fraction (section 4.2.3), presumably by phosphorylation of A2. This remodelling of the granule structure most likely allows polysome assembly and translation initiation. The proposed signalling cascade is summarised in Figure 5-1.

Stimulation of oligodendrocytes through L1 may also be accomplished by binding to CAMs other than F3 (question mark-labelled in Figure 5-1), for example integrins (Oleszewski et al., 1999). Interestingly, maximal Fyn activation appears to be achieved by synergistic action of L1 and the ECM protein laminin-2 which accumulates around axons in the CNS. Contributing to the findings illustrated in Figure 5-1, laminin-2 was found to collaborate with L1 in binding and activation of an oligodendroglial signalling complex consisting of β1-integrin and F3, which are targeted by laminin-2 and L1, respectively (Laursen et al., 2009). Moreover, integrin stimulation recently was shown to additionally enhance translation of MBP mRNA. This was hypothesised to be mediated by phosphorylation of hnRNP K which was found associated with hnRNP A2 as an additional member of cytoplasmic granules containing MBP mRNA (Laursen et al., 2011). In erythroid precursor cells, hnRNP K was implicated in translation regulation triggered by Src kinase (Ostareck-Lederer et al., 2002). Laursen and colleagues assumed Fyn kinase to be mediating the effects of hnRNP K in oligodendrocytes. Thus, several extracellular signal pathways appear to converge (via Fyn, see also section 5.2) on the A2 pathway influencing local synthesis of MBP.

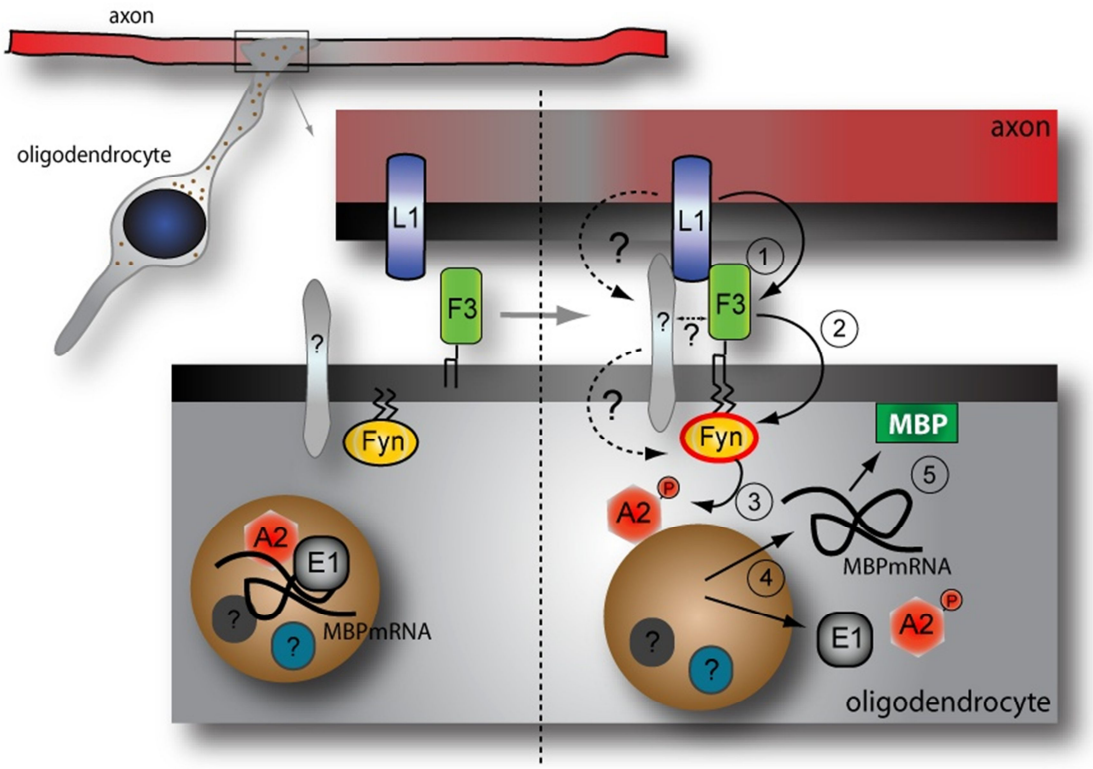


Figure 5-1 Model: Axon-oligodendroglial signalling triggers local translation of MBP mRNA

After export from the nucleus, MBP mRNA is transported into the processes of oligodendrocytes in a translationally silenced state. Upon axon-glial contact, the axonal cell adhesion molecule L1 binds the oligodendroglial cell adhesion molecule F3 (1) resulting in activation of the membrane-tethered Fyn kinase (2). Fyn phosphorylates the RNA-binding protein hnRNP A2 (3) which is considered as a main component of MBP mRNA transport granules (brown). Following phosphorylation, both hnRNP A2 and the associated hnRNP E1, which was shown to inhibit MBP mRNA translation, dissociate from the granule (4) allowing translation to ensue at the axon-glial contact site (5). L1 binding to oligodendrocytes could also involve other adhesion molecules additional to F3 (labelled with question mark). The drawing was prepared in collaboration with Dr. Robin White (White et al., 2008).

The DEAD box-family RNA helicase DDX5 was identified as a potential cytoplasmic binding partner of hnRNP A2 in oligodendrocytes by immunoprecipitation of A2 and mass spectrometry analysis of co-purifying proteins (White, 2007). The interaction of both proteins in the oligodendroglial cytoplasm was confirmed here as in the reciprocal co-immunoprecipitation hnRNP A2 turned out also to co-purify with cytoplasmic DDX5 (Figure 4-10). Furthermore, immunostainings showed that DDX5 is strongly expressed in the nucleus of oligodendroglial cells and in lower amounts in the cytoplasm where it also localises in granule-like structures in the distal processes (Figure 4-9). Together with the finding that DDX5 was isolated as component of RNA granules from total brain and neuronal cultures (Elvira et al., 2006; Kanai et al., 2004), this suggests DDX5 to be a constituent of hnRNP A2-dependent transport granules in oligodendrocytes. DEAD box helicases are known to function in modification of RNA secondary

structures and to influence RNA-protein interactions. Hence, DDX5 could be instrumental in remodelling structure and composition of hnRNP A2-containing transport granules and participate in the regulation of MBP mRNA translation. DDX5 was shown to be phosphorylated at several threonine and tyrosine residues and its activity was observed to depend on the phosphorylation status (Yang et al., 2005). As Fyn activation leads to translation of MBP mRNA, Fyn-dependent tyrosine-phosphorylation of DDX5 in addition to hnRNP A2 was regarded as possible regulatory means for translation regulation. However, DDX5 did not turn out to be phosphorylated in response to Fyn activity here (Figure 4-11). This does not necessarily mean however that DDX5 is not involved in translation regulation in the A2 pathway. Moreover, it could function in several other steps of the A2 pathway where its activity could be controlled by other kinases. The role of DDX5 in oligodendrocytes was further investigated by Peter Hoch-Kraft (Hoch-Kraft, 2010) and first results support an involvement of DDX5 in post-transcriptional regulation of MBP mRNA.

SGs are large cytoplasmic complexes consisting of translationally silent mRNAs and associated proteins. They form under a variety of stress conditions, such as nutrient deprivation or oxidative stress, and serve to store and protect mRNAs until the stress is relieved (Anderson and Kedersha, 2006). In diseases of the nervous system, like multiple sclerosis (MS), aberrant physiology may lead to the formation of deleterious and stressful environments. Thus, SGs could play an important role in such situations allowing the cell to adapt and endure damaging events. Almost nothing is known about how SGs impinge on the physiology of oligodendrocytes. Recently, MBP mRNA was found to be sorted to SGs in oligodendroglial cells (Wang et al., 2010). Here, hnRNP A2 which is known as a central controlling element in MBP mRNA metabolism also was shown to localise to oligodendroglial SGs (Figure 4-26). Thus, regulation of A2 pathway elements including translation of MBP mRNA could be a crucial factor in oligodendrocytes for efficient protection and recovery after stress. For example, remyelination events in MS could be affected by an insufficient stress management leading to compromised MBP mRNA metabolism and thereby to the inability of the cells to effectively contribute to myelin repair. Interestingly, not all cells displayed an association of hnRNP A2 with SGs. It is unclear whether this is an effect of time or of cellular conditions. The composition of SGs is known to vary dependent on cellular and environmental conditions (Buchan and Parker, 2009). On the one hand, sorting of hnRNP A2 to SGs could depend on the metabolic state of the cell. On the other hand, localisation of A2 to SGs may be transient and only occur during a certain phase of SG lifetime. Either way, the cause of the observed selectivity could be an important indicator for hnRNP A2 function in the cellular stress response.

5.2 The versatile contribution of Fyn kinase to oligodendroglial physiology and myelination

The importance of Fyn kinase for myelination was demonstrated by Fyn knock-out mice which display a pronounced hypomyelination phenotype in the forebrain (Sperber et al., 2001; Goto et al., 2008). A main contribution to this phenotype could be the Fyn-dependent regulation of MBP expression, MBP being the only structural myelin protein in the CNS which was demonstrated to be indispensable for myelination. Fyn was shown to stimulate transcription of MBP (Umemori et al., 1999) and as discussed in section 5.1, it plays an important role in regulation of MBP mRNA translation, by integrating extracellular signals coming from axonal processes. Intriguingly, Fyn-dependent translation of MBP was recently demonstrated to be triggered by neuronal electric activity, providing a mechanistic link between neural activity and myelin-dependent modulation of signal transduction speed as a kind of synaptic plasticity (Wake et al., 2011). Several further oligodendroglial "receptors" have been implicated in building distinct signalling complexes with Fyn and regulating its activity, including MAG, deleted in colorectal carcinoma (Dcc), immunoglobulin Fc receptor γ-chain (FcRγ) and LINGO-1 (reviewed by Krämer-Albers and White, 2010).

Additional downstream effects of Fyn involve target-dependent survival signalling (Colognato et al., 2004; Laursen et al., 2009) which matches oligodendrocyte numbers to numbers of axons to be myelinated. Important for the wrapping process, it regulates process formation of oligodendrocytes (Osterhout et al., 1999) by manipulating the actin cytoskeleton (Wolf et al., 2001; Liang et al., 2004) and it is able to recruit the microtubule cytoskeleton to the site of Fyn activation (Klein et al., 2002). Thereby, Fyn activity could control RNA granule transport towards the axon-glial contact site and finally localised translation. Thus, Fyn integrates many signals and affects oligodendroglial functions that potentially feed the same purpose: myelination (reviewed by Krämer-Albers and White, 2010).

Some of these downstream steps could be mediated by Fyn-dependent phosphorylation of p130Cas in oligodendrocytes. This multiadaptor protein has been shown to participate in various signalling pathways, mainly controlled by extensive phosphorylation through Src-family kinases including Fyn (Manie et al., 1997; reviewed by Defilippi et al; 2006). Here, p130Cas was shown to be tyrosine-phosphorylated in the presence of active Fyn in oligodendroglial cells. Moreover, immunostainings and co-immunoprecipitations suggest a direct interaction of p130Cas and Fyn in oligodendrocytes (section 4.4.2). As mentioned above, Fyn kinase links many external signals to many cellular functions. The participation of p130Cas in specific pathways depends on

its recruitment to distinct signalling complexes and the set of associated factors, both being governed by its tyrosine-phosphorylation status (Defilippi et al., 2006; Janostiak et al., 2011). Hence, p130Cas could help to direct Fyn signalling to different effectors by its diversity in binding partners. The here observed co-localisation of p130Cas and Fyn in the tips of oligodendroglial cell protrusions (Figure 4-13 C) indicates that p130Cas participates in Fyn-dependent actin-cytoskeleton remodelling and process outgrowth, as previously shown in neurons (Huang et al., 2006). Furthermore, p130Cas has been implicated in cell survival signalling in several cell types (Defilippi et al., 2006). A possible role in promoting oligodendroglial survival was confirmed here, as depletion of p130Cas in primary oligodendrocytes led to increased levels of activated caspase 3, an early indicator of apoptosis (section 4.4.3). This function could involve formation of a complex with Fyn as both proteins were shown to regulate cell survival downstream of integrin and growth factor signalling (Colognato et al., 2004; Defilippi et al., 2006).

5.3 The RNA-binding protein hnRNP F is a novel (MBP) mRNA granule component in oligodendrocytes

HnRNP F is a ubiquitously expressed RNA-binding protein (Honore et al., 1995). Here, its expression was demonstrated for all stages of oligodendrocyte development by immunostaining and Western analysis of primary oligodendrocytes (Figure 4-15 and Figure 4-16). It is present in NG2-positive progenitor cells as well as more mature PLP- and MBP-positive oligodendrocytes. According to previous findings (Wang et al., 2007), the expression of hnRNP F appears to decrease during oligodendroglial development, implying that distinct levels of hnRNP F are critical for defined aspects of oligodendroglial development. In fact, hnRNP F was implicated in the regulation of alternative splicing of PLP/DM20 in oligodendroglial cells, where the switch from high to low levels of hnRNP F (approximately 50% reduction observed here, Figure 4-16) contributes to the switch from DM20-expressing progenitors to PLP-expressing mature oligodendrocytes (Wang et al., 2007).

So far, hnRNP F has primarily been linked to nuclear functions such as splicing (Dominguez et al., 2010), which is also the only published function for hnRNP F in oligodendrocytes to date (see above). In agreement, hnRNP F was found here to be primarily localised to the nucleus of oligodendroglial cells. Furthermore, it was also observed to be present in the cytoplasm (Figure 4-15 and Figure 4-17), indicating novel cytoplasmic functions of hnRNP F in oligodendrocytes, additionally to its known role in splicing. Cytoplasmic localisation of hnRNP F has been shown for several cell types

(Matunis et al., 1994; Honore et al., 2004). However, a cytoplasmic function in mammalian cells was only recorded once: the translational control of *mu*-opioid receptor mRNA in a neuronal cell line (Song et al., 2011).

The cytoplasmic distribution of hnRNP F reaches into the oligodendroglial processes and appears granular (Figure 4-15 and Figure 4-17). Additionally, hnRNP F was shown here to associate with a cytoplasmic RNA granule fraction (Figure 4-18 A), indicating that it is part of oligodendroglial cytoplasmic RNA granules. Moreover, hnRNP F was demonstrated here to co-localise with hnRNP A2 in granular structures in the oligodendroglial cytoplasm and to co-immunoprecipitate with cytoplasmic hnRNP A2 and MBP mRNA (Figure 4-17 and Figure 4-18 B). This not only confirms the association of hnRNP F with cytoplasmic granules in oligodendrocytes, but also suggests it to be a novel factor acting in hnRNP A2-dependent transport granules containing MBP mRNA. The observed interaction of hnRNP F and hnRNP A2 is independent of RNA. Thus, both proteins could conceivably bind directly in the granule. It is also possible that A2 and F function together in the regulation of other mRNAs (in addition to MBP mRNA) in oligodendrocytes. Furthermore, the partial co-localisation of A2 and F in cytoplasmic granular structures indicates heterogeneity of oligodendroglial RNA granules. Those which are only positive for A2 or F could represent intermediates in the dynamic granule remodelling process. Alternatively, they could be distinct types of granules fulfilling different functions. The smallest splice variant of the hnRNP A2/B1 gene (see section 2.6.1) is hnRNP A2b which lacks exon 9. Recently, A2b was proposed as the major hnRNP A2/B1 isoform to be localised in the cytoplasm of neural cells (Han et al., 2010). The antibody used in this study does not discriminate between the different hnRNP A2/B1 isoforms (particularly important for immunostainings). Moreover, hnRNP A2b is present in the co-immunoprecipitation experiment with hnRNP A2 and F described above. Hence, one cannot exclude that hnRNP F additionally binds to A2b. HnRNP F seems to exhibit some specificity in respect to participation in oligodendroglial RNA transport granules as it does not show significant association with β-actin mRNA (Figure 4-18 B) which is known to be transported in granules, too.

Interestingly, recent results suggest that hnRNP F can bind to MBP mRNA independently of hnRNP A2: a 358 nucleotide section of the first region of the 3'UTR of MBP seems to be sufficient to mediate the interaction with hnRNP F (see section 5.4; White, Gonsior et al., 2011, in revision). The binding element for hnRNP F in MBP mRNA remains to be elucidated. For translational control of *mu*-opioid receptor mRNA (see above), F appears to bind to a poly(G) run in the 5'UTR. Two such poly(G) runs are located in the previously mentioned 358 nucleotide region of the 3'UTR of MBP. An-

other possibility is that the binding site for F is rather than a short linear sequence, a conformational epitope which comprises several sections of the mRNA. Such a binding mode was shown for Glorund, the homologue of hnRNP F in *Drosophila* which is involved in translational regulation of nanos mRNA in oocytes (Kalifa et al., 2006).

5.4 Efficient synthesis of MBP requires defined levels of hnRNP F

HnRNP F is associated with hnRNP A2-containing cytoplasmic transport granules and MBP mRNA in oligodendrocytes. Its *Drosophila* homologue plays a role in translation regulation and recently, an involvement of mammalian hnRNP F in translation regulation in neuronal cells was discovered (see previous sections). Furthermore, it was shown here that siRNA-mediated knockdown of hnRNP F leads to reduced levels of MBP protein in primary oligodendrocytes whereas MBP mRNA levels are unaltered (Figure 4-19), strongly suggesting a function of hnRNP F in post-transcriptional regulation of MBP mRNA. The impact on MBP seems to be specific and not a general effect on myelin proteins, as those tested (CNP and MOG) were unaffected by hnRNP F manipulation. It is unlikely that protein degradation is responsible for the observed reduction of MBP, as inhibition of the proteasome was not able to reduce this effect (Figure 4-22). Thus, hnRNP F is involved in the synthesis of MBP and is most likely acting at the level of translation regulation. This finding was further corroborated by translational reporter assays using luciferase reporter constructs fused to 378 nucleotides of the first region of the 3'UTR of MBP containing the A2RE (bases 359-369) (Figure 4-21). Intriguingly, reporter expression was impaired by either knockdown or overexpression of hnRNP F, suggesting a need for defined levels of hnRNP F for "normal" synthesis of MBP.

The region downstream of the A2RE does not appear to be important for the interaction of hnRNP F with MBP mRNA, as with a reporter construct containing the whole 3'UTR of MBP, comparable effects were obtained following hnRNP F manipulation. Recent experiments using the MBP reporter constructs lacking the A2RE (i.e. without bases 359-378) indicate that hnRNP F affects MBP mRNA translation even independently of hnRNP A2 and that the interaction of F and MBP mRNA occurs in the first part of the 3'UTR, comprising 358 nucleotides (White, Gonsior et al., 2011, in revision). This and the finding that hnRNP F siRNA treatment does not alter expression of hnRNP A2, support the notion that hnRNP F acts directly on MBP mRNA.

Interestingly, downregulation of other components of MBP mRNA-containing granules such as hnRNP A2, TOG or hnRNP K also leads to diminished levels of MBP (Francone et al., 2007; Laursen et al., 2011). Depletion of hnRNP A2 leads to cytoplasmic reten-

tion of MBP mRNA and hnRNP K depletion leads to an accumulation at process branch points. In contrast, despite an impact on MBP expression, downregulation of TOG does not impair localisation of MBP mRNA (Francone et al., 2007). Apparently, these granule components exhibit distinct responsibilities in the metabolism of MBP mRNA-containing transport granules. Neither localisation of hnRNP A2 nor of MBP appears to be affected by hnRNP F knockdown (Figure 4-23), suggesting that hnRNP F is not involved in assembly or transport of granules containing MBP mRNA. Depletion of hnRNP F in oligodendrocytes results in a reduction of MBP protein to approximately 60% while the number of MBP-positive cells is only reduced to around 80% of controls (Figure 4-20). Taking into consideration the substantial knockdown of hnRNP F, one can deduce that loss of hnRNP F rather impairs the efficiency of MBP synthesis, without completely blocking it in siRNA-affected cells. HnRNP F has been implicated in affecting proliferation and survival in different cell types (see section 2.6.2). As oligodendroglial cell numbers do not change here upon hnRNP F siRNA treatment of primary oligodendrocytes (Figure 4-20), there is no indication that hnRNP F is involved in these functions in oligodendrocytes.

After hnRNP F manipulation, the reduction in MBP luciferase reporter expression is not as pronounced as the reduction in MBP protein levels in primary oligodendrocytes (Figure 4-19 and Figure 4-21). As mentioned before, a possible explanation for this discrepancy is the use of strong viral promoters in the luciferase constructs. The vast abundance of reporter mRNA could overstrain the cells' capacities in translational regulation and the resulting "leaky" translation could explain the observed results. Another possibility is that hnRNP F affects additional regulatory elements in MBP mRNA besides the 3'UTR, as it was shown for translation regulation of the *mu*-opioid receptor in neuronal cells where hnRNP F acts via sequences in the 5'UTR (Song et al., 2011).

Not much is known about the differences between the classic isoforms of MBP. Analysis of MBP mRNA metabolism mainly comprised effects that depend on the 3'UTR and/or the (included) A2RE which are identical for the classic MBP isoforms. The localisation of the different isoforms (mRNAs and proteins) has been shown to vary in time and space, primarily dependent on the presence of exon II (de Vries et al., 1997; Karthigasan et al., 1996; Allinquant et al., 1991). The mRNAs of the isoforms lacking exon II are transported to the distal processes. The corresponding proteins are the predominant MBP isoforms in mature oligodendrocytes and myelin and localise to the plasma membrane in cultured cells. In contrast, isoforms containing exon II prevail early during oligodendrocyte development. The mRNAs and proteins remain perinuclear and exon II-containing MBP was even shown to enter the nucleus which was suggested to

be a signalling event in oligodendrocytes. Nevertheless, it was shown here that all isoforms are affected by hnRNP F knockdown (Figure 4-19 A) as they also were by hnRNP A2 and K manipulation (Laursen et al., 2011). This suggests regulation of the MBP mRNAs by both isoform-specific factors (depending on exon II) and common factors such as hnRNP F, A2 and K (affecting the 3'UTR and maybe other regions). One can further conclude that MBP exon II (mRNA) either confers a signal inhibiting A2 pathway elements and thereby granule transport to the periphery or an additional localisation signal which overrules the hnRNP A2-dependent transport. Such a regulation could include post-translational modifications of granule components and/or the action of additional components recruited by exon II.

HnRNP F does not appear to influence levels of MBP mRNA (see above). Nevertheless, as MBP mRNA analysis performed here did not discriminate between the different isoforms, one cannot exclude that hnRNP F is involved in splicing of MBP. Developmental regulation of hnRNP F in oligodendrocytes contributes to the alternative splicing of PLP/DM20 (Wang et al., 2007). Similarly, hnRNP F could participate in the developmentally regulated alternative splicing of the MBP mRNA isoforms (see also section 2.4.3), additionally to its role in post-transcriptional control of MBP. Interestingly, hnRNP A2, F and K and DDX5 have recently been identified to interact in a splicing complex in human myoblasts (Paul et al., 2011). It is likely that these RNA-binding proteins also form splicing complexes containing MBP mRNA in oligodendrocytes and together modulate MBP mRNA processing during several steps from splicing to translation. However, Western analysis does not support a role of hnRNP F in alternative splicing of MBP as it did not reveal an obvious change in isoform expression upon hnRNP F depletion (Figure 4-19 A). To clarify this point, investigations should be carried out with isoform-specific detection of MBP mRNAs and proteins.

The findings presented here share intriguing similarity with findings from *Drosophila*. Glorund, the fly homologue of hnRNP F, was found in a splicing complex with Hrp48, an hnRNP A2/B1 homologue. Both proteins are also present in the cytoplasm of *Drosophila* oocytes where they contribute to localisation and translation regulation of distinct mRNAs (Kalifa et al., 2009).

5.5 Fyn kinase phosphorylates hnRNP F in the cytoplasm, affecting its granule association

In a screen, hnRNP F was identified as potential target of Fyn kinase in oligodendrocytes (White, 2007). Fyn-dependent phosphorylation of hnRNP F was confirmed subsequently as a GFP-F fusion protein was found to be tyrosine-phosphorylated in re-

sponse to Fyn activation (Gonsior, 2007). Here, these findings were further strengthened showing Fyn-dependent phosphorylation of endogenous hnRNP F. Moreover, tyrosine-phosphorylation of hnRNP F seems to occur in the cytoplasm of oligodendroglial cells (Figure 4-24 A + B), suggesting a direct interaction between the cytoplasmic, plasma membrane tethered Fyn and hnRNP F. Motif prediction analysis of hnRNP F revealed a potential binding site (amino acids 311 – 316) for SH3 domains such as those present in Fyn. Furthermore, an *in vitro* kinase assay demonstrated that Fyn is able to phosphorylate hnRNP F directly (Figure 4-24 C). Taken together, the results strongly suggest that hnRNP F is a direct substrate of Fyn kinase in the cytoplasm of oligodendrocytes.

Probability analysis and database search identified several putative tyrosine phosphorylation sites in hnRNP F. Here, three of these sites were tested for phosphorylation using mutated recombinant hnRNP F-myc (F-myc) proteins. Similar to a previous analysis where GFP-tagged hnRNP F mutants had been used (Gonsior, 2007), none of the chosen sites could be proven as responsible for the Fyn-dependent phosphorylation of hnRNP F. The tyrosine 306 mutant displayed the least phosphorylation compared to the other mutants (Figure 4-24 D). Furthermore, tyrosine 306 is part of a tyrosine phosphorylation consensus sequence and is located close to the aforementioned SH3-binding domain (Figure 2-10). However, the previous investigations using GFP-F mutants suggested the tyrosine 298 mutant to be less phosphorylated. Hence, identification of the Fyn-dependent tyrosine phosphorylation site(s) in hnRNP F, and if one of the investigated tyrosines 246, 298 or 306 contributes to hnRNP F phosphorylation, remains the subject of further analysis.

Translation of MBP mRNA 3'UTR reporter constructs is enhanced by Fyn activation (White, 2007). Moreover, active Fyn leads to a release of hnRNP F from the oligodendroglial cytoplasmic granule fraction and reduces its binding to MBP mRNA (Figure 4-25). Thus, Fyn-dependent removal of hnRNP F from MBP mRNA-containing RNA transport granules appears to be an additional way to initiate translation of MBP mRNA. This suggests that Fyn kinase can initiate MBP mRNA translation by several means, including phosphorylation of the hnRNPs A2 and F and by forcing the dissociation of hnRNP A2, F and E1 from the transport granules, presumably allowing polysome assembly. Phosphorylation of hnRNP K could contribute to granule reorganisation and translation initiation; even though this RNA-binding protein so far was not shown to be a target of Fyn in oligodendrocytes (see also section 5.1). The targeting of several MBP mRNA granule components by Fyn could provide a mechanism for the cell to fine tune MBP translation. Distinct signalling to Fyn can cause differential activation

patterns of the kinase (Laursen et al., 2009). It remains to be elucidated whether variable Fyn activation and/or variations in Fyn signalling complexes (time, localisation, binding partners) confer specificity in the targeting of Fyn substrates and whether this would affect translation efficiency.

It was shown here, that either diminished or elevated levels of hnRNP F impair translation of the MBP reporter constructs (section 5.4). Therefore, it is likely that hnRNP F levels have to be critically balanced as long as the granules containing MBP mRNA are en route through the cell, and that membrane-attached Fyn kinase triggers translation through phosphorylation and the release of hnRNP F and other factors only when the granule reaches the periphery of the cell.

Based on the findings of this study, the putative involvement of hnRNP F in MBP mRNA metabolism is illustrated in Figure 5-2.

5.6 HnRNP F, an important factor in CNS myelin forming cells with implications for leukodystrophies

As mentioned in section 5.3, expression levels of hnRNP F decrease during oligodendrocyte differentiation which allows the switch of alternative splicing of the plp1 transcripts. Higher amounts of hnRNP F contribute to the generation of DM20 earlier in the oligodendroglial lineage, while lower amounts favour production of the later emerging PLP which is expressed by myelinating oligodendrocytes (Wang et al., 2007). As shown here, expression of hnRNP F does not disappear entirely during oligodendrocyte development but rather is reduced to approximately 50% (Figure 4-16). Furthermore, complete absence of hnRNP F in myelinating oligodendrocytes would probably compromise efficient synthesis of MBP by interfering with formation of fully functional MBP mRNA transport granules (section 5.4). Thus, it appears that oligodendrocytes regulate the levels of hnRNP F during their maturation to ensure efficient expression of the 2 major myelin proteins of the CNS.

Mutations in the eukaryotic translation initiation factor eIF2B are the cause for a group of inherited leukodystrophies known as “vanishing white matter” (VWM) or “childhood ataxia with central nervous system hypomyelination” (CACH) (Schiffmann and van der Knaap, 2004; Pavitt and Proud, 2009). VWM/CACH patients initially myelinate normally but suffer from early onset demyelination followed by neuronal degeneration which is exacerbated by various kinds of stress conditions.

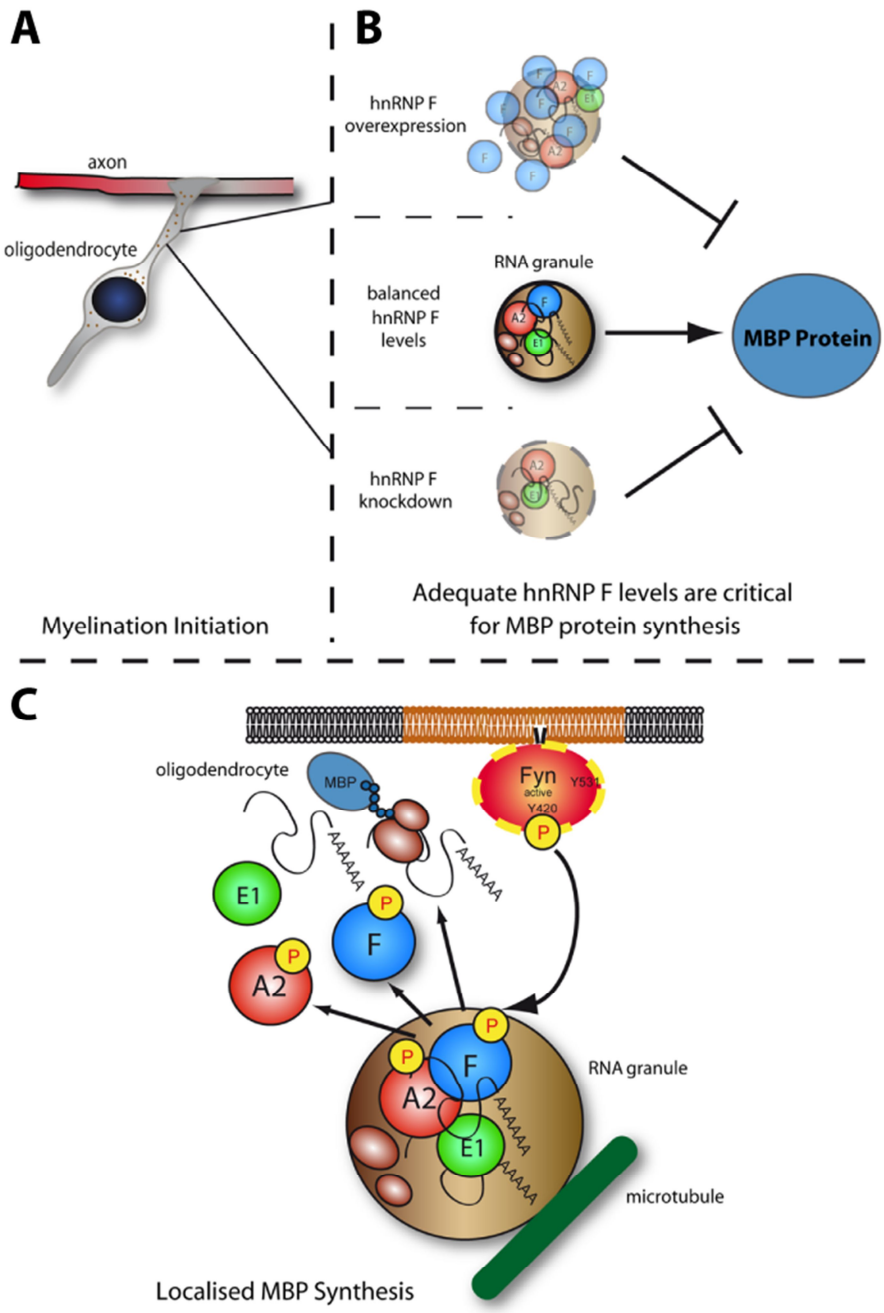


Figure 5-2 Model of the putative involvement of hnRNP F in MBP mRNA metabolism

MBP mRNA is transported in RNA granules towards the periphery of the cell (A) where Fyn-mediated translational initiation occurs by phosphorylation of RNA binding proteins, their subsequent liberation from the granule and a dissociation of MBP mRNA from hnRNP F (C). Distinct levels of hnRNP F seem to be required to form fully functional MBP mRNA granules, thus facilitating efficient protein synthesis (B). The drawing was prepared in collaboration with Dr. Robin White (White, Gonsior et al., 2011, in revision).

It was proposed that glial cells are highly susceptible to eIF2B mutations as VWM/CACH strongly affects CNS white matter tracts. Knock-in mice carrying eIF2B mutations display abnormal numbers of glial cells, reduced levels of the major myelin

proteins PLP and MBP and fail to remyelinate upon brain injury (Geva et al., 2010). EIF2B is part of the general translation initiation machinery and serves as a guanine nucleotide exchange factor (GEF) in the eIF2 complex. Recently, transcriptome analyses of fibroblasts or foetal white matter from VWM/CACH patients revealed significant deregulation of several genes. One of them was hnRNP F which was found to be considerably downregulated (Horzinski et al., 2009). As levels of hnRNP F are important for efficient synthesis of MBP which in turn is crucial for generation and maintenance of the myelin sheath, a dysregulation of the level of hnRNP F in VWM/CACH patients could negatively contribute to the course of the disease. Moreover, eIF2B mutations induce constant activation of stress response mechanisms in oligodendroglial cells including phosphorylation of eIF2α (Kantor et al., 2008) which is a prerequisite and an indicator of stress granule formation. MBP mRNA and hnRNP A2 are sorted to oligodendroglial stress granules, which could not be shown for hnRNP F here (section 4.6). Nevertheless, hnRNP F interacts with hnRNP A2 and MBP mRNA during "normal" conditions. Thus, abnormal abundance of hnRNP F in VWM/CACH could affect stress-dependent processing of MBP mRNA such as entry to or exit from stress granules and thereby additionally (or alternatively) impair production of MBP, especially during recovery from white matter damage when remyelination is required (see also section 5.1).

5.7 Outlook

Investigations on post-transcriptional regulation of MBP mRNA were performed here using biochemical assays and immunostainings of hnRNP A2 as a marker for MBP mRNA containing transport granules. In order to get a better understanding of the mechanisms of MBP mRNA localisation, future analyses could involve direct visualisation of MBP mRNA by *in situ* hybridisation techniques. Moreover, MBP mRNA dynamics could be investigated by live-cell imaging using Fluorochrome-tagged mRNA. A suitable system would be the so called "MS2-system" (Lionnet et al., 2011). It allows specific and very sensitive detection of even single mRNAs in living cells. This could be combined with the use of fluorescently tagged RNA-binding proteins, such as hnRNP A2 and F or DDX5, for simultaneous visualisation. Very little is known about the different isoforms of MBP. The corresponding mRNAs were observed to be diversely distributed in cultured cells dependent on the presence of exon II. Isoform-specific analysis of spatial and temporal dynamics of exon II-including and exon II-lacking mRNAs and associated factors could shed light on their specific functions.

To investigate MBP mRNA metabolism in a more physiological environment, neuron-oligodendroglial co-cultures could be used. It would be interesting to manipulate neuronal and oligodendroglial factors involved in MBP mRNA metabolism, for example by siRNA-mediated knockdown of one or more components of the pathway (neuron- or oligodendrocyte-specific) and subsequently analyse effects on MBP mRNA localisation and translation and eventually myelination. Furthermore, utilisation of the mutant Fyn constructs in such co-culture systems could help to further elucidate the role of this kinase in myelination and, particularly, in the distribution and translation of MBP mRNA.

It would be useful to identify the respective tyrosine residue(s) that are phosphorylated in hnRNP A2 and F in response to Fyn activation, as was begun here for hnRNP F. The target sites could be mutated to mimic the phosphorylated state or to produce mutants that cannot be phosphorylated. Such mutants could be introduced into cells to be able to distinguish between the specific functions of Fyn-dependent phosphorylation of these proteins.

Another interesting subject is the regulation of the oligodendroglial mRNA metabolism in response to stress. Understanding of the mechanisms and the exact functional impact of stress granule dynamics in oligodendrocytes could help in developing strategies supportive to oligodendrocytes in stressful situations such as brain damage or neurological diseases. Therefore, oligodendrocyte stress granule components and their roles in the regulation of the oligodendroglial stress response, especially with regard to MBP mRNA, should be analysed.

The composition of oligodendroglial cytoplasmic RNA granules under different conditions could be investigated to gain further insight into the oligodendrocyte-specific network of mRNA regulation. This could be achieved by purification of granular fractions over density gradients and subsequent immunoprecipitations, to define the granule components.

Regarding a possible involvement of hnRNP F in the glial phenotype of VWM/CACH diseases, one could study the effects of eIF2B mutations on oligodendrocyte physiology with a particular focus on hnRNP F-dependent mechanisms. It would be interesting to clarify if deregulation of MBP expression contributes to the disease course.

6 SUMMARY

In the central nervous system (CNS), oligodendrocytes form the multilamellar and compacted myelin sheath by spirally wrapping around defined axons with their specialised plasma membrane. Myelin is crucial for the rapid saltatory conduction of nerve impulses and for the preservation of axonal integrity. The absence of the major myelin component Myelin Basic Protein (MBP) results in an almost complete failure to form compact myelin in the CNS. The mRNA of MBP is sorted to cytoplasmic RNA granules and transported to the distal processes of oligodendrocytes in a translationally silent state. A main mediator of MBP mRNA localisation is the *trans*-acting factor heterogeneous nuclear ribonucleoprotein (hnRNP) A2 which binds to the *cis*-acting A2 response element (A2RE) in the 3'UTR of MBP mRNA. A signalling cascade had been identified that triggers local translation of MBP at the axon-glial contact site, involving the neuronal cell adhesion molecule (CAM) L1, the oligodendroglial plasma membrane-tethered Fyn kinase and Fyn-dependent phosphorylation of hnRNP A2. This model was confirmed here, showing that L1 stimulates Fyn-dependent phosphorylation of hnRNP A2 and a remodelling of A2-dependent RNA granule structures. Furthermore, the RNA helicase DDX5 was confirmed here acting together with hnRNP A2 in cytoplasmic RNA granules and is possibly involved in MBP mRNA granule dynamics.

Lack of non-receptor tyrosine kinase Fyn activity leads to reduced levels of MBP and hypomyelination in the forebrain. The multiadaptor protein p130Cas and the RNA-binding protein hnRNP F were verified here as additional targets of Fyn in oligodendrocytes. The findings point at roles of p130Cas in the regulation of Fyn-dependent process outgrowth and signalling cascades ensuring cell survival. HnRNP F was identified here as a novel constituent of oligodendroglial cytoplasmic RNA granules containing hnRNP A2 and MBP mRNA. Moreover, it was found that hnRNP F plays a role in the post-transcriptional regulation of MBP mRNA and that defined levels of hnRNP F are required to facilitate efficient synthesis of MBP. HnRNP F appears to be directly phosphorylated by Fyn kinase what presumably contributes to the initiation of translation of MBP mRNA at the plasma membrane.

Fyn kinase signalling thus affects many aspects of oligodendroglial physiology contributing to myelination. Post-transcriptional control of the synthesis of the essential myelin protein MBP by Fyn targets is particularly important. Deregulation of these Fyn-dependent pathways could thus negatively influence disorders involving the white matter of the nervous system.

7 ABBREVIATIONS

A2RE	HnRNP A2 response element
ALLN	Acetyl-L-Leucyl-L-Leucyl-L-Norleucinal
ATP	Adenosine triphosphate
BSA	Bovine serum albumine
CACH	Childhood ataxia with central nervous system hypomyelination
CAM	Cell adhesion molecule
Cas	Crk-associated substrate
cDNA	Complementary DNA
CDS	Coding sequence
CNP	2',3'-Cyclic Nucleotide 3'-Phosphodiesterase
CNS	Central nervous system
DAPI	4',6-diamidino-2-phenylindole
dbcAMP	Dibutyryl cyclic adenosine monophosphate
DDX5	DEAD box RNA helicase 5
(d)dH_2O	(Double) Distilled H_2O
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
EDTA	Ethylene-diamine-tetraacetic acid
eIF	Eukaryotic initiation factor
FCS	Foetal calf serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase

(e)GFP	(Enhanced) Green Fluorescent Protein
golli	Genes of oligodendrocyte lineage
HBSS	Hank's balanced salt solution
hnRNP	Heterogeneous nuclear ribonucleoprotein
HRP	Horseradish peroxidase
HS	Horse serum
IgG	Immunoglobulin G
IP	Immunoprecipitation
kDa	Kilodalton
LB	Lysogeny broth
M	Molar
MAG	Myelin-Associated Glycoprotein
MBP	Myelin Basic Protein
miRNA	Micro RNA
MOG	Myelin Oligodendrocyte Glycoprotein
mRNA	Messenger RNA
mRNP	Messenger ribonucleoprotein particle
MS	Multiple sclerosis
NA	Numerical aperture
NG2	Nerve-Glia antigen 2
NP-40	Nonidet P-40
OD	Optical density
OPC	Oligodendrocyte precursor cell
PBS	Phosphate buffered saline
P-body	Processing body
PCR	Polymerase chain reaction
PEI	Polyethylenimine
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)
PLL	Poly-L-lysine

PLP	Proteolipid Protein
PNS	Peripheral nervous system
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
rpm	Rounds per minute
(q)RRM	(Quasi) RNA Recognition Motif
RT	Room temperature
(q)RT-PCR	(Quantitative) Reverse transcription-PCR
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SG	Stress granule
SH	Src homology
siRNA	Small interfering RNA
SV40	Simian Virus 40
TAE	Tris-acetate-EDTA
TBS(T)	Tris buffered saline (Tween 20)
Tia-1	T-cell intracellular antigen 1
T_m	Melting temperature
TOG	Tumor Overexpressed Gene
U	Unit
UTR	Untranslated region
v/v	Volume per volume
VWM	Vanishing white matter
w/v	Weight per volume
YFP	Yellow Fluorescent Protein

8 REFERENCES

Abdelhaleem M (2005) RNA helicases: Regulators of differentiation. Clin Biochem 38:499-503.

Aggarwal S, Yurlova L, Simons M (2011a) Central nervous system myelin: structure, synthesis and assembly. Trends Cell Biol 21:585-93.

Aggarwal S, Yurlova L, Snaidero N, Reetz C, Frey S, Zimmermann J, Pähler G, Janshoff A, Friedrichs J, Müller DJ, Goebel C, Simons M (2011b) A size barrier limits protein diffusion at the cell surface to generate lipid-rich myelin-membrane sheets. Dev Cell 21:445-56.

Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK (2003) RNA interference: biology, mechanism and applications. Microbiol Mol Biol Rev 67:657-85.

Ainger K, Avossa D, Diana AS, Barry C, Barbarese E, Carson JH (1997) Transport and localization elements in myelin basic protein mRNA. J Cell Biol 138:1077-1087.

Ainger K, Avossa D, Morgan F, Hill SJ, Barry C, Barbarese E, Carson JH (1993) Transport and localization of exogenous myelin basic protein mRNA microinjected into oligodendrocytes. J Cell Biol 123:431-441.

Alkan SA, Martincic K, Milcarek C (2006) The hnRNPs F and H2 bind to similar sequences to influence gene expression. Biochem J 393:361-371.

Allen NJ, Barres BA (2009) Neuroscience: Glia - more than just brain glue. Nature 457:675-7.

Allinquant B, Staugaitis SM, D'Urso D, Colman DR (1991) The ectopic expression of myelin basic protein isoforms in Shiverer oligodendrocytes: implications for myelinogenesis. J Cell Biol 113:393-403.

Anderson P, Kedersha N (2009) RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol 10:430-6.

Anderson P, Kedersha N (2006) RNA granules. J Cell Biol 172:803-8.

Araque A, Navarrete M (2010) Glial cells in neuronal network function. Philos Trans R Soc Lond B Biol Sci 365:2375-81.

Ballabh P, Braun A, Nedergaard M (2004) The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis 16:1-13.

Barbarese E, Brumwell C, Kwon S, Cui H, Carson JH (1999) RNA on the road to myelin. J Neurocytol 28:263-70.

Baron W, Hoekstra D (2009) On the biogensis of myelin membranes: sorting, trafficking and cell polarity. FEBS Lett 584:1760-70.

Barres BA, Raff MC (1994) Control of oligodendrocyte number in the developing rat optic nerve. Neuron 12:935-42.

Bashkirov VI, Scherthan H, Solinger JA, Buerstedde JM, Heyer WD (1997) A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates. J Cell Biol 136:761-73.

Boggs JM (2006) Myelin basic protein: a multifunctional protein. Cell Mol Life Sci 63:1945-61.

Boggs JM, Rangaraj G, Heng YM, Liu Y, Harauz G (2011) Myelin basic protein binds microtubules to a membrane surface and to actin filaments in vitro: effect of phosphorylation and deimination. Biochim Biophys Acta 1808:761-73.

Bonifacino JS, Dell'Angelica EC, Springer TA (2001) Immunoprecipitation. Curr Protoc Immunol 8.3.1-8.3.28.

Bottenstein JE, Sato GH (1979) Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc Natl Acad Sci U S A 76:514-517.

Brown MT, Cooper JA (1996) Regulation, substrates and functions of src. Biochim Biophys Acta 1287:121-149.

Brumwell C, Antolik C, Carson JH, Barbarese E (2002) Intracellular trafficking of hnRNP A2 in oligodendrocytes. Exp Cell Res 279:310-20.

Buchan JR, Parker R (2009) Eukaryotic stress granules: the ins and outs of translation. Mol Cell 36:932-41.

Caenepeel S, Charydczak G, Sudarsanam S, Hunter T, Manning G (2004) The mouse kinome: discovery and comparative genomics of all mouse protein kinases. Proc Natl Acad Sci USA 101:11707-12.

Campagnoni AT, Pribyl TM, Campagnoni CW, Kampf K, Amur-Umarjee S, Landry CF, Handley VW, Newman SL, Garbay B, Kitamura K (1993) Structure and developmental regulation of Golli-mbp, a 105-kilobase gene that encompasses the myelin basic protein gene and is expressed in cells in the oligodendrocyte lineage in the brain. J Biol Chem 268:4930-8.

Campagnoni AT, Carey GD, Yu YT (1980) In vitro synthesis of the myelin basic proteins: subcellular site of synthesis. J Neurochem 34:677-686.

Campbell NA, Reece JB (2002) Biology. Pearson Education, Inc./Benjamin Cummings.

Caputi M, Zahler AM (2001) Determination of the RNA binding specificity of the heterogeneous nuclear ribonucleoprotein (hnRNP) H/H'/F/2H9 family. J Biol Chem 276:43850-9.

Carpenter B, MacKay C, Alnabulsi A, MacKay M, Telfer C, Melvin WT, Murray GI (2006) The roles of heterogeneous nuclear ribonucleoproteins in tumour development and progression. Biochim Biophys Acta 1765:85-100.

Carré JL, Goetz BD, O'Connor LT, Bremer Q, Duncan ID (2002) Mutations in the rat myelin basic protein gene are associated with specific alterations in other myelin gene expression. Neurosci Lett 330:17-20.

Carson JH, Barbarese E (2005) Systems analysis of RNA trafficking in neural cells. Biol Cell 97:51-62.

Chaudhury A, Chander P, Howe PH (2010) Heterogeneous nuclear ribonucleoproteins (hnRNPs) in cellular processes: Focus on hnRNP E1's multifunctional regulatory roles. RNA 16:1449-62.

Colman DR, Kreibich G, Frey AB, Sabatini DD (1982) Synthesis and incorporation of myelin polypeptides into CNS myelin. J Cell Biol 95:598-608.

Colognato H, Ramachandrappa S, Olsen IM, ffrench-Constant C (2004) Integrins direct Src family kinases to regulate distinct phases of oligodendrocyte development. J Cell Biol 167:365-75.

Colognato H, ffrench-Constant C (2004) Mechanisms of glial development. Curr Opin Neurobiol 14:37-44.

Czopka T, von Holst A, ffrench-Constant C, Faissner A (2010) Regulatory mechanisms that mediate tenascin C-dependent inhibition of oligodendrocyte precursor differentiation. J Neurosci 30:12310-22.

Davidson D, Viallet J, Veillette A (1994) Unique catalytic properties dictate the enhanced function of p59fynT, the hemopoietic cell-specific isoform of the Fyn tyrosine protein kinase, in T cells. Mol Cell Biol 14:4554-64.

DeBruin LS, Harauz G (2007) White matter rafting--membrane microdomains in myelin. Neurochem Res 32:213-28.

Decorsiere A, Cayrel A, Vagner S, Millevoi S (2011) Essential role for the interaction between hnRNP H/F and a G quadruplex in maintaining p53 pre-mRNA 3'-end processing and function during DNA damage. Genes Dev 25:220-5.

Defilippi P, Di Stefano P, Cabodi S (2006) p130Cas: a versatile scaffold in signaling networks. Trends Cell Biol 16:257-63.

Del Bigio MR (2010) Ependymal cells: biology and pathology. Acta Neuropathol 119:55-73.

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C (1996) Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci USA 93:9887-92.

de Vries H, de Jonge JC, Schrage C, van der Haar ME, Hoekstra D (1997) Differential and cell development-dependent localization of myelin mRNAs in oligodendrocytes. J Neurosci Res 47:479-88.

Dominguez C, Allain FH (2006) NMR structure of the three quasi RNA recognition motifs (qRRMs) of human hnRNP F and interaction studies with Bcl-x G-tract RNA: a novel mode of RNA recognition. Nucleic Acids Res 34:3634-3645.

Dreyfuss G, Matunis MJ, Pinol-Roma S, Burd CG (1993) hnRNP proteins and the biogenesis of mRNA. Annu Rev Biochem 62:289-321.

Dreyfuss G, Kim VN, Kataoka N (2002) Messenger-RNA-binding proteins and the messages they carry. Nat Rev Mol Cell Biol 3:195-205.

Elvira G, Wasiak S, Blandford V, Tong XK, Serrano A, Fan X, del Rayo Sánchez-Carbente M, Servant F, Bell AW, Boismenu D, Lacaille JC, McPherson PS, DesGroseillers L, Sossin WS (2006) Characterization of an RNA granule from developing brain. Mol Cell Proteomics 5:635-51.

Emery B (2010) Regulation of oligodendrocyte differentiation and myelination. Science 330:779-82.

Erickson SL Lykke-Andersen J (2011) Cytoplasmic mRNA granules at a glance. J Cell Sci 124:293-7.

Fancy SP, Chan JR, Baranzini SE, Franklin RJ, Rowitch DH (2011) Myelin regeneration: a recapitulation of development? Annu Rev Neurosci 34:21-43.

Fields RD (2010) Neuroscience. Change in the brain's white matter. Science 330:768-9.

Fields RD (2005) Myelination: an overlooked mechanism of synaptic plasticity? Neuroscientist 11:528-31.

Francone VP, Maggipinto MJ, Kosturko LD, Barbarese E (2007) The microtubule-associated protein tumor overexpressed gene/cytoskeleton-associated protein 5 is necessary for myelin basic protein expression in oligodendrocytes. J Neurosci 27:7654-7662.

Fulton D, Paez PM, Campagnoni AT (2010) The multiple roles of myelin protein genes during the development of the oligodendrocyte. ASN Neuro 2:e00027.

Gao Y, Tatavarty V, Korza G, Levin MK, Carson JH (2008) Multiplexed dendritic targeting of alpha calcium calmodulin-dependent protein kinase II, neurogranin and activity-regulated cytoskeleton-associated protein RNAs by the A2 pathway. Mol Biol Cell 19:2311-2327.

Garneau D, Revil T, Fisette JF, Chabot B (2005) Heterogeneous nuclear ribonucleoprotein F/H proteins modulate the alternative splicing of the apoptotic mediator Bcl-x. J Biol Chem 280:22641-22650.

Geva M, Cabilly Y, Assaf Y, Mindroul N, Marom L, Raini G, Pinchasi D, Elroy-Stein O (2010) A mouse model for eukaryotic translation initiation factor 2B-leucodystrophy reveals abnormal development of brain white matter. Brain 133:2448-61.

Goh ET, Pardo OE, Michael N, Niewiarowski A, Totty N, Volkova D, Tsaneva IR, Seckl MJ, Gout I (2010) Involvement of heterogeneous ribonucleoprotein F in the regulation of cell proliferation via the mammalian target of rapamycin/S6 kinase 2pathway. J Biol Chem 285:17065-76.

Gonsior C (2007) Expression und Phosphorylierung des heterogenen nukleären Ribonukleoproteins F durch die Tyrosin-Kinase Fyn in Oligodendrozyten. Diploma thesis, Mainz, Germany.

Gordon GR, Mulligan SJ, MacVicar BA (2007) Astrocyte control of the cerebrovasculature. Glia 55:1214-21.

Goto J, Tezuka T, Nakazawa T, Sagara H, Yamamoto T (2008) Loss of Fyn tyrosine kinase on the C57BL/6 genetic background causes hydrocephalus with defects in oligodendrocyte development. Molecular and cellular neurosciences 38:203-212.

Gould RM, Byrd AL, Barbarese E (1995) The number of Schmidt-Lantermann incisures is more than doubled in shiverer PNS myelin sheaths. J Neurocytol 24:85-98.

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280:1610-3.

Han SP, Friend LR, Carson JH, Korza G, Barbarese E, Maggipinto M, Hatfield JT, Rothnagel JA, Smith R (2010) Differential subcellular distributions and trafficking functions of hnRNP A2/B1 spliceoforms. Traffic 11:886-98.

Harauz G, Ladizhansky V, Boggs JM (2009) Structural polymorphism and multifunctionality of myelin basic protein. Biochemistry 48:8094-104.

Hartline DK, Colman DR (2007) Rapid conduction and the evolution of giant axons and myelinated fibers. Curr Biol 17:R29-35.

Hatfield JT, Rothnagel JA, Smith R (2002) Characterization of the mouse hnRNP A2/B1/B0 gene and identification of processed pseudogenes. Gene 295:33-42.

He Y, Smith R (2009) Nuclear functions of heterogeneous nuclear ribonucleoproteins A/B. Cell Mol Life Sci 66:1239-56.

Herculano-Houzel S (2011) Scaling of brain metabolism with a fixed energy budget per neuron: implications for neuronal activity, plasticity and evolution. PLoS One 6:e17514.

Hoch-Kraft P (2010) Charakterisierung der RNA-bindenden Proteine FUS und DDX5 in Oligodendrozyten. Diploma thesis, Mainz, Germany.

Hoek KS, Kidd GJ, Carson JH, Smith R (1998) hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. Biochemistry 97:7021-9.

Honda H, Oda H, Nakamoto T, Honda Z, Sakai R, Suzuki T, Saito T, Nakamura K, Nakao K, Ishikawa T, Katsuki M, Yazaki Y, Hirai H (1998) Cardiovascular anomaly, impaired actin bundling and resistance to Src-induced transformation in mice lacking p130Cas. Nat Genet 19:361-5.

Honore B, Rasmussen HH, Vorum H, Dejgaard K, Liu X, Gromov P, Madsen P, Gesser B, Tommerup N, Celis JE (1995) Heterogeneous nuclear ribonucleoproteins H, H', and F are members of a ubiquitously expressed subfamily of related but distinct proteins encoded by genes mapping to different chromosomes. J Biol Chem 270:28780-28789.

Honore B, Baandrup U, Vorum H (2004) Heterogeneous nuclear ribonucleoproteins F and H/H' show differential expression in normal and selected cancer tissues. Exp Cell Res 294:199-209.

Horzinski L, Henaut A, Bertini E, Schiffmann R, Rodriguez D, Dantal Y, Boespflug-Tanguy O, Fogli A. (2009) Developmental genes are specifically involved in severe forms of eIF2B-pathies. Glia S13:S50 Euroglia 2009 poster abstract P-102.

Huang J, Hamasaki H, Nakamoto T, Honda H, Hirai H, Saito M, Takato T, Sakai R (2002) Differential regulation of cell migration, actin stress fiber organization and cell transformation by functional domains of Crk-associated substrate. J Biol Chem 277:27265-72.

Huang J, Sakai R, and Furuichi T (2006) The docking protein Cas links tyrosine phosphorylation signaling to elongation of cerebellar granule cell axons. Mol Biol Cell 17:3187-96.

Huttelmaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M, Meng X, Bassell GJ, Condeelis J, Singer RH (2005) Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature 438:512-515.

Jahn O, Tenzer S, Werner HB (2009) Myelin proteomics: molecular anatomy of an insulating sheath. Mol Neurobiol 40:55-72.

Janostiak R, Tolde O, Bruhová Z, Novotny M, Hanks SK, Rösel D, Brábek J (2011) Tyrosine Phosphorylation within the SH3 domain Regulates CAS Subcellular Localization, Cell Migration and Invasiveness. Mol Biol Cell, Sep 30 Epub ahead of print.

Jordan CA, Friedrich VL Jr, de Ferra F, Weismiller DG, Holmes KV, Dubois-Dalcq M (1990) Differential exon expression in myelin basic protein transcripts during central nervous system (CNS) remyelination. Cell Mol Neurobiol 10:3-18.

Jung M, Kramer E, Grzenkowski M, Tang K, Blakemore W, Aguzzi A, Khazaie K, Chlichlia K, von Blankenfeld G, Kettenmann H, et al. (1995) Lines of murine oligodendroglial precursor cells immortalized by an activated neu tyrosine kinase show distinct degrees of interaction with axons in vitro and in vivo. Eur J Neurosci 7:1245-1265.

Kalifa Y, Huang T, Rosen LN, Chatterjee S, Gavis ER (2006) Glorund, a Drosophila hnRNP F/H homolog, is an ovarian repressor of nanos translation. Dev Cell 10:291-301.

Kalifa Y, Armenti ST, Gavis ER (2009) Glorund interactions in the regulation of gurken and oskar mRNAs. Dev Biol 326:68-74.

Kanai Y, Dohmae N, Hirokawa N (2004) Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. Neuron 43:513-25.

Kantor L, Pinchasi D, Mintz M, Hathout Y, Vanderver A, Elroy-Stein O (2008) A point mutation in translation initiation factor 2B leads to a continuous hyper stress state in oligodendroglial-derived cells. PLoS One 3:e3783.

Karthigasan J, Garvey JS, Ramamurthy GV, Kirschner DA (1996) Immunolocalization of 17 and 21.5 kDa MBP isoforms in compact myelin and radial component. J Neurocytol 25:1-7.

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91:461-553.

Kimura M, Sato M, Akatsuka A, Saito S, Ando K, Yokoyama M, Katsuki M (1998) Overexpression of a minor component of myelin basic protein isoform (17.2 kDa) can restore myelinogenesis in transgenic shiverer mice. Brain Res 785:245-52.

Kirby BB, Takada N, Latimer AJ, Shin J, Carney TJ, Kelsh RN, Appel B (2006) In vivo time-lapse imaging shows dynamic oligodendrocyte progenitor behaviour during zebrafish development. Nat Neurosci 9:1506-11.

Klein C, Kramer EM, Cardine AM, Schraven B, Brandt R, Trotter J (2002) Process outgrowth of oligodendrocytes is promoted by interaction of fyn kinase with the cytoskeletal protein tau. J Neurosci 22:698-707.

Koch T, Brugger T, Bach A, Gennarini G, Trotter J (1997) Expression of the immunoglobulin superfamily cell adhesion molecule F3 by oligodendrocyte-lineage cells. Glia 19:199-212.

Kosturko LD, Maggipinto MJ, D'Sa C, Carson JH, Barbarese E (2005) The microtubule-associated protein tumor overexpressed gene binds to the RNA trafficking protein heterogeneous nuclear ribonucleoprotein A2. Mol Biol Cell 16:1938-47.

Kosturko LD, Maggipinto MJ, Korza G, Lee JW, Carson JH, Barbarese E (2006) Heterogeneous nuclear ribonucleoprotein (hnRNP) E1 binds to hnRNP A2 and inhibits translation of A2 response element mRNAs. Mol Biol Cell 17:3521-3533.

Kramer EM, Klein C, Koch T, Boytinck M, Trotter J (1999) Compartmentation of Fyn kinase with glycosylphosphatidylinositol-anchored molecules in oligodendrocytes facilitates kinase activation during myelination. J Biol Chem 274:29042-29049.

Krämer-Albers EM, Gehrig-Burger K, Thiele C, Trotter J, Nave KA (2006) Perturbed interactions of mutant proteolipid protein/DM20 with cholesterol and lipid rafts in oligodendroglia: implications for dysmyelination in spastic paraplegia. J Neurosci 26:11743-52.

Krämer-Albers EM, White R (2011) From axon-glial signalling to myelination: the integrating role of oligodendroglial Fyn kinase. Cell Mol Life Sci 68:2003-12.

Krecic AM, Swanson MS (1999) hnRNP complexes: composition, structure, and function. Curr Opin Cell Biol 11:363-371.

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA (2003) Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 33:366-74.

Laursen LS, Chan CW, ffrench-Constant C (2009) An integrin-contactin complex regulates CNS myelination by differential Fyn phosphorylation. J Neurosci 29:9174-85.

Laursen LS, Chan CW, Ffrench-Constant C (2011) Translation of myelin basic protein mRNA in oligodendrocytes is regulated by integrin activation and hnRNP-K. J Cell Biol 192:797-811.

Lawrence JB, Singer RH (1986) Intracellular localization of messenger RNAs for cytoskeletal proteins. Cell 45:407-15.

Lazzarini RA (2004) Myelin Biology and Disorders. Elsevier Academic Press, San Diego, London.

Liang X, Lu Y, Wilkes M, Neubert TA, Resh MD (2004) The N-terminal SH4 region of the Src family kinase Fyn is modified by methylation and heterogeneous fatty acylation: role in membrane targeting, cell adhesion, and spreading. J Biol Chem 279:8133-9.

Lionnet T, Czaplinski K, Darzacq X, Shav-Tal Y, Wells AL, Chao JA, Park HY, de Turris V, Lopez-Jones M, Singer RH (2011) A transgenic mouse for in vivo detection of endogenous labeled mRNA. Nat Methods 8:165-70.

Lu Z, Ku L, Chen Y, Feng Y (2005) Developmental abnormalities of myelin basic protein expression in fyn knock-out brain reveal a role of Fyn in posttranscriptional regulation. J Biol Chem 280:389-95.

Lyons DA, Naylor SG, Scholze A, Talbot WS (2009) Kif1b is essential for mRNA localization in oligodendrocytes and development of myelinated axons. Nat Genet 41:854-8.

Maggipinto M, Rabiner C, Kidd GJ, Hawkins AJ, Smith R, Barbarese E (2004) Increased expression of the MBP mRNA binding protein HnRNP A2 during oligodendrocyte differentiation. J Neurosci Res 75:614-23.

Manie SN, Astier A, Haghayeghi N, Canty T, Druker BJ, Hirai H, Freedman AS (1997) Regulation of integrin-mediated p130(Cas) tyrosine phosphorylation in human B cells. A role for p59(Fyn) and SHP2. J Biol Chem 272:15636-41.

Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. Science 298:1912-1934.

Martin GS (2001) The hunting of the Src. Nat Rev Mol Cell Biol 2:467-75.

Matunis MJ, Xing J, Dreyfuss G (1994) The hnRNP F protein: unique primary structure, nucleic acid-binding properties, and subcellular localization. Nucleic Acids Res 22:1059-1067.

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304:700-3.

Miller RH (2002) Regulation of oligodendrocyte development in the vertebrate CNS. Prog Neurobiol 67:451-467.

Min Y, Kristiansen K, Boggs JM, Husted C, Zasadzinski JA, Israelachvili J (2009) Interaction forces and adhesion of supported myelin lipid bilayers modulated by myelin basic protein. Proc Natl Acad Sci USA 106:3154-9.

Miyamoto Y, Yamauchi J, Tanoue A (2008) Cdk5 phosphorylation of WAVE2 regulates oligodendrocyte precursor cell migration through nonreceptor tyrosine kinase Fyn. J Neurosci 28:8326-37.

Moore MJ (2005) From birth to death: the complex lives of eukaryotic mRNAs. Science 309:1514-8.

Moser JJ, Fritzler MJ (2009) Cytoplasmic ribonucleoprotein (RNP) bodies and their relationship to GW/P bodies. Int J Biochem Cell Biol 42:828-43.

Munro TP, Magee RJ, Kidd GJ, Carson JH, Barbarese E, Smith LM, Smith R (1999) Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking. J Biol Chem 274:34389-34395.

Murray N, Steck AJ (1984) Impulse conduction regulates myelin basic protein phosphorylation in rat optic nerve. J Neurochem 43:243-8.

Nave KA (2010a) Myelination and support of axonal integrity by glia. Nature 468:244-52.

Nave KA (2010b) Myelination and the trophic support of long axons. Nat Rev Neurosci 11:275-83.

Okada M, Nakagawa H (1989) A protein tyrosine kinase involved in regulation of pp60c-src function. J Biol Chem 264:20886-20893.

Oleszewski M, Beer S, Katich S, Geiger C, Zeller Y, Rauch U, Altevogt P (1999) Integrin and neurocan binding to L1 involves distinct Ig domains. J Biol Chem 274:24602-10.

Ostareck-Lederer A, Ostareck DH, Cans C, Neubauer G, Bomsztyk K, Superti-Furga G, Hentze MW (2002) c-Src-mediated phosphorylation of hnRNP K drives translational activation of specifically silenced mRNAs. Mol Cell Biol 22:4535-4543.

Osterhout DJ, Wolven A, Wolf RM, Resh MD, Chao MV (1999) Morphological differentiation of oligodendrocytes requires activation of Fyn tyrosine kinase. J Cell Biol 145:1209-1218.

Palacios EH, Weiss A (2004) Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene 23:7990-8000.

Paul S, Dansithong W, Jog SP, Holt I, Mittal S, Brook D, Morris GE, Comai L, Reddy S (2011) Expanded CUG repeats dysregulate RNA splicing by altering the stoichiometry of the muscleblind 1 complex. J Biol Chem, Sep 7 Epub ahead of print.

Pavitt GD, Proud CG (2009) Protein synthesis and its control in neuronal cells with a focus on vanishing white matter disease. Biochem Soc Trans 37:1298-310.

Pedraza L, Fidler L, Staugaitis SM, Colman DR (1997) The active transport of myelin basic protein into the nucleus suggests a regulatory role in myelination. Neuron 18:579-89.

Rasband MN, Peles E, Trimmer JS, Levinson SR, Lux SE, Shrager P (1999) Dependence of nodal sodium channel clustering on paranodal axoglial contact in the developing CNS. J Neurosci 19:7516-28.

Readhead C, Hood L (1990) The dysmyelinating mouse mutations shiverer (shi) and myelin deficient (shiml d). Behav Genet 20:213-34.

Relucio J, Tzvetanova ID, Ao W, Lindquist S, Colognato H (2009) Laminin alters fyn regulatory mechanisms and promotes oligodendrocyte development. J Neurosci 29:11794-806.

Resh MD (1998) Fyn, a Src family tyrosine kinase. Int J Biochem Cell Biol 30:1159-62.

Richardson WD, Kessaris N, Pringle N (2006) Oligodendrocyte wars. Nat Rev Neurosci 7:11-18.

Rocak S, Linder P (2004) DEAD-box proteins: the driving forces behind RNA metabolism. Nat Rev Mol Cell Biol 5:232-41.

Rodriguez AJ, Czaplinski K, Condeelis JS, Singer RH (2008) Mechanisms and cellular roles of local protein synthesis in mammalian cells. Curr Opin Cell Biol 20:144-9.

Roskoski R, Jr. (2004) Src protein-tyrosine kinase structure and regulation. Biochem Biophys Res Commun 324:1155-1164.

Roskoski R, Jr. (2005) Src kinase regulation by phosphorylation and dephosphorylation. Biochem Biophys Res Commun 331:1-14.

Roth AD, Ivanova A, Colman DR (2006) New observations on the compact myelin proteome. Neuron Glia Biol 2:15-21.

Salzer JL (1997) Clustering sodium channels at the node of Ranvier: close encounters of the axon-glia kind. Neuron 18:843-6.

Salzer JL, Brophy PJ, Peles E (2008) Molecular domains of myelinated axons in the peripheral nervous system. Glia 56:1532-40.

Sambrook J, Russell D (2001) Molecular Cloning - A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, USA.

Schiffmann R, van der Knaap MS (2004) The latest on leukodystrophies. Curr Opin Neurol 17:187-92.

Shav-Tal Y, Singer RH (2005) RNA localization. J Cell Sci 118:4077-81.

Sherman DL, Brophy PJ (2005) Mechanisms od axon ensheathment and myelin growth. Nat Rev Neurosci 6:683-90.

Shier D, Butler J, Lewis R (1999) Human Anatomy and Physiology, 8th edition. McGraw-Hill.

Simons M, Trotter J (2007) Wrapping it up: the cell biology of myelination. Curr Opin Neurobiol 17:533-40.

Singh R, Valcárcel J (2005) Building specificity with nonspecific RNA-binding proteins. Nat Struct Mol Biol 12:645-53.

Song KY, Choi HS, Law PY, Wei LN, Loh HH (2011) Post-transcriptional regulation of mu-opioid receptor: role of the RNA-binding proteins heterogeneous nuclear ribonucleoprotein H1 and F. Cell Mol Life Sci, Jul 8 Epub ehead of print.

Songyang Z, Cantley LC (1995) SH2 domain specificity determination using oriented phosphopeptide library. Methods Enzymol 254:523-35.

Sperber BR, Boyle-Walsh EA, Engleka MJ, Gadue P, Peterson AC, Stein PL, Scherer SS, McMorris FA (2001) A unique role for Fyn in CNS myelination. J Neurosci 21:2039-2047.

Susuki K, Rasband MN (2008) Molecular mechanisms of node of Ranvier formation. Curr Opin Cell Biol 20:616-23.

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P, Chao MV, Falls DL, Role L, Salzer JL (2005) Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 47: 681-694.

Taylor CM, Marta CB, Claycomb RJ, Han DK, Rasband MN, Coetzee T, Pfeiffer SE (2004) Proteomic mapping provides powerful insides into functional myelin biology. Proc Natl Acad Sci USA 101:4643-8.

Trotter J, Bitter-Suermann D, Schachner M (1989) Differentiation-regulated loss of the polysialylated embryonic form and expression of the different polypeptides of the neural cell adhesion molecule by cultured oligodendrocytes and myelin. J Neurosci Res 22:369-83.

Umemori H, Sato S, Yagi T, Aizawa S, Yamamoto T (1994) Initial events of myelination involve Fyn tyrosine kinase signalling. Nature 367:572-576.

Umemori H, Kadowaki Y, Hirosawa K, Yoshida Y, Hironaka K, Okano H, Yamamoto T (1999) Stimulation of myelin basic protein gene transcription by Fyn tyrosine kinase for myelination. J Neurosci 19:1393-1397.

Van Dusen CM, Yee L, McNally LM, McNally MT (2010) A glycine-rich domain of hnRNP H/F promotes nucleocytoplasmic shuttling and nuclear import through an interaction with transportin 1. Mol Cell Biol 30:2552-62.

Velumian AA, Samoilova M, Fehlings MG (2010) Visualization of cytoplasmic diffusion within living myelin sheaths of CNS white matter axons using microinjection of the fluorescent dye Lucifer Yellow. Neuroimage 56:27-34.

Veraldi KL, Arhin GK, Martincic K, Chung-Ganster LH, Wilusz J, Milcarek C (2001) hnRNP F influences binding of a 64-kilodalton subunit of cleavage stimulation factor to mRNA precursors in mouse B cells. Mol Cell Biol 21:1228-1238.

Verkhratsky A (2010) Physiology of neuronal-glial networking. Neurochem Int 57:332-43.

Wake H, Lee PR, Fields RD (2011) Control of local protein synthesis and initial events in myelination by action potentials. Science 333:1647-51.

Wang PS, Wang J, Xiao ZC, Pallen CJ (2009) Protein-tyrosine phosphatase alpha acts as an upstream regulator of Fyn signaling to promote oligodendrocyte differentiation and myelination. J Biol Chem 284:33692-702.

Wang E, Dimova N, Cambi F (2007) PLP/DM20 ratio is regulated by hnRNPH and F and a novel G-rich enhancer in oligodendrocytes. Nucleic Acids Res 35:4164-78.

Wang Y, Lacroix G, Haines J, Doukhanine E, Almazan G, Richard S (2010) The QKI-6 RNA binding protein localizes with the MBP mRNAs in stress granules of glial cells. PLoS One 5 pii:e12824.

Weighardt F, Biamonti G, Riva S (1996) The roles of heterogeneous nuclear ribonucleoproteins (hnRNP) in RNA metabolism. Bioessays 18:747-56.

White R (2007) The central role of Fyn kinase in axon-glial signalling and translation of myelin proteins. Doctoral thesis, Mainz, Germany.

White R, Gonsior C, Krämer-Albers EM, Stöhr N, Hüttelmaier S, Trotter J (2008) Activation of oligodendroglial Fyn kinase enhances translation of mRNAs transported in hnRNP A2-dependent RNA granules. J Cell Biol 181:579-86. (Parts of this doctoral thesis were originally published in this article)

White R & Gonsior C, Bauer NM, Krämer-Albers EM, Luhmann HJ, Trotter J (2011) HnRNP F is a novel component of oligodendroglial RNA transport granules contributing to the regulation of MBP protein synthesis. J Biol Chem, 287(3):1742-54. (Parts of this doctoral thesis were originally published in this article, © the American Society for Biochemistry and Molecular Biology)

Williams SK, Spence HJ, Rodgers RR, Ozanne BW, Fitzgerald U, Barnett SC (2005) Role of Mayven, a kelch-related protein in oligodendrocyte process formation. J Neurosci Res 81:622-31.

Wolf RM, Wilkes JJ, Chao MV, Resh MD (2001) Tyrosine phosphorylation of p190 RhoGAP by Fyn regulates oligodendrocyte differentiation. J Neurobiol 49:62-78.

Yang L, Lin C, Liu ZR (2005) Signaling to the DEAD box--regulation of DEAD-box p68 RNA helicase by protein phosphorylations. Cell Signal 17:1495-504.

Zrihan-Licht S, Lim J, Keydar I, Sliwkowski MX, Groopman JE, Avraham H (1997) Association of csk-homologous kinase (CHK) (formerly MATK) with HER-2/ErbB-2 in breast cancer cells. J Biol Chem 272:1856-1863.

9 ACKNOWLEDGEMENTS

10 APPENDIX

10.1 DNA sequences of generated expression vectors

Mutant variants of the below listed constructs are described in section 3.3.13.

10.1.1 pFynB

GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCA
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10.1.2 pFyn-myc

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10.1.3 pFyn-YFP

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10.1.6 pFirefly MBP 3'UTR

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10.2 CURRICULUM VITAE

Persönliche Daten

Name:	Constantin Johannes Gonsior
Anschrift:	Löhrstr. 17, 55116 Mainz
E-mail:	gonsior@uni-mainz.de
Geburtstag:	06.04.1978
Geburtsort:	Heidelberg
Staatsangehörigkeit:	deutsch
Familienstand:	ledig

Ausbildung

09/1984 - 07/1986	Grundschule Rotenberg
09/1986 - 07/1987	Grundschule Georgsheil
09/1987 - 07/1988	Grundschule Rauenberg
09/1988 - 02/1993	Gymnasium Wiesloch
02/1993 - 05/1997	Leibniz Gymnasium Östringen
05/1997	Abitur
04/1999 - 07/2006	Studium an der Johannes Gutenberg Universität Mainz im Fachbereich Biologie Vordiplom mit Note „sehr gut“ bestanden
08/2006 - 04/2007	Diplomarbeit in der Abteilung Molekulare Zellbiologie an der Johannes Gutenberg Universität Mainz: „Expression und Phosphorylierung des heterogenen nukleären Ribonukleoproteins F durch die Tyrosin-Kinase Fyn in Oligodendrozyten“ Diplom mit Note „sehr gut“ bestanden
Seit 07/2007	Dissertation in der Abteilung Molekulare Zellbiologie an der Johannes Gutenberg Universität Mainz: „Fyn kinase targets in oligodendroglial physiology and myelination“

Tätigkeiten

08/2005 - 08/2006	Wissenschaftliche Hilfskraft am Universitätsklinikum Mainz Mitarbeit beim *ESCAPE TRIAL: Molecular Mechanisms of Disease Progression and Renoprotective Pharmacotherapy in Children with Chronic Renal Failure*
11/2006 - 02/2007, 11/2007 - 02/2008, 04/2008 - 07/2008, 11/2008 - 02/2009	und
04/2009 - 07/2009	Wissenschaftliche Hilfskraft an der Johannes Gutenberg Universität Mainz Betreuung des Praktikums „Biologie für Mediziner“
11/2008 - 02/2009, 04/2009 - 07/2009, 11/2009 - 02/2010, 04/2010 - 07/2010, 11/2010 - 02/2011	und
04/2011 - 07/2011	Leiter des Tutoriums „Biologie für Mediziner“ an der Johannes Gutenberg Universität Mainz

Präsentationen

12/2007	*Hirschegg* (Österreich), Jahresarbeitstreffen Graduiertenkolleg: „Fyn kinase substrates in oligodendrocytes“ (Vortrag)
05/2008	*Pisa* (Italien), Gordon Research Conference (Myelin): “HnRNP F is a downstream target of oligodendroglial Fyn kinase” (Poster)
09/2008	*Bochum*, Vernetzungstreffen der Neuro-Graduiertenkollegs Bochum und Mainz: „Fyn kinase substrates in oligodendrocytes“ (Vortrag)
12/2008	*Mainz*, Jahrestagung des Interdisziplinären Arbeitskreises für „Molekulare und zelluläre Neurobiologie“ der Johannes Gutenberg Universität Mainz: “HnRNP F is a downstream target of oligodendroglial Fyn kinase” (Poster)
12/2009	*Mainz*, Jahrestagung des Interdisziplinären Arbeitskreises für „Molekulare und zelluläre Neurobiologie“ der Johannes Gutenberg Universität Mainz: „HnRNP F, a Fyn-dependent regulator of MBP translation in oligodendrocytes“ (Poster)

03/2010 *Hirschegg* (Österreich), Jahresarbeitstreffen Graduiertenkolleg: „HnRNP F, a Fyn-dependent regulator of MBP translation in oligodendrocytes“ (Vortrag)

11/2010 *Ein Gedi* (Israel), EMBO workshop RNA control of cell dynamics: “The Fyn kinase substrate hnRNP F is a regulator of MBP translation in oligodendrocytes” (Poster)

11/2010 *Oberwesel*, Annual meeting Rhine-Main Neuroscience Network: “Post-transcriptional regulation of Myelin Basic Protein expression” (Vortrag)

Publikationen

White R., Gonsior C., Krämer-Albers EM., Stöhr N., Hüttelmaier S. and Trotter J. (2008). Activation of oligodendroglial Fyn kinase enhances translation of mRNAs transported in hnRNP A2-dependent RNA granules. J. Cell Biol., 181 (4), 579-586.

White R.*, Gonsior C.*, Kraemer-Albers E.M., Luhmann H.J. and Trotter J. (2011, in Revision beim J Biol Chem) HnRNP F is a novel component of oligodendroglial RNA transport granules contributing to the regulation of MBP protein synthesis.
*equal contribution.

Auszeichnungen und Stipendien

2007 Promotionsstipendium der Deutschen Forschungsgemeinschaft im Graduiertenkolleg „Entwicklungsabhängige und krankheitsinduzierte Modifikationen im Nervensystem“

2008 Ernennung zum Sprecher des Graduiertenkollegs „Entwicklungsabhängige und krankheitsinduzierte Modifikationen im Nervensystem“

2009 Auszeichnung für die beste Posterpräsentation der 9. Jahrestagung des Interdisziplinären Arbeitskreises für „Molekulare und zelluläre Neurobiologie“ der Johannes Gutenberg Universität Mainz

Besondere Weiterbildungen und Kenntnisse

Kurse: Webseiten gestalten: HTML
Webseiten gestalten II: HTML für Fortgeschrittene
Rhetorik (Vorträge, Präsentationen, Postersessions)
Statistik

Sehr gute Kenntnisse im Umgang mit MS Office und Adobe Photoshop

Englischkenntnisse fließend in Wort und Schrift

Wehr- oder Ersatzdienst

09/1997 - 09/1998 Zivildienst im Kraichgauheim (Bad Schönborn)

10.3 Eidesstattliche Erklärung

Hiermit erkläre ich, Constantin Johannes Gonsior, geboren am 06.04.1978 in Heidelberg, dass ich meine Dissertation selbstständig und nur unter Verwendung der angegebenen Hilfsmittel angefertigt habe. Ich habe keinen anderen Promotionsversuch unternommen.

_____ _____

Ort, Datum (Constantin Gonsior)