Extracellular regulators of axonal growth in the adult central nervous system

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Robust axonal growth is required during development to establish neuronal connectivity. However, stable fibre patterns are necessary to maintain adult mammalian central nervous system (CNS) function. After adult CNS injury, factors that maintain axonal stability limit the recovery of function. Extracellular molecules play an important role in preserving the stability of the adult CNS axons and in restricting recovery from pathological damage. Adult axonal growth inhibitors include a group of proteins on the oligodendrocyte, Nogo-A, myelin-associated glycoprotein, oligodendrocyte-myelin glycoprotein and ephrin-B3, which interact with axonal receptors, such as NgR1 and EphA4. Extracellular proteoglycans containing chondroitin sulphates also inhibit axonal sprouting in the adult CNS, particularly at the sites of astroglial scar formation. Therapeutic perturbations of these extracellular axonal growth inhibitors and their receptors or signalling mechanisms provide a degree of axonal sprouting and regeneration in the adult CNS. After CNS injury, such interventions support a partial return of neurological function.

Keywords: spinal cord injury; axon regeneration; myelin; Nogo; Nogo receptor; chondroitin sulphate proteoglycans

1. INTRODUCTION

During development, growth-inhibitory interactions mediated by membrane-bound and diffusible cues regulate the guidance of axons towards their targets and contribute to synapse selection and formation. The last decade and a half has witnessed an explosion of molecular knowledge regarding these events. It is now clear that extracellular semaphorins, ephrins, netrins, slits and repulsive guidance molecules interact with their axonal receptors, plexins, neuropilins, Eph kinases, deleted in colorectal carcinoma, Unc5s, robos and neogenin, to generate the precision of axonal guidance required for the formation of a functional nervous system (Tessier-Lavigne & Goodman 1996; Yu & Bargmann 2001; Monnier *et al.* 2002; Matsunaga *et al.* 2004; Rajagopalan *et al.* 2004; figure 1). Growth-inhibitory mechanisms are at least as crucial as growth-stimulating effects during the axonal guidance period. Once axonal target zones are identified, synaptic connections are refined in juveniles during a period of experience-dependent plasticity that relies heavily on *N*-methyl-D-aspartate (NMDA) receptors and neurotrophins (Fox & Wong 2005; Hensch 2005; Taha & Stryker 2005).

After the establishment and refinement of central nervous system (CNS) tracts during embryonic and early postnatal periods, the mammalian CNS maintains a relatively stable pattern of connectivity. The growth and rearrangement of adult fibres over distances greater than 1 mm is rare (Holtmaat *et al.*

2005). While gross axonal stability is necessary to maintain higher order CNS function, this fixed 'wiring' of neurons limits the ability of the adult CNS to recover from traumatic, ischaemic and inflammatory injury.

It is of fundamental biological interest and crucial therapeutic implication to define the molecular basis for disparities in the stability of neuronal connections as a function of developmental epoch and anatomical region. For injured adult CNS neurons, axonal growth is limited by both the lack of cell-autonomous intrinsic growth promoters and the presence of extrinsic environmental inhibitors. In contrast, damage to the peripheral nervous system (PNS) results in successful axonal regeneration, which relies on both the induction of a neuronal growth response and the exposure to a permissive environment. Those cell-autonomous factors, which promote peripheral axonal regeneration, but are lacking from injured adult central neurons, have been reviewed elsewhere (Skene 1989; Bomze *et al.* 2001). This paper focuses on the environmental inhibitors located in CNS myelin and astroglial scar, which restrict the plasticity and regeneration of adult CNS axons.

The non-permissive nature of the mature CNS was first appreciated by the observations made by Cajal in 1927. Subsequently, Aguayo & Richardson demonstrated that providing damaged CNS axons with a permissive substrate allows them to regenerate over long distances (David & Aguayo 1981; Richardson *et al.* 1982). The permissive environment used in these studies was a peripheral nerve graft devoid of CNS glia, thereby implicating these cells as pivotal in mediating the inhibition of axon regeneration in the adult CNS.

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Figure 1. Molecular regulation of axonal guidance, plasticity and regeneration. A schematic summarizes the shift of CNS axons through three phases of development. In the embryonic period, there is rapid axonal growth and the premium is on guidance. In the neonatal and juvenile phase, connectivity is plastic and undergoes refinement. In the third adult phase, axons are stable with myelin and CSPGs playing crucial roles in limiting rearrangements. The extracellular molecules and receptors most critical in each stage of this developmental progression are listed. Axonal growth, plasticity and regeneration in the injured adult CNS may be achieved by perturbing the function of those extracellular molecules limiting axonal growth in the adult setting.

Subsequent experiments revealed that CNS myelin homogenates were capable of collapsing growth cones and blocking neurite outgrowth *in vitro* (Savio & Schwab 1989; Bandtlow *et al.* 1990). Furthermore, axons regenerating *in vivo* appeared to become dystrophic and cease elongation when entering areas of astrogliosis (Davies *et al.* 1999). These investigations led to the hypothesis that axon growth inhibitors associated with adult CNS myelin and glial scar exist and are responsible for restricting CNS axon regeneration. Several such inhibitors have been identified in the last several years. These include chondroitin sulphate proteoglycans (CSPGs) and the myelin-associated molecules, Nogo-A, myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp) and ephrin-B3.

2. MYELIN-DERIVED INHIBITORS

(a) Nogo

Nogo's inhibitory activity was characterized by Schwab and colleagues over 17 years ago through size fractionation of adult CNS myelin by SDS-PAGE (Caroni & Schwab 1988). Membrane-associated proteins of 35 and 250 kDa, termed NI-35/250, inhibited neurite outgrowth. Following the publication of six partial peptide sequences derived from a proteolytic digest of the bovine homologue of rat NI250 (Spillmann *et al.* 1998), three groups independently identified a cDNA encoding NI-250, termed Nogo-A (Chen *et al.* 2000; GrandPre *et al.* 2000; Prinjha *et al.* 2000).

Alternative transcription of the Nogo gene results in the three different variants: Nogo-A (1162 amino acids), Nogo-B (373 amino acids) and Nogo-C (199 amino acids). They have a common carboxyl terminus of 188 amino acids that is homologous to members of the reticulon gene family (Chen *et al.* 2000; GrandPre *et al.* 2000; Prinjha *et al.* 2000). Reticulons constitute a family of endoplasmic reticulum-associated proteins with largely uncharacterized functions. Nogo-A expression is high in CNS oligodendrocytes and

minimal in peripheral myelinating Schwann cells, consistent with a role in mediating axonal growth inhibition specifically in the CNS.

All the three Nogo isoforms contain two hydrophobic domains that render these macromolecules integral membrane proteins. The loop between the two transmembrane domains, termed Nogo-66, can be detected in part at the extracellular surface and has axon growth-inhibitory effects (GrandPre *et al.* 2000; Prinjha *et al.* 2000). Nogo-66 binds to a neuronal receptor termed the Nogo-66 receptor that transduces its inhibitory activity (discussed in detail below). The amino-terminal domain of Nogo-A contains a unique sequence ($\Delta 20$) that inhibits both axon outgrowth and fibroblast spreading *in vitro*, independently of the Nogo-66 receptor (Fournier *et al.* 2001; Oertle *et al.* 2003). The monoclonal antibody IN-1 neutralizes the amino-terminal domain of Nogo-A (Fiedler *et al.* 2002). The neuronal receptor for this amino-terminal domain of Nogo-A is not yet known.

Two main topologies have been proposed for Nogo-A, one with amino-Nogo localized intracellularly and the other with amino-Nogo facing extracellularly (figure 2). Full characterization of these topologies is critical for understanding the Nogo-A access to intact and regenerating axons. Antibodies directed against Nogo-66 and amino-Nogo stain the surface of differentiated oligodendrocytes in culture, indicating that both Nogo-66 and amino-Nogo can be extracellular (Oertle *et al.* 2003). Additional evidence for a topology oriented with both domains in the extracellular space derives from the observation that an amino-Nogo-A-24, can bind to the receptor for Nogo-66 (Hu *et al.* 2005). The combination of Nogo-66 with this amino-Nogo-A-24 domain creates a substantially higher affinity for the NgR1, with a dissociation constant in the subnanomolar range. It is possible that Nogo-A can assume different topologies in different cell types and that its topology might be regulated in response to cellular signals such as contact with axons or in response to axon damage (Hu & Strittmatter 2004).

adult neuron types: retinal, superior cervical ganglion, spinal and hippocampal neurons. However, it promotes neurite outgrowth from neonatal dorsal root ganglion neurons (Mukhopadhyay *et al.* 1994), suggesting that MAG may provide a permissive substrate for axon outgrowth during early development, but restrict aberrant growth in adulthood. Since MAG is expressed in both the peripheral and central myelin fractions, its expression does not correlate precisely with axon regenerative permissiveness, suggesting that it might be less relevant than Nogo-A. However, MAG expression in Schwann cells might be compensated for by more rapid clearance of MAG in the periphery than in the CNS during Wallerian degeneration. Furthermore, MAG expression is upregulated in response to cortical axotomy, indicating that it may limit axonal regeneration after CNS injury (Mingorance *et al.* 2005).

MAG, like Nogo-66, can exert its axon-inhibitory activity by binding to the Nogo-66 receptor (Domeniconi *et al.* 2002; Liu *et al.* 2002). MAG also interacts with Nogo receptor 2 (NgR2; Venkatesh *et al.* 2005). As a siglec protein, MAG has affinity for ganglioside GT1b, and in some model systems this glycolipid serves as a principal cell surface-binding site for MAG (Vinson *et al.* 2001). The role of these gangliosides in mediating axonal growth inhibition by MAG is not clear (Tang *et al.* 1997).

(c) *Oligodendrocyte-myelin glycoprotein*

By focusing selectively on the glycosylphosphatidylinositol (GPI)-linked proteins of CNS myelin as inhibitors of axonal growth, He and colleagues (Wang *et al.* 2002b) described the activity of a third myelin protein, termed OMgp. OMgp had been identified by Mickol & Stefansson in 1988 as a peanut agglutinin-binding protein derived from CNS white matter (Vourc'h & Andres 2004). It is a 120 kDa glycoprotein with five tandem leucine-rich repeats that is anchored to the outer leaflet of the plasma membrane by GPI linkage site (figure 2). Although OMgp was originally purified from CNS myelin, immunohistochemical studies have shown that OMgp is also highly expressed on neurons in the hypothalamus, brainstem, hippocampus and cerebellum (Habib *et al.* 1998). Recent studies demonstrate that OMgp is localized to perinodal regions and plays a role in determining the spacing of nodes of Ranvier (Huang *et al.* 2005). The Nogo-66 receptor has been shown to provide a high-affinity binding site for OMgp, a site that is competent to initiate signal transduction to inhibit axonal growth (Wang *et al.* 2002b).

3. RECEPTOR MECHANISMS FOR MYELIN INHIBITORS

(a) *NgR1*

A receptor for the Nogo-66 fragment of Nogo-A, termed Nogo-66 receptor 1 (NgR1; figure 2), was identified through an expression-cloning strategy with Nogo-66 alkaline phosphatase fusion protein as a ligand (Fournier *et al.* 2001). NgR1 binds Nogo-66 with nanomolar affinity. As described above, the fusion amino-Nogo-A-24 to Nogo-66 yields a subnanomolar

affinity for NgR1 (Hu *et al.* 2005). This receptor is localized to the axolemma and found in many classes of CNS neurons (Fournier *et al.* 2001; Wang *et al.* 2002c; Hasegawa *et al.* 2004). Transfection of NgR1 confers a Nogo-66 response in otherwise non-responsive neurons, demonstrating that NgR1 functions as a receptor for Nogo-66. The human homologue of mouse NgR1 shares 89% amino acid identity (Fournier *et al.* 2001). NgR2 and NgR3 were later identified and found to possess 55% identity in the leucine rich repeat (LRR) domains to NgR1 (Barton *et al.* 2003; He *et al.* 2003; Lauren *et al.* 2003; Pignot *et al.* 2003). NgR2 and NgR3 do not bind to Nogo-66 (Barton *et al.* 2003).

Surprisingly, two other myelin-associated inhibitors, MAG and OMgp, also bind to NgR1 and can signal through this receptor despite their lack of sequence similarity to Nogo-A (Domeniconi *et al.* 2002; Liu *et al.* 2002; Wang *et al.* 2002b). In the recent work by Giger and colleagues, it has been reported that MAG can bind to both NgR1 and NgR2, but not NgR3 (Venkatesh *et al.* 2005). We have since confirmed that NgR2 has preferential affinity for clustered MAG (B. P. Liu & S. M. Strittmatter 2005, unpublished studies) as compared to AP-MAG (Barton *et al.* 2003).

The necessity of NgR1 for responses to myelin ligands has been explored in several *in vitro* experiments. An anti-NgR1 monoclonal antibody, 7E11, blocks Nogo, MAG and OMgp binding to NgR1 and effectively promotes neurite outgrowth from neurons cultured on CNS myelin substrates (Li *et al.* 2004b; Schimmele & Pluckthun 2005). Suppression of NgR1 expression by siRNA alleviates Nogo-66 and myelin inhibition (Ahmed *et al.* 2005). Truncated NgR1 protein in a soluble or membrane-bound form functions as a dominant negative to block myelin ligand responses (Fournier *et al.* 2002; Wang *et al.* 2002a). To explore the necessity of NgR1 genetically, NgR1 $-/-$ mice were generated (Kim *et al.* 2004; Zheng *et al.* 2005). Mice lacking NgR1 are viable but display mild hypoactivity and motor impairment. Of note, Nogo-A levels are increased in these mice, strongly supporting the notion that Nogo/NgR1 form a ligand/receptor pair *in vivo*. Dorsal root ganglia (DRG) neurons lacking NgR1 do not bind Nogo-66, and their growth cones are not collapsed by Nogo-66 or myelin (Kim *et al.* 2004). Despite the potential of NgR2 for binding some forms of MAG (Venkatesh *et al.* 2005), NgR1 $-/-$ DRG neurons are not inhibited by MAG (Kim *et al.* 2004). Studies on a second line of NgR1 $-/-$ mice confirmed the upregulation of Nogo-A, but yielded neurons that were inhibited by myelin *in vitro* (Zheng *et al.* 2005). Differences in myelin preparation or presentation may account for these disparities between *in vitro* assays (e.g. soluble native protein in growth cone collapse assays versus dried and partially denatured protein in outgrowth assays). At this point, we conclude that NgR1 is partially responsible for mediating myelin inhibition of axonal growth.

Owing to the observation that NgR1 can bind multiple myelin-associated inhibitors, an understanding of NgR1 interactions could lead to the design of specific receptor antagonists. NgR1 contains eight LRRs flanked by an amino terminal LRR domain (LRRNT) and a carboxyl terminal cysteine-rich LRR

(LRRCT). A unique domain consisting of 100 amino acid residues that link the LRRCT to the GPI anchor is the least conserved among the three NgR family members (Fournier *et al.* 2002; Wang *et al.* 2002a; figure 2). We analysed the function of various NgR1 domains in outgrowth inhibition. The leucine-rich repeat domain is necessary and sufficient for Nogo-66 binding and NgR1 multimerization heterologous cells (Fournier *et al.* 2002). Viral-mediated expression of mutated NgR1 in primary neurons demonstrates that the NgR1 C-terminal domain is required for inhibitory signalling but not for ligand binding. The NgR1 GPI domain is not essential for signalling, but appears to facilitate Nogo-A responses. A similar deletion analysis demonstrates that the binding of MAG and OMgp to cell-surface NgR1 also requires the entire LRR region of NgR1, but not other portions of the protein (Fournier *et al.* 2002; Barton *et al.* 2003).

The crystal structure of NgR1 has been determined (Barton *et al.* 2003; He *et al.* 2003) and most closely resembles that of platelet glycoprotein Ib α (Huizinga *et al.* 2002). The LRR segments are aligned in a parallel fashion to form a beta pleated sheet, creating a banana-shaped structure with a concave and convex surface capable of protein-protein interactions (Barton *et al.* 2003; He *et al.* 2003). Once the structure of the receptor in complex with different ligands has been solved, then the molecular basis for multiple binding sites can be fully appreciated.

(b) Signal transduction

NgRs do not possess intracellular signalling domains and therefore require co-receptors to transduce inhibitory activity. The low-affinity neurotrophic factor receptor, p75NTR, has been reported to transduce growth inhibitory signals through a membrane complex involving NgR1 and another transmembrane protein, LINGO-1 (Wang *et al.* 2002a; Wong *et al.* 2002; Mi *et al.* 2004). Furthermore, TAJ/TROY, an orphan tumour necrosis factor receptor family member expressed in many adult neurons, can serve as an alternative co-receptor for NgR1, substituting for p75NTR (Park *et al.* 2005; Shao *et al.* 2005). Recently, epidermal growth factor receptor (EGFR) activation has also been linked to NgR1-ligand binding (Koprivica *et al.* 2005). Transactivation of EGFR after NgR1 engagement was shown to be necessary for axonal growth inhibition, but the molecular basis for this linkage has not been defined.

RhoA stimulation is critical for myelin-derived inhibitory signalling downstream from NgR1 activation. RhoA activation in neurons inhibits neurite formation by stimulating Rho-associated kinase (ROCK) and actinomyosin contractility. This, in turn, leads to neurite retraction and growth cone collapse. Blocking RhoA activity with either dominant-negative RhoA or C3 exoenzyme, or inhibiting ROCK with Y27632, alleviates myelin substrate inhibition *in vitro* (Jin & Strittmatter 1997; Lehmann *et al.* 1999; Vinson *et al.* 2001; Dergham *et al.* 2002; Fournier *et al.* 2003; Schweigreiter *et al.* 2004). Measurement of *in vitro* RhoA activity in neurons also confirmed RhoA activation following exposure to myelin components (Winton *et al.* 2002; Fournier *et al.* 2003; Schweigreiter

et al. 2004). In one study, modulation of Rho-GDI activity by p75NTR coupling to NgR1 was linked to Rho activation (Yamashita & Tohyama 2003). Furthermore, several *in vivo* spinal cord injury (SCI) studies in which RhoA signalling was inhibited showed improved axon regeneration and functional recovery (see below).

Protein kinase C (PKC) activation has been associated with NgR1-based signalling (Sivasankaran *et al.* 2004). As for the EGFR activation, the molecular steps linking PKC activation to NgR1 engagement are not delineated. Another signalling mechanism reported downstream of NgR1 activation is regulated intramembranous proteolysis of p75NTR (Domeniconi *et al.* 2005). Sequential extracellular metalloproteinase and presenilin-dependent γ -secretase-dependent cleavage of a small fraction of p75NTR has been implicated in myelin/NgR1 signalling. Whether signalling via TAJ is associated with protease activation has not been studied.

4. MYELIN-TARGETED INTERVENTIONS FOR AXONAL REGENERATION IN VIVO

(a) Nogo

The identification of Nogo-A as a CNS myelin inhibitor of axonal growth *in vitro* has led to the design of Nogo-targeted intervention studies *in vivo* (table 1). Rodent models of SCI allow anatomical and functional assessment of pharmacological and genetic approaches to inhibit the action of Nogo. Schwab and colleagues pioneered the delivery of anti-Nogo strategies *in vivo* by transplanting hybridoma cells modified to secrete the IN-1 monoclonal antibody directed against the amino-terminal of Nogo-A. IN-1-treated animals that underwent a dorsal over-hemisection injury exhibited significant axonal regeneration and functional recovery (Schnell & Schwab 1990). Further studies from this group illustrated the efficacy of IN-1 and other anti-Nogo preparations in other models of experimental SCI (Raineteau *et al.* 1999; Brosamle *et al.* 2000; Buffo *et al.* 2000; Merkler *et al.* 2001; Raineteau *et al.* 2001; Bareyre *et al.* 2002, 2004; Fiedler *et al.* 2002) cortical lesions (Wenk *et al.* 1999; Emerick *et al.* 2003) and ischaemic lesions (Papadopoulos *et al.* 2002) after middle cerebral artery occlusion (MCAO).

Genetic studies have also shed light on the role of Nogo in limiting CNS regeneration. To assess the sufficiency of Nogo for limiting axonal regeneration, transgenic mice expressing Nogo-A or Nogo-C in peripheral Schwann cells were generated. Axon regeneration is delayed after sciatic nerve crush in mice with peripheral Nogo-A or C expression, demonstrating that Nogo can partially override the permissiveness of the PNS environment (Pot *et al.* 2002; Kim *et al.* 2003a).

While there may be additional myelin-derived inhibitors, *in vitro* experiments with cells or myelin from Nogo-A null mice have demonstrated that Nogo-A plays a detectably significant role in myelin blockade of axonal outgrowth. CNS myelin prepared from Nogo-A/B knockout mice in our laboratory, and in two other laboratories, exhibits reduced inhibition of neurite outgrowth (Kim *et al.* 2003b; Simonen *et al.* 2003; Zheng *et al.* 2003). Nogo-A and Nogo-A/B mutant mice have normal brain histology and myelin distribution. There are no obvious defects in axons

Table 1. *In vivo* studies of extracellular axon growth inhibitors in adult CNS injury. (N.T., not tested.)

target	intervention	mechanism	model		injury type	outcome			
			species	model		CST	5HT	function	comments
Nogo	antibody	monoclonal antibody against amino-Nogo	rat		middle cerebral artery occlusion (Papadopoulos <i>et al.</i> 2002)	N.T.	N.T.	yes	Nogo-A limit dendritic plasticity after stroke
			rat		middle cerebral artery occlusion (Seymour <i>et al.</i> 2005)	N.T.	N.T.	yes	efficacy in delayed treatment
			monkey		unilateral thoracic lesion (Fouad <i>et al.</i> 2004)	yes	N.T.	yes	neutralization of Nogo-A in primate
	genetic deletion	Nogo-A/B gene trap	rat		dorsal hemisection (Schnell & Schwab 1990)	yes	N.T.	yes	first study describing IN-1 treatment <i>in vivo</i>
			rat		dorsal hemisection (Brosamle <i>et al.</i> 2000)	yes	N.T.	N.T.	function of a humanized IN-1-Fab fragment
			mouse		dorsal hemisection (Kim <i>et al.</i> 2003b)	yes	N.T.	yes	extensive CST regeneration
			mouse		dorsal hemisection (Simonen <i>et al.</i> 2003)	yes	N.T.	yes	modest CST regeneration
			mouse		dorsal hemisection (Zheng <i>et al.</i> 2003)	no	N.T.	no	no CST regeneration
			mouse		focal brain infarction (Lee <i>et al.</i> 2004)	N.T.	N.T.	yes	plasticity mediated functional recovery
	genetic deletion	gene targeted	mouse		dorsal hemisection (Bartsch <i>et al.</i> 1995)	no	N.T.	no	no CST regeneration
			—		—	—	—	—	under investigation
	OMgp	—	N/A		various (Teng & Tang 2005)	N.T.	N.T.	N/A	reviewed in Teng & Tang 2005
	myelin	immunoprotection	mouse		dorsal hemisection/transsection (Kim <i>et al.</i> 2004)	no	yes	yes	anatomical and electrophysiological recovery
NgR1	genetic deletion	NgR1 targeted	mouse		dorsal hemisection (Zheng <i>et al.</i> 2005)	no	N.T.	N.T.	no CST regeneration
			mouse		focal brain infarction (Lee <i>et al.</i> 2004)	N.T.	N.T.	yes	plasticity mediated functional recovery
			mouse		dorsal hemisection (GrandPre <i>et al.</i> 2002)	yes	N.T.	yes	CST regeneration
	NEP 1-40	peptide	mouse		dorsal hemisection (Li & Strittmatter 2003)	yes	N.T.	yes	CST regeneration after delayed s.c. infusion
			rat		dorsal hemisection (Li <i>et al.</i> 2004a)	yes	yes	yes	CST/raphe spinal regeneration (infusion)
			mouse		dorsal hemisection (Li <i>et al.</i> 2005)	yes	yes	yes	CST/raphe spinal regeneration (transgene)
	NgR1 (310)ecto	recombinant NgR1	mouse		dorsal hemisection (Ji <i>et al.</i> 2005)	yes	N.T.	yes	combined efficacy with methylprednisolone
			mouse		rhizotomy (MacDermid <i>et al.</i> 2004)	N.T.	N.T.	N.T.	plasticity of spinal tracts
			mouse		optic nerve crush (Fischer <i>et al.</i> 2004)	N.T.	N.T.	N.T.	transgenic NgR1 inactivation enhances RGC regeneration
			rat		optic nerve crush (Lehmann <i>et al.</i> 1999)	N.T.	N.T.	N.T.	optic nerve regeneration
Rho	C3 transferase	inhibits RhoA	mouse		dorsal hemisection (Dergham <i>et al.</i> 2002)	yes	N.T.	yes	CST regeneration, cell-permeable C3
			rat		dorsal hemisection (Fournier <i>et al.</i> 2003)	no	N.T.	no	no CST regeneration
			mouse		dorsal hemisection (Dergham <i>et al.</i> 2002)	yes	N.T.	yes	CST regeneration
	Y-27632	inhibits Rho kinase	rat		dorsal hemisection (Fournier <i>et al.</i> 2003)	yes	N.T.	yes	CST regeneration
			rat		dorsal hemisection (Sivasankaran <i>et al.</i> 2004)	no	N.T.	N.T.	sensory but not motor regeneration
			mouse		optic nerve crush (Koprivica <i>et al.</i> 2005)	N.T.	N.T.	N.T.	optic nerve regeneration
	Gö6976	Inhibit PKC α/β	rat		nigrostriatal lesion (Moon <i>et al.</i> 2001)	N.T.	N.T.	N.T.	regeneration of nigrostriatal tract
			rat		dorsal column crush (Bradbury <i>et al.</i> 2002)	yes	N.T.	yes	electrophysiological and behavioral recovery
			rat		dorsal hemisection (Davies <i>et al.</i> 2004)	N.T.	N.T.	N.T.	sprouting of transplanted DRG across scar
	decorin	gene targeted	mouse		lateral hemisection (Goldshmit <i>et al.</i> 2004)	N.T.	N.T.	yes	spinal axon regeneration
			—		—	—	—	—	—

or oligodendrocytes, demonstrating that Nogo is dispensable (or compensated for) in the development of the mouse CNS. *In vivo* SCI studies with different Nogo-A/B mouse strains have yielded different results with regard to the importance of this one molecule in limiting corticospinal regeneration after SCI (Kim *et al.* 2003b; Simonen *et al.* 2003; Zheng *et al.* 2003). After SCI, corticospinal axons of one Nogo-A/B $-/-$ strain sprout rostral to a dorsal hemisection and some fibres regenerate into distal cord segments in about 50% of lesioned mice (Kim *et al.* 2003b). This growth is associated with recovery of locomotor function. Complimentary studies utilizing the same experimental paradigm yielded either moderate (Simonen *et al.* 2003) or a complete lack of corticospinal tract (CST) regeneration (Zheng *et al.* 2003). However, the exchange of mouse strains between the laboratories involved in these studies has made it clear that such CST growth is only seen with certain Nogo mutant alleles regardless of surgical technique (J. K. Lee, J. E. Kim & S. M. Strittmatter, unpublished studies). Moreover, strain background and age modulate the CST regeneration phenotype. The determinants of Nogo-A $-/-$ phenotypic variation and penetrance remain a subject of investigation (see Woolf (2003) for comment).

(b) Myelin-associated glycoprotein

The analysis of mice lacking MAG has provided insight into its role in the maintenance of myelin architecture as well as its role in limiting plasticity in the adult CNS (table 1). CNS myelin formation is delayed in MAG knockout mice and the cytoplasmic collars of mature CNS myelin are frequently missing or reduced (Li *et al.* 1994; Montag *et al.* 1994). Redundant myelination is common in the CNS of the adult MAG knockouts (Bartsch *et al.* 1995) and the mice display oligodendroglial pathology (Lassmann *et al.* 1997). Older mutant mice exhibit perturbations in axon integrity (Fruttiger *et al.* 1995; Weiss *et al.* 2000). MAG-null mice exhibit late-onset progressive PNS axonal atrophy and an increased rate of Wallerian degeneration (Bjartmar *et al.* 1999). Mice deficient in only L-MAG display most of the CNS abnormalities exhibited by the MAG null mice, but their PNS axon and myelin integrity appeared normal (Fujita *et al.* 1998). Thus, S-MAG is sufficient to maintain PNS myelin, and L-MAG may be selectively necessary for signalling within oligodendrocytes and for the maintenance of CNS myelin.

The role of MAG in limiting *in vivo* CNS axon regeneration was explored by dorsal hemisection (Bartsch *et al.* 1995). MAG $-/-$ mice failed to exhibit axonal regeneration after SCI. However, in *Wld^s*, MAG $-/-$ mice there is a moderate increase in peripheral axonal regeneration (Schafer *et al.* 1996). The *Wld^s* mice exhibit slow Wallerian degeneration so that peripheral nerve myelin (and MAG) persists for much longer distal to a nerve injury. The absence of a definitive phenotype may indicate that other ligands that bind NgR1, such as Nogo or OMgp, may compensate for the absence of MAG. In order to explore this issue further, experiments are underway in our laboratory to assess the regenerative phenotype of mice triple mutant for Nogo-A, MAG and OMgp.

(c) NgR1

The Nogo66 receptor 1 binds three myelin-associated ligands, Nogo, MAG and OMgp. The role of NgR1 in limiting recovery from CNS trauma has been studied in several experiments using various strategies in animal SCI models (table 1). A peptide antagonist of NgR1, NEP1-40, is a subfragment of Nogo-66 and competes for binding of Nogo-66 to NgR1 (GrandPre *et al.* 2002). The peptide blocks Nogo, but not MAG/OMgp, action *in vitro*. This peptide has shown significant efficacy in promoting regeneration and recovery after experimental SCI (GrandPre *et al.* 2002). When infused locally at the time of a lesion (GrandPre *et al.* 2002) or subcutaneously after a one week delay (Li & Strittmatter 2003), the NgR1 antagonist promotes corticospinal and raphespinal axon sprouting. Fibre growth is correlated with a recovery in locomotor function. A recombinant protein consisting of the ecto-domain of the NgR1 (27–310) inhibits Nogo-66, MAG and OMgp binding to immobilized NgR1 *in vitro* and promotes neurite outgrowth on myelin. Transgenic expression or intrathecal delivery of the NgR1 (27–310) *in vivo* significantly improves histological and functional recovery after dorsal hemisection injury of the thoracic spinal cord (Li *et al.* 2004a). Ramer and colleagues have demonstrated that soluble NgR1-Fc infusion promotes post-injury sprouting of intact serotonergic and tyrosine hydroxylase-positive fibres in the spinal cord (MacDermid *et al.* 2004). In addition, expression of a truncated, dominant negative NgR1 in retinal ganglion cells improves optic nerve regeneration *in vivo*, particularly when combined with manipulations that enhance the intrinsic growth state of these neurons (Fischer *et al.* 2004). The NgR1-ecto protein also promotes corticofugal sprouting and functional recovery when infused i.c.v. after a hemispherical stroke created by MCAO (Lee *et al.* 2004). Thus, a range of studies has demonstrated a role for NgR1 and/or its ligands in limiting axonal growth in the injured adult rodent CNS.

Two genetic studies assessed the necessity of NgR1 in limiting axonal regeneration after SCI (Kim *et al.* 2004; Zheng *et al.* 2005). In both studies, the CST did not grow after a dorsal hemisection of the spinal cord. Of note, the upregulation of Nogo-A and the increased NgR1-independent actions of amino-Nogo may explain the lack of CST regeneration here as compared to the NgR1-ecto and NEP1-40 experiments. A complete SCI assessment of NgR1 $-/-$ mice in our laboratory revealed that the mice recover better locomotor function significantly when compared to wild-type littermates (Kim *et al.* 2004). Mice which are lacking NgR1 exhibit regeneration of rubrospinal tract axons and significant sprouting of dorsal raphe fibres after either dorsal hemisection or complete spinal transection (Kim *et al.* 2004). The study from Zheng *et al.* (2005) did not assess animals functionally and concentrated solely on CST anatomy. The recovery of NgR1 $-/-$ mice from focal ischaemic lesions in the cerebral cortex has also been examined (Lee *et al.* 2004). The absence of NgR1 allows greater corticofugal fibre sprouting from the non-ischaemic cortex and greater behavioural recovery. In summary, NgR1 has a partial role in limiting axonal growth after CNS trauma.

5. ASTROCYTE-DERIVED INHIBITORS

(a) *Chondroitin sulphate proteoglycans*

Aside from oligodendrocytes, other CNS glia such as astrocytes and oligodendrocyte precursors (OPs) have been the focus of many regeneration studies due to their key role in the establishment of the glial scar. The glial scar is a dynamic entity that forms at the site of a CNS lesion almost immediately after damage (Berry *et al.* 1983; Fitch & Silver 1997). Hypertrophic astrocytic processes enmesh the lesion site and begin to express and deposit an inhibitory extracellular matrix (ECM) consisting primarily of CSPGs, (Levine 1994; Davies *et al.* 1997; 1999, 2004; Fitch *et al.* 1999; Asher *et al.* 2000; Bradbury *et al.* 2002; Jones *et al.* 2002; Tang *et al.* 2003). This tissue reaction ultimately results in the formation a dense complicated structure that is exquisitely inhibitory to regenerating axons (Davies *et al.* 1997, 1999; Bradbury *et al.* 2002).

The ECM of CNS scar tissue consists principally of collagens, glycoproteins (such as tenascin-C, tenascin-R), CSPGs and heparan sulphate proteoglycans. These components can interact with cell adhesion molecules and integrins. CSPGs present in the ECM have been shown to be inhibitory to axonal growth both *in vitro* (Snow *et al.* 1990; McKeon *et al.* 1991; Dou & Levine 1994; Friedlander *et al.* 1994; Smith-Thomas *et al.* 1994, 1995; McKeon *et al.* 1995; Fidler *et al.* 1999, 2002; Niederost *et al.* 1999; Ughrin *et al.* 2003) and *in vivo* (Davies *et al.* 1997, 1999; Moon *et al.* 2001; Bradbury *et al.* 2002). However, conflicting reports have emerged to suggest that CSPGs may also promote neurite outgrowth *in vitro* (Streit *et al.* 1993; Faissner *et al.* 1994) and *in vivo* (Oakley & Tosney 1991; Yaginuma *et al.* 1991; Jones *et al.* 2003), perhaps reflecting the diversity and versatility of this class of proteins. It now appears clear that the chondroitin sulphate moieties contribute a substantial fraction of the axon-inhibitory function of CSPGs, although the core proteins may contribute additional specificity and alternative mechanisms of action.

CSPGs comprise a range of complex molecules that have been shown to have crucial roles in cell adhesion, cell migration, pathfinding and barrier formation (Margolis & Margolis 1997; Bandtlow & Zimmermann 2000). CSPGs are characterized as having a central core protein to which long unbranched glucosaminoglycan (GAG) side chains are covalently attached. Modification by epimerases and sulphotransferases result in structural heterogeneity of bound GAGs, and dictate their function. The large aggregating proteoglycans, also known as the hyalactans or lecticans, include aggrecan (Paulsson *et al.* 1987; Krueger *et al.* 1992; Domowicz *et al.* 1995; Li *et al.* 1996), versican (Krusius *et al.* 1987; Dours-Zimmermann & Zimmermann 1994), the CNS-specific proteoglycan, neurocan (Rauch *et al.* 1992; Friedlander *et al.* 1994; Grumet *et al.* 1994; Oohira *et al.* 1994) and brevican (Yamada *et al.* 1994, 1997). They all share an N-terminal globular domain followed by a hyaluronic-binding region, a C-terminal EGF motif, lectin and complement regulatory protein domains and several regions upon which CS side chains are attached. The CSPG NG2 shares no sequence homology with the lecticans and is a transmembrane proteoglycan

(Levine & Card 1987; Stallcup & Beasley 1987). Phosphacan/DSD-1 is the extracellular CSPG domain of a receptor-type protein tyrosine phosphatase (Grumet *et al.* 1993, 1994; Maeda *et al.* 1994; Maurel *et al.* 1994).

(b) *Neurocan*

The prototypic lectican, neurocan, is produced as a 275 kDa secreted protein that can be proteolytically processed into two smaller fragments (Rauch *et al.* 1992; Matsui *et al.* 1994). Both the C-terminal (neurocan-C) and N-terminal fragment (neurocan-130) are sulphated. They are deposited by astrocytes and oligodendrocyte progenitors (OPs) exclusively in the CNS, and are concentrated in white matter. Neurocan has been shown to be inhibitory to neurite outgrowth *in vitro* (Asher *et al.* 2000). In keeping with its role as a key inhibitor of axonal regeneration, neurocan expression is elevated at the site of mechanical (Asher *et al.* 2000; Tang *et al.* 2003; Davies *et al.* 2004) or ischaemic (Deguchi *et al.* 2005) injury to the adult CNS. Neurocan is known to interact with NCAM, Ng-CAM/L1, TAG-1 and tenascin (Friedlander *et al.* 1994; Milev *et al.* 1994, 1996, 1998) and to inhibit axon growth by Ng-CAM/L1. Neurocan has also been implicated in pathfinding during development as it is transiently expressed in the optic chiasm during retino-tectal mapping and in the dorsal midline of the spinal cord (Sango *et al.* 2003; Leung *et al.* 2004; Popp *et al.* 2004). However, mice which are null mutant for neurocan appear to develop normally, with only mild LTP deficits (Zhou *et al.* 2001), illustrating that neurocan may have a redundant or subtle role during development.

(c) *Versican*

Versican is a large proteoglycan, which has the same N and C terminal motifs as the other members of the lectican family, but also contains unique α and β domains. The mRNA is alternatively spliced to result in three isoforms of versican, full length V0, which possess both α and β domains, V1, which lacks the α domain, and V2, which lacks the β domain (Dours-Zimmermann & Zimmermann 1994; Milev *et al.* 1998; Schmalfeldt *et al.* 1998). Recently, versican was shown to be expressed in a subset of interneurons in cytoarchitectonically distinct laminae of the embryonic tectum (Yamagata & Sanes 2005). It was theorized that graded versican expression in the tectum might restrict retinal axons from inappropriate pathways. Versican expression has also been implicated in neural crest cell migration (Perissinotto *et al.* 2000). The role of versican in restricting axonal projections and cell migration during development may parallel a role in restricting plasticity and regeneration after injury to the adult CNS. Expression of versican is elevated within two weeks after SCI and remains chronically elevated in the lesion perimeter (Jones *et al.* 2003; Tang *et al.* 2003).

(d) *Phosphacan*

Phosphacan is another CSPG found throughout the adult CNS (Snow *et al.* 1990; Maurel *et al.* 1994; Tang *et al.* 2003). It is expressed in secreted and membrane-bound forms (RPTP- β). Phosphacan has been shown to block the growth-promoting effects of N-CAM,

TAG-1 and tenascin. Phosphacan is highly expressed by radial glial cells and consequently has been shown to be crucial in the oriented movement of post-mitotic cells during cortical development. Phosphacan expression after SCI initially declines within the first few weeks post-injury and peaks after one month, in contrast to neurocan expression, which is elevated within days of the injury (Tang *et al.* 2003). Consequently, protracted phosphacan expression may contribute to chronic inhibition of axonal regeneration.

(e) NG2

NG2 is a 300 kDa proteoglycan, an integral membrane protein cloned in 1991 by Stallcup and colleagues (Nishiyama *et al.* 1991) characterized by a short 25 residue transmembrane domain, a cytoplasmic domain of 76 amino acids and a large extracellular domain (2225 residues), with a single chondroitin sulphate-binding site at serine 999 (Stallcup & Dahlin-Huppe 2001). NG2 was initially identified as a cell surface antigen of neural tumour-derived cell lines that had properties intermediate between neurons and glial cells (therefore nerve glia (NG)). Later investigations revealed that NG2 was expressed by a subset of small stellate cells within the CNS parenchyma and thought to represent a group of progenitors. Subsequent studies inspired by the identification of O2A progenitors by Raff *et al.* (1983, 1998) confirmed the identity of NG2 expressing cells as OPs (Levine & Card 1987; Stallcup & Beasley 1987).

NG2 was shown to be inhibitory to neurite outgrowth *in vitro* independent of the integrity of its chondroitin sulphate side chain as pre-treatment of NG2-rich substrata with chondroitinase ABC (ChABC) failed to alleviate inhibition. However, treatment of these cultures with polyclonal antibodies raised against NG2 was able to partially reverse inhibition (Dou & Levine 1994). Similarly, membrane preparations from cultured OPs were inhibitory to neurite outgrowth from cerebellar explants (Chen *et al.* 2002) in a manner reversed in the presence of NG2 polyclonal antibodies. A more thorough investigation of three separate domains within the extracellular portion of the membrane-bound form of NG2 has implicated independent inhibitory regions which may have implications on the function of this CSPG in a membrane bound or secreted form (Ughrin *et al.* 2003).

NG2 is upregulated at the lesion margins after brain lesion (Levine 1994), spinal cord hemisection (Jones *et al.* 2002) and spinal transection (de Castro *et al.* 2005), areas correlated with axon regeneration failure and consistent with the role for NG2 in limiting axonal regeneration. Indeed, preliminary reports infusing monoclonal antibodies against NG2 appeared to enhance primary afferent regeneration after experimental SCI (Tang *et al.* 2003). However, mice null mutant for NG2 failed to show enhanced axonal regeneration after complete transection (de Castro *et al.* 2005). Moreover, wild-type mice illustrated a more robust axonal regeneration. These results implicate a detrimental effect of the loss of NG2. Therefore, in the absence of a specific receptor, the action of NG2 may be mediated by interactions with other ECM molecules (Burg *et al.* 1996; Dou & Levine 1997).

6. PROTEOGLYCAN-TARGETED INTERVENTION FOR AXONAL REGENERATION

Early *in vitro* reports identified CSPGs as the potent inhibitory species expressed by astrocytes (Snow *et al.* 1990; McKeon *et al.* 1991; Dou & Levine 1994; Smith-Thomas *et al.* 1994). Subsequent investigations revealed that cultured retinal ganglion cells were capable of enhanced growth on CSPG-rich substrate, if the substrate was pre-treated with the bacterial enzyme ChABC (McKeon *et al.* 1995). ChABC liberates CS GAGs from CSPG core proteins and thus ultimately identified the GAG moiety of CSPGs as crucial in mediating axonal growth arrest *in vitro*. More recently, ChABC has been used with great effect *in vivo* to enhance regeneration of damaged CNS pathways. ChABC was capable of enhancing regeneration of microlesioned nigrostriatal axons back to their targets (Moon *et al.* 2001). Intrathecal delivery of ChABC restored functional corticospinal connectivity after bilateral dorsal column lesion in the rat, with concomitant restoration of sensory and propriospinal function (Bradbury *et al.* 2002). Furthermore, Clarke's nucleus neurons regenerate into a peripheral nerve graft (Yick *et al.* 2000) and past the lesion site after dorsal hemisection (Yick *et al.* 2003). These studies have spearheaded the use of ChABC in a number of combinatorial studies that have used a multi-dimensional stratagem to overcome regeneration failure. ChABC has been successfully combined with Schwann cell seeded guidance channels (Chau *et al.* 2004), Schwann cell and olfactory ensheathing cell bridges (Fouad *et al.* 2005) and neural stem/progenitor cells with resultant enhanced axonal regeneration. An alternative strategy to the use of ChABC in reducing the effects of GAG moiety-associated inhibition was the use of a DNA enzyme that targets the mRNA of xylosyltransferase-1, an enzyme critical for the addition of GAG side chains to core proteins. Administration of this enzyme reduced the expression of GAGs on CSPG core proteins on astrocytes *in vitro* and in areas undergoing reactive gliosis after SCI *in vivo* (Grimpe & Silver 2004).

Another approach to targeting CSPG-associated inhibition has focused on modulating the ability of the damaged CNS glial cells to proliferate and produce inhibitory ECM (Rhodes *et al.* 2003). The molecular mechanisms that cause upregulation of CSPG after injury have been partially described (Asher *et al.* 2000). Intraspinal delivery of the small CSPG, decorin, after spinal stab injury has been shown to negate the upregulation of neurocan, phosphacan and NG2, suppress the formation of the glial limitans of the scar and enhance axon regeneration while reducing expression of the cytokine transforming growth factor (TGF) beta (Davies *et al.* 2004). Inflammation is known to exacerbate the production of CSPGs (Asher *et al.* 2000) and therefore anti-inflammatory therapy provides another mechanism whereby extrinsic inhibitory factors can be controlled.

The diverse structure and function of CSPGs has made them complicated targets for intervention, and to date no general chondroitin sulphate receptors have been identified that mediate their function. Activation of RhoA is a necessary step in mediating CSPG-associated

inhibition *in vitro*. Several laboratories have reported disinhibition of neurite outgrowth on CSPG-rich substrates in the presence of ROCK inhibitors (Borisoff *et al.* 2003; Monnier *et al.* 2003). Therefore, blockade of the Rho/ROCK axis holds the potential to block signalling by myelin, CSPG and ephrins. *In vivo* studies targeting RhoA with C3 exoenzyme have yielded little evidence of regeneration (Fournier *et al.* 2003) except in studies with a modified cell permanent version of the protein (Winton *et al.* 2002). ROCK inhibitors have yielded a degree of axonal growth and functional improvement in several studies, but a mixture of beneficial and deleterious effects may ultimately limit their usefulness (Borisoff *et al.* 2003; Fournier *et al.* 2003; Park *et al.* 2005). One study has implicated PKC activation as critical in transducing CSPG-mediated RhoA activation and hence growth cone collapse (Sivasankaran *et al.* 2004). Intrathecal delivery of the PKC inhibitor Go6976 resulted in modest regeneration of ascending dorsal column afferents after dorsal hemisection (Sivasankaran *et al.* 2004). More recently, EGF receptor activation (Koprivica *et al.* 2005) has emerged as another intermediate in transducing the action of both CSPG and myelin inhibitory function. These investigators showed that inhibition of EGF kinase function was capable of ablating the neurite outgrowth-inhibitory effects of CSPGs. Furthermore, local delivery of EGF receptor inhibitors enhanced axon growth after optic nerve crush.

7. EPHRINS: DEVELOPMENT, MYELIN AND SCAR TISSUE

The ephrins and their cognate Eph receptors play major roles in axonal pathfinding and target recognition during CNS development (Klein 2004). Recent studies of Eph/ephrins expression after CNS injury have elucidated a range of roles for this receptor-ligand family after CNS trauma. Several of these proteins appear to be expressed by reactive astrocytes. Bidirectional signalling between ephrin-B2-expressing astrocytes and EphB2-expressing meningeal fibroblasts may regulate the interaction between these two cell types during CNS scar formation (Bundesen *et al.* 2003). Since scar tissue is clearly inhibitory for axonal outgrowth, this ephrin/Eph interaction may secondarily alter axonal growth after trauma. There is also evidence that EphA4 is upregulated in reactive astrocytes after SCI. In mice lacking EphA4, astrogliosis is reduced and inhibition of axonal growth both *in vivo* and *in vitro* is reduced. Furthermore, functional recovery from spinal hemisection is enhanced in EphA4 $-/-$ mice (Goldshmit *et al.* 2004).

Ephrins have also been implicated in myelin-dependent inhibition of axonal growth. Ephrin-B3 is selectively expressed on mature myelinating oligodendrocytes (Benson *et al.* 2005). Furthermore, myelin derived from ephrin-B3 knockout mice is less inhibitory than wild-type myelin for neurite outgrowth. During development, ephrin-B3 is known to function as a midline glial repellent for descending EphA4 receptor-expressing corticospinal tract axons (Kullander *et al.* 2001). Thus, at least for EphA4-expressing adult axonal tracts, ephrin-B3 expression in oligodendrocytes

probably contributes to limited adult CNS axonal growth. *In vitro* studies with tissue and cells from gene targeted lines suggest that both ephrin-B3 and NgR1 ligands play detectable roles in CNS myelin inhibition of adult axon growth (Benson *et al.* 2005).

8. SEMAPHORINS AND OTHER AXON GUIDANCE MOLECULES: DEVELOPMENT, MYELIN AND SCAR TISSUE

Like the ephrins, the semaphorins have prominent axon repulsive functions during early neuronal development (Nakamura *et al.* 2000; Fujisawa 2004). These functions are mediated largely through plexin-class receptors. A number of soluble semaphorin ligands use neuropilins as high-affinity binding co-receptors together with plexins (He & Tessier-Lavigne 1997; Kolodkin *et al.* 1997; Winberg *et al.* 1998; Takahashi *et al.* 1999; Tamagnone *et al.* 1999). Our understanding of the role of semaphorins and their receptors in limiting adult axonal growth is incomplete. Expression of inhibitory sema3 proteins by meningeal fibroblasts at sites of CNS scar has been documented, but their functional role is unclear (De Winter *et al.* 2002). Oligodendrocytes, especially those near injury sites, have been shown to express a transmembrane sema4D, which is capable of inhibiting axonal growth from several neuronal types (Moreau-Fauvarque *et al.* 2003). In optic nerve, oligodendrocytes also express another axon-inhibitory transmembrane semaphorin, sema5A (Oster *et al.* 2003).

Netrins are secreted proteins with both axonal attractive and repulsive roles during development (Tessier-Lavigne & Goodman 1996). In the adult, netrin-1 expression has been documented in mature oligodendrocytes (Manitt *et al.* 2001). Since the inhibitory netrin receptors, Unc5H1-3, are expressed in adult spinal cord, this protein may also have an axonal growth-limiting action after CNS injury (Manitt *et al.* 2004).

9. ROLE OF MYELIN/CSPG INHIBITORS IN NORMAL PHYSIOLOGY

In vivo injury studies demonstrate a *pathological* role for myelin/NgR1 and CSPGs in limiting axonal growth in the adult CNS after injury. An obvious issue is the *physiological* function of these molecular pathways. Since local sprouting and plasticity from uninjured fibres contribute to the positive effects of NgR1 and CSPG perturbations in the recovery from injury, it is important to consider whether these targets might participate in physiological, experience-dependent plasticity. The visual system provides an ideal *in vivo* model in which this question can be addressed (McGee *et al.* 2005). It is well established that the visual system exhibits a striking ability to rearrange in response to sensory input during a defined window in the late juvenile phase of development (Hensch 2005; Hensch & Fagioli 2005; Taha & Stryker 2005; Yang *et al.* 2005). Monocular deprivation (MD) during this time period leads to reduce responsiveness to the deprived eye without any damage to the nervous system itself. The reduced visual acuity in the deprived eye has a clinical correlation in amblyopia. After the critical period for

ocular dominance plasticity (P19–32 in mice), MD produces only minimal ‘sub-threshold’ changes in cortical function (Hensch 2005) that can be detected by visually evoked potentials.

In mice lacking NgR1, the visual cortex develops normally, exhibiting electrical response properties and cortical maps indistinguishable from those in wild-type mice (McGee *et al.* 2005). Closing one eye for several days shifts the responses to the open eye to the same degree in both NgR1 $-/-$ and wild-type mice during the critical period. However, at P45 or P120, well after the closure of the critical period, the NgR1 $-/-$ visual cortex continues to exhibit full ocular dominance plasticity to 4 day MD while the wild-type cortex exhibits no plasticity of single unit recordings to such deprivation. Thus, NgR1 plays a role in the normal closure of the critical period. At the anatomical and cellular level, the basis for continued ocular dominance plasticity in the NgR1 $-/-$ mouse visual cortex is not yet defined.

CSPGs have also been implicated in limiting plasticity and closing of the critical period (Pizzorusso *et al.* 2002). These ECM components are expressed most highly in condensed lattice-like structures called perineuronal nets (PNNs) surrounding parvalbumin-positive GABAergic neurons, and reach maximum expression as the critical period closes. Removal of GAG side chains from CSPGs by enzymatic digestion with chondroitinase ABC partially restores ocular dominance plasticity to older animals after closure of the critical period (Pizzorusso *et al.* 2002). Two different mechanisms for CSPG-mediated plasticity have been proposed. In one, CSPG may dampen structural changes through their inhibitory actions on neurite outgrowth. In the other, the negatively charged ECM milieu created by these molecules may buffer requisite ionic and transmitter concentrations (Pizzorusso *et al.* 2002; Hensch & Fagiolini 2005). Structures akin to PNNs have been located in the dorsal horn of the spinal cord termed *periaxonal* nets where they appear to serve a similar function to their counterparts in the cortex. Primary afferent terminals within spinal grey matter are decorated with CSPGs. Intraspinal delivery of ChABC degrades these intrinsically expressed CSPGs and encourages primary afferent re-organization after deafferentation of lesions to the spinal cord (W. B. J. Cafferty & S. McMahon 2005, unpublished observations).

10. SUMMARY

Historically, the failure of axonal regeneration within the damaged CNS has been ascribed to two limiting factors, an intrinsically reduced growth capacity of mature CNS neurons and the presence of inhibitory molecules produced by non-neuronal cells unique to the CNS: oligodendrocytes and astrocytes. CNS axons have been shown to grow considerable distances *in vivo* if presented with a permissive environment lacking oligodendrocytes and astrocytes. The most instructive demonstration of this capacity came from seminal experiments illustrating the ability of damaged CNS axons to regenerate into tissue bridges consisting of transplanted peripheral nerve (David & Aguayo 1981;

Richardson *et al.* 1982). Thus, while adult CNS neurons may have a diminished growth capacity (Kalil & Reh 1982; Schreyer & Jones 1983; Schreyer & Skene 1993; Goldberg & Barres 1998), they retain adequate intrinsic growth potential to extend if the environment is acceptable.

Much research over the last decade has focused on identifying the factors produced by CNS glial cells that arrest axonal sprouting and regeneration. Earlier studies from Schwab and colleagues identified damaged CNS myelin as a substrate inhibitory to injured axons (Schwab & Thoenen 1985; Schwab & Caroni 1988; Savio & Schwab 1989). Later studies identified Nogo, MAG and OMgp as factors present in CNS myelin that mediate its inhibitory nature (McGee & Strittmatter 2003). All the three of these proteins can interact with an axonal NgR1 protein. Perturbation of these molecules or downstream signalling events permits a degree of CNS axonal growth and repair.

Derived primarily from the reactive astrocytes, CSPGs also play a crucial inhibitory role in the adult CNS (Silver & Miller 2004; Carulli *et al.* 2005). *In vivo* evidence indicates that CSPG digestion can promote CNS axonal growth and functional recovery (Bradbury *et al.* 2002). A number of protein factors important for embryonic axonal guidance may also contribute to limiting axonal growth in the adult. While this area requires further investigation, the data implicating members of the ephrin family are the most advanced (Goldshmit *et al.* 2004; Benson *et al.* 2005).

Those myelin proteins and CSPGs that play a prominent role in limiting axonal growth after CNS injury also play a crucial role during late juvenile development to consolidate connectivity optimized by experience-dependent plasticity in the nervous system. Thus, the evidence for improved functional recovery after adult CNS injury may be ascribed to the ability of myelin or CSPG perturbations to reverse the last stages of brain development and to reinstate the plasticity of the juvenile brain. In this sense, further investigations of the penultimate stages of brain development may be most useful for developing therapeutic interventions for adult CNS injuries.

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