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Nerve Regeneration: Regrowth Stumped by Shared Receptor

Dispatch

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Three different myelin proteins, Nogo, MAG, and OMgp, inhibit regenerating axons after CNS injury. New work reveals that they all share a common receptor and that blockade of this receptor promotes CNS repair and functional recovery.

Unlike peripheral nervous system (PNS) axons, severed central nervous system (CNS) axons are unable to regenerate. Why do CNS and PNS axons differ so dramatically in their regenerative abilities? Differences in CNS and PNS glial cells appear to be crucial, as some CNS axons are able to regenerate through a peripheral nerve graft [1]. Axons are tightly wrapped by myelin membrane produced by oligodendrocytes in the CNS and Schwann cells in the PNS. Following axonal injury in the PNS, myelin is rapidly cleared by macrophages as the axons degenerate in a process known as Wallerian degeneration. In contrast, myelin is cleared much more slowly after CNS injury. This difference suggests that CNS myelin strongly inhibits regenerating axons, a possibility that was directly confirmed in elegant experiments by Martin Schwab and his colleagues [2].

An important first step towards identifying myelin inhibitors of axon regeneration was taken many years ago with the development of an anti-CNS myelin monoclonal antibody, IN-1, which promotes CNS regeneration and a limited degree of functional recovery in animal models [2]. Only in the last few years, however, have researchers begun to elucidate the key inhibitors and their receptors, uncovering a few surprises along the way. The target of the IN-1 antibody turned out to be Nogo, a reticulon homolog with three isoforms: Nogo-A is expressed primarily in oligodendrocytes, and the other two isoforms are more widely distributed ([reviewed in [3]). Interestingly, Nogo-A has two separate domains that can inhibit growing axons: the amino-terminal portion (Amino-Nogo), which is unique to Nogo-A, and a 66 amino acid peptide (Nogo-66) located between the two transmembrane regions, which is common to all isoforms.

How does Nogo inhibit regenerating axons? The functional receptor for Nogo-66, NgR, is a 473 amino acid protein with an attached glycosylphosphatidylinositol (GPI) lipid. This GPI linkage serves to anchor the protein to the outer surface of the cell membrane, where it can interact with its ligand. Binding studies and the effects of ectopic expression both confirmed that this protein is the functional receptor for Nogo-66 [4]. The expression patterns of Nogo and NgR are consistent with the interactions of these proteins at points of contact between axons and myelin, as well as at synapses [5]. It is unlikely, however, that NgR works alone: its lack of a transmembrane region implies the presence of an as yet unidentified transmembrane co-receptor that transduces the inhibitory signal.

These new findings have already led to efforts to develop drugs that enhance CNS regeneration. By analyzing small peptide fragments of Nogo-66, Strittmatter and colleagues [6] found that, although residues 1–31 of Nogo-66 bind with high affinity to NgR, only a peptide containing residues 31–55 inhibits axon outgrowth. On the basis of these results, they developed a competitive antagonist peptide, termed NEP1-40 for 'Nogo extracellular peptide residues 1–40', which specifically blocks Nogo-66, but not Amino-Nogo, activity [6]. The delivery of NEP1-40 into an injured rat spinal cord produces significant axon regrowth and a modest but noteworthy promotion of locomotor recovery. This finding supports the view that NgR plays an important role in CNS regenerative failure and is an encouraging target for new therapeutics.

While Nogo has attracted much of the spotlight of late, additional inhibitory myelin proteins are known. One of these, myelin-associated glycoprotein (MAG), shares with Nogo the interesting property that it inhibits postnatal and adult, but not embryonic, neurons. MAG binds to a sialic acid-bearing glycosphingolipid called ganglioside GT1b and closely related gangliosides on the neuronal surface. This ganglioside has been suspected of helping to form a MAG receptor, because clustering of GT1b by antibody complexes is sufficient to inhibit neurite outgrowth by activating a Rho kinase [7,8], but it has been unclear how a ganglioside could help to transmit a signal across the plasma membrane. Unexpectedly, the ganglioside GT1b turns out to associate with the neurotrophin receptor p75 to form a MAG receptor complex which transmits an inhibitory signal via the small GTPase Rho [9]. As no inhibitory effect of MAG is seen in the absence of p75, this complex appears to be necessary and sufficient to mediate MAG's inhibitory effects.

In striking contrast to this report, a mutant MAG that is unable to bind to gangliosides still inhibits neurite outgrowth when expressed on Schwann cells, implying the presence of an additional MAG receptor [10]. Intriguing new studies have revealed that the Nogo-66 receptor NgR fits the bill and, moreover, is itself necessary and sufficient to mediate MAG's inhibitory actions [11,12]. These latest findings show that MAG binding to NgR is independent of GT1b, that dominant-negative forms of NgR block MAG activity, and that NgR expression confers both MAG and Nogo-66 responsiveness on embryonic neurons. Importantly, however, the NgR antagonist peptide NEP1-40 does not interfere with MAG's inhibitory activity [12], suggesting that additional functional recovery may be achieved by improved antagonists.

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How can GT1b-p75 and NgR each be necessary and sufficient to mediate MAG's inhibitory effects? The resolution of these seemingly conflicting reports may lie in the propensity of both GPI-anchored proteins and gangliosides to partition into cholesterol-rich microdomains of the cell membrane known as lipid rafts. These are gel-like collections of particular lipids and proteins that float like tiny rafts in the more fluid surrounding membrane. These microdomains can promote signaling by clustering specific proteins together [13]. One possibility is that NgR and GT1b share the ability to cluster and activate the same raft-associated signal transduction apparatus, perhaps p75. Therefore, it will be interesting to see whether Nogo-A directly or indirectly interacts with GT1b and p75, whether inhibition by Nogo-66 requires p75, and whether antibody-mediated clustering of GT1b can still inhibit axon outgrowth in neurons that lack NgR.

Are MAG and Nogo the only inhibitory proteins found in myelin? Recognizing that the myelin sheath is enriched in GPI-anchored proteins [14], Wang and colleagues [15] searched for GPI-linked proteins from myelin that might be regeneration inhibitors. They enzymatically released all GPI-anchored proteins from purified myelin and found that this fraction contained inhibitory activity. From its molecular weight, they inferred that this activity is oligodendrocyte-myelin glycoprotein (OMgp), a known myelin protein initially isolated from human white matter, and then confirmed that recombinant OMgp is in fact a potent neurite inhibitor. Interestingly, like Nogo, OMgp is also made by neurons [16]. Expression cloning revealed that the binding partner of OMgp is none other than NgR, the same receptor responsible for mediating the inhibitory action of Nogo-66 and MAG. Ectopic NgR expression confers OMgp sensitivity on embryonic neurons. Nogo-66 and OMgp have similar affinities for NgR, bind to overlapping regions, and compete for binding [15]. So, remarkably, three very different myelin proteins inhibit axon regeneration through a common receptor, NgR (Figure 1).

A considerable amount of work remains to ascertain the importance of these recent findings in injury models. Continuing gene knockout studies in mice should help to clarify the relative roles of Nogo, OMgp and MAG in the blockade of axon outgrowth, as well as their normal roles in development. Similarly, NgR-deficient mice will allow assessment of myelin's contribution to regenerative failure, as well as the therapeutic potential of disrupting this shared receptor. While these new advances clearly make NgR a very inviting target, there is plenty of evidence that myelin inhibitors are only one of several obstacles that regenerating CNS axons face. They must also overcome growth inhibitors produced by other glial cells called astrocytes, as well as a lack of appropriate substrates and trophic factors. Furthermore, recent studies have shown that CNS neurons undergo apparently irreversible changes as the CNS matures that limit their intrinsic regenerative ability [17]. Despite these impediments, the success of NEP1-40 at promoting axon growth and functional recovery gives good reason for optimism. The development and testing of the next generation of NgR antagonists, screened for the ability to block the inhibitory activity of Nogo, MAG and OMgp, will provide important insight into myelin's role in blocking regeneration and may, with a little luck, even prove to be useful clinically.

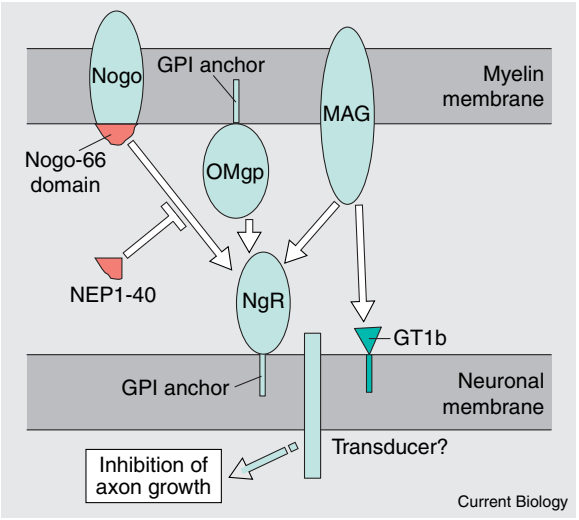

Figure 1. NgR is the shared receptor of three myelin-associated inhibitors of axon growth.

The myelin proteins Nogo, OMgp and MAG block the regeneration of axons. All three inhibit axon outgrowth by binding to the axonal GPI-anchored Nogo-66 receptor, NgR. MAG also binds the ganglioside GT1b, which may be important for signaling, perhaps by helping to bring together NgR and an as-yet-unknown transmembrane signal transducer, which may be the neurotrophin receptor p75. The antagonist peptide NEP1–40, derived from the first 40 residues of Nogo-66, blocks inhibition by Nogo-66 but not MAG (with OMgp yet to be tested). NEP1–40 promotes recovery in a spinal cord injury model, inspiring hope for future drugs that more completely block the activation of NgR or its transducer.

References

1. David, S. and Aguayo, A.J. (1981). Axonal elongation into peripheral nervous system 'bridges' after central nervous system injury in adult rats. Science *214*, 931–933.
2. Caroni, P. and Schwab, M.E. (1988). Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. Neuron *1*, 85–96.
3. Goldberg, J.L. and Barres, B.A. (2000). Nogo in nerve regeneration. Nature *403*, 369–370.
4. Fournier, A.E., GrandPre, T. and Strittmatter, S.M. (2001). Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature *409*, 341–346.
5. Wang, X., Chun, S.J., Treloar, H., Vartanian, T., Greer, C.A. and Strittmatter, S.M. (2002). Localization of Nogo-A and Nogo-66 receptor proteins at sites of axon-myelin and synaptic contact. J. Neurosci. *22*, 5505–5515.
6. GrandPre, T., Li, S. and Strittmatter, S.M. (2002). Nogo-66 receptor antagonist peptide promotes axonal regeneration. Nature *417*, 547–551.
7. Vyas, A.A., Patel, H.V., Fromholt, S.E., Heffer-Lauc, M., Vyas, K.A., Dang, J., Schachner, M. and Schnaar, R.L. (2002). Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. Proc. Natl. Acad. Sci. U.S.A. *99*, 8412–8417.
8. Vinson, M., Strijbos, P.J., Rowles, A., Facci, L., Moore, S.E., Simmons, D.L. and Walsh, F.S. (2001). Myelin-associated glycoprotein interacts with ganglioside GT1b: a mechanism for neurite outgrowth inhibition. J. Biol. Chem. *276*, 20280–20285.
9. Yamashita, T., Higuchi, H. and Tohyama, M. (2002). The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. J. Cell Biol. *157*, 565–570.

10. Tang, S., Shen, Y.J., DeBallard, M.E., Mukhopadhyay, G., Salzer, J.L., Crocker, P.R. and Filbin, M.T. (1997). Myelin-associated glycoprotein interacts with neurons via a sialic acid binding site at ARG118 and a distinct neurite inhibition site. J. Cell Biol. *138*, 1355–1366.
11. Domeniconi, M., Cao, Z., Spencer, T., Sivasankaran, R., Wang, K.C., Nikulina, E., Kimura, N., Cai, H., Deng, K., Gao, Y. *et al*. (2002). Myelin-associated glycoprotein interacts with the Nogo-66 receptor to inhibit neurite outgrowth. Neuron *35*, 283–290.
12. Liu, B.P., Fournier, A., GrandPre, T. and Strittmatter, S.M. (2002). Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *297*, 1190-1193,
13. Simons, K. and Toomre, D. (2000). Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. *1*, 31–41.
14. Kramer, E.M., Kock, T., Niehaus, A. and Trotter, J. (1997). Oligodendrocytes direct glycosyl phosphatidylinositol-anchored proteins to the myelin sheath in glycosphingolipid-rich complexes. J. Biol. Chem. *272*, 8937–8945.
15. Wang, K.C., Koprivica, V., Kim, J.A., Sivasankaran, R., Guo, Y., Neve, R.L. and He, Z. (2002). Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature *417*, 941–944.
16. Habib, A.A., Marton, L.S., Allwardt, B., Gulcher, J.R., Mikol, D.D., Hognason, T., Chattopadhyay, N. and Stefansson, K. (1998). Expression of Oligodendrocyte-myelin glycoprotein by neurons of the mouse central nervous system. J. Neurochem. *70*, 1704–1711.
17. Goldberg, J.L., Klassen, M.P., Hua, Y. and Barres, B.A. (2002). Retinal maturation triggers retinal ganglion cells to switch from an axonal to a dendritic growth mode. Science *296*, 1860–1864.