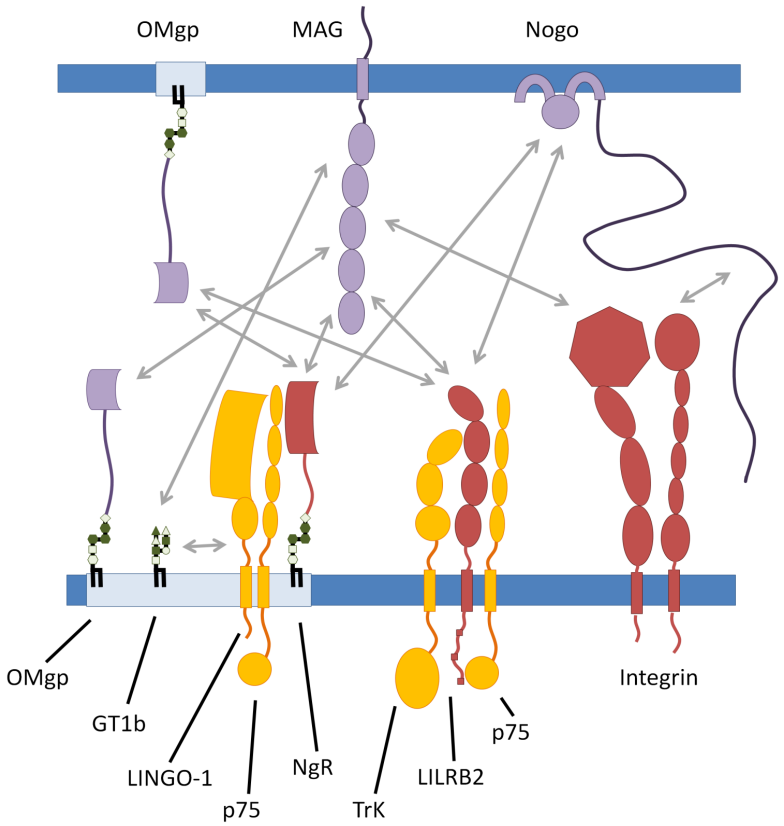
Myelin associated inhibitors; molecular mechanisms and therapeutic potential



Master Thesis

for the
Molecular and Cellular Life Sciences
Master Program

Universiteit Utrecht

Matti F. Pronker, BSc

2012

Myelin associated inhibitors; molecular mechanisms and therapeutic potential

This literature review is written as the Master Thesis for the Molecular and Cellular Life Sciences master program (7.5 ECTS) at Utrecht University by Matti F. Pronker (student number 3240932), under the supervision of Bert J.C. Janssen in the period of June-July 2012.

Summary

Whereas the peripheral nervous system can readily regenerate after injury, regeneration is very limited in the central nervous system of adult vertebrates. Over the past three decades, it has become clear that this lack of regeneration has a molecular basis. The myelin that provides electrical insulation of neuronal fibers has a different composition in the central nervous system, as compared to peripheral nervous system myelin. Several proteins expressed on myelin have been found to have inhibitory effects for neuronal regeneration and were dubbed myelin-associated inhibitors. The three classical myelin-associated inhibitors (Nogo, MAG and OMgp) were found to signal all three through two distinct receptor complexes, providing a puzzling redundancy for these interactions. This signaling is speculated to be important for stabilizing neuronal circuitry in healthy adult organisms. Other proteins known to be involved in axonal guidance, such as semaphorins, ephrins, netrins and Wnts, as well as extracellular matrix components such as the chondroitin sulfate proteoglycans, have also been shown to have regeneration inhibitory effects. Downstream signaling by neuronal effector proteins culminates in modulation of the cytoskeleton and transcription, explaining the morphological changes of the neurons that are observed upon signaling. The fact that the lack of regeneration has a molecular basis provides prospects for therapeutic intervention to stimulate regeneration for injuries of the central nervous system, like spinal cord injury or stroke. Indeed, a substantial body of different proteins, peptides and small molecules that intervene with the different steps involved in the inhibition of regeneration shows promising effects, both *in vitro* and *in vivo*.

This review will discuss the advances made on understanding the lack of regeneration in the central nervous system. After an introduction on the nervous system, injury and regeneration, the molecular mechanisms of inhibition will be discussed. A special focus will be on the three classical myelin associated inhibitors and their receptor complexes, but other molecules that are inhibitory for regeneration will be discussed as well. The current understanding of the downstream signaling cascades of the myelin associated inhibitors will be reviewed and finally, different strategies that demonstrate the therapeutic potential of interfering with these mechanisms will be discussed.

Table of content

Introduction

The Nervous System

The vertebrate nervous system is the network of nerve cells (neurons) and fibers that transmits nerve impulses between parts of the body. It is divided in the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain, spine cord and retina, whereas the PNS consists of the nerves, sensory neurons and neuron clusters called ganglia that connect the CNS to the different parts of the body. Neurons are electrically excitable cells that process and transmit information by electrical and chemical signals. They consist of a cell body, containing the nucleus and other organelles, an axon and dendrites (see fig. 1).

Both the dendrites and the axon are neuronal projections that can branch to form numerous contacts with other neurons. However, the axon differs from the dendrites in that it conducts electrical signals away from the cell body, whereas dendrites conduct electrical signals the neuron receives from other neurons towards the cell body. A neuron can sprout many dendrites, but only a single axon extends from the cell body at a site called the axon hillock. However, the axon can be very branched, allowing signal transmission to many other neurons. Neurite is a term that can refer to both axons and dendrites when it is not clear whether an extension is an axon or a dendrite. Dendrites show small protrusions, known as dendritic spines, that are often involved in a contact with a terminating axon. The contacts that terminating axons make with target neurons (either via dendritic spines or directly to the cell body) are called synapses. This is where electrical signals called action potentials are transmitted from one neuron to another[1].

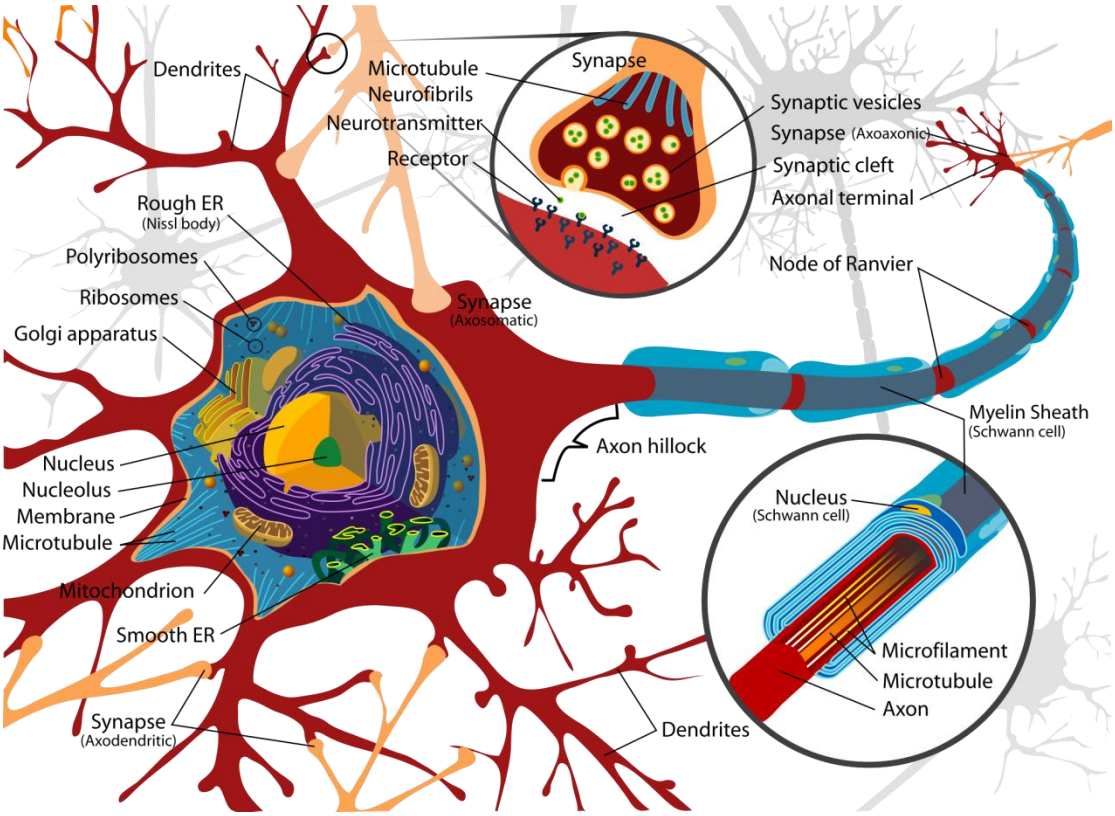


Fig. 1: A neuron with a myelinated axon, showing architecture and components[325]

The action potentials are traveling along axons by voltage-gated ion channels, membrane protein channels that allow ion-flux through the membrane. Like other cell membranes, the neuronal membrane is subject of an electrochemical gradient of sodium, potassium, calcium and chloride ions called the resting potential. Shifting the resting potential of the neuronal plasma membrane over a threshold causes a chain reaction, resulting in a propagating wave of sodium ion influx and potassium ion efflux through their respective voltage-gated channels. This way the electrical signals are transmitted from dendrites to the axonal synapses of a neuron[1–3].

When an axon potential reaches a synaps, it can trigger the release of synaptic vesicles containing neurotransmitters – small molecules that chemically transmit the electrical signal from the signaling to the target neuron - upon reaching the synaps. These neurotransmitters are released into the synaptic cleft, the extracellular space that is present between the axon terminal and the target neuron, where they can diffuse to reach receptors in the target neuron. This again can result in the opening of ion channels, which results in an ion current across the membrane in the target neuron. If this ion current can reduce the membrane potential enough to reach the threshold value, a new action potential can be generated. Some synapses called electrical synapses do not chemically transmit the signal, but instead a direct connection between the two neurons is formed by connexin proteins. These connexins form pores called connexons that can be in an open or closed state. These allow direct ion flux between the two neurons, thereby fastening the transmission of the action potential by bypassing the release of vesicles and diffusion of neurotransmitters[1–5].

To fasten the speed at which action potentials travel, vertebrates ensheath their axons with myelin, an electrically insulating wrapping of multiple layers of (lipid bilayer) membrane. The myelin sheath is not continuous, it is intermitted by the regularly spaced nodes of Ranvier at approximately every micrometer. Myelin of the PNS is made up by Schwann cells, whereas the CNS axons are ensheathed by oligodendrocyte cells. Although the myelin composition is similar, Schwann cells only enwrap a small piece of a single axon enclosed by nodes of Ranvier, whereas oligodendrocytes can provide many of these nodes-of-Ranvier-enclosed wrappings (internodes), on the same or on different axons (see fig. 2). The insulating myelin sheath increases the speed of action potential propagation by reducing the capacitance of the cell membrane[1–3].

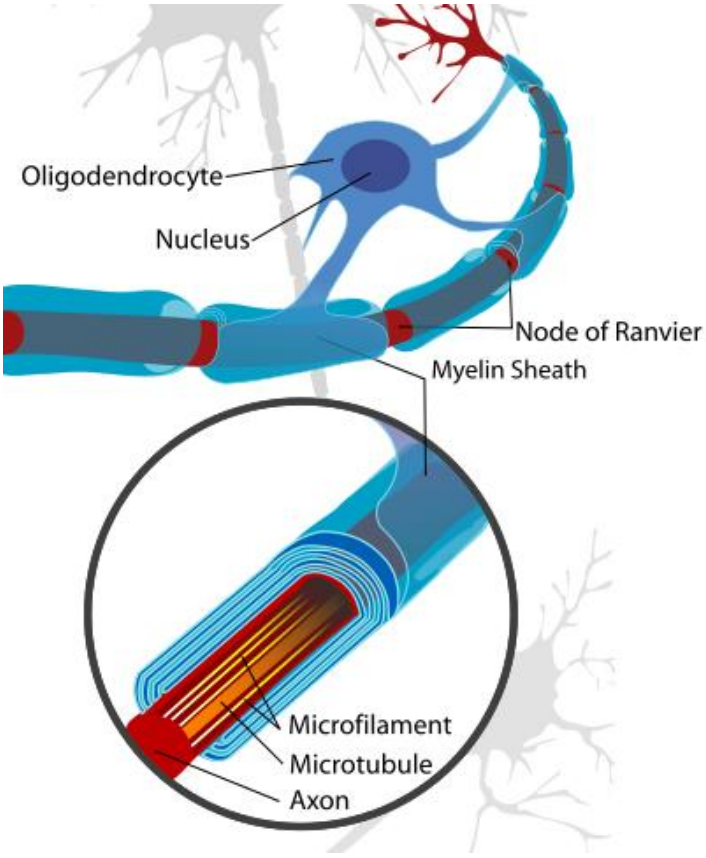


Fig. 2: Myelination of an axon by an oligodendrocyte[326]

Apart from providing insulation and enhancing the speed of axon potential propagation, myelination also reduces the energy it takes to generate axon potentials. Furthermore, oligodendrocytes and Schwann cells also protect the axons from damage and maintain their long-term structural integrity and survival, as wel as the providing trophic support. These functions may be independent of the myelination itself[6,7].

The myelin sheath has a high lipid content of almost 50% (75% dry weight). The primary lipids of myelin are glycoceramides, also known as cerebrosides. Cholesterol is also highly abundant in the myelin sheath[8]. The high lipid content of myelinated axons gives neuronal tissue a white appearance. This led to the distinction of white matter and grey matter, neuronal tissue in which axons are not myelinated. The remainder of the dry mass of CNS myelin is made up of proteins, of which the most abundant are proteolipid protein (PLP, 17%) and myelin basic protein (MBP, 8%), which are responsible for the wrapped up shape of myelin[9].

Apart from the brain, the vertebrate CNS consists of the spinal cord. This is where the nerve fibers involved in the sensory and motor systems are conducted to and from the brain respectively. The sensory nerves in the spinal cord together form the somatosensory organization, whereas the motor nerves make up the corticospinal tract. A cut-through of the spinal cord reveals a grey matter core surrounded by white matter, with a symmetrical arrangement of an exit and entry for motor and sensory nerves respectively at the left and right side of each vertebra. These are called the ventral/anterior (the exit of motory neurons) and dorsal/posterior (the entry of sensory neurons) roots (see fig. 3). The ascending sensory and the descending motor fibers are bundled in distinct regions of the spinal tract (see fig. 4)[1].

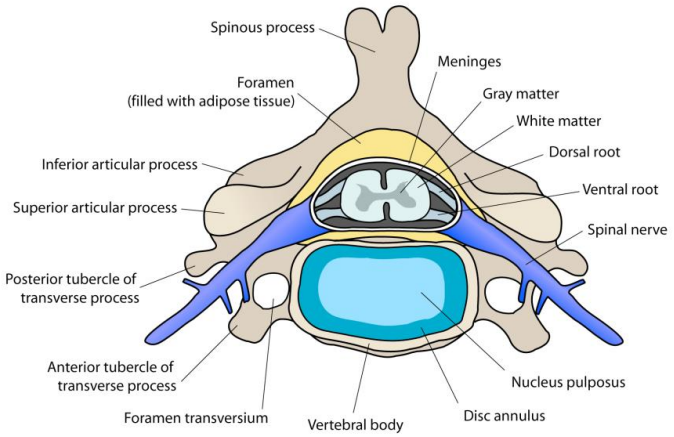


Fig. 3: Horizontal cut-through of the vertebra, showing the spinal cord in the middle with the entry of sensory nerves at the dorsal root and the exit of motor nerves at the ventral root[327]

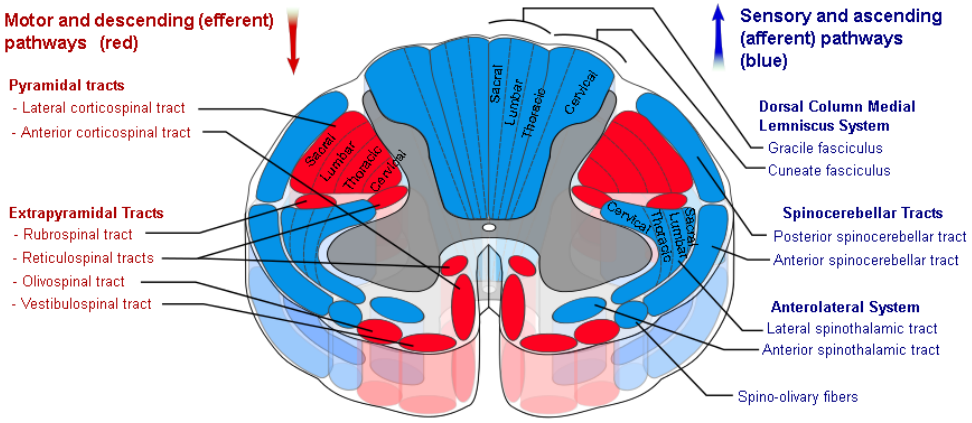


Fig. 4: Horizontal cut-through of the spinal cord, showing the locations of the different ascending (blue) and descending (red) nerve tracts[328].

Close to the entry point of each dorsal root in the spinal cord, a swelling known as the dorsal root ganglion (DRG) sits. Each DRG contains the cell bodies of the sensory neurons, also known as afferent neurons, that conduct the signals from the distal sense organs and receptors of the PNS to the spinal cord[1].

The grey matter in the center of the spinal cord contains interneurons (also known as relay neurons) that form connections between motor and sensory neurons, without being either themselves. Certain reflexes such as the knee-jerk reflex happen through neuronal short-cuts formed by these interneurons, so that the signal does not need to travel all the way to and from the brain[1].

Injury and Regeneration

Whereas it has long been recognized that the CNS is not supporting regeneration, nerves in the PNS can readily regenerate upon injury. Neuronal regeneration happens by extension of growth cones, the dynamic tips of neurites that guide the extending axons and dendrites by sensing attractive and repulsive guidance cues. Once contact is made with target neurons, synapses can be formed creating new circuitry. However, injury often results in the formation of a glial scar, containing reactive astrocytes, macrophages, extracellular matrix components and other debris. In the PNS, this debris is cleared much faster by macrophages. Schwann cells are also rapidly cleared by macrophages, whereas oligodendrocytes persist much longer[1].

Remarkably, CNS neurons were shown to be able to regenerate in a PNS environment and a PNS nerve graft could be used to bridge an injury site in the CNS, showing there are cues in the CNS that are not present in the PNS that prevent regeneration[10,11]. It was found that CNS white matter and not gray matter is inhibitory towards regeneration, suggesting that the inhibitory properties are myelin-associated[12,13]. Two fractions of CNS myelin membrane proteins of approximate molecular weights of 35 and 250 kDa were isolated with inhibitory properties after reconstitution in vesicles, showing that the inhibitory properties of CNS myelin have a molecular basis[14]. Furthermore, regeneration-permissive PNS myelin supplemented with these protein fractions also became non-permissive for regeneration[14]. Two antibodies were raised against these protein fractions, called IN-1 and IN-2. Both showed the property to overcome regeneration inhibition for sensory and sympathetic neurons in the CNS[15]. Inhibition by several other CNS white matter tissues could also be reduced by the IN-1 antibody[12]. Subsequent investigations found that these inhibitory proteins were expressed by fully differentiated oligodendrocytes, since growth cones of newborn DRG neurons encountering oligodendrocytes or astrocytes were only stopped by oligodendrocytes[16,17]. Filopodial contact of axons with oligodendrocytes resulted in growth inhibition and often to subsequent growth cone collapse. This growth cone arrest could be reduced by treatment with the IN-1 antibody as well[16]. Finally, intracerebrally applied IN-1-expressing tumours cells induced neuronal sprouting and axons crossing a complete spinal cord lesion in rats[18].

Further investigations showed that suppression of myelination by neonatal x-ray irradiation lead to increased sprouting of sensory neurons into the spinal cord, suggesting a function of the inhibitory proteins expressed by oligodendrocytes in (negatively) regulating neuronal plasticity[19]. This was confirmed by the fact that the IN-1 antibody induced spontaneous sprouting of neurites from uninjured Purkinje neurons[20]. The function of these proteins was also hypothesized to be the guidance of axons in the CST during early development. IN-1 application or oligodendrocyte elimination by x-ray irradiation resulted in anatomical abnormalities of CST fibers[21].

However, it was found later that DRG neurons were able to grow in the adult CNS and project long-distance axons. It was suggested that a reactive extracellular matrix that is the result of injury causes axonal growth-cone collapse, whereas CNS white matter itself supports regeneration and is permissive for axonal growth cones[22]. Although sensory axons were able to regenerate in damaged CNS white matter even after three months post-injury, they were acutely stopped by the molecular barrier at the so-called glial scar, where the lesion happened[23].

Neuronal regenerative capacity changes after a so-called critical period shortly after birth. Whereas newborn vertebrates show high neuronal plasticity, this is lost as the CNS maturates[24]. A famous (Noble prize-winning) example of this phenomenon is ocular dominance plasticity: monocular deprivation during this critical period results in an irreversible shift of ocular dominance in favor of the non-deprived eye. This shift however does not occur in adult vertebrates[25–28]. Newborn rats also show enhanced ability to regenerate after SCI, as compared to adults[29].

Thus, whereas the peripheral nervous system supports regeneration after injury, in the adult central nervous system regeneration is inhibited by molecular cues present on the myelinating oligodendrocytes and at the glial scar.

Molecular Mechanisms

The discovery that the lack of regeneration after injury in the CNS was caused by molecular (protein) determinants present in CNS myelin, raised the opportunity that the proteins and their interactions that cause the signaling responsible for this inhibition of regeneration could be therapeutic targets for spinal cord or brain injury. Identification of these myelin associated inhibitors (MAIs), their neuronal receptors and their downstream signalers has been the goal of many studies in the past two decades. Indeed, three MAIs and two receptor complexes have been successfully identified, as well as many downstream signalers in the neuronal cytosol. These have indeed been linked to cytoskeletal rearrangements, causing morphological changes such as inhibition of neurite outgrowth or growth cone collapse. Transcription-mediated signaling pathways have been identified as well. The three MAIs, their receptors and other extracellular molecules involved in the inhibition of regeneration will be discussed in this chapter, whereas the downstream signaling in the neuronal cytosol is the subject of the next.

Myelin-associated glycoprotein

The first MAI to be identified was Myelin-associated glycoprotein (MAG). Two groups independently identified MAG as an inhibitor of neurite outgrowth[30,31]. Paradoxally, MAG had earlier been identified as a promoter of neurite outgrowth. Recombinant MAG had been shown to bind to neurites in DRG or spinal cord cultures and fibroblasts expressing MAG enhanced DRG neurite outgrowth[32]. However, the difference was caused by the use of newborn neurons, on which MAG has a stimulating effect for neurite outgrowth, as opposed to adult CNS neurons, to which it is inhibitory[30,33,34]. This bifunctionality is in accordance with the difference in ability to regenerate during the postnatal critical period.

MAG is a single-pass type I transmembrane protein, with a glycosylated N-terminal extracellular domain consisting of 5 Ig-like repeats, a transmembrane helix and a 90 residue cytosolic C-terminal domain. Its molecular weight consists of ca. 30% carbohydrate, with 8 N-linked glycosylation sites (Asparagine (Asn) residues 99, 106, 223, 246, 315, 406, 450 and 454), of which one is partially glycosylated (Asn106)[35]. A 19-residue N-terminal signal peptide is removed in the ER. Alternative splicing produces two different forms of MAG, named L-MAG and S-MAG for the longer and shorter forms. These only differ in their cytosolic C-terminal domain[36]. MAG is present both in the CNS and PNS and is shown to be expressed in oligodendrocytes[37]. MAG is subject to proteolysis, which releases a soluble form of MAG called dMAG from myelin in vivo[38]. This fragment consists of the entire extracellular domain, cleaved just before it enters the membrane. Like MAG, dMAG is an inhibitor of neurite outgrowth[39]. The function of dMAG is not entirely clear.

MAG contains an FRGD-motif (residues 117-120) in the first Ig-like domain that enables it to bind to sialic acid moieties like those present on gangliosides, as well as integrins[40,41]. The inhibitory activity of MAG has been shown to reside in the Ig-like domains 4 and 5, which are closest to the plasma membrane[42]. However, this result is conflicting with a study published a month later, in which it is shown that the first three domains are necessary and sufficient to bind to its receptor[43]. The former study however claims that other unidentified receptors mediate the inhibitory effects of MAG.

Earlier studies have also suggested roles for MAG regulation of axonal caliber (i.e. the diameter of the axon)[44]. MAG was also found to function as a myelin-associated stabilizer of axons, protecting it against damage and degeneration. The effect was independent of the signaling that inhibits regeneration, but was

dependent on Arginine 118, suggesting it might involve neuronal ganglioside or integrin ligands. This implicates MAG in the role of myelin in stabilizing axonal integrity[45]. Recently, MAG has been suggested to function as a protector against neuronal excitotoxicity[46]. Knockout studies on mice indicate that MAG is not the only MAI[32,47,48]. This indicates that other (myelin-associated) molecules are also inhibitory to axonal regrowth.

Nogo

Already in the early nineties a monoclonal antibody (IN-1) was generated against an inhibitory protein component of myelin, which showed the ability to enhance regeneration after SCI[18]. This antibody also induced spontaneous sprouting in uninjured purkinje cell axons, suggesting an inhibitory role for the antigen[20]. However, it was not before the year 2000 that the antigen was characterised as a product of the Nogo gene[49–51], after proteolytic peptides of the purified protein component had been sequenced[52]. The NOGO gene codes for three different proteins. RNA splicing results in a long, intermediate and a short protein construct (Nogo-A, -B and –C respectively). Nogo-A consists of 1192 amino acids, whereas Nogo-B lacks residue 186-1004. Nogo-C shares the conserved C-terminal domain (residue 1004-1192) with Nogo-A and –B, but completely misses the N-terminal domain of the others and also has an alternative short N-terminal sequence (MDGQKKNWKDK). The Nogo proteins are members of the Reticulon family. The Nogo gene is also referred to as the Reticulon 4 (Rtn4) gene, with Rtn4-A, -B and –C corresponding to the Nogo-A, -B and –C proteins. Other reticulon genes like Rtn1, Rtn2 and Rtn3 all encode for different splice variants like Rtn4, all of which possess an homologous C-terminal domains of ca. 200 residues[50].

A 66 residue stretch in this conserved C-terminal domain (Nogo-66) is enclosed by two hydrophobic stretches which are thought to be embedded in the membrane. Nogo-66 has been shown to be exposed to the extracellular side of the membrane and to have an inhibitory effect on axonal extension. Furthermore, it collapses DRG axonal growth cones. The C-terminal 200 residues (including the Nogo-66 region) of the other four reticulon family proteins have a sequence conservation of 70%. However, only Nogo-66 and not the corresponding regions in the other reticulon proteins have inhibitory activity[49,50]. In another report, Rtn2 and Rtn3 have been shown to bind to the Nogo receptor (NgR), but no inhibitory effects are mentioned[53].

Although there is inhibitory activity in different regions of the amino-terminal domains of Nogo-A and –B, Nogo-C can also delay nerve degeneration, confirming the importance of the Nogo-66 region[54]. Epitope mapping of Nogo-A for the IN-1 antibody using overlapping 15 residue oligopeptides, combined with truncation and substitution analysis, revealed an IKxLRRL (residues 1103-1109 in human Nogo, x is not P) motif that is present in the Nogo-66 region as the binding epitope for both IN-1 and its receptor[55]. However, another report describes the Nogo residues 1055-1095 are the essential residues, based on AP-fusion peptides. These are also present in the Nogo-66 region though[56].

Two solution-structures of the Nogo-66 region have been determined by NMR spectroscopy[57,58]. Although the structures agree on an alpha-helical secondary structure, the tertiary structure is completely different (see fig. 5). Both structures are measured on similarly prepared protein in the same buffer, although the first report uses a 60 residue Nogo sequence instead of all 66 residues. Both studies use a protein construct containing an N-terminal His_6-tag. The latter study finds that Nogo-66 is disordered in solution, but adapts a defined conformation when binding to a lipid bilayer. This makes sense because of the many surface-exposed hydrophobic residues. Paramagnetic resonance

enhancement (PRE) measurements using three different labels and nuclear Overhauser effect spectroscopy (NOESY) with phospholipids were employed to determine the orientation with respect to the membrane. These more elaborate measurements in a more native-like environment (in contact with the lipid bilayer), as well as the longer construct make the structure that the latter study finds more convincing (right in fig. 5 and fig. 7). Another study investigated the structure of the N- and C-termini of Nogo using bioinformatics, NMR and CD spectroscopy. This study finds that both the N- and C-termini of Nogo-B as well as Nogo-A are intrinsically unstructured. However, binding motifs for several other protein domains are present, such as WW, PDZ, SH3 and SH2 domains[59].

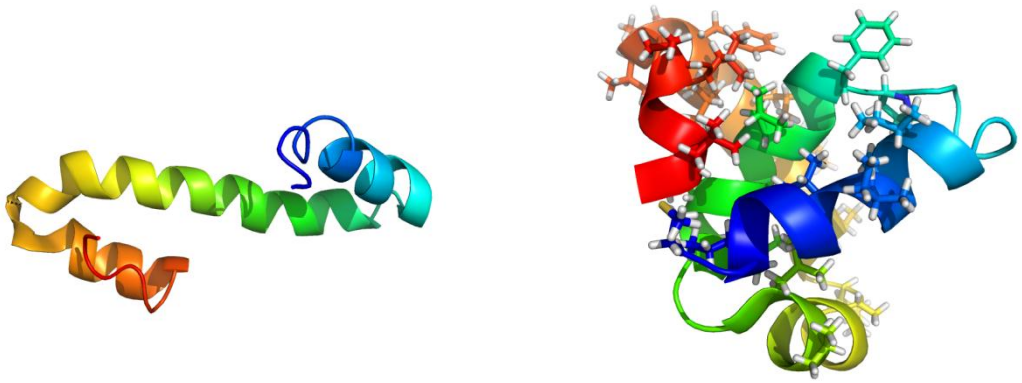


Fig. 5: Cartoon representations of NMR structures from two different studies on Nogo-66. Side chains of surface-exposed hydrophobic residues are shown as sticks in the right structure. Based on PDB accession codes 2g31[58] and 2ko12[57].

C-terminal of the second hydrophobic stretch, a double lysine endoplasmatic reticulum (ER) retention motif is present, which can only be "read" if on the cytoplasmic side. The availability of Nogo-66 on the extracellular side, flanked by two hydrophobic strectches, suggests an orientation where the domains N-terminal and C-terminal of these stretches are facing the cytoplasmic side[49,50]. This is confirmed by a large number of phosphorylation sites in the N-terminal domain of Nogo-A, which would not be expected for an extracellular domain[60,61]. Apart from the many phosphorylation sites in the N-terminal domain of Nogo-A, a binding site for the ubiquitin ligase WWP1 is also present here (Nogo-A residues 650-666). Although ubiquitylation of Nogo has not been demonstrated, the authors state they confirmed the interaction *in vivo*, although these observations are unpublished. The specific interaction of this region of Nogo with a cytosolic protein further strengthens the evidence for a topology with the N-terminal domain of Nogo-A on the cytosolic side[62]. Nck2, a cytoplasmic adaptor protein was also shown to be able to bind to N-terminal Nogo-A sequences. However, this interaction has not been shown *in vivo* either[63].

However, residues 1-172 and 544-725 in the N-terminal domain of Nogo-A have also been shown to have myelin inhibitory activity and are exposed on the cell surface. Furthermore, the Nogo-66 loop has also been shown to be available on the cytoplasmic side, suggesting multiple possible topological conformations for Nogo[64].

Different reports suggest that the hydrophobic stretches enclosing Nogo-66 are too long to be single-pass transmembrane helices (35 and 36 residues for the N- and C-terminal stretch respectively)[53,65]. This implies that both the N-terminus and C-terminus of Nogo might be surface-exposed as well. Apart from Nogo-66, residues N-terminal and C-terminal of the hydrophobic stretches enclosing Nogo-

66 have also been implicated in binding to NgR. Both the C-terminal 39 residues of Nogo-A and a 24-residue Nogo-A oligopeptide N-terminally of the first hydrophobic stretch (Nogo-A residues 995–1018) showed binding to NgR, though not as strong as Nogo-66. Fusion of the different interacting regions resulted in a higher-affinity ligand for NgR than Nogo-66 on itself[53,66,67]. However, contribution of these regions to NgR-binding of Nogo has not been confirmed *in vivo*. The inability of cells to deal with the long hydrophobic stretches of the Nogo proteins in a consistent fashion might explain the different topologies that have been revealed.

In agreement with the myelin inhibition hypothesis, Nogo is expressed in the oligodendrocytes of the CNS, but not in the Schwann cells of the PNS. It is most prevalent in the ER membrane, but is also present on the plasma membrane of oligodendrocytes[50,68]. Surprisingly, it is also present on the surface of neurons.[69] Nogo-A is increasingly upregulated for at least 28 days after focal ischemic stroke in adult rat brains. Both excitatory and inhibitory neurons upregulate Nogo-A expression after stroke[68,70].

Different knockout studies for Nogo-A, Nogo-A/B and Nogo–A/B/C have been performed in mice[71–73]. The results however were inconsistent in the ability of knockout mice to regenerate after SCI compared to wild-type mice. This might have been caused by the different knockout strategies that were employed[74]. The lack of regeneration can also be explained by the availability of MAG and other MAIs.

Oligodendrocyte myelin glycoprotein

Shortly after the identification of Nogo as an MAI, a third MAI was identified. Oligodendrocyte myelin glycoprotein (OMgp), a glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed on CNS myelin, was found to be an inhibitor of neurite outgrowth[75,76]. The protein was already identified in 1988 as a GPI-anchored glycoprotein expressed by oligodendrocytes, which also release it as a soluble 105 kDa protein[77]. Mature OMgp lacks a 24 residue signal peptide for secretion, that is proteolytically removed in the ER. It is predicted to have a Leucine-rich repeat (LRR) fold, with an N-terminal LRR capping domain followed by 8 LRRs and a 200 residue stretch with a serine/threonine-rich compositional bias. It has ten potential sites for N-linked glycosylation, although these have not been confirmed experimentally. The inhibitory function of OMgp is dependent on the LRR domain and not on the GPI anchor[78].

It has been reported that OMgp is not expressed in compact myelin, but by oligodendroglia-like cells, which encircle the nodes of Ranvier. Knockout of OMgp in mice resulted in unusually wide nodes and increased collateral sprouting from these nodes, suggesting a function of OMgp in stabilizing the nodes of Ranvier and thereby preventing collateral sprouting[79,80]. However, these results have been disputed by a more recent report[81]. The results of earlier studies may have been the result of unspecific anti-OMgp antiserum, which also appears to recognize versican V2 chondroitin sulfate proteoglycan (CSPG). Using a more specific antibody, no enrichment of OMgp was detected at the nodes of Ranvier, nor did knockout of OMgp affect the formation or stability of the nodes of Ranvier[81].

Another OMgp knockout study showed less inhibitory myelin as compared to wild-type, but no significant functional recovery or axonal sprouting following dorsal hemisection[82]. An early study on OMgp expression patterns in mice showed that the protein is not confined to oligodendrocytes and myelin, but instead is more prevalent on neurons like the pyramidal cells of the hippocampus, the Purkinje cells of the Cerebellum, motor neurons in the brain stem and anterior horn cells

of the spinal cord. OMgp levels gradually increased during development, until the 21st day of life[83]. A peak in the expression levels of OMgp was seen during the late stages of myelination, which was independent of axonal cues[84]. Studies that analyzed the function of OMgp expression during development show effects suggesting OMgp to be a regulator of neurogenesis and myelination[85,86]. OMgp expression has been found to be upregulated upon SCI[87].

The Nogo Receptor is a receptor for all MAIs

Shortly after the identification of Nogo as a MAI, the neuronal receptor was discovered and dubbed Nogo Receptor (NgR, also known as NgR1 or Rtn4R)[88]. Surprisingly, this protein also appeared to be the receptor for both MAG[89,90] and OMgp[75]. Mature NgR consists of a 288 residue N-terminal leucine-rich repeat (LRR) domain, responsible for binding Nogo, MAG and OMgp, followed by a 137 residue C-terminal domain responsible for signal transduction, with its C-terminus being GPI-anchored for membrane attachment[75,89,91,92]. A 26 residue N-terminal signal peptide for secretion and a 26 residue C-terminal propeptide are proteolytically removed in mature NgR. The C-terminal domain is necessary, but not sufficient for signal transduction[91]. NgR has been shown to be capable of self-association, with the complete N-terminal domain being responsible for multimerisation[91,92].

Since all three MAIs share the same receptor and even the same binding domain in the receptor, it is relevant whether these interactions are competitive. Indeed, MAG and Nogo-66 appear to compete for NgR binding[89]. Furthermore, OMgp and Nogo-66 competed for the same binding site as well[75]. Therefore it is likely that OMgp and MAG also compete for the same binding site, although this has not been verified experimentally[89]. Conversely, in one of the two reports describing MAG as an NgR ligand, it is described that excess Nogo-66 did not prevent MAG binding[90]. These observations however are unpublished. The competitive binding of different ligands to the same receptor with the same downstream effects is indicative of a redundant ligand model, meaning that multiple ligands can independently signal through the same receptor.

Two paralogs of NgR have been characterized: NgR-like 1 and 2 (henceforth NgRL1 and NgRL2, also known as NgR2 and NgR3 or NgRH1 and NgRH2 respectively), both being GPI-anchored like NgR[92–94]. Whereas their amino acid sequence is very similar in the N-terminal LRR-domain, the C-terminal domain shows little sequence conservation. Apart from their primary sequence similarity and GPI anchor, NgRL1 and NgRL2 are also predominantly expressed on the surface of neurons of the CNS, like NgR. However, their expression patterns differ in that NgRL1 and NgRL2 are also expressed outside the CNS. NgRL1 is also expressed in the mammary gland, muscle and salivary gland, whereas NgRL2 is additionally expressed in the mammary and thyroid glands[94]. Proteolytically cleaved soluble N-terminal forms of all three NgR variants were released from cultured cells expressing the respective receptors. However, this phenomenon has not been demonstrated *in vivo*[93]. It is interesting to know whether these homologues are also capable of inhibition and whether they bind the same ligands. However, the reports in literature are conflicting. Two reports suggest that only NgR is a receptor for Nogo-66, MAG and OMgp[92,93], but another group does report a strong interaction of MAG with NgRL1[95]. This report shows no binding of Nogo-66 or OMgp to NgRL1 and NgRL2, nor did they see MAG binding to NgRL2. The experiments performed in the latter report are however more elaborate and even show a higher affinity of NgRL1 for MAG as compared to NgR[95]. Combined, these results suggest that NgR is a receptor for all three MAIs, whereas NgRL1 only is a receptor for MAG.

Two crystal structures of the N-terminal LRR domain of NgR were published shortly after each other[92,96]. Although crystallized in different space groups, the structures almost completely overlap when aligned. The structures show the location of two N-linked glycans, as well as three disulfide bridges. The LRR domain consists of 8 similar LRRs, enclosed by an N-terminal and a C-terminal LRR-capping domain (see fig. 6). It shows a high similarity to the structure of the platelet receptor glycoprotein 1bα (Gp1bα), which binds to the A1 domain of von Willebrand factor (vWF) blood glycoprotein[97]. Other features of the NgR structure are a "phenyl-spine"; a stack of conserved phenylalanine residues in each LRR and an acidic cavity of three aspartic acid side chains on the concave surface.

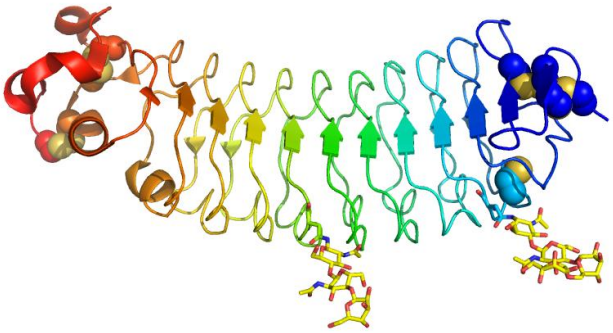


Fig. 6: Cartoon representation of a crystal structure of the N-terminal LRR domain of NgR, with the side chains of cysteine residues shown as spheres and the N-linked glycans as sticks. Based on PDB accession code 1ozn[96].

The concave surface of NgR contains a number of evolutionarily conserved aromatic residue patches that might be involved in ligand binding. These are not present or less conserved in NgRL1 and NgRL2. The overall conservation is also much higher on the concave as compared to the convex surface, apart from a chain of conserved proline residues on the convex surface. An extensive mutagenesis study confirms binding to the concave surface for all three MAIs, whereas no difference in binding affinity was seen for mutating residues on the convex surface. On the other hand, many residues on the concave surface were sensitive to mutation for binding the different MAIs. Remarkably, Nogo-binding was generally much less sensitive to mutations than MAG or OMgp binding[53]. Furthermore, a monoclonal antibody against NgR that prevented MAG, Nogo-66 and OMgp binding was shown to bind to the concave surface of NgR[98].

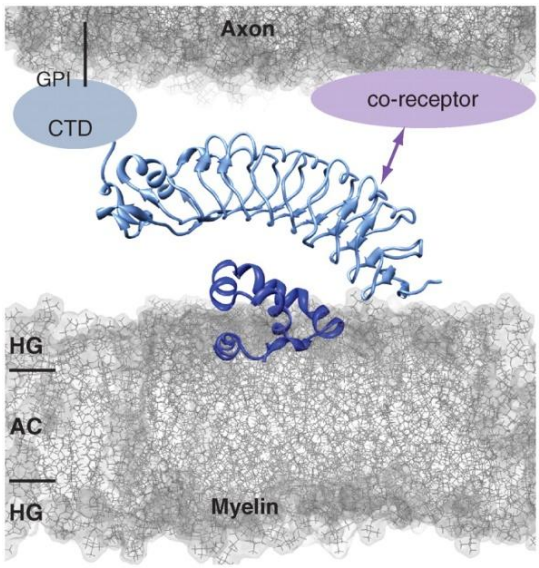


Fig. 7: Model of the NgR/Nogo-66 interaction, based on the Nogo NMR structure, the NgR structure and the predicted binding interface. Adapted from [57].

The disulfide pattern reported in both crystal structures appeared to be an artifact of truncation, as a slightly longer construct that includes cysteine residues 335 and 336 (the crystallized constructs are truncated at residues 310/311) has a

different disulfide bridging pattern, as shown by mass spectrometry analysis after proteolytic digestion[99]. Cystine disulfide bonds were formed between residues 266-335 and 309-336. The other cystine disulfide bonds in the LRR were found to be correct (between cysteines 264-287, 27-33 and 31-43) and a new disulfide bond was found in the C-terminal stalk between cysteine residues 419 and 429. Cysteine residues 80 and 140 were free thiols as in the crystal structures, which show they are buried in the LRR region. The list of glycosylation sites that were found in the crystal structure at asparagine residues 82 and 179 (both N-linked) was extended by N-linked glycosylation of Asn237 and extensive O-glycosylation in the C-terminal stem domain[99].

Comparison of different NgR/NgRL1/NgRL2 fusion constructs showed that the N-terminal region of the stem domain of NgR was inhibitory towards Nogo-66 and OMgp binding, whereas the stem domain of NgRL1 enhanced MAG binding when fused to the LRR domain of NgR[43,95].

NgR is also involved in reducing ocular dominance plasticity after the critical period. Knockout of NgR in mice resulted in normal ocular dominance plasticity during this critical period. However, the critical period appeared to be extended, so that after 45 or even 120 days postnatal, plasticity remained the same as in newborns. This result supports the inhibitory function of NgR-dependent signaling of reducing plasticity after trauma, like stroke or SCI[100].

A number of other knockout and knockdown studies have been performed for NgR[101–103]. The results are inconsistent in the ability of knockout mice to regenerate after SCI. In one study, improved functional recovery is seen for knockout mice after dorsal hemisection or complete transection, as well as regeneration of raphespinal and rubrospinal fibres[103]. Conversely, another study sees no improvement in neurite outgrowth of knockout DRG or cerebellar granule neurons (CNGs) on a myelin or Nogo-66 substrate[101]. The second study sees an upregulation of Nogo transcripts, suggesting a feedback loop from NgR to regulate Nogo expression. Both studies agree on the lack of enhanced regeneration for CST neurons, suggesting involvement of other receptors for MAIs. A third study combined classical knockout with transient knockdown using short hairpin RNA (shRNA). Their results indicate that NgR is only required for the acute growth-cone collapsing effects of MAIs, but not for their chronic inhibitory effects[102]. Taken together, these studies confirm that NgR is a receptor for MAIs, but likely not the only one. Furthermore, the fact that NgR does not have an intracellular domain, but instead is GPI-anchored, suggests that other transmembrane proteins might be involved in mediating downstream signaling to proteins in the neuronal cytosol[88].

$p75^{NTR}$ is a co-receptor for NgR

The first co-receptor of NgR that was identified as a mediator for downstream signaling induced by the three MAIs, was the neurotrophin receptor $p75^{NTR}$ (also known as low-affinity nerve growth factor receptor (LNGFR) and tumor necrosis factor receptor superfamily member 16 (TNFRSF16), henceforth p75). MAG stimulation resulted in enhanced complex formation of NgR-p75, although association also occurred without MAG stimulation[104–106]. Interestingly, p75 was already known to function as a low-affinity receptor for all four neurotrophins (nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophins 3 and 4 (NT3 and NT4)). These can paradoxically either induce apoptosis or promote cell survival by interacting with and thereby dimerising p75[107–109]. Whereas one crystal structure of p75 in complex with an NGF dimer suggested a 1:2 (p75:NGF) stoichiometry of the complex,[110] a later report disproves this[109]. The latter study reports a crystal structure of an NT3-p75

complex with a 2:2 stoichiometry (see fig. 8) and shows that 2:2 complexes are formed in solution for both NT3 and NGF. They show that 2:1 complexes are an experimental artifact caused by artificial deglycosylation. However, the presence of preformed p75 dimers on the surface of cells in the absence of any ligands indicates that the mechanism might involve dimerization of dimers, i.e. multimerization/clustering[111].

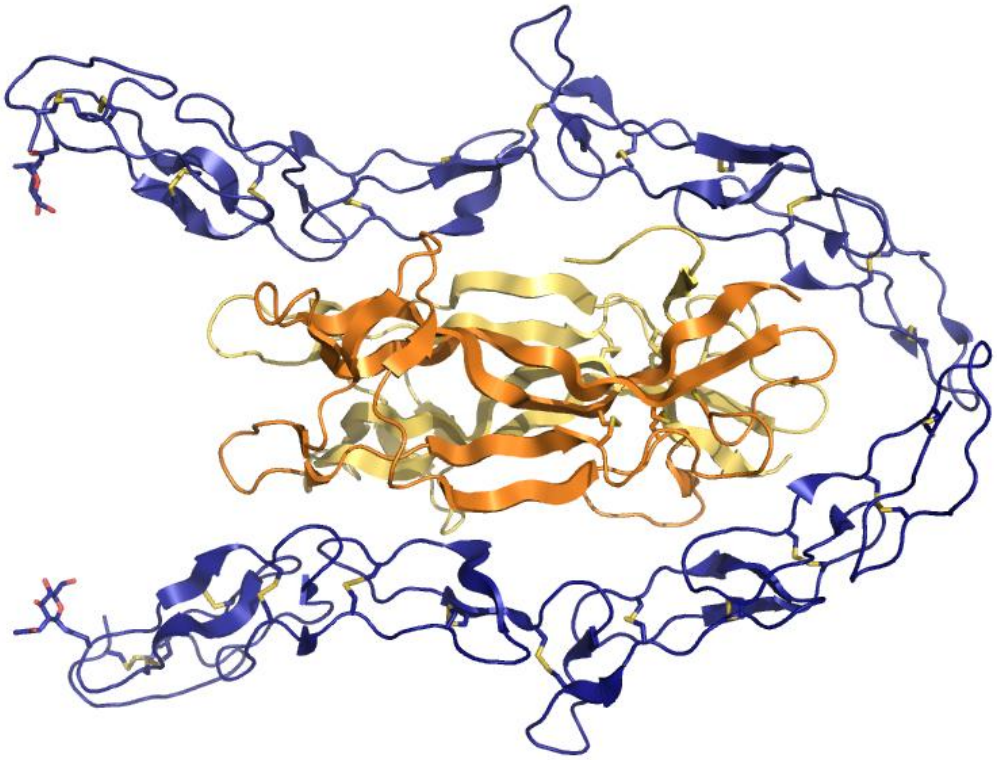


Fig. 8: Cartoon representation of the Crystal structure of two p75 molecules (blue) in complex with an NT3 dimer (orange). Cysteines and N-linked glycans are shown as sticks. Based on PDB accession code 3buk[109].

It has been hypothesized that the function of p75 in nerve growth factor signaling is to regulate the responsiveness of other nerve growth factor receptors (receptors of the tropomyosin receptor kinase family; Trk's) by enhancing their specificity towards their ligands (NGF for TrkA, BDNF and NT4 for TrkB and NT3 for TrkC)[112,113]. These Trk's have a higher affinity for certain nerve growth factors than p75. The neurotrophins also induce dimerization of Trks. Signal transduction is attained by their cytosolic tyrosine kinase domains, which can cross-phosphorylate tyrosine residues on nearby Trk molecules[112].

An early study on the influence of a monoclonal α-p75 antibody and BDNF on p75 signaling and neurite growth already demonstrated a relation between p75 signaling and inhibition of neurite growth[114]. However, although prior incubation of neurons with neurotrophins could prevent inhibition by MAG and myelin, neurotrophin binding after injury could not interfere anymore with inhibitory signaling of MAG[115,116]. Knockout studies on p75 in mice show less inhibition by myelin, MAG, OMgp or Nogo-66 as compared to wild-type mice[101,104–106]. Furthermore, overexpression of a truncated version of p75 lacking the cytosolic

domain in neurons, as well as antibodies preventing a p75-NgR interaction, attenuate the inhibitory functions of the three MAIs, supporting a central role for p75 in the signal transduction[104]. Apart from neurons, p75 is also expressed by Schwann cells in the PNS upon injury. However, expression by oligodendrocytes remains similarly low after injury[117]. This has been suggested to contribute to the ability of PNS nerves to regenerate, perhaps by providing neurotrophin gradients for Trk signaling or by competing for NgR with neuronal p75[118].

The p75 gene codes for a single-pass type I transmembrane protein with a 28 residue signal peptide for secretion that is proteolytically removed in the ER. The mature protein consists of four TNFR-type cysteine repeats (CR) (also known as Ig-like C2 domains), followed by a stretch of ca. 50 residues with a serine/threonine-rich compositional bias, a transmembrane helix and a 155 residue cytoplasmic domain, which also contains a 78 residue death domain (DD). Each of the CR domains contains 3 disulfide bonds and the extracellular domain is N-glycosylated at least once, at asparagine residue 31[109]. A solution structure of the DD determined by NMR spectroscopy shows it consists of six alpha-helices. It is also shown that this DD does not self-associate in solution[119].

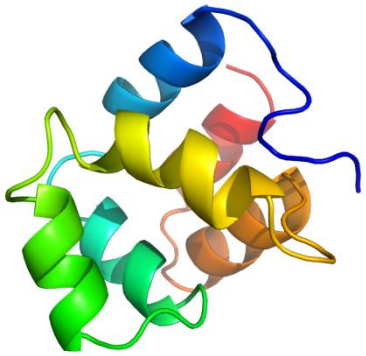


Fig. 9: Cartoon representation of an NMR structure of the p75 C-terminal intracellular death domain. Based on PDB accession code 1ngr. [119]

p75-AP fusions only bind to cells expressing full-length NgR and not to cells expressing truncated constructs lacking (parts of) the LRR N-terminal or C-terminal domains[104], suggesting that interaction is happening along the whole extracellular domain. This is in agreement with an earlier study that suggests that the C-terminal domain of NgR is necessary but not sufficient for signal transduction[91]. However, the truncation of the LRR-domain in a way that causes an artificial disulfide bonding pattern[99] might have caused the lack of binding of p75 to the LRR domain (without the C-terminal domain). The fact that the C-terminal domain is heavily O-glycosylated makes it unlikely that specific interactions are present in this domain, so perhaps p75 only binds to the LRR N-terminal domain.

p75 is only present in subpopulations of mature neurons, whereas NgR and NgRL1 are expressed ubiquitously in the CNS[120]. This suggests that alternative co-receptors are present in the neurons lacking p75, to facilitate signal transduction.

TROY can substitute for p75 as a co-receptor for NgR

Two groups simultaneously identified TROY (also known as TAJ or TNFRSM19), an orphan TNF receptor family member, as an alternative NgR binding facilitator of MAI induced signal transduction[121,122].

Like p75, TROY is a single-pass type I transmembrane protein of the TNFR family, but it has three (instead of four for p75) TNFR-type cysteine repeats in the extracellular domain. It has a unique 230 residue C-terminal cytosolic domain, with no similarity to the cytosolic domains of other TNFRs. The eight C-terminal residues VRQRLGSL are replaced by EA in an isoform. TROY is known to interact with TRAF family members and can activate the Jun N-terminal kinase (JNK)

pathway and the apoptosis inducing transcription factor NFκB, despite lacking a DD[123].

Both knockout of TROY and exogenous addition of a soluble form of the extracellular N-terminal domain of TROY enhanced regeneration in the presence of Nogo-66 or OMgp. TROY binds to NgR with a higher affinity than p75 and was able to mediate signal transduction[121,122]. The studies identifying TROY as an alternative NgR signal transducer note a more widespread distribution of TROY as compared to p75[121,122]. However, another study on expression levels of several MAI receptor complex components found that the expression patterns of TROY in the CNS is even more limited than that of p75 and does not complement p75 expression[120]. Furthermore, no TROY was detected in the neurons projecting to the spinal cord, suggesting a limited role in inhibition of regeneration after SCI[120].

LINGO-1 is another co-receptor necessary for MAI signaling

Whereas it is clear that NgR and p75 are involved in the signaling of MAIs, it was found that after reconstitution of both receptors in non-neuronal COS-7 cells, no downstream signaling was detected upon addition of OMgp. This lead to the discovery of a third component of the MAI receptor complex, which was called LINGO-1 (LRR- and Ig-domain containing Nogo receptor-interacting protein 1)[124]. The single-pass type I transmembrane protein LINGO-1 was shown to be abundantly expressed in the brain and spinal cord, as well as in the DRGs[120,125]. It was shown to be able to directly interact with NgR as well as p75 and TROY. The interaction of LINGO-1 with NgR was dependent on both the N-terminal LRR and the C-terminal stalk domain of NgR, as truncation abrogated binding[126]. Again, this might also have been caused by artifactual disulfide patterns as the result of truncation[99]. NgR forms a complex with LINGO-1 with a 2:1 stoichiometry. The three components of the complex can interact with each other in a non-competitive way, so a ternary complex is possible. Unlike a p75-NgR complex or other binary complexes of NgR, p75 and LINGO-1, the ternary complex was able to transduce the inhibitory signals of OMgp, Nogo and MAG. However, binding of the inhibitors to the cells did not increase if either LINGO-1 or p75 was present in combination with NgR, as compared to NgR alone[124,126]. This indicates that NgR is the MAI interacting component of the ternary receptor complex.

After removal of a 41 residue N-terminal secretion signal peptide in the ER, mature LINGO-1 consists of a LRR domain of 11 LRRs enclosed by N- and C-terminal LRR capping domains, followed by a short stretch containing 6 positively charged residues (RRARIRDRK), an Ig-like domain, a stalk of ca. 50 residues, a transmembrane helix and a 38 residue cytosolic domain[127]. Surprisingly, a crystal structure of the extracellular domain of LINGO-1 indicates that it can homo-tetramerize, with the C-termini oriented in the same direction, indicating that this structure might be physiologically relevant (see fig. 10). Tetramers of soluble ectodomain are also present in solution, showing that it is not a crystal packing artifact[128]. This is surprising because of the 2:1 complex that NgR forms with LINGO-1. Although the method for stoichiometry determination for the NgR-LINGO-1 complex is not extremely accurate (size exclusion chromatography), it is at least clear that it is not a 4:4 complex[126].

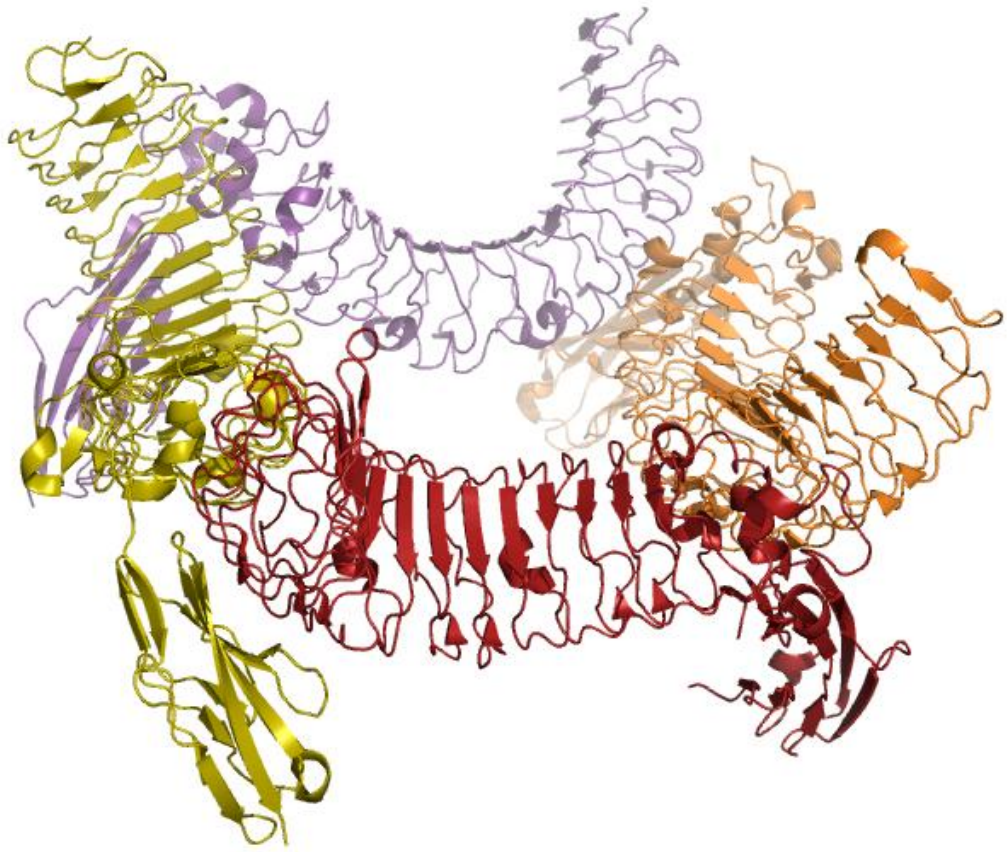


Fig. 10: Cartoon representation of the crystal structure of a LINGO-1 tetramer. The C-terminal Ig-like domains are oriented in the same direction. Based on PDB accession code 2id5[128].

The crystal structure also shows that it has seven confirmed N-linked glycosylation sites at asparagine residues 105, 163, 225, 235, 254, 302 and 453. In the stalk region, another 3 putative N-linked glycosylation sites are present, although these have not been verified experimentally. Five disulfide bridges are present in the extracellular domain. It was also found that the soluble form of the LINGO-1 ectodomain shows a higher affinity for p75-expressing than for NgR-expressing Chinese hamster ovarian (CHO) cells[128]. Analysis of the binding site of NgR for different ligands including LINGO-1 by site-directed mutagenesis suggests a similar interacting region for LINGO-1 on NgR as for the three MAIs[53]. This suggests that there might be competition of the MAIs with LINGO-1 for NgR and that MAIs might induce dissociation of NgR and LINGO-1. However, these hypotheses are speculative and require further experiments for validation.

Fig. 11: Surface representation of the N-terminal LRR domain of NgR, based on PDB accession code 1ozn[96]. Residues that are implicated in LINGO binding are marked yellow, residues that showed no effect upon mutation are green[53].

The cytosolic domain contains a canonical EGFR-like tyrosine phosphorylation site, suggesting involvement in signal transduction for the cytosolic domain of LINGO-1. Indeed, neurons expressing a truncated LINGO-1 receptor lacking the C-terminal cytosolic domain showed reduced response to different MAIs and myelin[124]. Furthermore, exogenous soluble LINGO-1 extracellular domain, either monomeric with a His-tag or dimerised as an Fc-fusion, disrupts neurite outgrowth inhibition by myelin components, confirming its importance in signal transduction[124,128].

LINGO-1 also forms a complex with the BDNF receptor TrkB and negatively regulates its activity upon injury of RGCs by preventing TrkB phosphorylation[129,130]. Remarkably, NGF stimulates neuronal expression of LINGO-1 through TrkA, so as to negatively regulate myelination of TrkA-expressing cells[131]. Analyses of LINGO-1 expression in purified populations of CNS cells revealed that apart from neurons, oligodendrocytes express LINGO-1 as well. Different experiments suggest LINGO-1 to function as an inhibitor of myelination *in vivo*[132]. The ligand for LINGO-1 on oligodendrocytes or oligodendrocyte precursor cells appears to be neuronal LINGO-1, by homophilic intercellular interaction[131,133]. The fact that LINGO-1 can be both receptor and ligand makes sense with regard to its ability to tetramerize, although the orientation of the C-termini in the crystal structure suggests that the receptors have to be embedded in the same cell membrane.

PirB/LILRB2 is another receptor for the three MAIs

The fact that NgR knockouts did not completely abolish inhibitory effects of the MAIs suggests the presence of other receptors to mediate inhibitory signaling. The single-pass type I transmembrane protein Leukocyte immunoglobulin (Ig)–like receptor B2 (LILRB2) and its mouse homologue paired Ig-like receptor B (PirB) are able to bind Nogo-66. Furthermore, OMgp and MAG also bound to cells expressing PirB. Treatment of mouse CGNs with function-blocking antibodies to PirB lead to reduced inhibition by an AP-Nogo-66 fusion or myelin. Furthermore, neurons from mutant mice expressing a PirB variant lacking the transmembrane domain and part of the intracellular domain were less inhibited by AP-Nogo-66 or myelin as compared to wild-type neurons. When comparing PirB and NgR, there was a difference in growing on an inhibitory substrate versus the induction of growth cone collapse by MAIs. Whereas no enhanced neurite outgrowth was seen for NgR knockouts growing on a myelin or AP-Nogo-66 substrate, there was a complete relieve of growth-cone collapse for NgR knockouts as compared to wild-type neurons. For PirB, both growth-cone collapse and inhibition of neurite outgrowth by Nogo-66 or myelin could be partially overcome by α-PirB. However, NgR knockout did augment the disinhibitory function of α-PirB when growing on an AP-Nogo-66 or myelin substrate[134].

The fact that MAG inhibition could be mediated by receptors other than NgR and NgRL1 can also be explained by the discovery of PirB as an MAI receptor[42]. If PirB would bind to MAG domain 4 and 5 and NgR to domain 1-3, this could also explain the discrepancy between the reports about different MAG domains being responsible for inhibition[42,43].

PirB mRNA and protein levels were shown to increase upon damage of cortical neurons in newborn rats and PirB antibody treatment resulted in enhanced regeneration *in vivo*[135]. MAI binding to PirB has been shown to recruit both Trk neurotrophin receptors and p75, both of which are necessary to mediate downstream signaling. Whereas Trks interacted with PirB only via the intracellular domain, both the intracellular and extracellular parts of p75 contribute to the binding of p75 to PirB[136,137].

Whereas human LILRB2 has four N-terminal extracellular Ig-like domains, mouse PirB possesses six. These are preceded by a 24 residue signal peptide for secretion that is proteolytically removed in the ER. The six Ig-like domains are followed by a transmembrane helix and a 178 residue cytosolic C-terminal domain, which contains 4 immunoreceptor tyrosine-based inhibitory motifs (ITIMs)[138]. PirB on B-cells and myeloid cells was earlier identified as an inhibitor of Major Histocompatibility Complex (MHC) class I signaling, whereas its counteracting homologue PirA was found to act as an activator for MHC class I signaling[139,140]. The human PirA homologue LILRA2 also shares a high similarity with the PirB homologue LILRB2. Since both PirA and LILRA2 both lack the ITIMs, LILRA2 likely also is an antagonist of LILRB2.

Crystal structures of the two N-terminal Ig-like domains of LILRB1, LILRB2 and PirB in different complexes with binding partners of the MHC class are available[141–143]. The co-crystallized binding partners are the MHC class I molecule HLA-A2 for LILRB1, the non-classical MHC molecule HLA-G for LILRB2 and the human cytomegalovirus class I MHC mimic protein UCL18 for PirB. All structures show a sharp angle between the two Ig-like domains (see fig. 12), but important differences are present between PirB/LILRB2 and LILRB1. For example, the D1 domain of LILRB2 has a larger binding interface on the α3 domain of HLA-G, as compared to that of LILRB1 with HLA-A2. Other remarkable features are two polyproline type II helices in PirB and one or two 3_{10} helices in all the structures. Similar to NgR binding of Nogo, the interaction of Nogo with PirB is enhanced by binding of the same oligopeptides N- and C-terminal of the two hydrophobic stretches enclosing Nogo-66[66].

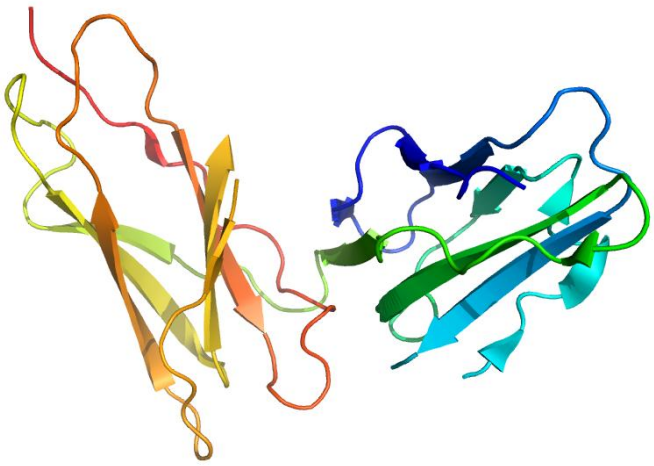


Fig. 12: Cartoon representation of a crystal structure of LILRB2. Based on PDB accession code 2gw5[142].

PirB knockout also results in an extension of the critical period for ocular dominance plasticity, confirming a role in the restriction of neuronal plasticity similar to NgR[144]. However, two PirB knockout studies in mice by the same group report no enhanced regeneration after SCI *in vivo*[145,146]. No functional recovery was seen and neither was there a difference in the number of sprouting fibres in the corticospinal or corticorubral tract. This can be explained by a redundant receptor model, where the NgR-p75-LINGO-1 complex and PirB can act independently as receptors for the MAIs. Another possible explanation is that there are even more receptors for inhibitory cues.

Other receptors for MAIs

The N-terminal domain of Nogo-A (Amino-Nogo) inhibits axonal outgrowth and cell adhesion via a mechanism independent of NgR. It was found that Amino-Nogo does so by a preventing integrin activation, by means of a direct physical interaction. It was shown that $\alpha v\beta 3$, $\alpha 5$, and $\alpha 4$ integrins, but not $\alpha 6$ integrins are

sensitive to Amino-Nogo. Since both αv and α5 integrins are prevalent in the brain and in axonal growth cones, this interaction may contribute to the inhibitory activity of Nogo-A[147]. MAG also contains the canonical integrin-recognition motif RGD, which overlaps with the FRG motif responsible for ganglioside interaction (the sequence is FRGD).[40] Indeed MAG is also able to bind β1-integrins and mutating residues in this tripeptide abolishes this interaction. OMgp showed no interaction with integrins. MAG on the other hand was able to stimulate tyrosine phosphorylation of focal adhesion kinase (FAK), a downstream target of integrins. [41,148]. The fact that the integrin interaction site of MAG overlaps with the sialic acid binding site, raises the question of whether there is competition between gangliosides and integrin for MAG and if so, whether this would be functionally relevant.

Remarkably, MAG was found to bind OMgp and the interaction was stronger than that of OMgp or MAG for NgR[53]. The fact that OMgp is expressed to higher levels on neurons than on oligodendrocytes[83], suggests that this signaling might be relevant. Studies identifying NgR as the principal receptor for MAG, use phosphatidyl-inositol–specific phospholipase C (PI-PLC) treatment to demonstrate that the MAG-receptor was GPI-anchored[89,90]. Indeed, MAG-binding was strongly reduced after PI-PLC treatment. However, the fact that OMgp is also GPI-anchored, has a high affinity for MAG and might have been present on neurons as well puts these results in a different perspective. No signal transducers like p75/TROY and LINGO-1 for NgR have been identified for OMgp as of yet. OMgp might also function as a negative regulator of MAG signaling, by scavenging free MAG and thereby preventing signaling through the NgR-p75/TROY-LINGO-1 or PirB-p75-Trk complexes. The function of the OMgp-MAG interaction may also be solely structural, providing cell adhesion between myelin and the axon. Another possibility is that neuronal OMgp binds NgR, either supporting or preventing interaction with MAG. In the first scenario, a ternary complex of OMgp, MAG and NgR can be formed. This is possible since no competition between MAG and OMgp for NgR has been demonstrated. In the second scenario, MAG may distort the interaction of neuronal OMgp with NgR by binding OMgp, making NgR available for other MAIs. However, these hypothetical mechanisms are highly speculative and need further investigation.

Gangliosides

Shortly after the identification of MAG as an MAI, MAG was also shown to be a member of a sialoadhesin family of sialic acid-binding proteins. It was shown that sialylated glycans mediate MAG binding to neurons[149]. It was first proposed that sialylated glycoproteins mediated MAG-induced inhibition[150]. However, the neuronal molecules carrying these sialic acids were later identified as gangliosides; glycosphingolipids based on a ceramide hydrophobic platform, which indeed contain terminal sialic acids. Only the GT1b, GD1a and GQ1ba gangliosides showed specific MAG-mediated cell adhesion. Neuraminidase treatment, which removes terminal sialic acids, abolished MAG-mediated adhesion. Based on a comparison of 19 gangliosides, a negatively charged α2,3-N-acetylneuraminic acid (Neu5Ac, which is a sialic acid) appeared to be essential for MAG binding[151,152]. A clue for *in vivo* functionality for this interaction was provided by knockout mice that could not produce complex gangliosides. These suffered from distorted myelination and Wallerian-like degeneration, similar to MAG knockouts[153]. A double knockout of MAG and the Galgt1 gene responsible for complex ganglioside formation only showed a modest increase in phenotype as compared to the similar phenotypes of single knockouts, supporting the hypothesis that interaction between MAG and gangliosides contributes to axon-myelin stability[154].

Subsequently, it was shown that the GT1b is a functional ligand for MAG. Neuraminidase treatment, α-ganglioside monoclonal antibodies and blocking of ganglioside biosynthesis, either by inhibitors or by genetically modifying the terminal structures of nerve cell surface gangliosides, attenuated MAG-mediated neurite outgrowth inhibition. Furthermore, GT1b clustering (and not GD1a clustering) mediated by antibodies resulted in a MAG-like inhibitory response for neurite outgrowth[155,156].

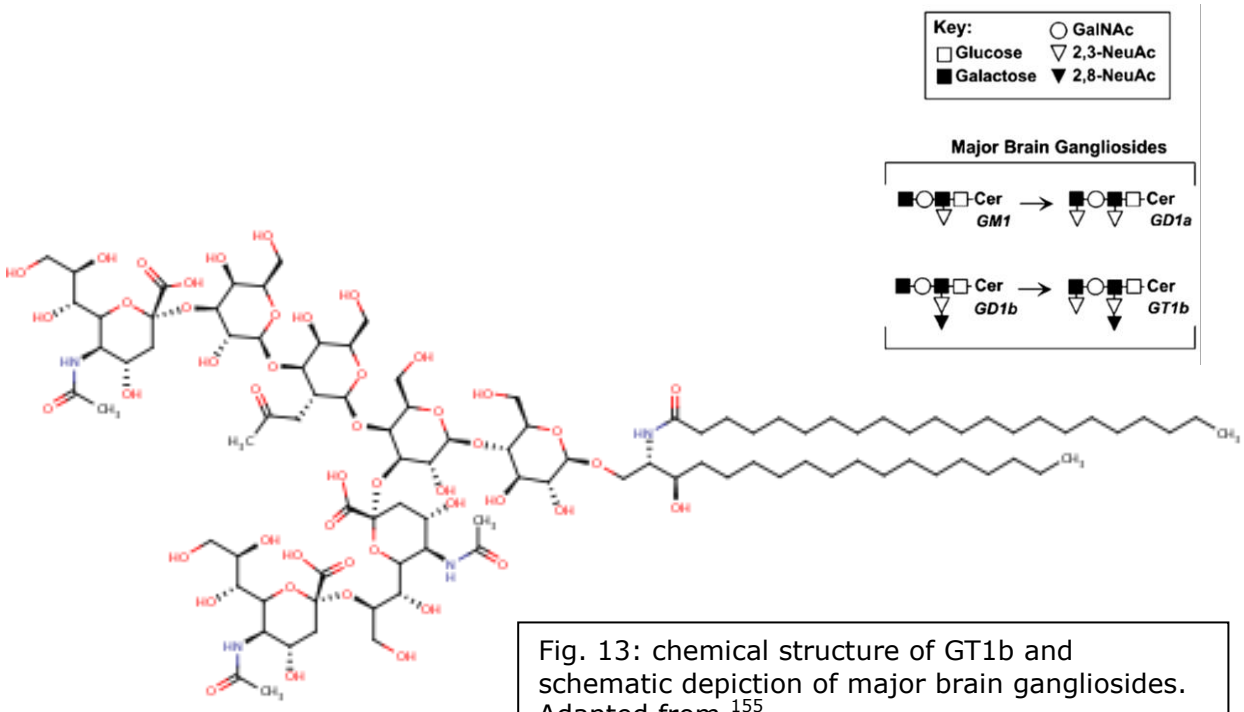


Fig. 13: chemical structure of GT1b and schematic depiction of major brain gangliosides. Adapted from [155].

Site-directed mutagenesis, X-ray crystallography and NMR spectroscopy have been employed to investigate sialic acid binding of the MAG homologue sialoadhesin[157–159]. Structures of complexes with sialic acid-containing molecules (sialyllactose and a glycopeptide) by X-ray crystallography show how proteins of the sialoadhesin family recognize sialic acid. Arg97 (corresponding to R118 in MAG) forms a salt bridge with the carboxylate group of Neu5Ac. Other amino acids important in recognition are Trp2 and Trp106, the former of which is conserved and the latter is replaced by a tyrosine in MAG. The V-type Ig-like domain of sialoadhesin itself show no drastic conformational changes upon binding. It was shown that R118 in the N-terminal Ig-domain, which is located in an FRGD motif, is essential for the interaction of MAG with sialic acid-containing gangliosides[156,160]. This binding site overlaps with the integrin binding motif of MAG (RGD, residues 118-120)[41], so simultaneous binding of gangliosides and integrin to MAG is excluded.

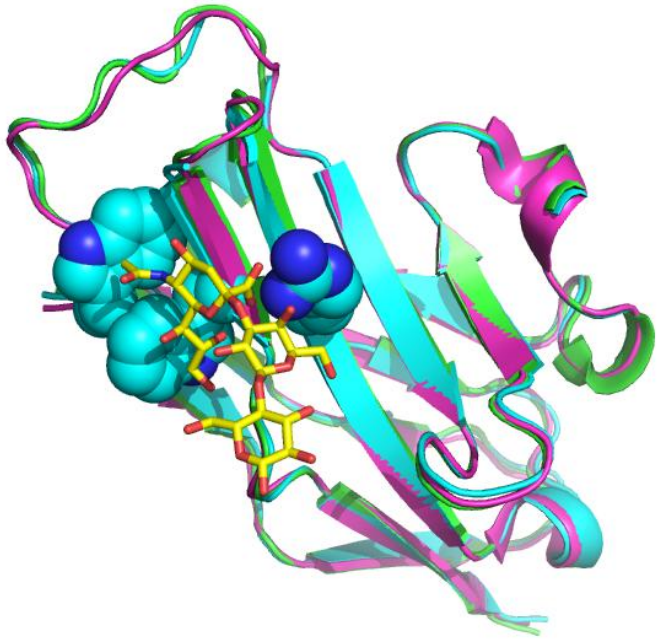


Fig. 14: Cartoon representations of crystal structures of the V-type Ig-domain of sialoadhesin. Residues R97, W2 and W106 are shown as spheres, sialyllactose as sticks (yellow). Based on PDB accession codes 1qfo, 1qfp and 1url.[158]

Paradoxically, it was also shown that MAG can signal independently from gangliosides (and integrins), because abolishing the ganglioside interaction by mutating R118 did not impair MAG-mediated inhibition. Furthermore, a construct consisting of the first three domains (containing the sialic acid binding site at R118) fused to an Fc-domain could not inhibit neurite outgrowth, suggesting a dependence of the fourth and fifth Ig-like domain[160]. This was confirmed by a study demonstrating that the inhibitory function of MAG resides in the fourth and fifth Ig-like domain[42]. However, this study also finds that inhibition is largely independent of NgR and NgRL1. This result could be explained by the presence of PirB/LILRB2, of which the identification was only published when this study was under review. A report describing the direct interaction between NgR and MAG also claims that the interaction is sialic acid independent, as sialidase treatment did not impair binding[89]. A study that compared the ability of different neurons to grow on a MAG expressing substrate, showed that MAG can signal independently via gangliosides or NgR. DRG neurons were less inhibited after both sialidase treatment (abolishing ganglioside-mediated inhibition) and PI-PLC treatment (removing GPI-anchored proteins like NgR from the cells). The latter however exhibited a stronger effect than sialidase treatment. Combined treatment augmented the relief of inhibition, suggesting a cumulative effect. However, hippocampal neuron outgrowth was more sensitive to sialidase treatment than PI-PLC treatment. CGN neurite outgrowth inhibition was only sensitive to sialidase treatment, implying that MAG inhibitory signaling can happen via different

receptors in different cell types. These results were confirmed by treatment with ganglioside biosynthesis or NgR inhibitors[161].

Similar to MAG, NgR also contains FRG motifs (three in total, at residues 150-152, 198-200 and 278-280). Indeed, NgR is also able to bind GT1b and mutating the arginine residues in either of these three triplets reduces binding. However, the phenylalanine residues of the first two FRG motifs seem to be located in the core of the protein as part of the phenylalanine spine, so might not contribute to GT1b binding. As with MAG, the interaction happens via the terminal sialic acid residues of GT1b. A cyclic peptide containing the FRG motif abolished the inhibition of CGN neurite outgrowth caused by MAG or α-ganglioside antibodies. However, Nogo-binding appeared to be independent of gangliosides.[162] Similar to NgR, binding of its homologue NgRL1 to MAG was also shown to be sialic acid-dependent[95].

Later, it was found that gangliosides can recruit p75 to lipid rafts, if induced by either MAG-Fc or α-ganglioside antibodies. Recruitment of p75 was shown to be necessary for signal transduction. It was shown that both gangliosides and GPI-anchored proteins (e.g. NgR or OMgp) are necessary for signal transduction[163]. Another report described a direct interaction between p75 and GT1b (but not GD1a), that is SDS resistant[106].

LINGO-1 has been found to bind gangliosides too in a sialic acid-dependent manner. The presence of GT1b enhanced the affinity of LINGO-1 for NgR, but did not influence the affinities of LINGO-1 or NgR for p75, which also bind to GT1b. This might be explained by the quaternary structure of the complexes. Remarkably, p75 presence enhanced the interaction of Nogo with the NgR-LINGO-1-GT1b complex, but reduced the affinity if GT1b was missing[126]. These results suggest that GT1b may serve as a glue holding the different receptor components together, as well as enhancing the affinity of MAG for the receptor complex.

Combined knockout of NgR1, NgR2 and desialylation by *Vibrio cholerae* neuraminidase (VCN) only decreased MAG-induced inhibition by 65%[42]. However, the remaining inhibitory activity may have been mediated by PirB, which was only found to be a MAG receptor during publication of this report.

Summarizing, both MAG, NgR, p75 and LINGO-1 can bind gangliosides with terminal sialic acids like GT1b, although for the last two it remains unclear which residues are responsible for binding. No interactions have been reported for Nogo, OMgp or PirB. Presence of GT1b seems to enhance the signaling of MAG, although it is not essential.

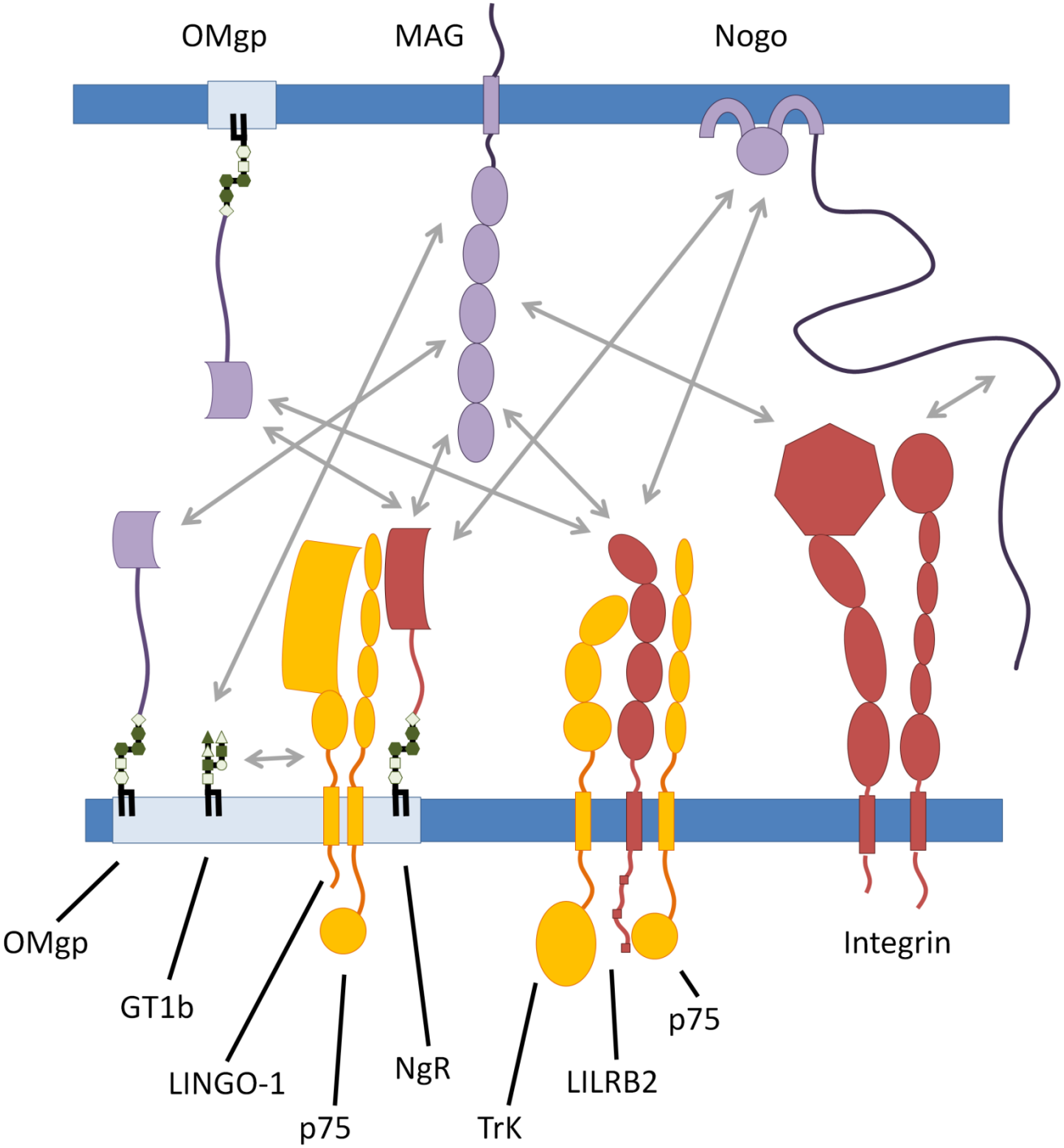


Fig. 15: Overview of the three MAIs and their neuronal receptors, showing the different interactions by grey arrows. MAIs are colored purple, receptors red and co-receptors yellow. Membranes are blue and sugar groups (of the GPI anchors and of GT1b) are green. Lipid rafts are indicated in light blue for molecules that are expected to localize these membrane patches. Nogo-A/B is shown with an all extracellular topology, but other topologies might also be possible. The long unstructured N-terminal tail is missing in Nogo-C. TROY can substitute for p75 in the NgR-p75-LINGO-1 complex. Note that GT1b interacts with al three receptor components of the NgR receptor complex.

Injury-induced CSPG secretion by astrocytes inhibits regeneration.

Gangliosides appeared not to be the only sugar-based molecules involved in myelin inhibition. Chondroitin sulfate proteoglycans (CSPGs) are a component of the extracellular matrix (ECM) in different tissues, including the CNS. They consist of a protein core with a varying number of (up to 150) chondroitin sulphate glycosaminoglycan chains, each of which can consist of over 100 individual sugars. Chondroitin sulfate is a linear polymer composed of a chain of alternating glucuronic acid sugars and either N-acetylgalactosamine or N-acetylglucosamine, which can be sulphated at different positions. This results in high-molecular weight (in the megadalton order of magnitude) and highly negatively charged molecules[164]. CSPGs present in the CNS include aggrecan, neurocan, versican, brevican, NG2 and phosphacan/DSD-1. They are abundant in the ECM and have been shown to interact with other ECM components such as laminin, fibronectin, tenascin, hyaluronic acid (HA) and collagen. These interactions can happen both via the protein or the chondroitin sulphate chains[165].

A substantial body of evidence shows upregulation of the CNS CSPGs neurocan, brevican, versican, phosphacan and NG2 upon injury. They are secreted by astrocytes, oligodendrocyte progenitor cells and microglia[166–176]. Furthermore, versican, neurocan, brevican and NG2 have been shown to be inhibitory for neurite outgrowth[168,177–183].

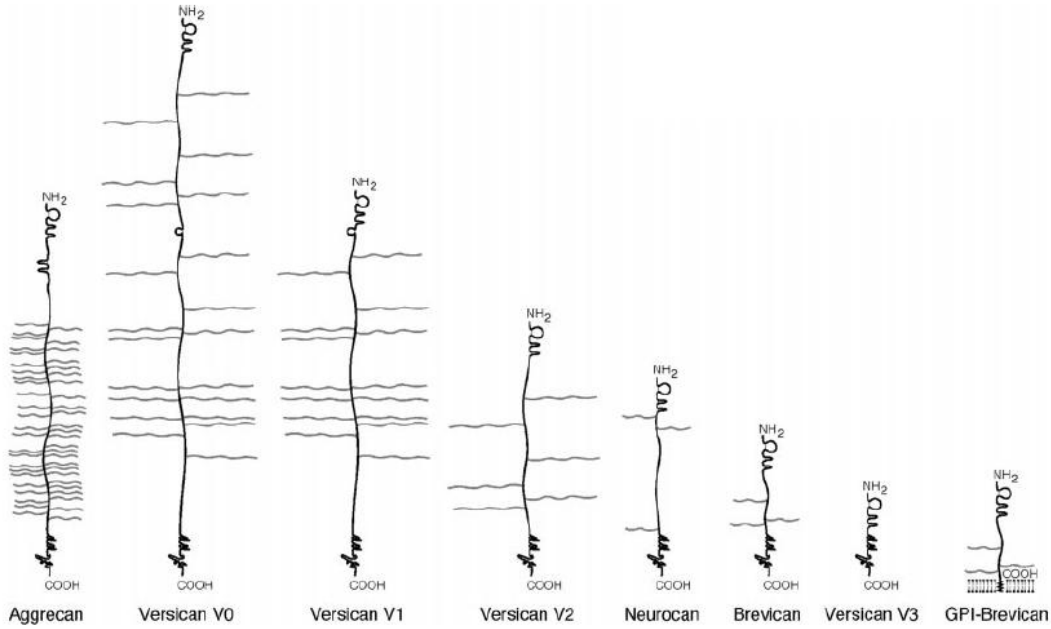


Fig. 16: Architecture of Major brain CSPGs; the glycosaminoglycan chains are indicated horizontally. Adapted from [164].

Recently, the protein tyrosine phosphatase sigma (PTPσ) receptor was identified as a receptor for neurocan, aggrecan and possibly other CSPGs[184]. Also, the repulsive axonal guidance receptor Semaphorin 5A was shown to directly bind to CSPGs, providing another mechanism by which CSPGs can mediate inhibition of neurite growth[185].

Similar to NgR and PirB, CSPGs appear to be involved in inhibiting ocular dominance plasticity after the critical period. ChonABC treatment could reactivate cortical plasticity, similar to NgR or PirB inhibition[186]. Indeed, NgR, as well as NgRL2, appeared to be receptors for the chondroitin sulfate chains of CSPGs too.

Combined knockout of these receptors and PTPσ resulted in a strong reduction of CSPG-mediated inhibition. Unlike the interaction with MAIs, the CSPG interaction with NgR and NgRL2 happened at the C-terminal stalk domain instead of the N-terminal LRR-domain[187]. This report demonstrates a direct link between MAI- and CSPG-mediated inhibition of neurite outgrowth and shows a function for NgRL2.

Other inhibitors and their receptors

MAIs and CSPGs are not the only inhibitors of neurite growth. Several other proteins and protein families are also inhibitory to neurite growth. Their signaling is generally not considered as canonical MAI signaling. Therefore, these will be summarised and not discussed as elaborately as the other MAIs. Their receptors will be discussed as well.

Semaphorins are a family of dimeric growth guidance signaling molecules, their general function in the CNS being the guidance of axonal growth cones[188]. They signal to their neuronal plexin receptors by inducing oligomerisation[189]. Sema6A was found to be upregulated at lesions in the CNS, suggesting a role in the inhibition of regeneration[190]. Sema3A (also known as Collapsin-1, Sema D or Sema III) is shown to repel axons,[191] is inhibitory for axon growth *in vitro*[192] and *in vivo*[193] and can induce axonal growth cone collapse[194] via its neuropilin/plexin A1 receptor complex[195,196]. Remarkably, p75 is recruited to the neuropilin/plexin complex upon Sema3A stimulation. It interacts with both receptor components and reduces the interaction of the two receptor components. Based on different single and combined knockouts of p75 and Sema3A in mice, p75 is suggested to be a negative modulator of Sema3A signaling to the neuropilin plexin complex[197]. Downstream signaling is thought to happen via the cytosolic collapsin response mediator proteins (CRMPs) like the chicken CRMP-62[198]. Cyclic nucleotides like cGMP and cAMP could convert the repulsive signal of Sema3A to attractive[199].

Similarly, the oligodendrocyte-expressed transmembrane semaphorin Sema4D/CD100 was shown to be inhibitory for axon growth. Furthermore, expression was upregulated after lesion[200]. Another oligodendrocyte-expressed semaphorin implicated in inhibition of axonal regereration is Sema5A. It is able to inhibit growth and collapse growth cones of retinal ganglion cells (RGCs)[201]. Notably, Sema5A appeared to bind to the glycosaminoglycan chains of CSPGs and heparan sulphate proteoglycans (HSPGs). CSPG binding to Sema5A was found to convert Sema5A from an attractive to an inhibitory cue for axon guidance, similar to the effect of cyclic nucleotides on Sema3A[185].

Another family of proteins inhibitory for neurite outgrowth and regeneration are the ephrins. Ephrin-B3 is known to act as a midline repellant for axonal growth during embryonic axonal pathfinding in the corticospinal tract. However, it is still expressed by postnatal oligodendrocytes and indeed is inhibitory for neurite outgrowth in the mature CNS[202]. Furthermore, knockout of the EphA4 neuronal receptor for Ephrin-B3 results in enhanced axonal regeneration and functional recovery after spinal cord hemisection[203]. Whereas no EphA4 protein could be detected in the uninjured adult rat corticospinal tract, EphA4 was upregulated in axon stumps proximal to a dorsal column hemisection. Another ligand for EphA4, Ephrin-B2 is also upregulated in astrocytes in the glial scar.[204] Other Ephrin receptors like EphA3 and EphA7 also show marked upregulation in neurons and astrocytes after injury[205,206].

A third family of axonal guidance cues are the netrins. Like semaphorins and ephrins, they can repel neuronal growth cones, but they can also acts as chemoattractants[188]. Netrin-1 is a secreted glycoprotein that associates with membrane patches by means of a positively charged alpha helical C-terminus[207].

In adult organisms, netrin-1 is expressed by oligodendrocytes, cells of the central canal and the meninges. The neuronal receptors UNC5 and neogenin mediate repellant signaling by Netrin-1. Similar to the MAIs, semaphorins and ephrins, signaling of netrin-1 through UNC5 mediates inhibition of axonal regeneration after SCI in adult rats[208].

Repulsive guidance molecule a (RGMa) is another repelling axonal guidance cue, that is upregulated upon injury of the brain and spinal cord[209,210]. Like the aforementioned proteins, RGMa is an inhibitor of neurite growth in the CNS. It is expressed by oligodendrocytes, in myelinated fibres and in neurons of the spinal cord. Signaling is dependent on the Netrin-1 receptor neogenin[211].

Wnt proteins are a family of secreted developmental regulators that also function as axonal guidance cues in the CNS. They can both attract ascending axons and repel descending axons in the spinal cord. Although their expression is not detectable in the adult spinal cord, Wnt1, Wnt4 and Wnt5a are upregulated upon SCI[212]. Indeed, the signaling of Wnt5a to its neuronal receptor Ryk was found to inhibit axon growth and functional recovery in rats[213].

Lynx1 appeared to be a negative regulator of ocular dominance plasticity after the critical period. It signals through the nicotinic acetyl choline receptor (nAChR), suggesting a mechanism distinct from CSPG, NgR or PirB regulation of ocular dominance plasticity[214].

However, several other proteins do bind to NgR. B lymphocyte stimulator (BLyS), a tumor necrosis factor family protein essential for B-cell development, was identified as another functional ligand for NgR in the CNS. Its inhibitory effect was significantly reduced in NgR knockout mice. However, unlike the MAIs, BLyS is not expressed by oligodendrocytes but instead by astrocytes and microglial cells at injury sites[215]. Leucine-rich glioma inactivated 1 (LGI1) is a secreted protein that binds to NgR and antagonises MAI-mediated inhibition. It recruits ADAM22, which directly interacts with NgR to form a ternary receptor complex[216].

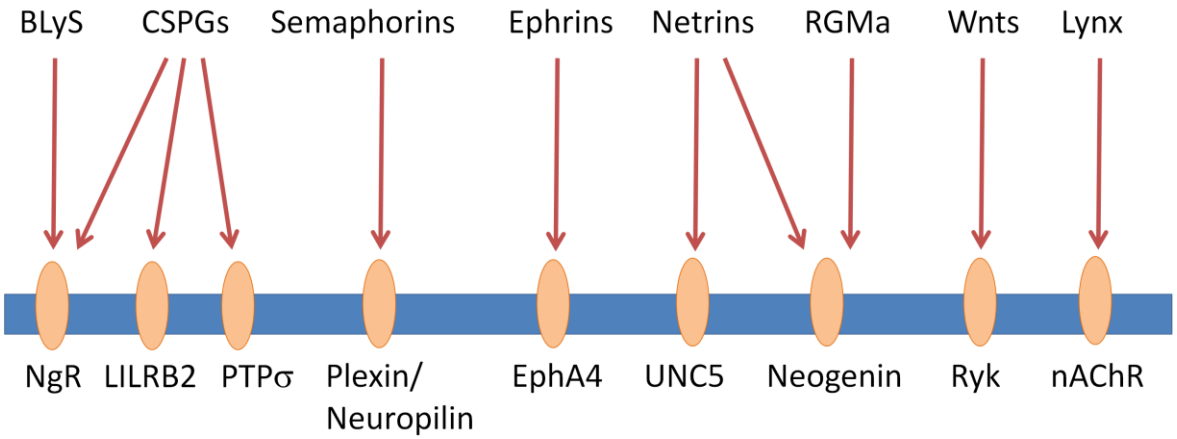


Fig. 17: Other molecules inhibitory for neurite outgrowth and their neuronal receptors.

Combined knockouts and in vivo function of MAIs

Apart from the knockout and knockdown studies of inhibitors and receptors mentioned in their respective paragraphs before, a number of combined knockout and knockdown studies have been performed as well. These show insight in the *in vivo* functions of these proteins.

A mouse knockout study that investigated the roles of Nogo and MAG in myelin sheath formation in the developing optic nerve found a delay in oligodendrocyte differentiation, myelin sheath formation and axonal caliber growth in Nogo knockouts. Even more severe hypomyelination was the result of combined Nogo/MAG double knockouts. MAG knockout also resulted in malformation of the structure of the myelin sheath and the nodes of Ranvier. MAG knockout also led to upregulation of NgR, whereas Lingo-1 expression levels remained unchanged.

A knockout study of NgR and p75 in mice, that also investigated the role of gangliosides by VCN desialylation, found that neither knockout of p75 or NgR, nor VCN treatment was sufficient to release MAG-mediated inhibition. TROY could not substitute for p75 in the RGCs. However, combination of NgR knockout and VCN treatment resulted in enhanced neurite growth[217].

Another mouse knockout study investigated the roles of NgR and Nogo in the regulation of plasticity after ischemic stroke. Knockout of either NgR or Nogo-A/B leads to enhanced recovery after stroke, as well as enhanced axonal plasticity and regeneration[218]. These results implicate that the inhibitory roles of NgR and Nogo are not restricted to the spinal cord, but are also present in the brain.

A p75 and NgR knockdown demonstrated an increased ability of DRG neurons to regenerate upon knockdown, as well as an increase in βIII-tubulin protein expression levels. It was also demonstrated that CNS myelin impedes FGF2-induced neurite outgrowth[219].

One study compared single, double and triple knockouts of the three MAIs Nogo, MAG and OMgp in mice. This showed a prominent role for inhibition by Nogo, whereas combined knockout of OMgp and MAG did not result in a strong relief of inhibitory effects. However, triple knockout of Nogo, MAG and OMgp did result in even better regeneration and functional recovery, compared to either Nogo single or MAG-OMgp double knockouts[220]. Shortly after publication of this study, another knockout study also assessed Nogo-MAG-OMgp single and triple knockout mice for their ability to regenerate after injury. Although they also found inhibitory effects that were strongest for Nogo, they did not see enhanced regeneration in triple knockout mice[221]. Differences may have been caused by the fact that the two studies employed different injury models (single dorsal hemisection in the former, dorsal hemisection, complete transection, pyramidotomy and lateral hemisection in the latter) as well as a different Nogo knockout strategy (resulting in knockout of Nogo-A and –B in the former and of all three isoforms in the latter). Also, the functional recovery was assessed by different assays and the former study did not distinguish sprouting from regeneration, whereas the latter used separate definitions[221]. The fact that numerous other signaling systems (e.g. semaphorins, ephrins, netrins, Wnts etc.) have also been shown to contribute to inhibition after injury might explain the general lack of complete relief of inhibition in the knockout studies.

Both knockout of Nogo-A, treatment with the IN-1 antibody against Nogo, antibodies against LINGO-1 or NgR or inhibiting ROCK leads to longer neurites, increased fasciculation and decreased branching of cultured DRG neurons. Knockout of Nogo-A also results in increased fasciculation and reduced branching

of peripheral nerves in mouse embryos. Injection of IN-1 in chicken ova leads to atypical innervation of the hindlimb. These results clearly demonstrate a role for Nogo-NgR signalling in the developing nervous system.[222] Developmental effects in differentiation, neurogenesis and myelination were also seen for p75, LINGO-1, MAG and OMgp[33,85,86,132,133,223].

MAI signaling regulates neuronal plasticity

Fibroblast growth factor 1 and 2 (FGF1 and FGF2) are secreted proteins that bind with high affinity to NgR, but not NgRL1 or NgRL2. The interaction is mediated by the N-terminal LRR domain of NgR. NgR1 and the FGF receptor (FGFR) are shown to colocalise at synapses and the interaction is hypothesized to regulate dendritic spine morphology and activity-dependent synaptic strength[224]. The function of NgR as a regulator of synaptic plasiticity sheds light on the *in vivo* function of the receptor and suggests a mechanism for its role in inhibiting ocular dominance plasticity after the critical period[100]. The function of PirB and CSPGs in ocular dominance plasticity regulation further solidifies the hypothesis that these molecules negatively regulate neuronal plasticity[144,186].

A function of NgR in plasticity was also demonstrated by a genetic approach employing a constitutively overexpressed NgR transgene in mice. This resulted in normal short-term memory (24 hours) and long-term potentiation, but long-term memory (months) was severely impaired[225]. Furthermore, knockout studies on NgR and PirB also showed a role for these proteins in coordinating structural and functional plasticity. Nogo-66 and OMgp were found to negatively regulate plasticity by suppressing long-term potentiation, a mechanism for altering the stability of synapses[226]. Studies investigating the function of Nogo-NgR signaling in the brain used antibody treatment, Nogo-A and NgR knockout and shRNA-mediated knockdown of Nogo-A and NgR to interfere with Nogo-NgR signaling. These experiments had similar results of distorted dendrite structure, increased axonal complexity and length. Dendritic spines showed an immature distribution phenotype[218,227]. Application of the anti-Nogo antibody IN-1 induced spontaneous sprouting from uninjured Purkinje cells and spontaneous sprouting by afferent neurons also occurred in a demyelinated spinal cord[19,20]. These results suggest a role for MAI signaling in stabilizing and maintaining the architecture of neuronal circuitry.

Downstream Events

Whereas the triggering of signaling of MAIs and other molecules inhibitory for axonal regeneration happens in the extracellular space, the downstream effects must happen intracellularly. p75, TROY, Lingo-1 and PirB have cytoplasmic domains that are responsible for downstream effects. The exact mechanisms that lead to downstream signaling are however not entirely clear yet, but several mechanisms and downstream effectors have been identified.

MAIs recruit p75 to lipid rafts to initiate signaling

GPI-anchored proteins are known for their property to preferentially reside in lipid rafts, microdomains in the plasma membrane that are enriched in cholesterol and sphingolipids. Indeed, NgR and p75 were present in lipid raft extracts from the rat brain. In postnatal day (PND) 8 brain samples, p75 was detected abundantly in detergent sensitive membrane fractions, whereas NgR was localized exclusively in the lipid raft fractions. In adult rat brain fractions, p75 was relatively more prevalent in the lipid raft fractions compared to the PND 8 brain samples, suggesting a correlation between p75 localization in lipid rafts and developmental loss of plasticity. Direct binding of NgR to p75 was confirmed in the lipid rafts. Furthermore, it was shown that disruption of the lipid rafts prevented Nogo-signaling[228].

Gangliosides are also known to preferentially reside in lipid rafts, because of their ceramide/sphingomyelin lipid part. Both MAG-Fc and anti-ganglioside antibodies were able to recruit p75 to lipid rafts. Knockout of GalNAcT, an enzyme responsible for the biosynthesis of complex gangliosides, resulted in insensitivity to MAG. This lead to the hypothesis that gangliosides might mediate the recruitment of p75 to lipid rafts in the case of MAG inhibition. This study also showed that lipid rafts were necessary for both MAG and Nogo mediated inhibition of neurite growth and axonal growth cone collapse[163]. Furthermore, receptor clustering using preclustered Nogo, MAG or polyclonal anti-p75 antibodies enhanced downstream signaling and growth cone collapsing effects[115,229].

p75 activates RhoA

The fact that p75 seems to be vital for signal transduction, but lacks a cytoplasmic domain with enzymatic activity (as opposed to other neurotrophin receptors, such as the tyrosin receptor kinases TrkA and TrkB), raises the question of how it can mediate this signal transduction. Already before p75 was identified as a signal tranducer for MAI signaling, RhoA was known as a downstream effector of neurotrophin signaling to p75.[106] RhoA is a cytosolic small (193 residue) guanine triposphatase (GTPase) of the Ras homology (Rho) family, that can associate with membranes by means of a geranylgeranylated (a 20-carbon isoprenoid chain PTM) cysteine. Small GTPases are considered to be molecular switches, which can be in an active GTP bound state or an inactive GDP bound state.[230]

A direct interaction of the cytoplasmic DD of p75 with RhoA was demonstrated. Furthermore, p75 acts as an activator of RhoA, since its presence results in an increase of cellular RhoA-bound GTP. Neurotrophin binding to p75 prevents the RhoA-activator function of p75[231]. RhoA was also found to be significantly upregulated after SCI[232].

These results suggested that RhoA activation might also be involved in MAI-mediated signaling through p75. Indeed, treatment of PC12 cells with the *Clostridium botulinum* toxine C3, which inactivates RhoA (as well as the

homologous small GTPAses RhoB and RhoC) by ADP-ribosylation of Asn41, elicits neurite growth on myelin or MAG substrates. Furthermore, C3 treatment stimulated regeneration in crushed optic nerves *in vivo*[233]. Another report showed a MAG-induced activation of RhoA mediated by p75 in DRG neurons, as well as in CGN wild-type mice[106]. Furthermore, it was shown that NgR, but not a truncated form that could bind the MAIs but not p75, was necessary for MAG-induced activation of RhoA[104]. Stimulation with amino-nogo did not result in altered RhoA activity levels as with Nogo-66[234].

The mechanism by which p75 stimulates RhoA activation, was reported to happen via an interaction of Rho GDP dissociation inhibitor (Rho-GDI) with p75[235]. Rho-GDI is a binding partner of RhoA that prevents GDP exchange for GTP and binds to its isoprenoid carbon tail[236]. p75 was shown to simultaneously interact with both RhoA and Rho-GDI. Both interactions could be abolished by neurotrophin treatment, whereas treatment with Nogo and MAG enhances the interactions. Furthermore, an interaction of endogenous p75 with Rho-GDI was only seen after stimulation with Nogo or MAG. Different experiments suggest that the cytosolic p75 DD functions by dissociating RhoA from Rho-GDI, by means of an interaction with the 5th alpha helix of the DD, thereby activating RhoA[235].

Kalirin9 is a RhoGEF involved in regulating neuronal morphology and neurite outgrowth. Apart from a RhoGEF domain, it also has a Rac-specific guanine exchange factor (RacGEF) domain[237]. Kalirin9 was found to bind p75-NgR via two distinct domains (not the RhoGEF or RacGEF domains) both *in vitro* and *in vivo*. It was shown to compete with Rho-GDI for p75 binding. However, MAG treatment strongly reduced the association of p75 with Kalirin9, concomitant with an increased interaction with Rho-GDI. Different experiments suggest that the released Kalirin9 binds to the RhoA that is also released upon association of the Rho-GDI-RhoA complex with p75, after which it can perform its guanine exchange function. Knockdown of Kalirin9 in CGN neurons also allows neurite outgrowth on a myelin substrate[238].

Although LINGO-1 possesses only a short 38 residue cytoplasmic domain, its necessity for MAI signaling suggests this domain might also be important for cytosolic signal transduction. This domain was shown to bind to the protein kinase "with no lysine 1" (WNK1) both *in vitro* and *in vivo*. Nogo-66 treatment enhances the interaction of endogenous WNK1 and LINGO-1. Knockdown of WNK1 in different neurons resulted in abrogation of the inhibition of neurite outgrowth by Nogo-66, as well as a decrease of Nogo-66-induced activation of RhoA. A specific interaction of WNK1 with Rho-GDI was demonstrated and Nogo-66 treatment of cortical rat neurons reduced the interaction of WNK1 with Rho-GDI. This suggests a mechanism in which MAI-induced recruitment of p75 (with Kalirin9) to NgR and LINGO-1 brings LINGO-1-WNK1-asssociated Rho-GDI/RhoA in the proximity of p75, resulting in dissociation and release of both RhoA and Kalirin9[239].

Since neurotrophin binding has opposite effects as MAI-mediated recruitment of p75 to NgR (and LINGO-1), one could speculate that the binding interface of neurotrophins on p75 overlaps with that for NgR or LINGO-1. This way, neurotrophins could compete with NgR/LINGO-1 for p75, resulting in less recruitment of p75 to lipid raft-localized NgR and LINGO-1. However, this hypothesis needs experimental verification.

MAI signaling is dependent on PKC activation and elevation of calcium levels

It was found that MAI signaling through RhoA depends on conventional protein kinase C (PKC) activation. Furthermore, it is demonstrated that both the MAIs and CSPG could activate PKC. PKC is a family of cytosolic regulatory protein kinases, of which the conventional PKC subfamily is activated by calcium and diacylglycerol (DAG), the product of phopholipase degradation of phosphatidylinositol 4,5-bisphosphate (PIP_2)[240].

MAG signaling causes an NgR- and p75-dependent influx of calcium, one of the prerequisites for PKC activation[105]. Calcium influx is suggested by different experiments to be dependent on activation of a G_i protein and its major downstream effector phospholipase C (PLC). PLC hydrolyzes PIP_2 to DAG and 1,4,5-triphosphate (IP_3). IP_3 stimulates calcium release from the ER. Indeed, inhibition of the ER-embedded IP_3 receptor prevents MAG-induced elevation of calcium levels. The elevated calcium and DAG levels can activate PKC, allowing RhoA signaling. However, despite its effect on calcium levels, inhibition of the IP_3 receptor ameliorated the inhibitory effects of MAG and Nogo in cerebellar neurons instead of preventing them[241]. This suggests that additional regulatory or feedback pathways exist. Moreover, the question remains how MAG stimulates G_i. It is known that many G protein-coupled receptors (GPCRs) couple to G_i, including neurotransmitter receptors like adrenergic, acetylcholine, adenosine, dopamine, glutamate, melatonin, serotonin, opioid and $GABA_b$ (gamma-aminobutyric acid) receptors. However, it remains unclear whether a GPCR and if so, which, is necessary for MAI signaling.

MAG signaling is also dependent on the presence of the calcium channel TRPC1 and on the peptidyl-prolyl isomerase (PPIase) activity of FKBP52. FKBP52 catalyses cis/trans isomerisation of proline residues in regions of TRPC1. The isomerisation of TRPC1 proline residues by FKBP52 PPIase activity is thought to regulate calcium-influx, suggesting a role for these proteins in the calcium-dependent signaling mechanism[242,243].

MAI- or CSPG-induced calcium influx also leads to epidermal growth factor receptor (EGFR) phosphorylation. Inhibiting the (intracellular) kinase function of EGFR blocks the inhibitory effects of MAIs and CSPG[244,245]. The exact mechanisms are not clear, but it is notable that the intracellular domain of LINGO-1 contains an EGFR-dependent phosphorylation site[124]. It has also been shown that LINGO-1 directly binds to EGFR extracellularly and that LINGO-1 suppresses EGFR activation[246]. Furthermore, activated EGFR is also localized to lipid rafts[247]. However, a more recent report disputes these findings and claims that only in astrocyte-expressed EGFR, phophorylation is detected and not in DRG neurons. Furthermore, this report argues that EGFR phosphorylation does not correlate with axonal regeneration[248].

Summarizing, MAIs activate G_i by an as of yet unknown mechanism, resulting in PLC activation, subsequent DAG and IP_3 production and calcium release, either from the ER through the IP_3 receptor or from the extracellular space through the TRPC1 calcium channel, which in turn is opened by FKBP52. Calcium and DAG allow PKC activation, which is necessary for MAI signaling and RhoA activation.

cAMP elevation reverses inhibition

The involvement of G_i, an inhibitor of adenylate cyclase-mediated conversion of ATP to the second messenger cyclic adenine monophosphate (cAMP), in the downstream signaling of MAIs suggests that cAMP signaling might also play a role in the downstream signaling cascade induced by MAIs. Indeed, it was found that a cAMP analog that is readily uptaken by cells (dibutyryl cAMP; db-cAMP) could reverse inhibition of neurite outgrowth on MAG-expressing cells. Inhibition of the cAMP-dependent protein kinase A (PKA) inhibits this block of inhibition, suggesting a signaling pathway through PKA[116]. Subsequently, it was reported that db-cAMP could induce regeneration of DRG neurons in CNS myelin *in vivo* after SCI[249,250]. Another interesting observation was that cAMP levels were dramatically higher in young neurons, but decreased steeply after 3 to 4 PNDs. This correlated with the switch of MAG from promoting to inhibiting neurite growth and PKA inhibition prevented MAG-induced promotion of neurite growth[33,34]. Earlier reports had described conditioning lesions; lesions in the PNS that stimulate regeneration of DRG neurons into the CNS after dorsal column lesion, presumably by affecting intrinsic neuronal molecular mechanisms[251]. It was found that these conditioning lesions resulted in cAMP levels of DRG neurons that increased up to 200% after a day. Myelin inhibitors did not affect DRG generation at these cAMP levels, whereas PKA inhibition could completely reverse this effect. After a week, cAMP levels return to normal and PKA inhibition only attenuates regeneration[250]. cAMP also stimulates transcription of arginase I to overcome inhibition of regeneration by MAIs. This enzyme catalyses the hydrolysis of arginine to ornithin and urea. This is the last step in the urea cycle, but also the first step in the polyamine synthesis pathway. It was demonstrated that cAMP-stimulated arginase I expression and subsequent initiation of polyamine synthesis prevented inhibition by MAG or myelin[252]. The cAMP responsive element binding protein (CREB) transcription factor was later shown to mediate cAMP induced transcription of arginase I. Elevated cAMP levels resulted in phosphorylation of Ser133 in CREB, activating arginase I transcription and polyamine synthesis[253]. How polyamines influence neurite outgrowth remains to be determined. However, elevation of polyamine levels after stroke have been linked to cytotoxicity and concomitant neuronal injury[254].

p75 proteolysis by secretases

Before the function of p75 in MAI signaling was identified, a study reported the release of a 50 kDa fragment of the p75 extracellular domain from neurons[255]. A more recent study aimed at resolving the mechanisms of MAI signal transduction, found that MAG causes sequential proteolytic cleavage of p75 by α- and γ-secretases. These proteolytic cleavages release an extracellular and a cytosolic domain from the membrane respectively. Both reactions are dependent on PKC activation. Most importantly, cleavage was necessary for both activation of RhoA and inhibition of outgrowth. This is not in conflict with the earlier reports of p75 signaling by Rho-GDI interaction, because in this study soluble cytosolic DD of p75 was used instead, which is similar to the released cytosolic fragment after γ-secretase cleavage[256]. Another report disputes these results and claim that secretase-mediated proteolysis of p75 promotes neurite outgrowth on a myelin substrate, instead of being a step in the signal transduction of MAI-induced inhibition of neurite outgrowth. They argue their results are more native-like (whole myelin extracts instead of MAG-expressing cells) and the fact that the α-secretase-cleaved extracellular domain might compete with the transmembrane form of p75 for NgR also supports their view. These findings suggest that the secretases provide a negative feedback loop, regulating the NgR signaling through p75[257].

MAIs deactivate the RhoA antagonist Rac1

Similar to RhoA activation, the different MAIs also exert effects on other small GTPases of the Rho family. Both Nogo and MAG stimulation of CGNs resulted in a decrease of the cellular levels of Rac1-bound GTP, as opposed to the increase that was seen for RhoA. These results suggest that the MAIs have antagonistic effects on RhoA and Rac1, activating the former but suppressing the latter[229]. This result makes sense, since Rac1 was already known as a regulator of neurite outgrowth with antagonistic (stimulating) effects compared to RhoA[258]. The mechanism by which MAG suppresses Rac1 activity might involve β-1 integrins, since competition by the RGD motif containing integrin ligand lamellin with MAG resulted in reduced suppression of Rac1[148].

RhoA activates ROCK, which initiates cytoskeletal remodeling

One downstream target of RhoA was found to be Rho-associated coiled-coil kinase (ROCK, also known as Rho-kinase or p160ROCK). It specifically binds GTP-bound RhoA, which activates it so as to allow autophosphorylation. Once activated, it can also phosphorylate other targets. Apart from an N-terminal Serine/Threonine kinase domain, it contains a long coiled-coil domain that contains the Rho-binding site, followed by a cysteine-rich zinc finger-like motif and a pleckstrin homology region in the C-terminus[259,260]. RhoA to ROCK signaling was found to regulate actin dynamics, by phosphorylating myosin light chain (MLC) and myosin light chain phosphatase (MLCP)[261]. Furthermore, RhoA to ROCK signaling enhances the formation of actin stress fibers and focal adhesions[262]. ROCK activation was found to be sufficient and necessary for neurite retraction. Apart from elevated myosin light chain phosphorylation levels, disassembly of microtubules and intermediate filaments was also observed[263]. Moreover, inhibition of ROCK resulted in enhanced neurite outgrowth on MAI substrates *in vitro* and in increased sprouting in CST fibers and functional recovery after lesion in adult rats[234]. ROCK activation also correlated with increased PirB expression in damaged cortical rat neurons, suggesting ROCK to be a downstream target[135]. Finally, CSPG inhibition of neurite outgrowth could be overcome by RhoA or ROCK inhibition, indicating that CSPGs make use of similar downstream pathways as the MAIs[264].

Other downstream signal transducers of ROCK are LIMK (Lin-11, Isl-1, and Mec-3 kinase), slingshot phosphatase 1 (SSH1) and the actin depolymerization factor cofilin. LIMK is a direct target of ROCK and ROCK-mediated activation by phosphorylation of LIMK stimulates cofilin inactivation by phosphorylation on a fast timescale (30 seconds). SSH1 subsequently reactivates cofilin by dephosphorylation on a slower timescale (30-60 minutes). These mechanisms were all induced by Nogo-66 in neurons and inhibition of LIMK circumvents myelin inhibition in chicken DRGs. Cofilin (in)activation by LIMK/SSH1 is another direct link to cytoskeletal rearrangements[265,266].

Amino-Nogo induces endocytosis

The Nogo-A-specific N-terminal peptide NogoΔ20 also inhibits axonal regeneration and neurite outgrowth by activating RhoA. A recent study found that NogoΔ20 is internalized by neurons in a Pincher and Rac1-dependent, but dynamin- and clathrin-independent fashion. Endocytosis results in RhoA activation and growth cone collapse. Endosomes are transported back from neurites to the cell body in DRGs, activating RhoA as it passes by. Once at the cell body, CREB phosphorylation is reduced. Endocytosis is not dependent on NgR[267].

PirB/LILRB2 downstream signaling

Although PirB/LILRB2 was only recently identified as a receptor for the MAIs, there is already some understanding about its downstream signaling. MAG-binding to PirB was shown to induce association with Trk neurotrophin receptors. Src homology 2-containing protein tyrosine phosphatase (SHP)-1 and SHP-2 are recruited to PirB upon MAG-binding. These then function as Trk tyrosine phosphatases to initiate downstream signaling[137,144]. p75 is also recruited to the complex of PirB and Trk upon MAG and OMgp binding. It was found that the intracellular domain of p75 was necessary for tyrosine dephosphorylation of Trk by SHP-1/2. A mechanism was proposed in which MAI binding to PirB leads to the recruitment of Trks and p75. Trk-mediated tyrosine phosphorylation of the ITIMs of PirB recruits the SHPs. This results in SHP activation by tyrosine phosphorylation mediated by the intracellular kinase domain of Trk that also needs the intracellular domain of p75, possibly as an adaptor. SHP subsequently dephosphorylates Trk and phosphorylated SHP and dephosphorylated Trk mediate further downstream effects. These effects were also seen in rat optical nerve injury models[136].

Another recent report identified three other downstream signalers for Nogo-66 induced PirB/LILRB2 signaling; plenty of SH3s (POSH), leucine zipper kinase (LZK) and Shroom3. Knockdown of any of these three proteins in cortical neurons with RNA interference (RNAi) results in release of myelin and Nogo-66 inhibition. LZK was found to function downstream of Nogo/PirB in a manner dependent on the scaffolding protein POSH[268]. An earlier report had already shown that POSH mediates inhibition of axonal growth by a pathway involving Shroom3, ROCK and myosin. The third SH3 domain of POSH was found to bind to Shroom3, whereas the ASD2 domain is thought to recruit ROCK. Myosin II was identified as a downstream target of the POSH-Shroom3-ROCK signaling[269]. It remains to be determined how SHP phosphorylation and/or Trk dephosphorylation influence LZK/POSH/Shroom3/ROCK signaling.

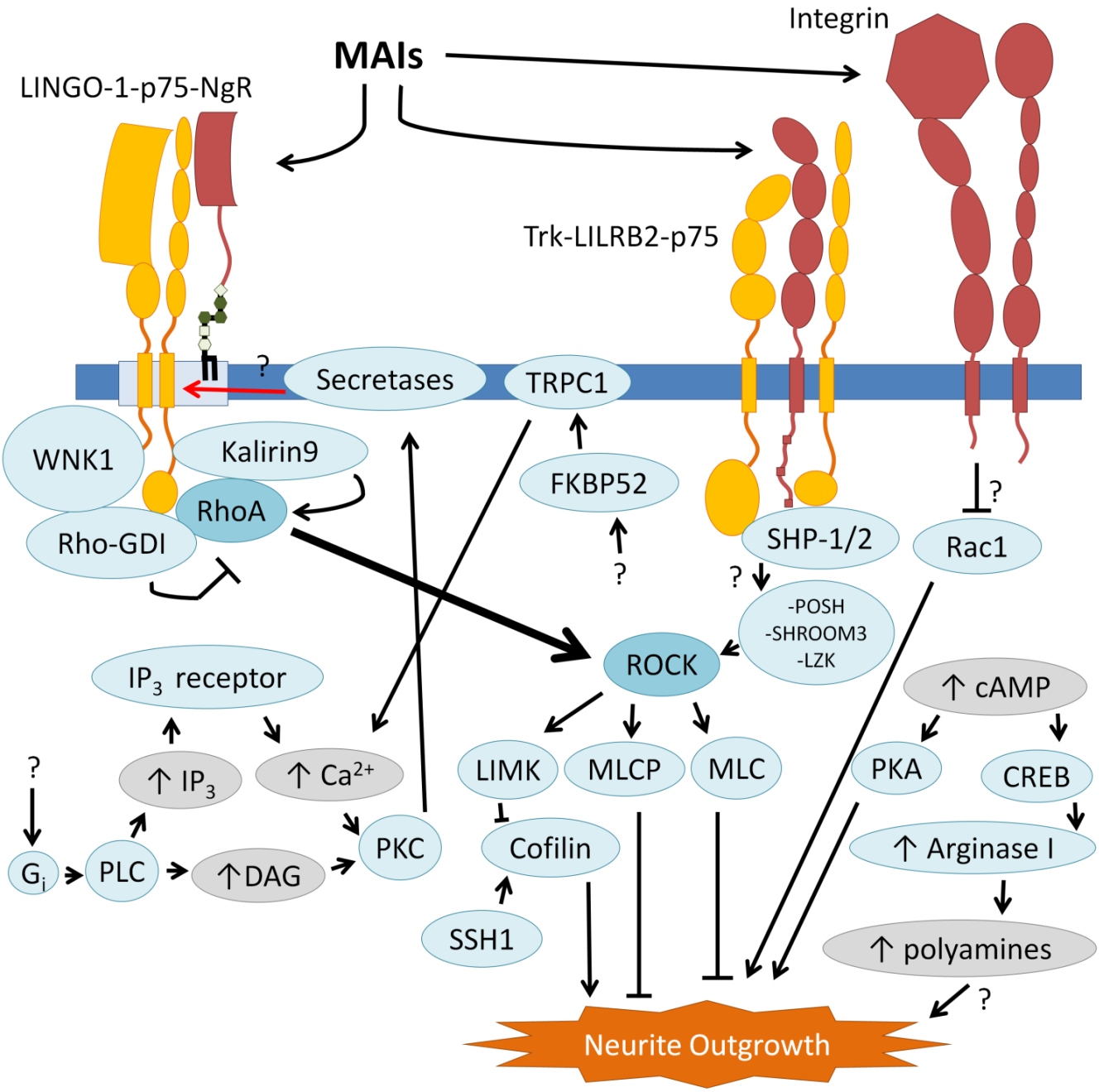


Fig. 18: Overview of the different downstream signaling pathways of MAI signaling. Proteins are indicated as turquoise elipses (size not representative), small molecules and ions are grey. The main signal transducers RhoA and ROCK are indicated in a darker shade of turquoise. Question marks indicate proteins or signaling mechanisms that are not identified or clear. The red arrow indicates proteolysis of p75 by α- and γ-secretases.

Therapeutic Potential

Although SCI is notorious for a low percentage of patients showing recovery, recovery does occur for the most common form of SCI; incomplete tetraplegia. 91% of patients under 50 years of age with this light form of SCI show recovery of ambulation, versus 42% for patients older than 50 years[270]. Furthermore, spontaneous neuronal rearrangements with concomitant functional recovery were also seen in an incomplete SCI model in adult rats[271]. These results show that rehabilitation is not excluded and any enhancement of regeneration could make important differences for patients suffering from SCI.

Anti-Nogo antibodies

The initial discovery of the molecular basis of regeneration inhibition[14] and the success of the IN-1 antibody against Nogo-A in enhancing regeneration[15,18,49] have made MAIs and their receptors attractive targets for therapeutic intervention for patients suffering from SCI. Subsequent investigations on IN-1 showed that intrathecal IN-1 administration after SCI did not just lead to enhanced regeneration, but also to recovery of reflex and motor function in rats, as well as improved sensory responses in the brain.[272–275] Surprisingly, IN-1 treatment in adult rats resulted in upregulation of growth factors, growth-related proteins and transcription factors[275]. A humanized recombinant Fab fragment of IN-1 also showed regenerating effects[276]. IN-1 administration after SCI also had both regeneration and functional recovery stimulating effects in primates, such as the marmoset (*Callitrix jacchus*), rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) monkeys[277–280].

Apart from SCI, IN-1 has also shown promising effects for recovery after brain injuries such as stroke. An early report demonstrates enhanced functional recovery and neuroanatomical plasiticity after injecting IN-1-secreting hybridoma cells at the injury site for middle cerebral artery occlusion in adult rats[281]. Subsequent studies found that IN-1 treatment in adult rats resulted in functional reorganization of the sensorimotor cortex after artificial lesion, again using hybridoma cells secreting IN-1[282]. Another purified monoclonal antibody against Nogo-A (7B12), was tested in two different stroke models (photothrombotic injury and middle cerebral artery occlusion) in adult rats. Both corticospinal plasticity and functional recovery were significantly enhanced in treated rats as compared to control rats[283]. Delayed administration with the 7B12 monoclonal antibody still resulted in brain reorganization and enhanced functional recovery[284]. Apart from axonal plasticity, IN-1 treatment for cerebral artery occlusion in adult rats also resulted in enhanced dendritic sprouting, arborization and increased spine density[285]. Another monoclonal anti-Nogo antibody (11C7) also stimulated cognitive recovery in adult rats with middle cerebral artery occlusion[286].

Other antibodies

Apart from the IN-1 antibody against Nogo-A, other antibodies have also shown promising effects in overcoming myelin inhibition. For example, polyclonal anti-MAG antibodies showed reversal of inhibition in cerebellar neurons by 50%[30]. An anti-NgR monoclonal antibody blocked binding of Nogo, MAG and OMgp with IC_{50} values of 120, 14 and 4.5 nM respectively. Furthermore, it stimulated neurite outgrowth of DRG neurons on CNS myelin[98]. A monoclonal antibody against p75 could abolish the interaction with NgR and prevent MAG-mediated growth cone turning[105]. Intrathecal administration of LINGO-1 polyclonal antiserum in a rat SCI model decreased RhoA activation, increased neuronal survival and was shown to promote functional recovery[287]. Anti-PirB antibodies also partially reversed myelin- or Nogo-66-mediated neurite outgrowth inhibition[134]. Furthermore, anti-

PirB antibodies could induce regeneration of damaged cortical neurons in rats, showing *in vivo* potential[135]. Monoclonal antibodies against gangliosides were shown to prevent MAG-mediated neurite outgrowth inhibition[151]. However, these antibodies caused direct inhibition of regeneration even in the PNS of a mouse injury model, instead of the desired opposite effect. This might have been caused by the clustering effects these antibodies have on the gangliosides[288]. Intrathecal delivery of polyclonal antibodies against the neuronal Wnt-receptor Ryk in a rat SCI model prevented retraction of CST axons and promoted their regrowth. Furthermore, it lead to enhanced sprouting of collateral branches across the lesion site[212]. Injection of polyclonal antibodies against the Sema3A downstream signal transducer CRMP-62 in DRG neurons prevented growth cone collapse by Sema3A[198]. However, intraneuronal injection might not be clinically feasible.

Soluble receptors

Shortly after the identification of NgR as the Nogo receptor, a soluble version of the NgR LRR domain (residues 27-310) was shown to block inhibition of axon growth by myelin[91]. It was shown to block both Nogo-66, MAG and OMgp binding to native NgR and intrathecal delivery promoted axonal sprouting and functional recovery in a rat SCI model[289]. Genetically modified mice that secreted this decoy receptor from astrocytes showed increased axonal sprouting after SCI as well.[290] Intrathecal delivery in a rat SCI model resulted in enhanced functional recovery even if delivery was delayed by 30 days after SCI[291]. Gene therapy using a herpes simplex viral vector to induce soluble NgR expression by DRG and hippocampal neurons prevented inhibition of neurite outgrowth *in vitro* and stimulated regeneration in a rat SCI model. Furthermore, it was shown to prevent RhoA activation and surprisingly, reduce the increase of Nogo-A expression upon injury[70]. A soluble NgR construct with the N-terminal 13 residues of the NgRL1 stalk fused in between the NgR LRR construct and the remainder of the NgR stalk, as well as deletion of cysteine residues 309 and 336 showed enhanced binding of Nogo, MAG and OMgp as compared to wild-type NgR and NgRL1. Furthermore, it promotes neurite outgrowth on CNS myelin and MAG substrates, making this artificial construct a promising candidate for therapeutic intervention[43]. Soluble Fc-fusion constructs of both p75 and LINGO-1 were also shown to attenuate the inhibitory activity of MAG, Nogo-66 and OMgp *in vitro*[104,124]. Furthermore, LINGO-1-Fc was shown to enhance axonal sprouting, reduce RhoA activity and stimulate functional recovery in rat SCI models[292].

Therapeutic peptides and aptamers

Several short peptide constructs have shown therapeutic potential in preventing regeneration inhibition. An antagonistic Nogo peptide of Nogo-A residues 1055-1095, corresponding to the Nogo-66 residues 1-40 and therefore present in Nogo-B and –C as well, showed blocking of Nogo-66 or CNS myelin inhibition of neurite outgrowth. Furthermore, intrathecal delivery resulted in enhanced axon growth in the CST, as well as functional recovery in a rat SCI model[56]. Both subcutaneous and intrathecal delivery were effective and delaying treatment for seven days after injury resulted in similarly enhanced axonal sprouting and functional recovery. Furthermore, treatment with this peptide resulted in sprouting of serotonergic fibers, extensive growth of corticospinal axons, upregulation of the axonal growth protein small proline-rich repeat protein 1A (SPRR1A) and synapse reformation[293]. This peptide and another Nogo-66 derived peptide (Nogo-66 residue 31-55 or Nogo-A residues 1085-1110) also prevented NGF-induced p75-mediated apoptosis in embryonic motor neurons, but this does not have direct therapeutic potential[294].

A 15 residue peptide (CFFRGGFFNHNPRYC) was shown to inhibit the interaction of the cytosolic DD domain of p75 with Rho-GDI. However, to inhibit the interaction, this peptide must be present in the cytosol. A human immunodeficiency virus (HIV) TAT peptide fusion with this inhibitory peptide provides entry to the cytosol and abolishes the MAG-induced interaction of p75 with Rho-GDI in cerebellar neurons. Furthermore, it abolished the inhibition of DRG and cerebellar neuron neurite outgrowth and the RhoA activation caused by MAG or Nogo-66[235].

A 16 residue cyclic peptide agonist of the TrkB neurotrophin receptor containing a tandem binding motif of BDNF (N-Ac-CSRRGELAASRRGELC-NH_2) showed the ability to promote neurite outgrowth on a MAG-substrate, even to an extent that MAG inhibition was completely overcome, whereas its normal ligand BDNF could not. It was proposed to do so by activating (dimerizing) TrkB without recruitment of p75, which would be overruling the growth stimulating effects in the case of MAG inhibition. Competition of BDNF binding to p75 with an excess of NGF (that does bind p75 but not TrkB) also could overcome MAG inhibitory signaling[115].

An FRG motif containing cyclic peptide (N-AcCLQKFRGSSC-NH_2) was designed as an antagonist of the GT1b-NgR interaction. It was shown to prevent MAG-mediated inhibition of neurite outgrowth. It also abolishes the inhibitory effects of antibody-mediated ganglioside clustering. The peptide however had no effect on basal neurite outgrowth levels. Structure-function analysis showed that the FRG motif was responsible for the inhibitory effects of this peptide. However, inhibition by myelin could not be overcome, probably because of the other inhibitors (Nogo and OMgp) and receptors (PirB/LILRB2) that can carry out inhibition independently of gangliosides[162].

Peptides that inhibit non-MAI inhibition of regeneration have been developed too. A peptoid that inhibits Sema3A-mediated axonal growth cone deflection by preventing interaction with the neuropilin/plexin A1 receptor complex also showed neuronal regeneration enhancing effects for damaged axons. A peptoid is an oligopeptide containing non-natural amino acids. The enhancement of regeneration effects were also seen in mice, showing *in vivo* relevance[295]. Another peptide inhibitor could block the ephrin-EphA4 interaction and prevent inhibition of regeneration. Furthermore, administration in rat SCI models resulted in reduced cavity formation, improved axon sprouting and functional recovery[296].

Apart from oligopeptides, oligonucleotides also have shown potential to overcome myelin inhibition. RNA aptamers have been generated that bind with high affinity to NgR and that compete with MAI binding. They also promote neurite outgrowth in the presence of MAIs, showing therapeutic potential[297].

Small molecule inhibitors

Apart from proteins, peptides and oligonucleotides, a number of small molecules have shown the potential to stimulate regeneration. These have a number of advantages, such as a cheap production price and generally good pharmacological properties. Especially the downstream signaler ROCK appeared to be a valuable target, because both NgR, PirB and CSPG signaling seems to happen via ROCK activation. Different ROCK inhibitors were already available before the molecular dissection of myelin inhibition. Fasudil for example has been used to treat cerebral vasospasm. Indeed, it appeared to promote neurological recovery after SCI in rats as well[232,298]. However, only immediate administration of fasudil and not delayed treatment after four weeks resulted in regenerating effects and functional recovery[299]. Another ROCK inhibitor known as Y27632 also could induce regenerating effects on axons and functional recovery after SCI in rats and mice. The Clostridium botulinum toxin C3 transferase is known to be able to inactivate

RhoA by ADP-ribosylating it. This toxin showed similar effects as Y27632[232,233,264,300–303]. However, another study in rats suffering from SCI found that only Y27632 and not C3 administration resulted in regenerating effects and functional recovery[234]. This might have been caused by the different delivery system used, since other studies injected C3 in neurons or used a toxin fusion to provide cell entry, which is necessary for C3 to exert its function. For an optical nerve crush injury model in adult cats, only Y27632 and not fasudil administration resulted in optic axonal extension beyond the crush site[304]. The differing results compared to earlier positive effects of fasudil are suggested by the authors to be the result of to the different extracellular environment in the optic nerve as compared to the CST and to the different species investigated (rats vs. cats)[304].

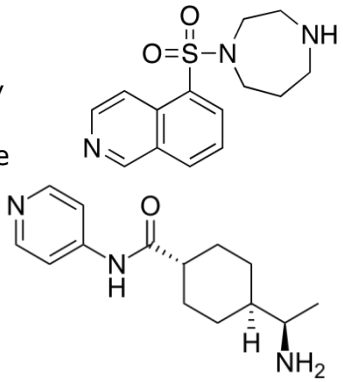


Fig. 19: Chemical structures of Fasudil (top) and Y27632 (bottom).

Another small molecule approach that showed regenerating effects for neurons is cAMP elevation. As discussed in the previous chapter, cAMP elevation resulted in regenerating effects through PKA and CREB binding, resulting in transcription of arginase I, an enzyme that is involved in polyamine synthesis[34,249,250,252]. Conditioning lesions in the peripheral branch of DRG neurons one to two weeks prior to injury have been shown to induce axonal growth across the lesion[251]. This effect was later explained by concomitant cAMP elevation[249]. cAMP analogs that can be uptaken by cells, such as db-cAMP, therefore provide an opportunity for therapeutic intervention. However, cAMP is a second messenger involved in many signaling pathways and therefore side effects are more likely to occur. Still, db-cAMP injection in rat DRGs after SCI resulted in enhanced regenerating effects, although functional recovery has not been investigated[249].

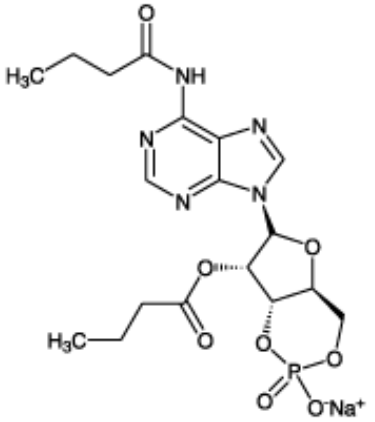


Fig. 20: Chemical structure of db-cAMP

The different MAIs have been suggested to cause EGFR phosphorylation in a calcium-dependent fashion. Inhibition of the intracellular kinase domain of EGFR that can transphosphorylate tyrosine residues in nearby EGFR molecules, was shown to stimulate neurite outgrowth in the presence of MAIs. The inhibitors AG1478 and PD168393 showed these effects, although complete ablation of the inhibitory effects of the MAIs was not seen. Regenerating effects were also seen in an optic nerve crush injury model in mice[244]. EGFR activation has also been implicated in making astrocytes reactive and stimulating their secretion of CSPGs[305,306]. Intrathecal delivery of the EGFR inhibitor PD168393, a drug already in clinical use for certain lung cancers, was also shown to enhance regeneration and functional recovery after SCI in rats[245].

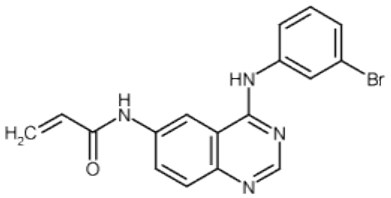


Fig. 21: Chemical structure of PD168393

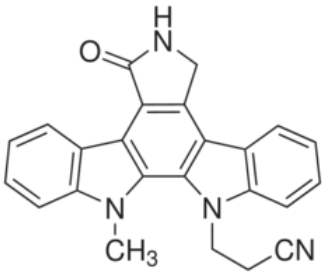


Fig. 22: Chemical structure of Gö6976

PKC was shown to be involved in the signal transduction of both MAI and CSPG-mediated inhibition of regeneration. Inhibition of PKC by Gö6976 was also shown to promote neuronal regeneration beyond the lesion site after dorsal hemisection in rats.[240] Functional recovery after Gö6976 treatment has not been investigated yet.

The implication of Sema3A in axonal growth cone collapse suggests this as a target for therapeutic intervention too[191–195]. An inhibitor for Sema3A signaling was developed (Xanthofulvin, also known as SM-216289) and shown to prevent its interaction with neuropilin. Furthermore, it could stimulate regeneration of olfactory neurons after axotomy in rats[309]. It also showed regenerating effects after intrathecal administration for a rat SCI model, as well as decreasing numbers of apoptotic cells. Finally, functional recovery was shown too, demonstrating the potential of inhibiting Sema3A mediated axonal growth cone collapse[310].

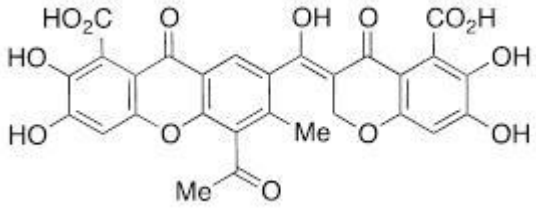


Fig. 23: Chemical structure of Xanthofulvin

Other possibilities for therapeutic intervention

Neurotrophins NGF, NT3 and glial-cell-line-derived neurotrophic factor (GDNF), but not BDNF were shown to enhance DRG axonal regrowth into the dorsal root entry zone of the spinal cord. This resulted in recovery of sensory functions like heat and pressure[311]. Although this effect has not been reported for SCI, it is promising for root avulsion injury. Neurotrophins have been shown to induce cAMP elevation and cAMP elevation itself also enhances regeneration (see previous paragraph)[116]. A combination of fetal spinal cord tissue transplantation in the lesion cavity of adult rat spinal cord transection and intrathecal neurotrophin administration (BDNF and NT3) showed regeneration across the lesion site after a two week delay. Surprisingly, this effect was not seen when the treatment was applied immediately after injury. Apart from regeneration, functional recovery was demonstrated when this combined therapy was applied too[312]. Injection of genetically modified Schwann cells that secrete BDNF also supported axonal regeneration across a spinal cord lesion[313].

CSPG glycosaminoglycan degradation by ChonABC could intervene with the inhibitory properties of CSPG for regeneration. Indeed, intrathecal delivery of ChonABC in a rat SCI model resulted in regeneration of both ascending projections and descending motor neurons. Furthermore, post-synaptic activity below the lesion was restored after electrical stimulation of corticospinal neurons and functional recovery was demonstrated[315]. These results were confirmed by another study, that also showed that *Clostridium perfringens* sialidase treatment to trim the terminal sialic acid residues from GT1b and GD1a had regeneration enhancing effects in different SCI models in rats. However, PI-PLC treatment to release GPI-anchored proteins like the NgR family of receptors and OMgp from the membrane did not result in enhanced regeneration[316].

Electrical stimulation has also been shown to be a factor of influence for regeneration, as it modulated the turning response of *Xenopus laevis* spinal neuron growth cones to guidance cues, such as MAG and netrin-1. Whereas netrin-1 induced attraction was enhanced, MAG and myelin-induced repulsion was converted to attraction. The effects were mediated by calcium. In addition, cAMP elevation was demonstrated[314].

The redundant ligands and receptors in MAI signaling suggest that the best approach to tackle inhibition of regeneration might be to combine multiple therapies. Indeed, such efforts have been made. For example, adenovirus-based gene therapy to introduce a truncated NgR receptor was not successful on itself, but when combined with induced lens injury to stimulate growth factor release, strongly enhanced regeneration was seen after optic nerve crush in rats[317]. Another study combined conditioning lesions, grafts of marrow stromal cells and NT3 neurotrophin administration in a rat SCI model. It was found that combinations of two of these therapies did not, but of all three did improve regeneration. Regeneration was achieved when treatment started six or even 15 weeks after SCI[318]. However, many other combinations of the therapies mentioned in this chapter are possible. Smart combinations of complementary inhibitors of the different signaling pathways might be a good approach to selectively intervene with all the inhibitory cues for axonal regeneration.

A completely different approach to circumvent the inhibitory environment of CNS lesions might be to employ artificial nervegrafts. Biodegradable polymeric (e.g. polylactate) tubes can be modified so that their inner surface contains growth stimulating cues like neurotrophins. The physical barrier for MAIs that the tubing across the lesion site provides for growth cones entering the tubing should prevent growth cone collapse.

MAI signaling in neurodegenerative diseases

MAIs and their receptors are not only of clinical significance when it comes to SCI or brain injury. Several of these proteins have also been implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. The β-amyloid converting enzyme 1 (BACE1) is the β-secretase responsible for releasing the Aβ amyloid peptide from the amyloid precursor protein (APP), which is thought to be important in Alzheimer's disease. The Rtn proteins appear to be negative regulators of BACE1, since their overexpression leads to reduced Aβ secretion. Binding was demonstrated by co-immunoprecipitation. Rtn3 colocalized with BACE1 in neurons and RNAi mediated knockdown of Rtn3 resulted in elevated Aβ secretion. Furthermore, Rtn3 was found to be the principal BACE1-interacting protein in the brain[319]. Nogo-B and Nogo-C (Rtn4B/C) binding to BACE1 and inhibition of Aβ secretion was also demonstrated in neurons[320]. A conserved C-terminal QID motif that is present in all the four Rtn proteins was shown to mediate the BACE1 interaction. Mutation of this triplet impedes the inhibitory effects of the Rtn proteins on BACE1-mediated Aβ secretion. It was also shown that whereas Rtn3 can normally dimerize, it exists as a monomer in the complex with BACE1. Disruption of the QID motif did not modify the propensity of Rtn3 to dimerize[321]. Nogo knockout mice with Alzheimer mutations suffered from aggravated learning and memory deficits. However, expression levels of markers for synapto–dendritic complexity and axonal sprouting, including synaptophysin, MAP2, GAP43 and neurofilament that are otherwise reduced in APP transgenic mice, were restored in Nogo knockout mice. Surprisingly, knocking out Nogo did not influence other Alzheimer symptoms like neuronal loss, astrogliosis, microgliosis or Aβ levels and amyloid deposits. It is suggested that Nogo

aggravates Alzheimer by inhibiting neurite outgrowth and axonal regeneration, whereas Rtn3 does so by directly inhibiting BACE1-mediated Aβ secretion[322].

Surprisingly, apart from the Rtn proteins, NgR also appears to be involved in Aβ release. Both Nogo and NgR were found to have altered subcellular localization in Alzheimer's disease. NgR was found to directly interact with APP and both proteins were localized to lipid rafts. Both cleavage products of BACE1-hydrolyzed APP show binding affinity for NgR. NgR was found to be a negative regulator of Aβ secretion both *in vitro* and in mice. It inhibits both α- and β-secretase proteolysis of APP[323]. Application of soluble NgR in a mouse Alzheimer model resulted in Aβ plaque formation, although the serum levels of Aβ increased. These changes correlated with improved spatial memory. This effect was thought to be the result of a direct interaction of Aβ with soluble NgR and provides a possible therapeutic for Alzheimer's disease[324].

Apart from Alzheimer's, MAI signaling proteins are involved in Parkinson's disease. Parkinson's disease is the result of dying dopamine-generating neurons in the substantia nigra, a region of the midbrain. LINGO-1 expression is present in these neurons. Knockout of LINGO-1 in mice, as well as treatment with LINGO-1-Fc or an anti-LINGO antibody all resulted in increased dopaminergic neuron survival. Parkinson symptoms were reduced as well and the effects correlated with an increase of Akt phosphorylation. LINGO-1 is suggested to inhibit EGFR-Akt signaling by binding to EGFR. A direct interaction of EGFR with LINGO-1 in the extracellular domain was demonstrated and EGFR activation was suppressed by LINGO-1[246].

These results indicate that MAIs are not only important therapeutic targets in SCI or brain injury, but might also be considered in Alzheimer's and Parkinson's diseases. Although soluble receptors or antibodies might not be very good candidates because of the blood brain barrier and the chronic nature of these diseases, small molecule inhibitors that interfere with the pathways involved might be successful.

Conclusion and Perspectives

Since the early investigations on inhibitors of regeneration, the field has expanded tremendously, resulting in the elucidation of many of the molecular mechanisms involved. It is well established that Nogo, MAG and OMgp signal through NgR-p75/TROY-LINGO-1 or PirB/LILRB2 resulting in inhibition of neurite outgrowth and growth cone collapse. Many other molecules known to be involved in axonal guidance have shown regeneration inhibiting effects too, such as CSPGs, semaphorins, ephrins, netrins, wnts etc. Downstream signaling pathways responsible for the inhibition of regeneration are successfully being elucidated too, with numerous players already identified, providing more therapeutic targets of intervention.

However, questions remain on different levels. For example, the structural determinants necessary for triggering of signaling by the MAIs remain unclear. Whereas it is shown that p75 is recruited to NgR in lipid rafts upon MAI stimulation, it is not yet known whether LINGO-1 is recruited to NgR in lipid rafts like p75 upon MAI binding. How exactly MAI binding changes the affinity to co-receptors, as well as the stoichiometry of the receptor complexes, remains to be determined. The reasons for the strong redundancy in this signaling (three ligands and two shared receptor complexes) remain puzzling. Do neurotrophins indeed compete with NgR or LINGO-1 for p75, resulting in opposite effects of neurotrophin and MAI signaling? How exactly GT1b functions in the receptor complexes is not yet completely clear either. Although both LINGO-1 and p75 contain intracellular phosphorylation sites, it has not been investigated whether these are involved in MAI signaling. Does LINGO-1 compete with NgR-binding of the MAIs, as suggested by site-directed mutagenesis studies? The structures of both MAG and OMgp are not determined, nor are there any structures of complexes of MAIs with receptors. The function of the interaction of MAG with OMgp is not clear yet either. Why neurons and oligodendrocytes release soluble forms of different MAIs and their receptors (MAG, OMgp, p75 and NgR) is not well understood. It remains unclear what G_i protein is involved in MAI signaling. Furthermore, the way MAIs stimulate G_i activation to induce calcium influx remains enigmatic. The molecular mechanisms of Rac1 deactivation by MAIs yet to be determained too. It is also not clear how MAG stimulation influences the activity of FKBP52 on the TRPC1 calcium channel. In addition, the way polyamines influence neurite outgrowth remains to be elucidated. The downstream signaling pathways of PirB are not fully understood yet either.

Solving these problems would help to get an idea about the complete mechanism and all the proteins involved. This way, selective combination of different therapies to systematically block all the inhibitory mechanisms of regeneration is possible. Furthermore, it might help in getting a better understanding in the function of these proteins in healthy (adult) organisms.

List of Abbreviations

ADP: Adenine Diphosphate
AP: Alkaline Phosphatase
APP: Amyloid Precursor Protein
ASIA: American Spinal Injury Association
Asn: Asparagine
ATP: Adenine Triphosphate
BACE: β-Amyloid Converting Enzyme
BLyS: B Lymphocyte Stimulator
cAMP: cyclic Adenine Monophosphate
CHO: Chinese Hamster Ovarian
CNG: Cerebellar Granule Neuron
CREB: cAMP Responsive Element Binding Protein
CRMP: Collapsin Response Mediator Protein
CRNF: Cysteine-Rich Neurotrophic Factor
CSPG: Chondroitin Sulfate Proteoglycan
CST: Corticospinal Tract
db-cAMP: dibutyryl cyclic Adenine Monophosphate
DD: Death Domain
DRG: Dorsal Root Ganglion
ECM: Extracellular Matrix
EGFR: Epidermal Growth Factor Receptor
ER: Endoplasmatic Reticulum
FAK: Focal Adhesion Kinase
FGF: Fibroblast Growth Factor
FGFR: Fibroblast Growth Factor Receptor
GABA: Gamma-Aminobutyric Acid
GDNF: Glial-cell-line-Derived Neurotrophic Factor
GEF: Guanine Exchange Factors
Gp1bα: Glycoprotein 1bα
GPCR: G protein-coupled receptor
HIV: Human Immunodeficiency Virus
HSPG: Heparan Sulphate Proteoglycans
Ig: Immunoglobulin
IP_3: inositol 1,4,5-triphosphate
ITIM: Immunoreceptor Tyrosine-based Inhibitory Motif
JNK: Jun N-terminal Kinase
LGI: Leucine-rich Glioma Inactivated
LILR: Leukocyte Ig–Like Receptor
LIMK: Lin-11, Isl-1, Mec-3 Kinase
LINGO: LRR- and Ig-domain containing Nogo receptor-interacting protein
LNGFR: Low-affinity Nerve Growth Factor Receptor
LRR: Leucine-Rich Repeat
LZK: Leucine Zipper Kinase
MAG: Myelin-Associated Glycoprotein
MAI: Myelin-Associated Inhibitor
MBP: Myelin Basic Protein
MHC: Major Histocompatibility Complex
MLC: Myosin Light Chain
MLCP: Myosin Light Chain Phosphatase
MOG: Myelin Oligodendrocyte Glycoprotein
NgBR: Nogo-B Receptor
NgR: Nogo Receptor
NgRL: Nogo Receptor-Like
NOESY: Nuclear Overhauser Effect Spectroscopy
NPC: Neural Progenitor Cell
OMgp: Oligodendrocyte-myelin glycoprotein

p75NTR: Neurotrophin Receptor p75
PIP_2: phosphatidylinositol 4,5-bisphosphate
PI-PLC: Phosphatidyl-Inositol–specific Phospholipase C
PirB: Paired Ig-like receptor B
PKA: Protein Kinase A
PKC: Protein Kinase C
PLC: Phospholipase C
PLP: Proteolipid Protein
PND: Postnatal Day
POSH: Plenty Of SH3s
PPIase: Peptidyl-Prolyl Isomerase
PRE: Paramagnetic Resonance Enhancement
PTEN: Phosphatase and Tensin homologue
RacGEF: Rac-specific Guanine Exchange Factor
RGC: Retinal Ganglion Cells
Rho: RAS Homology
RhoGEF: Rho-specific Guanine Exchange Factor
ROCK: Rho-associated Coiled-coil Kinase
SCI: Spinal Cord Injury
SDS: Sodium Dodecyl Sulphate
SHP: Src Homology 2-containing protein tyrosine Phosphatase
shRNA: short hairpin RNA
siRNA: short interfering RNA
SPRR: Small Proline-Rich Repeat protein
SSH: Slingshot phosphatase
STAT: Signal Transducers and Activators of Transcription
TNFRSM: Tumor Necrosis Factor Receptor Superfamily Member
Trk: Tropomyosin Receptor Kinase
TSC: Tuberous Sclerosis Complex
VCN: *Vibrio Cholerae* Neuraminidase
WNK: with no lysine

References

1. Purves, D. *et al. Neuroscience, fifth edition*. 1-106, 559-583 (2012).
2. Alberts, B. *et al. Molecular Biology of the Cell*. 615-657 (2002).
3. Brady, S.T. *Basic Neurochemistry: principles of molecular, cellular and medical neurobiology*. 66-67,585-592 (2012).
4. Brink, P.R., Cronin, K. & Ramanan, S.V. Gap junctions in excitable cells. *Journal of bioenergetics and biomembranes* **28**, 351-8 (1996).
5. Zoidl, G. & Dermietzel, R. On the search for the electrical synapse: a glimpse at the future. *Cell and tissue research* **310**, 137-42 (2002).
6. Nave, K.-A. Myelination and the trophic support of long axons. *Nature reviews. Neuroscience* **11**, 275-83 (2010).
7. Nave, K.-A. Myelination and support of axonal integrity by glia. *Nature* **468**, 244-52 (2010).
8. Williams, K. a & Deber, C.M. The structure and function of central nervous system myelin. *Critical reviews in clinical laboratory sciences* **30**, 29-64 (1993).
9. Jahn, O., Tenzer, S. & Werner, H.B. Myelin proteomics: molecular anatomy of an insulating sheath. *Molecular neurobiology* **40**, 55-72 (2009).
10. David, S. & Aguayo, A.J. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. *Science* **214**, 931-933 (1981).
11. Aguayo, A.J., David, S. & Bray, G.M. Influences of the glial environment on the elongation of axons after injury: transplantation studies in adult rodents. *Journal of Experimental Biology* **95**, 231-240 (1981).
12. Savio, T. & Schwab, M.E. Rat CNS white matter, but not gray matter, is nonpermissive for neuronal cell adhesion and fiber outgrowth. *The Journal of neuroscience* **9**, 1126-33 (1989).
13. Ng, W.P., Cartel, N., Roder, J., Roach, a & Lozano, a Human central nervous system myelin inhibits neurite outgrowth. *Brain research* **720**, 17-24 (1996).

14. Caroni, P. & Schwab, M.E. Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. *The Journal of cell biology* **106**, 1281-8 (1988).
15. Schwab, M.E. & Caroni, P. Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. *Neuron* **1**, 85-96 (1988).
16. Bandtlow, C., Zachleder, T. & Schwab, M.E. Oligodendrocytes arrest neurite growth by contact inhibition. *The Journal of neuroscience* **10**, 3837-48 (1990).
17. Schwab, M.E. & Caroni, P. Oligodendrocytes and CNS myelin are nonpermissive substrates for neurite growth and fibroblast spreading in vitro. *The Journal of neuroscience* **8**, 2381-2393 (1988).
18. Schnell, L. & Schwab, M.E. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. *Nature* **343**, 269-272 (1990).
19. Schwegler, G., Schwab, M. & Kapfhammer, J. Increased collateral sprouting of primary afferents in the myelin-free spinal cord. *The Journal of neuroscience* **15**, 2756-2767 (1995).
20. Buffo, A. *et al.* Application of Neutralizing Antibodies against NI-35/250 Myelin-Associated Neurite Growth Inhibitory Proteins to Purkinje Cell Axons. *The Journal of neuroscience* **20**, 2275-2286 (2000).
21. Schwab, M.E. & Schnell, L. Channeling of developing rat corticospinal tract axons by myelin-associated neurite growth inhibitors. *The Journal of neuroscience* **11**, 709-721 (1991).
22. Davies, S.J. *et al.* Regeneration of adult axons in white matter tracts of the central nervous system. *Nature* **390**, 680-683 (1997).
23. Davies, S.J., Goucher, D.R., Doller, C. & Silver, J. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. *The Journal of neuroscience* **19**, 5810-22 (1999).
24. Berardi, N., Pizzorusso, T. & Maffei, L. Critical periods during sensory development. *Current opinion in neurobiology* **10**, 138-45 (2000).
25. Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L. & Maffei, L. Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. *Vision research* **34**, 709-20 (1994).
26. Wiesel, T.N. & Hubel, D.H. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *Journal of neurophysiology* **28**, 1029-40 (1965).
27. Crair, M.C., Gillespie, D.C. & Stryker, M.P. The Role of Visual Experience in the Development of Columns in Cat Visual Cortex. *Science* **279**, 566-570 (1998).
28. Wiesel, T.N. & Hubel, D.H. Ordered arrangement of orientation columns in monkeys lacking visual experience. *The Journal of comparative neurology* **158**, 307-18 (1974).
29. Z'Graggen, W.J. *et al.* Compensatory sprouting and impulse rerouting after unilateral pyramidal tract lesion in neonatal rats. *The Journal of neuroscience* **20**, 6561-9 (2000).
30. Mukhopadhyay, G., Doherty, P., Walsh, F.S., Crocker, P.R. & Filbin, M.T. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. *Neuron* **13**, 757-67 (1994).
31. McKerracher, L. *et al.* Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron* **13**, 805-11 (1994).
32. Johnson, P.W. *et al.* Recombinant myelin-associated glycoprotein confers neural adhesion and neurite outgrowth function. *Neuron* **3**, 377-385 (1989).
33. Domeniconi, M. & Filbin, M.T. Overcoming inhibitors in myelin to promote axonal regeneration. *Journal of the neurological sciences* **233**, 43-7 (2005).
34. Cai, D. *et al.* Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. *The Journal of neuroscience* **21**, 4731-9 (2001).
35. Burger, D., Pidoux, L. & Steck, A.J. Identification of the glycosylated sequons of human myelin-associated glycoprotein. *Biochemical and biophysical research* **197**, 457-464 (1993).
36. Lai, C. *et al.* Two forms of 1B236/myelin-associated glycoprotein, a cell adhesion molecule for postnatal neural development, are produced by alternative splicing. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 4337-41 (1987).
37. Trapp, B.D. Myelin Associated Glycoprotein, Location and PotentialFunctions. *Annals New York Academy of Sciences* 29-43 (1995).
38. Möller, J.R. Rapid conversion of myelin-associated glycoprotein to a soluble derivative in primates. *Brain research* **741**, 27-31 (1996).
39. Tang, S. *et al.* Soluble Myelin-Associated Glycoprotein (MAG) Found in Vivo Inhibits Axonal Regeneration. *Molecular and Cellular Neuroscience* **346**, 333-346 (1997).
40. Vinson, M., Strijbos, P., Rowles, A. & Facci, L. Myelin-associated glycoprotein interacts with ganglioside GT1b. *Journal of Biological* **276**, 20280-20285 (2001).
41. Goh, E.L.K. *et al.* Beta1-Integrin Mediates Myelin-Associated Glycoprotein Signaling in Neuronal Growth Cones. *Molecular brain* **1**, 10 (2008).
42. Wörter, V. *et al.* Inhibitory activity of myelin-associated glycoprotein on sensory neurons is largely independent of NgR1 and NgR2 and resides within Ig-Like domains 4 and 5. *PloS one* **4**, e5218 (2009).
43. Robak, L.A. *et al.* Molecular Basis of the Interactions of the Nogo-66 Receptor and Its Homolog NgR2 with Myelin-Associated Glycoprotein : Development of NgR OMNI -Fc , a Novel Antagonist of CNS Myelin Inhibition. *The Journal of neuroscience* **29**, 5768-5783 (2009).
44. Yin, X. *et al.* Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. *The Journal of neuroscience* **18**, 1953-62 (1998).
45. Nguyen, T. *et al.* Axonal protective effects of the myelin-associated glycoprotein. *The Journal of neuroscience* **29**, 630-7 (2009).

46. Lopez, P.H.H. *et al.* Myelin-associated glycoprotein protects neurons from excitotoxicity. *Journal of Neurochemistry* **116**, 900-908 (2011).
47. Ng, W., Cartel, N., Li, C. & Roder, J. Myelin from MAG-deficient mice is a strong inhibitor of neurite outgrowth. *Neuroreport* **7**, 861-864 (1996).
48. Bartsch, U. *et al.* Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. *Neuron* **15**, 1375-81 (1995).
49. Chen, M.S. *et al.* Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* **403**, 434-9 (2000).
50. GrandPré, T., Nakamura, F., Vartanian, T. & Strittmatter, S.M. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature* **7199**, 439-444 (2000).
51. Prinjha, R. *et al.* Inhibitor of neurite outgrowth in humans. *Nature* **403**, 383-4 (2000).
52. Spillmann, A.A., Bandtlow, C.E., Lottspeich, F., Keller, F. & Schwab, M.E. Identification and characterization of a bovine neurite growth inhibitor (bNI-220). *The Journal of biological chemistry* **273**, 19283-93 (1998).
53. Laurén, J. *et al.* Characterization of myelin ligand complexes with neuronal Nogo-66 receptor family members. *The Journal of biological chemistry* **282**, 5715-25 (2007).
54. Kim, J.-E., Bonilla, I.E., Qiu, D. & Strittmatter, S.M. Nogo-C is sufficient to delay nerve regeneration. *Molecular and Cellular Neuroscience* **23**, 451-459 (2003).
55. Zander, H., Reineke, U., Schneider-mergener, J. & Skerra, A. Epitope mapping of the neuronal growth inhibitor Nogo-A for the Nogo receptor and the cognate monoclonal antibody IN-1 by means of the SPOT technique. *Journal of Molecular Recognition* **20**, 185-196 (2007).
56. GrandPré, T., Li, S. & Strittmatter, S.M. Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature* **417**, 547-51 (2002).
57. Vasudevan, S.V., Schulz, J., Zhou, C. & Cocco, M.J. Protein folding at the membrane interface, the structure of Nogo-66 requires interactions with a phosphocholine surface. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 6847-51 (2010).
58. Li, M., Liu, J. & Song, J. Nogo goes in the pure water: Solution structure of Nogo-60 and design of the structured and buffer-soluble Nogo-54 for enhancing CNS regeneration. *Protein science* **15**, 1835-1841 (2006).
59. Li, M. & Song, J. The N-and C-termini of the human Nogo molecules are intrinsically unstructured: Bioinformatics, CD, NMR characterization, and functional implications. *Proteins: Structure, Function, and Bioinformatics* **108**, 100-108 (2007).
60. Dephoure, N. *et al.* A quantitative atlas of mitotic phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 10762-7 (2008).
61. Gauci, S. *et al.* Lys-N and trypsin cover complementary parts of the phosphoproteome in a refined SCX-based approach. *Analytical chemistry* **81**, 4493-501 (2009).
62. Qin, H., Pu, H.X., Li, M., Ahmed, S. & Song, J. Identification and structural mechanism for a novel interaction between a ubiquitin ligase WWP1 and Nogo-A, a key inhibitor for central nervous system regeneration. *Biochemistry* **47**, 13647-58 (2008).
63. Liu, J., Li, M., Ran, X., Fan, J.-song & Song, J. Structural insight into the binding diversity between the human Nck2 SH3 domains and proline-rich proteins. *Biochemistry* **45**, 7171-7184 (2006).
64. Oertle, T. *et al.* Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. *The Journal of neuroscience* **23**, 5393-406 (2003).
65. Voeltz, G.K., Prinz, W. a, Shibata, Y., Rist, J.M. & Rapoport, T. a A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* **124**, 573-86 (2006).
66. Huebner, E. a, Kim, B.G., Duffy, P.J., Brown, R.H. & Strittmatter, S.M. A multi-domain fragment of Nogo-A protein is a potent inhibitor of cortical axon regeneration via Nogo receptor 1. *The Journal of biological chemistry* **286**, 18026-36 (2011).
67. Hu, F. *et al.* Nogo-A interacts with the Nogo-66 receptor through multiple sites to create an isoform-selective subnanomolar agonist. *The Journal of neuroscience* **25**, 5298-304 (2005).
68. Cheatwood, J.L., Emerick, A.J., Schwab, M.E. & Kartje, G.L. Nogo-A expression after focal ischemic stroke in the adult rat. *Stroke; a journal of cerebral circulation* **39**, 2091-8 (2008).
69. Mathis, C., Schröter, A., Thallmair, M. & Schwab, M.E. Nogo-a regulates neural precursor migration in the embryonic mouse cortex. *Cerebral cortex* **20**, 2380-90 (2010).
70. Peng, X., Zhou, Z., Hu, J., Fink, D.J. & Mata, M. Soluble Nogo receptor down-regulates expression of neuronal Nogo-A to enhance axonal regeneration. *The Journal of biological chemistry* **285**, 2783-95 (2010).
71. Simonen, M. *et al.* Systemic deletion of the myelin-associated outgrowth inhibitor Nogo-A improves regenerative and plastic responses after spinal cord injury. *Neuron* **38**, 201-11 (2003).
72. Zheng, B. *et al.* Lack of enhanced spinal regeneration in Nogo-deficient mice. *Neuron* **38**, 213-24 (2003).
73. Kim, J.E., Li, S., GrandPré, T., Qiu, D. & Strittmatter, S.M. Axon regeneration in young adult mice lacking Nogo-A/B. *Neuron* **38**, 187-99 (2003).
74. Woolf, C. No Nogo: now where to go? *Neuron* **38**, 153-156 (2003).
75. Wang, K., Koprivica, V., Kim, J. & Sivasankaran, R. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* **417**, 941-944 (2002).
76. Kottis, V. *et al.* Oligodendrocyte-myelin glycoprotein (OMgp) is an inhibitor of neurite outgrowth. *Journal of neurochemistry* **82**, 1566-9 (2002).

77. Mikol, D.D. & Stefansson, K. A phosphatidylinositol-linked peanut agglutinin-binding glycoprotein in central nervous system myelin and on oligodendrocytes. *The Journal of cell biology* **106**, 1273-9 (1988).
78. Vourc'h, P. *et al.* Oligodendrocyte myelin glycoprotein growth inhibition function requires its conserved leucine-rich repeat domain, not its glycosylphosphatidyl-inositol anchor. *Journal of Neurochemistry* **85**, 889-897 (2003).
79. Huang, J.K. *et al.* Glial membranes at the node of Ranvier prevent neurite outgrowth. *Science* **310**, 1813-7 (2005).
80. Nie, D.-yu *et al.* Oligodendrocytes regulate formation of nodes of Ranvier via the recognition molecule OMgp. *Neuron glia biology* 151-164 (2007).
81. Chang, K.-J., Susuki, K., Dours-Zimmermann, M.T., Zimmermann, D.R. & Rasband, M.N. Oligodendrocyte myelin glycoprotein does not influence node of ranvier structure or assembly. *The Journal of neuroscience* **30**, 14476-81 (2010).
82. Ji, B. *et al.* Assessment of functional recovery and axonal sprouting in oligodendrocyte-myelin glycoprotein (OMgp) null mice after spinal cord injury. *Molecular and cellular neurosciences* **39**, 258-67 (2008).
83. Habib, a a *et al.* Expression of the oligodendrocyte-myelin glycoprotein by neurons in the mouse central nervous system. *Journal of neurochemistry* **70**, 1704-11 (1998).
84. Vourc'h, P. *et al.* The oligodendrocyte-myelin glycoprotein gene is highly expressed during the late stages of myelination in the rat central nervous system. *Brain research. Developmental brain research* **144**, 159-68 (2003).
85. Lee, X. *et al.* Oligodendrocyte differentiation and myelination defects in OMgp null mice. *Molecular and cellular neurosciences* **46**, 752-61 (2011).
86. Martin, I. *et al.* Effect of the oligodendrocyte myelin glycoprotein (OMgp) on the expansion and neuronal differentiation of rat neural stem cells. *Brain research* **1284**, 22-30 (2009).
87. Guo, Q., Li, S. & Su, B. Expression of oligodendrocyte myelin glycoprotein and its receptor NgR after the injury of rat central nervous system. *Neuroscience letters* **422**, 103-8 (2007).
88. Fournier, A., GrandPre, T. & Strittmatter, S. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature* **409**, 341-346 (2001).
89. Domeniconi, M. *et al.* Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron* **35**, 283-90 (2002).
90. Liu, B.P., Fournier, A., GrandPré, T. & Strittmatter, S.M. Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *Science* **297**, 1190-3 (2002).
91. Fournier, A.E., Gould, G.C., Liu, B.P. & Strittmatter, S.M. Truncated soluble Nogo receptor binds Nogo-66 and blocks inhibition of axon growth by myelin. *The Journal of neuroscience* **22**, 8876-83 (2002).
92. Barton, W. a *et al.* Structure and axon outgrowth inhibitor binding of the Nogo-66 receptor and related proteins. *The EMBO journal* **22**, 3291-302 (2003).
93. Pignot, V. *et al.* Characterization of two novel proteins, NgRH1 and NgRH2, structurally and biochemically homologous to the Nogo-66 receptor. *Journal of Neurochemistry* **85**, 717-728 (2003).
94. Laurén, J., Airaksinen, M.S., Saarma, M. & Timmusk, T. Two novel mammalian nogo receptor homologs differentially expressed in the central and peripheral nervous systems☆. *Molecular and Cellular Neuroscience* **24**, 581-594 (2003).
95. Venkatesh, K. *et al.* The Nogo-66 receptor homolog NgR2 is a sialic acid-dependent receptor selective for myelin-associated glycoprotein. *The Journal of neuroscience* **25**, 808-22 (2005).
96. He, X.L. *et al.* Structure of the Nogo receptor ectodomain: a recognition module implicated in myelin inhibition. *Neuron* **38**, 177-85 (2003).
97. Huizinga, E.G. *et al.* Structures of glycoprotein Ibalpha and its complex with von Willebrand factor A1 domain. *Science (New York, N.Y.)* **297**, 1176-9 (2002).
98. Li, W. *et al.* A neutralizing anti-Nogo66 receptor monoclonal antibody reverses inhibition of neurite outgrowth by central nervous system myelin. *The Journal of biological chemistry* **279**, 43780-8 (2004).
99. Wen, D. *et al.* Disulfide structure of the leucine-rich repeat C-terminal cap and C-terminal stalk region of Nogo-66 receptor. *Biochemistry* **44**, 16491-501 (2005).
100. McGee, A.W., Yang, Y., Fischer, Q.S., Daw, N.W. & Strittmatter, S.M. Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. *Science* **309**, 2222-6 (2005).
101. Zheng, B. *et al.* Genetic deletion of the Nogo receptor does not reduce neurite inhibition in vitro or promote corticospinal tract regeneration in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 1205-10 (2005).
102. Chivatakarn, O., Kaneko, S., He, Z., Tessier-Lavigne, M. & Giger, R.J. The Nogo-66 receptor NgR1 is required only for the acute growth cone-collapsing but not the chronic growth-inhibitory actions of myelin inhibitors. *The Journal of neuroscience* **27**, 7117-24 (2007).
103. Kim, J.-E., Liu, B.P., Park, J.H. & Strittmatter, S.M. Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. *Neuron* **44**, 439-51 (2004).
104. Wang, K.C., Kim, J. a, Sivasankaran, R., Segal, R. & He, Z. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* **420**, 74-8 (2002).
105. Wong, S.T. *et al.* A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nature neuroscience* **5**, 1302-8 (2002).
106. Yamashita, T., Higuchi, H. & Tohyama, M. The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. *The Journal of cell biology* **157**, 565-70 (2002).

107. Lee, F.S., Kim, A.H., Khursigara, G. & Chao, M.V. The uniqueness of being a neurotrophin receptor. *Current Opinion in Neurobiology* **11**, 281-286 (2001).
108. Dechant, G. & Barde, Y. a Signalling through the neurotrophin receptor p75NTR. *Current opinion in neurobiology* **7**, 413-8 (1997).
109. Gong, Y., Cao, P., Yu, H.-jun & Jiang, T. Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. *Nature* **454**, 789-93 (2008).
110. He, X.-L. & Garcia, K.C. Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. *Science* **304**, 870-5 (2004).
111. Wehrman, T. *et al.* Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. *Neuron* **53**, 25-38 (2007).
112. Huang, E.J. & Reichardt, L.F. Trk receptors: roles in neuronal signal transduction. *Annual review of biochemistry* **72**, 609-42 (2003).
113. Barker, P. a & Shooter, E.M. Disruption of NGF binding to the low affinity neurotrophin receptor p75LNTR reduces NGF binding to TrkA on PC12 cells. *Neuron* **13**, 203-215 (1994).
114. Kimpinski, K., Jelinski, S. & Mearow, K. The anti-p75 antibody, MC192, and brain-derived neurotrophic factor inhibit nerve growth factor-dependent neurite growth from adult sensory neurons. *Neuroscience* **93**, 253-63 (1999).
115. Williams, G. *et al.* Overcoming the inhibitors of myelin with a novel neurotrophin strategy. *The Journal of biological chemistry* **280**, 5862-9 (2005).
116. Cai, D., Shen, Y., De Bellard, M., Tang, S. & Filbin, M.T. Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. *Neuron* **22**, 89-101 (1999).
117. Johnson, E.J., Taniuchi, M. & Distefano, P.S. Expression and possible function of nerve growth factor receptors on Schwann cells. *Trends in neurosciences* **11**, 299-304 (1988).
118. Zhou, X.-fu & Li, H.-yun Mini-Review Roles of Glial p75NTR in Axonal Regeneration. *Journal of neuroscience research* **85**, 1601-1605 (2007).
119. Liepinsh, E., Ilag, L.L., Otting, G. & Ibáñez, C.F. NMR structure of the death domain of the p75 neurotrophin receptor. *The EMBO journal* **16**, 4999-5005 (1997).
120. Barrette, B., Vallières, N., Dubé, M. & Lacroix, S. Expression profile of receptors for myelin-associated inhibitors of axonal regeneration in the intact and injured mouse central nervous system. *Molecular and cellular neurosciences* **34**, 519-38 (2007).
121. Shao, Z. *et al.* TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. *Neuron* **45**, 353-9 (2005).
122. Park, J.B. *et al.* A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. *Neuron* **45**, 345-51 (2005).
123. Eby, M.T., Jasmin, a, Kumar, a, Sharma, K. & Chaudhary, P.M. TAJ, a novel member of the tumor necrosis factor receptor family, activates the c-Jun N-terminal kinase pathway and mediates caspase-independent cell death. *The Journal of biological chemistry* **275**, 15336-42 (2000).
124. Mi, S. *et al.* LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nature neuroscience* **7**, 221-8 (2004).
125. Okafuji, T. & Tanaka, H. Expression pattern of LINGO-1 in the developing nervous system of the chick embryo. *Gene expression patterns* **6**, 57-62 (2005).
126. Saha, N., Kolev, M.V., Semavina, M., Himanen, J. & Nikolov, D.B. Ganglioside mediate the interaction between Nogo receptor 1 and LINGO-1. *Biochemical and biophysical research communications* **413**, 92-7 (2011).
127. Carim-Todd, L., Escarceller, M., Estivill, X. & Sumoy, L. LRRN6A/LERN1 (leucine-rich repeat neuronal protein 1), a novel gene with enriched expression in limbic system and neocortex. *European Journal of* **18**, 3167-3182 (2003).
128. Mosyak, L. *et al.* The structure of the Lingo-1 ectodomain, a module implicated in central nervous system repair inhibition. *The Journal of biological chemistry* **281**, 36378-90 (2006).
129. Fu, Q.-L. *et al.* Combined effect of brain-derived neurotrophic factor and LINGO-1 fusion protein on long-term survival of retinal ganglion cells in chronic glaucoma. *Neuroscience* **162**, 375-82 (2009).
130. Fu, Q.-L. *et al.* LINGO-1 negatively regulates TrkB phosphorylation after ocular hypertension. *The European journal of neuroscience* **31**, 1091-7 (2010).
131. Lee, X. *et al.* NGF regulates the expression of axonal LINGO-1 to inhibit oligodendrocyte differentiation and myelination. *The Journal of neuroscience* **27**, 220-225 (2007).
132. Mi, S. *et al.* LINGO-1 negatively regulates myelination by oligodendrocytes. *Nature neuroscience* **8**, 745-51 (2005).
133. Jepson, S. *et al.* LINGO-1, a Transmembrane Signaling Protein, Inhibits Oligodendrocyte Differentiation and Myelination through Intercellular Self-interactions. *The Journal of biological chemistry* **287**, 22184-95 (2012).
134. Atwal, J.K. *et al.* PirB is a functional receptor for myelin inhibitors of axonal regeneration. *Science* **322**, 967-70 (2008).
135. Wang, H., Xiong, Y. & Mu, D. PirB restricts neuronal regeneration in developing rat brain following hypoxia-ischemia. *Molecular Medicine Reports* **6**, 59-69 (2012).
136. Fujita, Y., Takashima, R., Endo, S., Takai, T. & Yamashita, T. The p75 receptor mediates axon growth inhibition through an association with PIR-B. *Cell death & disease* **2**, e198 (2011).
137. Fujita, Y., Endo, S., Takai, T. & Yamashita, T. Myelin suppresses axon regeneration by PIR-B/SHP-mediated inhibition of Trk activity. *The EMBO journal* **30**, 1389-401 (2011).

138. Kubagawa, H., Burrows, P.D. & Cooper, M.D. A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 5261-6 (1997).
139. Nakamura, A., Kobayashi, E. & Takai, T. Exacerbated graft-versus-host disease in Pirb-/- mice. *Nature immunology* **5**, 623-9 (2004).
140. Takai, T. & Ono, M. Activating and inhibitory nature of the murine paired immunoglobulin-like receptor family. *Immunological reviews* **181**, 215-22 (2001).
141. Willcox, B.E., Thomas, L.M. & Bjorkman, P.J. Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor. *Nature immunology* **4**, 913-9 (2003).
142. Willcox, B.E. *et al.* Crystal structure of LIR-2 (ILT4) at 1.8 Å: differences from LIR-1 (ILT2) in regions implicated in the binding of the Human Cytomegalovirus class I MHC homolog UL18. *BMC structural biology* **2**, 1-9 (2002).
143. Shiroishi, M. *et al.* Structural basis for recognition of the nonclassical MHC molecule HLA-G by the leukocyte Ig-like receptor B2 (LILRB2/LIR2/ILT4/CD85d). *Proceedings of the National Academy of Sciences of the United States of America* **103**, 16412-7 (2006).
144. Syken, J., Grandpre, T., Kanold, P.O. & Shatz, C.J. PirB restricts ocular-dominance plasticity in visual cortex. *Science* **313**, 1795-800 (2006).
145. Omoto, S., Ueno, M., Mochio, S., Takai, T. & Yamashita, T. Genetic deletion of paired immunoglobulin-like receptor B does not promote axonal plasticity or functional recovery after traumatic brain injury. *The Journal of neuroscience* **30**, 13045-52 (2010).
146. Nakamura, Y., Fujita, Y., Ueno, M., Takai, T. & Yamashita, T. Paired immunoglobulin-like receptor B knockout does not enhance axonal regeneration or locomotor recovery after spinal cord injury. *The Journal of biological chemistry* **286**, 1876-83 (2011).
147. Hu, F. & Strittmatter, S.M. The N-terminal domain of Nogo-A inhibits cell adhesion and axonal outgrowth by an integrin-specific mechanism. *The Journal of neuroscience* **28**, 1262-9 (2008).
148. Laforest, S., Milanini, J., Parat, F., Thimonier, J. & Lehmann, M. Evidences that beta1 integrin and Rac1 are involved in the overriding effect of laminin on myelin-associated glycoprotein inhibitory activity on neuronal cells. *Molecular and cellular neurosciences* **30**, 418-28 (2005).
149. Kelm, S., Pelz, A., Schauer, R., Filbin, M. & Tang, S. Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily. *Current Biology* **4**, (1994).
150. DeBellard, M.E., Tang, S., Mukhopadhyay, G., Shen, Y.J. & Filbin, M.T. Myelin-associated glycoprotein inhibits axonal regeneration from a variety of neurons via interaction with a sialoglycoprotein. *Molecular and cellular neurosciences* **7**, 89-101 (1996).
151. Yang, L.J. *et al.* Gangliosides are neuronal ligands for myelin-associated glycoprotein. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 8412-8417 (2002).
152. Collins, B.E. *et al.* Sialic acid specificity of myelin-associated glycoprotein binding. *The Journal of biological chemistry* **272**, 1248-55 (1997).
153. Sheikh, K. a *et al.* Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 7532-7 (1999).
154. Pan, B. *et al.* Myelin-associated glycoprotein and complementary axonal ligands, gangliosides, mediate axon stability in the CNS and PNS: neuropathology and behavioral deficits in single- and double-null mice. *Experimental neurology* **195**, 208-17 (2005).
155. Vyas, A. a *et al.* Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 8412-7 (2002).
156. Vinson, M. *et al.* Myelin-associated glycoprotein interacts with ganglioside GT1b. A mechanism for neurite outgrowth inhibition. *The Journal of biological chemistry* **276**, 20280-5 (2001).
157. Crocker, P.R., Vinson, M., Kelm, S. & Drickamer, K. Molecular analysis of sialoside binding to sialoadhesin by NMR and site-directed mutagenesis. *The Biochemical journal* **341**, 355-61 (1999).
158. May, a P., Robinson, R.C., Vinson, M., Crocker, P.R. & Jones, E.Y. Crystal structure of the N-terminal domain of sialoadhesin in complex with 3' sialyllactose at 1.85 A resolution. *Molecular cell* **1**, 719-28 (1998).
159. Vinson, M. *et al.* Characterization of the sialic acid-binding site in sialoadhesin by site-directed mutagenesis. *The Journal of biological chemistry* **271**, 9267-72 (1996).
160. Tang, S. *et al.* Myelin-associated glycoprotein interacts with neurons via a sialic acid binding site at ARG118 and a distinct neurite inhibition site. *The Journal of cell biology* **138**, 1355-66 (1997).
161. Mehta, N.R., Lopez, P.H.H., Vyas, A. a & Schnaar, R.L. Gangliosides and Nogo receptors independently mediate myelin-associated glycoprotein inhibition of neurite outgrowth in different nerve cells. *The Journal of biological chemistry* **282**, 27875-86 (2007).
162. Williams, G. *et al.* Ganglioside inhibition of neurite outgrowth requires Nogo receptor function: identification of interaction sites and development of novel antagonists. *The Journal of biological chemistry* **283**, 16641-52 (2008).
163. Fujitani, M. *et al.* Binding of soluble myelin-associated glycoprotein to specific gangliosides induces the association of p75NTR to lipid rafts and signal transduction. *Journal of neurochemistry* **94**, 15-21 (2005).
164. Bandtlow, C.E. & Zimmermann, D.R. Proteoglycans in the developing brain: new conceptual insights for old proteins. *Physiological reviews* **80**, 1267-90 (2000).

165. Rhodes, K.E. & Fawcett, J.W. Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? *Journal of anatomy* **204**, 33-48 (2004).
166. Haas, C. a, Rauch, U., Thon, N., Merten, T. & Deller, T. Entorhinal cortex lesion in adult rats induces the expression of the neuronal chondroitin sulfate proteoglycan neurocan in reactive astrocytes. *The Journal of neuroscience* **19**, 9953-63 (1999).
167. McKeon, R.J., Jurynec, M.J. & Buck, C.R. The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. *The Journal of neuroscience* **19**, 10778-88 (1999).
168. Asher, R. a *et al.* Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. *The Journal of neuroscience* **20**, 2427-38 (2000).
169. Asher, R. a *et al.* Versican is upregulated in CNS injury and is a product of oligodendrocyte lineage cells. *The Journal of neuroscience* **22**, 2225-36 (2002).
170. Levine, J.M. Increased Expression after Brain Injury of the NG2 Chondroitin-Sulfate Proteaglycan. *The Journal of neuroscience* **14**, 4716-4730 (1994).
171. Ong, W.Y. & Levine, J.M. A light and electron microscopic study of NG2 chondroitin sulfate proteoglycan-positive oligodendrocyte precursor cells in the normal and kainate-lesioned rat hippocampus. *Neuroscience* **92**, 83-95 (1999).
172. Rhodes, K.., Moon, L.D.. & Fawcett, J.. Inhibiting cell proliferation during formation of the glial scar: effects on axon regeneration in the CNS. *Neuroscience* **120**, 41-56 (2003).
173. Laywell, E.D. & Steindler, D.A. Boundaries and wounds, glia and glycoconjugates. *Annals of the New York Academy* 122-141 (1991).at <http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.1991.tb15603.x/abstract>
174. Mckeon, R.J., Hocke, A. & Silver, J. Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars. *Experimental neurology* **136**, 32-43 (1995).
175. Jones, L.L., Margolis, R.U. & Tuszynski, M.H. The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. *Experimental Neurology* **182**, 399-411 (2003).
176. Tang, X., Davies, J.E. & Davies, S.J.A. Changes in Distribution , Cell Associations , and Protein Expression Levels of NG2 , V2 , and Tenascin-C During Acute to Chronic Maturation of Spinal Cord Scar Tissue. *Journal of Neuroscience Research* **71**, 427-444 (2003).
177. Dou, C.L. & Levine, J.M. Identification of a neuronal cell surface receptor for a growth inhibitory chondroitin sulfate proteoglycan (NG2). *Journal of neurochemistry* **68**, 1021-30 (1997).
178. Dou, C.L. & Levine, J.M. Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. *The Journal of neuroscience* **14**, 7616-28 (1994).
179. Friedlander, D.R. *et al.* The neuronal chondroitin sulfate proteoglycan neurocan binds to the neural cell adhesion molecules Ng-CAM/L1/NILE and N-CAM, and inhibits neuronal adhesion and neurite outgrowth. *The Journal of cell biology* **125**, 669-80 (1994).
180. Smith-Thomas, L.C. *et al.* An inhibitor of neurite outgrowth produced by astrocytes. *Journal of cell science* **107**, 1687-95 (1994).
181. Yamada, H. *et al.* The brain chondroitin sulfate proteoglycan brevican associates with astrocytes ensheathing cerebellar glomeruli and inhibits neurite outgrowth from granule neurons. *The Journal of Neuroscience* **17**, 7784-7795 (1997).
182. Niederöst, B.P., Zimmermann, D.R., Schwab, M.E. & Bandtlow, C.E. Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. *The Journal of neuroscience* **19**, 8979-89 (1999).
183. Schmalfeldt, M., Bandtlow, C.E., Dours-Zimmermann, M.T., Winterhalter, K.H. & Zimmermann, D.R. Brain derived versican V2 is a potent inhibitor of axonal growth. *Journal of cell science* **113**, 807-16 (2000).
184. Shen, Y. *et al.* PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. *Science* **326**, 592-6 (2009).
185. Kantor, D.B. *et al.* Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. *Neuron* **44**, 961-75 (2004).
186. Pizzorusso, T. *et al.* Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* **298**, 1248-51 (2002).
187. Dickendesher, T.L. *et al.* NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. *Nature Neuroscience* **15**, (2012).
188. Culotti, J.G. & Kolodkin, A.L. Functions of netrins and semaphorins in axon guidance. *Current opinion in neurobiology* **6**, 81-88 (1996).
189. Janssen, B.J.C. *et al.* Structural basis of semaphorin-plexin signalling. *Nature* **467**, 1118-22 (2010).
190. Kury, P., Abankwa, D., Kruse, F., Greiner-Petter, R. & Muller, H.W. Gene expression profiling reveals multiple novel intrinsic and extrinsic factors associated with axonal regeneration failure. *European journal of* **19**, 32-42 (2004).
191. Messersmith, E.K. *et al.* Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord. *Neuron* **14**, 949-59 (1995).
192. Püschel, a W., Adams, R.H. & Betz, H. Murine semaphorin D/collapsin is a member of a diverse gene family and creates domains inhibitory for axonal extension. *Neuron* **14**, 941-8 (1995).
193. Pasterkamp, R.J., Anderson, P.N. & Verhaagen, J. Peripheral nerve injury fails to induce growth of lesioned ascending dorsal column axons into spinal cord scar tissue expressing the axon repellent Semaphorin3A. *The European journal of neuroscience* **13**, 457-71 (2001).

194. Luo, Y., Raible, D. & Raper, J. a Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. *Cell* **75**, 217-27 (1993).
195. Reza, J.N., Gavazzi, I. & Cohen, J. Neuropilin-1 Is Expressed on Adult Mammalian Dorsal Root Ganglion Neurons and Mediates Semaphorin3a/Collapsin-1-Induced Growth Cone Collapse by Small Diameter Sensory Afferents. *Molecular and Cellular Neuroscience* **14**, 317-326 (1999).
196. Takahashi, T. *et al.* Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. *Cell* **99**, 59-69 (1999).
197. Ben-Zvi, A., Ben-Gigi, L., Klein, H. & Behar, O. Modulation of semaphorin3A activity by p75 neurotrophin receptor influences peripheral axon patterning. *The Journal of neuroscience* **27**, 13000-13011 (2007).
198. Goshima, Y., Nakamura, F., Strittmatter, P. & Strittmatter, S.M. Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. *Nature* **376**, 509-514 (1995).
199. Song, H.-jun *et al.* Conversion of Neuronal Growth Cone Responses from Repulsion to Attraction by Cyclic Nucleotides. *Science* **281**, 1515-1518 (1998).
200. Moreau-Fauvarque, C. *et al.* The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. *The Journal of neuroscience* **23**, 9229-39 (2003).
201. Goldberg, J.L. *et al.* An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. *The Journal of neuroscience* **24**, 4989-99 (2004).
202. Benson, M.D. *et al.* Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 10694-9 (2005).
203. Goldshmit, Y., Galea, M.P., Wise, G., Bartlett, P.F. & Turnley, A.M. Axonal regeneration and lack of astrocytic gliosis in EphA4-deficient mice. *The Journal of neuroscience* **24**, 10064-73 (2004).
204. Fabes, J. *et al.* Accumulation of the inhibitory receptor EphA4 may prevent regeneration of corticospinal tract axons following lesion. *The European journal of neuroscience* **23**, 1721-30 (2006).
205. Figueroa, J.D. *et al.* Inhibition of EphA7 Up-Regulation After Spinal Cord Injury Reduces Apoptosis and Promotes Locomotor Recovery. *Journal of Neuroscience Research* **84**, 1438-1451 (2006).
206. Irizarry-Ramirez, M. *et al.* Upregulation of EphA3 receptor after spinal cord injury. *Journal of Neurotrauma* **22**, 929-935 (2005).
207. Serafini, T. *et al.* The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. *Cell* **78**, 409-24 (1994).
208. Löw, K., Culbertson, M., Bradke, F., Tessier-Lavigne, M. & Tuszynski, M.H. Netrin-1 is a novel myelin-associated inhibitor to axon growth. *The Journal of neuroscience* **28**, 1099-108 (2008).
209. Schwab, J.M. *et al.* Central nervous system injury-induced repulsive guidance molecule expression in the adult human brain. *Archives of neurology* **62**, 1561-8 (2005).
210. Schwab, J.M. *et al.* Spinal cord injury-induced lesional expression of the repulsive guidance molecule (RGM). *The European journal of neuroscience* **21**, 1569-76 (2005).
211. Hata, K. *et al.* RGMa inhibition promotes axonal growth and recovery after spinal cord injury. *The Journal of cell biology* **173**, 47-58 (2006).
212. Liu, Y. *et al.* Repulsive Wnt Signaling Inhibits Axon Regeneration after CNS Injury. *Journal of Neuroscience* **28**, 8376-8382 (2008).
213. Miyashita, T. *et al.* Wnt-Ryk signaling mediates axon growth inhibition and limits functional recovery after spinal cord injury. *Journal of neurotrauma* **26**, 955-64 (2009).
214. Morishita, H., Miwa, J.M., Heintz, N. & Hensch, T.K. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science* **330**, 1238-40 (2010).
215. Zhang, L. *et al.* Identification of BLyS (B lymphocyte stimulator), a non-myelin-associated protein, as a functional ligand for Nogo-66 receptor. *The Journal of neuroscience* **29**, 6348-52 (2009).
216. Thomas, R. *et al.* LGI1 is a Nogo receptor 1 ligand that antagonizes myelin-based growth inhibition. *The Journal of neuroscience* **30**, 6607-12 (2010).
217. Venkatesh, K., Chivatakarn, O., Sheu, S.-S. & Giger, R.J. Molecular dissection of the myelin-associated glycoprotein receptor complex reveals cell type-specific mechanisms for neurite outgrowth inhibition. *The Journal of cell biology* **177**, 393-9 (2007).
218. Lee, J.-K., Kim, J.-E., Sivula, M. & Strittmatter, S.M. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. *The Journal of neuroscience* **24**, 6209-17 (2004).
219. Ahmed, Z. *et al.* Disinhibition of neurotrophin-induced dorsal root ganglion cell neurite outgrowth on CNS myelin by siRNA-mediated knockdown of NgR, p75NTR and Rho-A. *Molecular and cellular neurosciences* **28**, 509-23 (2005).
220. Cafferty, W.B.J., Duffy, P., Huebner, E. & Strittmatter, S.M. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. *The Journal of neuroscience* **30**, 6825-37 (2010).
221. Lee, J.K. *et al.* Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. *Neuron* **66**, 663-70 (2010).
222. Petrinovic, M.M. *et al.* Neuronal Nogo-A regulates neurite fasciculation, branching and extension in the developing nervous system. *Development* **137**, 2539-50 (2010).
223. Ozdinler, P.H., Ulupinar, E. & Erzurumlu, R.S. Dose and age-dependent axonal responses of embryonic trigeminal neurons to localized NGF via p75NTR receptor. *Journal of neurobiology* **62**, 189-206 (2005).
224. Lee, H. *et al.* Synaptic function for the Nogo-66 receptor NgR1: regulation of dendritic spine morphology and activity-dependent synaptic strength. *The Journal of neuroscience* **28**, 2753-65 (2008).

225. Karlen, A. *et al.* Nogo receptor 1 regulates formation of lasting memories. *Proceedings of the* **106**, 20476-20481 (2009).
226. Raiker, S.J. *et al.* Oligodendrocyte-myelin glycoprotein and Nogo negatively regulate activity-dependent synaptic plasticity. *The Journal of neuroscience* **30**, 12432-45 (2010).
227. Zagrebelsky, M., Schweigreiter, R., Bandtlow, C.E., Schwab, M.E. & Korte, M. Nogo-A stabilizes the architecture of hippocampal neurons. *The Journal of neuroscience* **30**, 13220-34 (2010).
228. Yu, W., Guo, W. & Feng, L. Segregation of Nogo66 receptors into lipid rafts in rat brain and inhibition of Nogo66 signaling by cholesterol depletion. *FEBS letters* **577**, 87-92 (2004).
229. Niederöst, B., Oertle, T., Fritsche, J., McKinney, R.A. & Bandtlow, C.E. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *The Journal of neuroscience* **22**, 10368-76 (2002).
230. Etienne-Manneville, S. & Hall, A. Rho GTPases in cell biology. *Nature* **420**, 629-35 (2002).
231. Yamashita, T., Tucker, K.L. & Barde, Y. a Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron* **24**, 585-93 (1999).
232. Sung, J.K. *et al.* A possible role of RhoA/Rho-kinase in experimental spinal cord injury in rat. *Brain research* **959**, 29-38 (2003).
233. Lehmann, M. *et al.* Inactivation of Rho signaling pathway promotes CNS axon regeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **19**, 7537-47 (1999).
234. Fournier, A.E., Takizawa, B.T. & Strittmatter, S.M. Rho kinase inhibition enhances axonal regeneration in the injured CNS. *The Journal of neuroscience* **23**, 1416-23 (2003).
235. Yamashita, T. & Tohyama, M. The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nature neuroscience* **6**, 461-7 (2003).
236. Sasaki, T. & Takai, Y. The Rho small G protein family-Rho GDI system as a temporal and spatial determinant for cytoskeletal control. *Biochemical and biophysical research communications* **245**, 641-5 (1998).
237. Penzes, P., Johnson, R.C., Kambampati, V., Mains, R.E. & Eipper, B.A. Distinct roles for the two Rho GDP/GTP exchange factor domains of kalirin in regulation of neurite growth and neuronal morphology. *The Journal of neuroscience* **21**, 8426-34 (2001).
238. Harrington, A.W. *et al.* The role of Kalirin9 in p75/nogo receptor-mediated RhoA activation in cerebellar granule neurons. *The Journal of biological chemistry* **283**, 24690-7 (2008).
239. Zhang, Z. *et al.* LINGO-1 interacts with WNK1 to regulate nogo-induced inhibition of neurite extension. *The Journal of biological chemistry* **284**, 15717-28 (2009).
240. Sivasankaran, R. *et al.* PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. *Nature neuroscience* **7**, 261-8 (2004).
241. Hasegawa, Y. *et al.* Promotion of axon regeneration by myelin-associated glycoprotein and Nogo through divergent signals downstream of Gi/G. *The Journal of neuroscience* **24**, 6826-32 (2004).
242. Shim, S. *et al.* Peptidyl-prolyl isomerase FKBP52 controls chemotropic guidance of neuronal growth cones via regulation of TRPC1 channel opening. *Neuron* **64**, 471-83 (2009).
243. Shim, S. *et al.* XTRPC1-dependent chemotropic guidance of neuronal growth cones. *Nature neuroscience* **8**, 730-5 (2005).
244. Koprivica, V. *et al.* EGFR activation mediates inhibition of axon regeneration by myelin and chondroitin sulfate proteoglycans. *Science* **310**, 106-10 (2005).
245. Erschbamer, M., Pernold, K. & Olson, L. Inhibiting epidermal growth factor receptor improves structural, locomotor, sensory, and bladder recovery from experimental spinal cord injury. *The Journal of neuroscience* **27**, 6428-35 (2007).
246. Inoue, H. *et al.* Inhibition of the leucine-rich repeat protein LINGO-1 enhances survival , structure , and function of dopaminergic neurons in Parkinson ' s disease models. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 14430-14435 (2007).
247. Hofman, E.G. *et al.* EGF induces coalescence of different lipid rafts. *Journal of cell science* **121**, 2519-28 (2008).
248. Ahmed, Z., Read, M.L., Berry, M. & Logan, A. Satellite glia not DRG neurons constitutively activate EGFR but EGFR inactivation is not correlated with axon regeneration. *Neurobiology of disease* **39**, 292-300 (2010).
249. Neumann, S., Bradke, F., Tessier-Lavigne, M. & Basbaum, A.I. Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. *Neuron* **34**, 885-93 (2002).
250. Qiu, J., Cai, D., Dai, H., McAtee, M. & Hoffman, P. Spinal axon regeneration induced by elevation of cyclic AMP. *Neuron* **34**, 895-903 (2002).
251. Neumann, S. & Woolf, C.J. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron* **23**, 83-91 (1999).
252. Cai, D. *et al.* Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. *Neuron* **35**, 711-9 (2002).
253. Gao, Y. *et al.* Activated CREB is sufficient to overcome inhibitors in myelin and promote spinal axon regeneration in vivo. *Neuron* **44**, 609-21 (2004).
254. Kim, G.H. *et al.* The role of polyamine metabolism in neuronal injury following cerebral ischemia. *The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques* **36**, 14-9 (2009).
255. DiStefano, P.S. & Johnson, E.M. Identification of a truncated form of the nerve growth factor receptor. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 270-4 (1988).

256. Domeniconi, M. *et al.* MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. *Neuron* **46**, 849-55 (2005).
257. Ahmed, Z. *et al.* TACE-induced cleavage of NgR and p75NTR in dorsal root ganglion cultures disinhibits outgrowth and promotes branching of neurites in the presence of inhibitory CNS myelin. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 1939-41 (2006).
258. Luo, L. Rho GTPases in neuronal morphogenesis. *Nature Reviews Neuroscience* **1**, 3-10 (2000).
259. Ishizaki, T. *et al.* The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. *The EMBO journal* **15**, 1885-93 (1996).
260. Amano, M., Nakayama, M. & Kaibuchi, K. Rho-kinase/ROCK: A key regulator of the cytoskeleton and cell polarity. *Cytoskeleton* **67**, 545-54 (2010).
261. Kimura, K. *et al.* Regulation of Myosin Phosphatase by Rho and Rho-Associated Kinase (Rho-Kinase). *Science* **273**, 245-248 (1996).
262. Amano, M. *et al.* Formation of Actin Stress Fibers and Focal Adhesions Enhanced by Rho-Kinase. *Science* **275**, 1308-1311 (1997).
263. Hirose, M. *et al.* Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. *The Journal of cell biology* **141**, 1625-36 (1998).
264. Monnier, P.P., Sierra, A., Schwab, J.M., Henke-Fahle, S. & Mueller, B.K. The Rho/ROCK pathway mediates neurite growth-inhibitory activity associated with the chondroitin sulfate proteoglycans of the CNS glial scar. *Molecular and Cellular Neuroscience* **22**, 319-330 (2003).
265. Montani, L. *et al.* Neuronal Nogo-A modulates growth cone motility via Rho-GTP/LIMK1/cofilin in the unlesioned adult nervous system. *The Journal of biological chemistry* **284**, 10793-807 (2009).
266. Hsieh, S.H.-K., Ferraro, G.B. & Fournier, A.E. Myelin-associated inhibitors regulate cofilin phosphorylation and neuronal inhibition through LIM kinase and Slingshot phosphatase. *The Journal of neuroscience* **26**, 1006-15 (2006).
267. Joset, A., Dodd, D. a, Halegoua, S. & Schwab, M.E. Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. *The Journal of cell biology* **188**, 271-85 (2010).
268. Dickson, H.M., Zurawski, J., Zhang, H., Turner, D.L. & Vojtek, A.B. POSH is an intracellular signal transducer for the axon outgrowth inhibitor Nogo66. *The Journal of neuroscience* **30**, 13319-25 (2010).
269. Taylor, J. *et al.* The scaffold protein POSH regulates axon outgrowth. *Molecular Biology of the Cell* **19**, 5181-5192 (2008).
270. Burns, S.P., Golding, D.G., Rolle, W. a, Graziani, V. & Ditunno, J.F. Recovery of ambulation in motor-incomplete tetraplegia. *Archives of physical medicine and rehabilitation* **78**, 1169-72 (1997).
271. Bareyre, F.M. *et al.* The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nature neuroscience* **7**, 269-77 (2004).
272. Bregman, B., Kunkel-Bagden, E., Schnell, L. & Dai, H. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. *Nature* **378**, 498-501 (1995).
273. Liebscher, T. *et al.* Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. *Annals of neurology* **58**, 706-19 (2005).
274. Raineteau, O., Fouad, K., Noth, P., Thallmair, M. & Schwab, M.E. Functional switch between motor tracts in the presence of the mAb IN-1 in the adult rat. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 6929-34 (2001).
275. Bareyre, F.M., Haudenschild, B. & Schwab, M.E. Long-lasting sprouting and gene expression changes induced by the monoclonal antibody IN-1 in the adult spinal cord. *The Journal of neuroscience* **22**, 7097-110 (2002).
276. Brösamle, C., Huber, a B., Fiedler, M., Skerra, a & Schwab, M.E. Regeneration of lesioned corticospinal tract fibers in the adult rat induced by a recombinant, humanized IN-1 antibody fragment. *The Journal of neuroscience* **20**, 8061-8 (2000).
277. Fouad, K., Klusman, I. & Schwab, M.E. Regenerating corticospinal fibers in the Marmoset (Callitrix jacchus) after spinal cord lesion and treatment with the anti-Nogo-A antibody IN-1. *The European journal of neuroscience* **20**, 2479-82 (2004).
278. Freund, P. *et al.* Anti-Nogo-A antibody treatment promotes recovery of manual dexterity after unilateral cervical lesion in adult primates--re-examination and extension of behavioral data. *The European journal of neuroscience* **29**, 983-96 (2009).
279. Freund, P. *et al.* Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. *Nature medicine* **12**, 790-2 (2006).
280. Freund, P. *et al.* Anti-Nogo-A antibody treatment enhances sprouting of corticospinal axons rostral to a unilateral cervical spinal cord lesion in adult macaque monkey. *The Journal of Comparative Neurology* **502**, 644-659 (2007).
281. Papadopoulos, C.M. *et al.* Functional Recovery and Neuroanatomical Plasticity following Middle Cerebral Artery Occlusion and IN-1 Antibody Treatment in the Adult Rat. *Annals of neurology* **51**, 433-441 (2002).
282. Emerick, A.J., Neafsey, E.J., Schwab, M.E. & Kartje, G.L. Functional reorganization of the motor cortex in adult rats after cortical lesion and treatment with monoclonal antibody IN-1. *The Journal of neuroscience* **23**, 4826-30 (2003).

283. Wiessner, C. *et al.* Anti – Nogo-A Antibody Infusion 24 Hours After Experimental Stroke Improved Behavioral Outcome and Corticospinal Plasticity in Normotensive and Spontaneously Hypertensive Rats. *Journal of Cerebral Bloodflow and Metabolism* **23**, 154-165 (2003).
284. Markus, T.M. *et al.* Recovery and brain reorganization after stroke in adult and aged rats. *Annals of neurology* **58**, 950-953 (2005).
285. Papadopoulos, C.M. *et al.* Dendritic plasticity in the adult rat following middle cerebral artery occlusion and Nogo-a neutralization. *Cerebral cortex* **16**, 529-36 (2006).
286. Gillani, R.L. *et al.* Cognitive recovery in the aged rat after stroke and anti-Nogo-A immunotherapy. *Behavioural brain research* **208**, 415-24 (2010).
287. Lv, J. *et al.* Passive immunization with LINGO-1 polyclonal antiserum afforded neuroprotection and promoted functional recovery in a rat model of spinal cord injury. *Neuroimmunomodulation* **17**, 270-8 (2010).
288. Lehmann, H.C. *et al.* Passive immunization with anti-ganglioside antibodies directly inhibits axon regeneration in an animal model. *The Journal of neuroscience* **27**, 27-34 (2007).
289. Li, S. *et al.* Blockade of Nogo-66, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein by soluble Nogo-66 receptor promotes axonal sprouting and recovery after spinal injury. *The Journal of neuroscience* **24**, 10511-20 (2004).
290. Li, S., Kim, J.-E., Budel, S., Hampton, T.G. & Strittmatter, S.M. Transgenic inhibition of Nogo-66 receptor function allows axonal sprouting and improved locomotion after spinal injury. *Molecular and cellular neurosciences* **29**, 26-39 (2005).
291. Wang, X., Baughman, K.W., Basso, D.M. & Strittmatter, S.M. Delayed Nogo receptor therapy improves recovery from spinal cord contusion. *Annals of neurology* **60**, 540-9 (2006).
292. Ji, B. *et al.* LINGO-1 antagonist promotes functional recovery and axonal sprouting after spinal cord injury. *Molecular and cellular neurosciences* **33**, 311-20 (2006).
293. Li, S. & Strittmatter, S.M. Delayed systemic Nogo-66 receptor antagonist promotes recovery from spinal cord injury. *The Journal of neuroscience* **23**, 4219-27 (2003).
294. Dupuis, L. *et al.* Nogo receptor antagonizes p75NTR-dependent motor neuron death. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 740-5 (2008).
295. Montolio, M. *et al.* A semaphorin 3A inhibitor blocks axonal chemorepulsion and enhances axon regeneration. *Chemistry & biology* **16**, 691-701 (2009).
296. Fabes, J., Anderson, P., Brennan, C. & Bolsover, S. Regeneration-enhancing effects of EphA4 blocking peptide following corticospinal tract injury in adult rat spinal cord. *The European journal of neuroscience* **26**, 2496-505 (2007).
297. Wang, Y. *et al.* Aptamer antagonists of myelin-derived inhibitors promote axon growth. *PloS one* **5**, e9726 (2010).
298. Hara, M. *et al.* Protein kinase inhibition by fasudil hydrochloride promotes neurological recovery after spinal cord injury in rats. *Journal of neurosurgery* **93**, 94-101 (2000).
299. Nishio, Y. *et al.* Delayed treatment with Rho-kinase inhibitor does not enhance axonal regeneration or functional recovery after spinal cord injury in rats. *Experimental neurology* **200**, 392-7 (2006).
300. Dergham, P. *et al.* Rho signaling pathway targeted to promote spinal cord repair. *The Journal of neuroscience* **22**, 6570-7 (2002).
301. Ellezam, B. *et al.* Inactivation of intracellular Rho to stimulate axon growth and regeneration. *Progress in brain research* **137**, 371-80 (2002).
302. Borisoff, J.F. *et al.* Suppression of Rho-kinase activity promotes axonal growth on inhibitory CNS substrates. *Molecular and Cellular Neuroscience* **22**, 405-416 (2003).
303. Bertrand, J., Winton, M.J., Rodriguez-Hernandez, N., Campenot, R.B. & McKerracher, L. Application of Rho antagonist to neuronal cell bodies promotes neurite growth in compartmented cultures and regeneration of retinal ganglion cell axons in the optic nerve of adult rats. *The Journal of neuroscience* **25**, 1113-21 (2005).
304. Ichikawa, M. *et al.* Differential effects of two ROCK inhibitors, Fasudil and Y-27632, on optic nerve regeneration in adult cats. *Brain research* **1201**, 23-33 (2008).
305. Smith, G.M. & Strunz, C. Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. *Glia* **52**, 209-18 (2005).
306. Liu, B., Chen, H., Johns, T.G. & Neufeld, A.H. Epidermal growth factor receptor activation: an upstream signal for transition of quiescent astrocytes into reactive astrocytes after neural injury. *The Journal of neuroscience* **26**, 7532-40 (2006).
307. Park, K.K. *et al.* Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. *Science* **322**, 963-966 (2008).
308. Rosivatz, E. *et al.* A Small-Molecule Inhibitor for Phosphatase and Tensin Homologue Deleted on Chromosome 10 (PTEN). *ACS chemical biology* **1**, 780-790 (2006).
309. Kikuchi, K. *et al.* In vitro and in vivo characterization of a novel semaphorin 3A inhibitor, SM-216289 or xanthofulvin. *The Journal of biological chemistry* **278**, 42985-91 (2003).
310. Kaneko, S. *et al.* A selective Sema3A inhibitor enhances regenerative responses and functional recovery of the injured spinal cord. *Nature medicine* **12**, 1380-9 (2006).
311. Ramer, M.S., Priestley, J.V. & McMahon, S.B. Functional regeneration of sensory axons into the adult spinal cord. *Nature* **403**, 312-6 (2000).
312. Coumans, J.V. *et al.* Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins. *The Journal of neuroscience* **21**, 9334-44 (2001).

313. Menei, P., Montero-Menei, C., Whittemore, S.R., Bunge, R.P. & Bunge, M.B. Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord. *The European journal of neuroscience* **10**, 607-21 (1998).
314. Ming, G., Henley, J., Tessier-Lavigne, M., Song, H. & Poo, M. Electrical activity modulates growth cone guidance by diffusible factors. *Neuron* **29**, 441-52 (2001).
315. Bradbury, E.J. *et al.* Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* **416**, 636-40 (2002).
316. Yang, L.J.S. *et al.* Sialidase enhances spinal axon outgrowth in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 11057-62 (2006).
317. Fischer, D., Zhigang, H. & Benowitz, L.I. Counteracting the Nogo Receptor Enhances Optic Nerve Regeneration If Retinal Ganglion Cells Are in an Active Growth State. *Journal of Neuroscience* **24**, 1646-1651 (2004).
318. Kadoya, K. *et al.* Combined intrinsic and extrinsic neuronal mechanisms facilitate bridging axonal regeneration one year after spinal cord injury. *Neuron* **64**, 165-72 (2009).
319. He, W. *et al.* Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. *Nature medicine* **10**, 959-65 (2004).
320. Murayama, K.S. *et al.* Reticulons RTN3 and RTN4-B/C interact with BACE1 and inhibit its ability to produce amyloid beta-protein. *The European journal of neuroscience* **24**, 1237-44 (2006).
321. He, W. *et al.* Mapping of interaction domains mediating binding between BACE1 and RTN/Nogo proteins. *Journal of molecular biology* **363**, 625-34 (2006).
322. Masliah, E. *et al.* Genetic deletion of Nogo/Rtn4 ameliorates behavioral and neuropathological outcomes in amyloid precursor protein transgenic mice. *Neuroscience* **169**, 488-94 (2010).
323. Park, J.H. *et al.* Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-beta plaque deposition. *The Journal of neuroscience* **26**, 1386-95 (2006).
324. Park, J.H. *et al.* Subcutaneous Nogo receptor removes brain amyloid-beta and improves spatial memory in Alzheimer's transgenic mice. *The Journal of neuroscience* **26**, 13279-86 (2006).
325. LadyofHats http://en.wikipedia.org/wiki/File:Complete_neuron_cell_diagram_en.svg. (2007).
326. Andrew, C. http://en.wikipedia.org/wiki/File:Neuron_with_oligodendrocyte_and_myelin_sheath.svg. (2010).
327. Debivort http://en.wikipedia.org/wiki/File:Cervical_vertebra_english.png. (2007).
328. Häggström, S. & Häggström, M. http://en.wikipedia.org/wiki/File:Spinal_cord_tracts_-_English.svg. (2010).