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Nogo-A Represses Anatomical and Synaptic Plasticity in the Central Nervous System

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Nogo-A was initially discovered as a myelin-associated growth inhibitory protein limiting axonal regeneration after central nervous system (CNS) injury. This review summarizes current knowledge on how myelin and neuronal Nogo-A and its receptors exert physiological functions ranging from the regulation of growth suppression to synaptic plasticity in the developing and adult intact CNS.

Pioneering work in the late 1980s led to the discovery of specific neurite growth inhibitory factors in CNS myelin and to the identification of two membrane protein fractions that were highly inhibitory for neurite outgrowth in vitro (NI-35 and NI-250) (16). Antibody-mediated neutralization of these fractions was found to largely relieve their growth-inhibitory properties and to increase axonal regeneration and functional recovery in vivo (15, 102, 114). NI-250 was later identified as a new ~1,200-amino acids (aa)-long protein. It was renamed Nogo-A and appeared as a member of the reticulon (RTN) family (18, 41, 96, 115). Ensuing studies using a variety of blockers interfering with Nogo-A, its interaction partners, or associated signaling pathways confirmed the initial results and underlined its role in restricting anatomical plasticity and functional recovery after different types of CNS injuries (147). Surprisingly, an induction of structural plasticity was also observed upon genetic or antibody-mediated inactivation of Nogo-A in the intact CNS, pointing to an important physiological role of Nogo-A. To date, Nogo-A is thought to act as a tonic brake on CNS growth and plasticity, thereby stabilizing neuronal circuits.

This review aims at describing the currently known physiological roles of Nogo-A in the developing and adult CNS with particular emphasis on the underlying cellular mechanisms. The first part focuses on molecular features of Nogo-A and its known receptors, and on the signaling cascades that lead to a destabilization of the cytoskeleton and to a suppression of neurite growth. The second part describes physiological roles of Nogo-A in restricting different forms of plasticity in the adult CNS. The last section summarizes regulatory roles of Nogo-A during development.

Molecular and Cellular Characteristics of Nogo-A

Four mammalian reticulon genes (*rtn1*, *rtn2*, *rtn3*, *rtn4*) give rise to a wide range of different splice variants forming the RTN family of proteins (89). The protein isoforms Nogo-A, Nogo-B, and Nogo-C are encoded by the *rtn4*/*nogo* gene by alternative splicing or different promoter usage (18, 41, 96) (FIGURE 1A). All RTN members share a COOH-terminal reticulon homology domain (RHD) of 180–200 aa, which consists of two membrane-anchored hydrophobic regions spanned by a 60- to 70-aa-long hydrophilic region, also called Nogo-66 in Nogo-A, and followed by a short COOH terminus (89) (FIGURE 1A). In contrast to the highly conserved RHD, little or no homology can be found between the NH_2-terminal regions of RTNs or other proteins (89), suggesting that various RTN isoforms may interact with different proteins and thereby exert a wide range of biological functions.

In the adult CNS, Nogo-A is predominantly expressed in myelin-forming oligodendrocytes but also is found in neurons of highly plastic CNS regions such as the hippocampus or the cortex (50). In the developing CNS, Nogo-A is transiently expressed by different neuronal populations, in particular projection neurons (50). Like other RTN family members, Nogo-A is predominantly localized to the endoplasmatic reticulum (ER) with small (<10%) but functionally significant amounts found at the cell surface of oligodendrocytes, neurons, and some nonneuronal cell types (28, 90). Intracellularly, Nogo-A has a strong preference to localize to the tubular ER where it is required for the formation and maintenance of ER tubules in vitro (128). Unlike other ER proteins or transmembrane proteins delivered to the plasma membrane via the classic ER/Golgi transport route, Nogo-A lacks a signal peptide for its translocation into the ER membrane (90). Despite a COOH-terminal dilysine ER retention motif, several studies have now shown that the transmembrane domains of RTNs are responsible for their proper ER localization (47, 88, 110, 113, 123). These findings, however, need to be confirmed for Nogo-A.

Membrane Topology and Trafficking of Nogo-A

Given their unusually long hydrophobic segments (~35 aa), RTN proteins have been suggested to

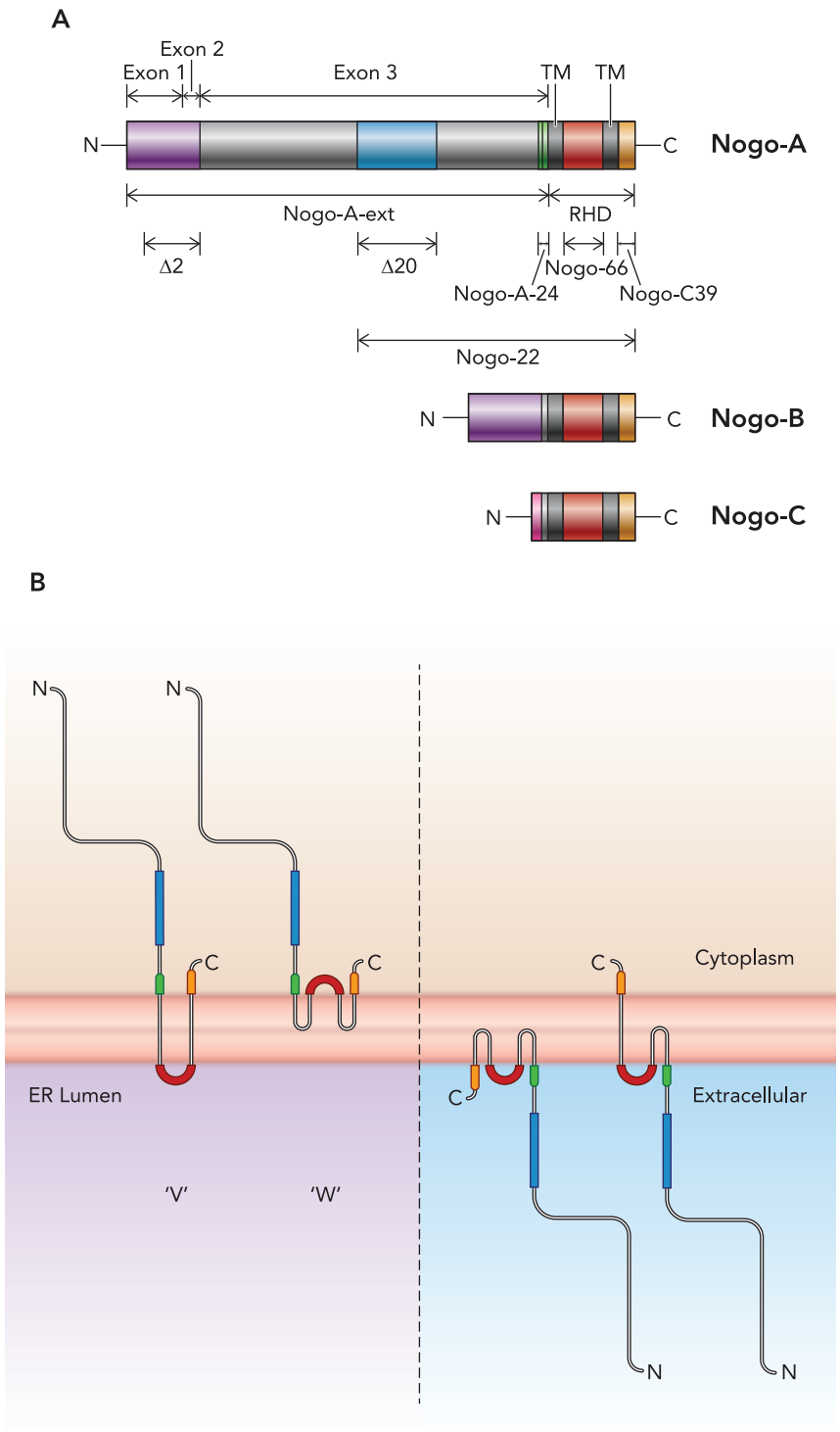


FIGURE 1. **Structure and membrane topology of RTN4/Nogo proteins**
A: schematic structure of the Nogo protein isoforms Nogo-A, Nogo-B, and Nogo-C. The functional domains Nogo-A/B-Δ2 (purple), Nogo-A-Δ20 (blue), Nogo-A-ext (amino-Nogo), Nogo-66 (red), Nogo-A-24 (green), Nogo-C39 (orange), and Nogo-22 are indicated. Nogo-66 is located between the transmembrane domains (TM) and found in all three isoforms. The Δ20 domain is encoded by parts of exon 3 and is specific to Nogo-A. *B*: multiple topologies proposed for RTN4 proteins in the ER (*left*) and plasma membrane (*right*) of different neuronal and nonneuronal cell types. Nogo-66 is found both inside and outside of the ER lumen, resulting in a *V* or *W* configuration of the protein. At the plasma membrane, both Nogo-A-Δ20 and Nogo-66 are facing the extracellular space. Different topologies in different cell types may reflect the functional diversity of RTN4 proteins.

adopt a hairpin topology different from classic transmembrane proteins. In the ER membrane, both the NH_2 and COOH terminus are located on the cytosolic side (FIGURE 1B), reminiscent of the scaffold-assembling proteins caveolin and reggie/flotillin (11, 47, 57, 90, 113, 128). In line with this, RTNs have been proposed to represent a novel class of membrane-shaping proteins, which generate membrane curvature through a local oligomerization of several hairpin loops inserted into one leaflet of the membrane (11, 110). The localization of the Nogo-66 loop might differ between different Nogo isoforms, resulting in a "V" or "W" configuration of the protein, in which each hydrophobic segment fully spans the membrane or adopts a hairpin structure, respectively (90, 128) (FIGURE 1B). Nogo-A is found in a different topology at the plasma membrane, in which the NH_2 terminus and the Nogo-66 loop face the extracellular space and thereby enable a functional engagement with several interaction partners in trans (see below) (41, 90, 105, 142) (FIGURE 1B). Alternate topologies allowing the expression of multiple biological functions in different cellular compartments have been described for a number of other proteins including ER-resident proteins such as the inositol 1,4,5-triphophate receptor (IP_3R) or the recently characterized Nogo-B receptor (NgBR) (44, 45, 67, 78, 120). Although bioinformatic models have predicted that the NH_2-terminal sequence of Nogo-A is a strong topological organizer, it is unclear how Nogo-A adopts its different topologies (47). Putative mechanisms may include dimerization, the association with different molecular chaperones known to determine the conformational flexibility of several membrane proteins, as well as the phospholipid composition of the membrane (12, 54, 61, 100). In line with this, Nogo-66 is known to require a phosphocholine lipid surface to fold properly (125).

Along its intriguing topology, it remains elusive how Nogo-A is transported from the ER to the cell surface. Being an unconventional membrane protein, Nogo-A trafficking is likely to occur in a Golgi-independent manner (90, 105). To date, several membrane proteins are known to bypass the Golgi such as the protein tyrosine phosphatase CD45 (9), the paranodal complex of F3/Contactin and Caspr/Paranodin (13), the cystic fibrosis transmembrane conductance regulator (CFTR) (37, 139), *Drosophila* αPS1 integrin (103, 104), and some connexins (Cx) such as Cx26 (74). Plasma membrane targeting might either involve a direct fusion between peripheral components of the ER, in which Nogo-A is strongly expressed, or a vesicle-mediated transport mechanism (128). The latter might be dependent on two recently identified critical components of a Golgi bypass pathway: Golgi reassembly stacking protein (GRASP) 55 and 65 (42). It will be interesting

Table 1. *Comparison of the different functional domains of Nogo-A*

Functional Domain	Function	Receptor/Signal Transducer(s)	Cell Type(s)	References
Nogo-A/B-Δ2 (aa 59–172)	Cell spreading inhibition	Unknown	3T3	91
Nogo-A-Δ20 (aa 544–725)	Growth cone collapse	Unknown	E13–15 cDRGs, E19 rHNs	54, 91
	Neurite outgrowth inhibition	Unknown/Integrin-dependent	E7–9 cRGCs, PC12, E9–11 cDRGs, P6–10 rDRGs, P6–8 CGNs	49–50, 54, 91
	Cell spreading inhibition	Unknown/Integrin-dependent	3T3, COS-7, CHO-K1, HUVEC	49–50, 91
Nogo-A-ext/amino-Nogo (aa 1–979)	Cell spreading inhibition		3T3, COS-7	34, 49
	Neurite outgrowth inhibition		E13 cDRGs, P4 msCGNs	34, 49
Nogo-66 (aa 1,026–1,091)	Growth cone collapse	NgR1, PirB	E12 cDRGs, P10 msDRGs	8, 34, 41
	Neurite outgrowth Inhibition	For soluble Nogo-66: NgR1-/p75-dependent	E12–13 cDRGs, P4 msCGNs, PC12	19, 34, 41, 132
		For substrate-bound Nogo-66: NgR1-independent, PirB-dependent/LINGO-1-, p75-, TROY-dependent	E12–13 cDRGs, P7 msCGNs, P10 or adult msDRGs	8, 19, 34, 41, 78, 93, 109, 132, 146
Nogo-A-24 (aa 966–989)	Increases the binding affinity of Nogo-66 to NgR 1; not inhibitory *per se*	NgR 1, PirB	E13 cDRGs, COS-7	49, 52
Nogo-C39 (aa 1126–1163)		NgR1, PirB		52
Nogo-22 (aa 966–1163)	Growth cone collapse	NgR1	E13 cDRGs	52
	Neurite outgrowth inhibition	NgR1, PirB-independent	Adult msDRGs, DIV21 CNs	52

Rat sequences are indicated. CNs, cortical neurons; CGNs, cerebellar granule neurons; DRGs, dorsal root ganglia; HNs, hippocampal neurons; RGCs, retinal ganglion cells; c, chicken; ms, mouse; r, rat; E, embryonic day; P, postnatal day; DIV, days in vitro.

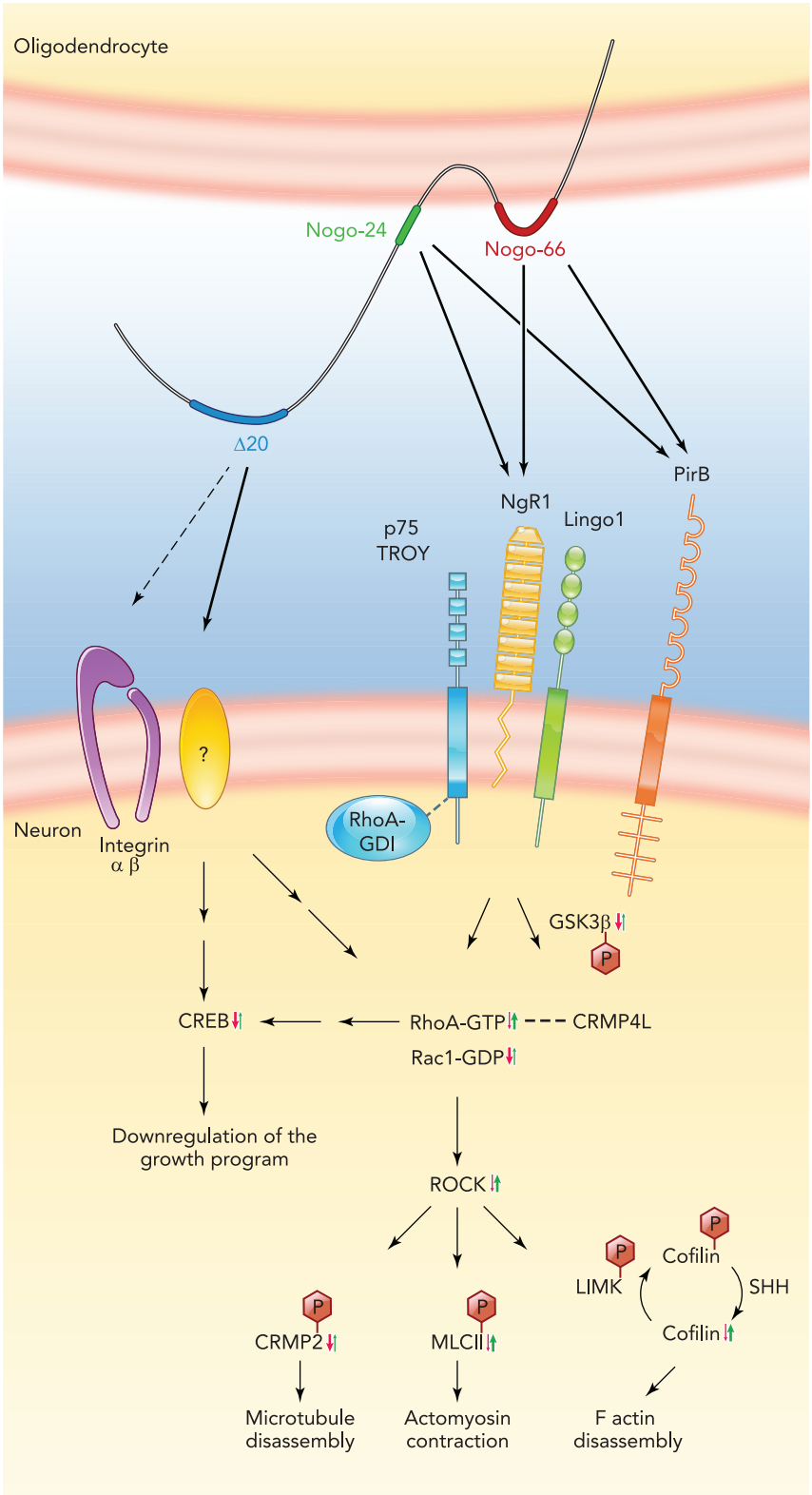
to study whether Nogo-A is trafficked in a GRASP-dependent manner to the cell surface and how GRASP is regulated at the onset of myelination.

Mechanisms of Nogo-A-Mediated Growth Inhibition

Functional Domains

Two different regions of Nogo-A induce growth-cone collapse and inhibit neurite outgrowth of primary neuronal cultures in vitro: Nogo-A-Δ20 (rat aa544–725) and Nogo-66 (rat aa1026–1,091) (41, 90) (FIGURES 1 AND 2; Table 1). Nogo-A-Δ20 additionally inhibits adhesion and cell spreading of various nonneuronal cell types, suggestive of a more ubiquitous function inside and outside the CNS (90). Although Nogo-A-Δ20 is encoded by parts of exon 3 and therefore specific to Nogo-A, the Nogo-66 region is encoded by exons 4 and 5 and found in all RTNs (87). However, the analysis of different RTN-Nogo-66 regions showed that neither RTN1- nor RTN2- nor RTN3-Nogo-66 induce growth cone collapse as opposed to RTN4-Nogo-66 (41). This has been partially linked to a sequence diversity in the COOH-terminal Nogo-66 residues of different RTN family members (62). A third region common to Nogo-A and Nogo-B, Nogo-A/B-Δ2 (rat aa59–172), also inhibits cell spreading but not neurite outgrowth (90) (FIGURE 1; Table 1). Interestingly, multi-domain fragments of Nogo-A comprising Nogo-66 or Nogo-A-Δ20 flanked by aa stretches that do not alter cell spreading or neurite outgrowth by themselves appear to be more inhibitory than Nogo-66 or Nogo-A-Δ20 alone. Herein, the multi-domain fragment Nogo-22 containing membrane-spanned Nogo-66 flanked by two regions extending NH_2 (Nogo-A-24) and COOH terminally (Nogo-C39) from either side is a more potent growth-inhibitory molecule than Nogo-66 alone (51). Similarly, the entire NH_2-terminal domain of Nogo-A (rat aa1–979; also called NiR-G or

amino-Nogo) possesses stronger inhibitory properties than Nogo-A-Δ20 (FIGURE 1; Table 1) (90). Together, these results suggest that multivalent interactions of different regions of Nogo-A with a heteromeric receptor complex might be critical for the activation of downstream signaling events.



Receptors

Two high-affinity interaction partners/receptors have been characterized for the Nogo-66 domain, whereas the molecular players interacting with Nogo-A-Δ20 are still largely unknown. Nogo-66 interacts with the Nogo-66 receptor 1 (NgR1) (34) and also with the paired immunoglobulin-like receptor B (PirB) (8) (FIGURE 2). NgR1 belongs to a family of three glycosylphosphatidylinositol (GPI)-anchored proteins (NgR1–3) lacking an intracellular signaling domain (126). To transduce intracellular signals, NgR1 interacts with the leucine-rich repeat (LRR) and Ig domain-containing Nogo receptor-interacting protein 1 (LINGO-1) (77) and with the low-affinity neurotrophin receptor $p75^{NTR}$ (131). Because the expression of $p75^{NTR}$ is temporally regulated and restricted in the adult CNS, the related tumor necrosis factor alpha (TNF-α) receptor superfamily member 19 (TROY) functionally substitutes for $p75^{NTR}$ (92, 108) (FIGURE 2). PirB also interacts with $p75^{NTR}$ for signal transduction (36). Surprisingly, two other structurally unrelated myelin proteins with growth inhibitory activity, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp), as well as the inhibitory non-myelin-associated protein B lymphocyte stimulator (BLyS) share the same receptor complex (29, 70, 131, 132, 137, 143). MAG binds the NgR1 homolog NgR2 with higher affinity than NgR1. NgR2, however, does not bind to Nogo-66 or OMgp (126). In addition, NgR1 and its third family member NgR3 bind to chondroitin sulfate proteoglycans (CSPGs) (26). NgR1 also interacts with the secreted proteins leucine-rich glioma inactivated (LGI1) and olfactomedin-1, which antagonize

FIGURE 2. **Nogo-A, its currently known receptors, and its associated signaling pathways**
Cell surface Nogo-A-Δ20 (blue) and Nogo-66 (red) bind to different receptors that converge on the activation of RhoA and on the suppression of Rac1, leading to growth inhibition via ROCK. Nogo-66 binds directly to NgR1 and PirB. NgR1 forms a complex with LINGO1 and p75 or TROY to transduce intracellular signals. Nogo-A-24 (green) increases the binding affinity of Nogo-66 to NgR1. Nogo-A-24 also binds NgR1 and PirB directly without affecting neurite outgrowth per se. Nogo-A-Δ20 interacts with a yet uncharacterized receptor and indirectly inhibits the activation of integrins. Activation of the RhoA/ROCK pathway results in a destabilization of the cytoskeleton affecting microtubules via CRMP2 and actin fibers via MLCII and Cofilin. Signaling events underlying RhoA activation include *1*) a strengthened interaction between $p75^{NTR}$ and Rho-GDI (dotted line) and *2*) an increased complex formation between CRMP4L and RhoA (dotted line) upon phosphorylation of GSK3β. The mechanisms by which Nogo-A-Δ20 transcriptionally downregulates the neuronal growth program involve inactivation of CREB but remain poorly understood. Green arrows indicate activation, and red arrows indicate inhibition. P, phosphorylation. The thickness of half-headed arrows illustrates an increase (green) or decrease (red). Abbreviations are explained in the text.

Nogo-66- and myelin-induced growth cone collapse (83, 122).

Functional evidence for NgR1 and PirB acting as Nogo-66-specific receptors was presented in numerous assays using different neuronal cell types. Acute blockade of NgR1 with a function-blocking antibody, a competitive antagonist of Nogo-66 (NEP1–40), or a soluble NgR1 ectodomain substantially antagonizes neurite outgrowth inhibition and growth cone collapse mediated by Nogo-66, MAG, OMgp, or crude myelin extracts in vitro (29, 33, 40, 70). Conversely, exogenous expression of NgR1 induces growth cone collapse in neurons that would otherwise be insensitive to any of the NgR1 ligands (34, 70, 132). However, studies using neurons derived from NgR1 knockout (KO) mutants have shown that NgR1 is required for acute Nogo-66-mediated growth cone collapse but not for chronic substrate-bound growth inhibition (19, 59, 127, 145). On the other hand, functional deletion of PirB is sufficient to rescue Nogo-66-mediated growth cone collapse and to partially release Nogo-66- and myelin-mediated substrate-bound growth inhibition (8). Together, these results suggest that the molecular mechanisms underlying acute and chronic growth-inhibitory effects can be dissociated and suggest the existence of NgR1-independent mechanisms for long-term growth-inhibitory effects (19).

Several studies have shown that Nogo-A-Δ20-mediated inhibitory effects occur independently of NgR1 (105). Functional receptors for Nogo-A-Δ20 are being studied in several laboratories at present but remain still largely unknown. One study reported that Nogo-A-Δ20 inhibits the activation of the integrin-associated focal adhesion kinase (FAK) and that Nogo-A-Δ20-mediated growth inhibition could be overcome by activating integrins (49, 119). However, a proof of interaction using purified proteins remains elusive, and it is unclear how far the observed effects require intermediate proteins (49). Furthermore, the orphan G-protein-coupled receptor 50 (GPR50) was recently found to interact with Nogo-A-Δ20, albeit intracellularly in cis and mediating opposite effects on neurite outgrowth (43). Thus high-affinity functional receptors mediating Nogo-A-Δ20-induced growth inhibition in trans remain to be discovered.

Intracellular Signaling Pathways

Both Nogo-66 and Nogo-A-Δ20 trigger the activation of the small GTPase RhoA and of its effector Rho-associated, coiled-coil containing protein kinase (ROCK), which results in a destabilization of the growth machinery (84, 85, 105) (FIGURE 2). Rho GTPases, including RhoA, Rac1, and Cdc42, integrate upstream directional cues and trigger downstream cytoskeletal rearrangements, e.g., actin polymerization for growth and protrusion of lamelli- and filopodia on growth cones and ruffling membranes (Rac1, Cdc42), or depolymerization and actomyosin contraction for retraction (RhoA) (72, 101). In line with this, Nogo-66 and Nogo-A-Δ20 not only activate RhoA but also decrease the activity of Rac1 (24, 85). Pharmacological blockade or dominant-negative forms of RhoA and ROCK substantially prevent Nogo-A- and myelin-mediated growth inhibition in vitro (4, 25, 35, 85). In vivo, application of the ROCK blocker Y-27632 stimulates fiber sprouting and regeneration after spinal cord or optic nerve injury but also accelerates functional motor recovery in different models of spinal cord injury (17, 25, 35, 98). Enzymatic inactivation of RhoA via C3 transferase leads to similar but more variable results, most probably due to an inconsistent cell penetration (25, 35, 117). More recently, genetic deletion of the brain-specific ROCK isoform ROCKII confirmed the functional importance of this signaling pathway (31). Given the convergence of multiple inhibitory cues other than Nogo-A onto RhoA and ROCK, e.g., CSPGs or members of the Ephrin and Semaphorin families of axon guidance molecules (38), it is very likely that several inhibitors may be affected by targeting this pathway in vivo.

Downstream cytoskeletal effectors of the RhoA/ROCK pathway include myosin light chain (MLC) II and cofilin, which induce growth cone retraction by either promoting myosin II contractile activity or F-actin depolymerization, respectively (4, 48, 72, 84) (FIGURE 2). More precisely, Nogo-66 modulates the phosphorylation levels of cofilin via a ROCK-dependent sequential activation of LIM (Lin-11, Isl-1, and Mec-3) kinase (LIMK) and Slingshot (SSH) phosphatase (48). Accordingly, a deactivation of the ROCK/LIMK/cofilin pathway has been associated with increased growth cone dynamics in Nogo-A KO mice (82). Besides affecting the actin cytoskeleton, Nogo-66 also destabilizes microtubule assembly through phosphorylation of the collapsin response mediator protein 2 (CRMP2) (79) (FIGURE 2). Upstream of Rho GTPases and their associated cytoskeletal effectors, CRMP4 and the Rho-guanine dissociation inhibitor (Rho-GDI) have been proposed to link Nogo-66 to RhoA activation (84). Nogo-66 is found to facilitate RhoA activation by strengthening the interaction of Rho-GDI with $p75^{NTR}$ and thereby releasing RhoA for conversion into its active form (138). In addition, Nogo-66 stimulation results in glycogen synthase kinase 3β (GSK3β) inactivation, thereby allowing an increased complex formation between CRMP4L and RhoA (5, 6) (FIGURE 2). Disruption of the CRMP4L-RhoA complex formation or knockdown of CRMP4L results in decreased growth inhibition on a myelin substrate (5, 6).

Other signaling components of Nogo-A include protein kinase C (PKC) and epidermal growth factor receptor (EGFR), whereby pharmacological blockade of PKCα/β and EGFR was shown to decrease Nogo-66 and myelin-mediated growth inhibition (46, 60, 112). In vivo, application of EGFR antagonists improved motor and sensory function after spinal cord injury (32), increased retinal ganglion cell (RGC) axon regeneration after optic nerve crush (60), and prevented RGC death in a glaucoma model (69). Intriguingly, the latter studies assigned phosphorylated EGFR expression in the adult CNS mainly to glial cells and not to neurons, suggesting that these effects might be glial and indirect (1, 30, 69). However, ensuing studies suggested that growth promotion induced by EGFR blockade might rely on off-target Trk activation by an increased secretion of neurotrophic factors rather than on EGFR inactivation (1, 2, 30). Thus these studies question a direct involvement of intraaxonal EGFR in the Nogo-A signaling axis.

Nogo-A Restricts Axonal Regeneration and Structural Plasticity After CNS Injury

Acute treatment with Nogo-A neutralizing antibodies was repeatedly shown to increase regenerative sprouting and growth of lesioned as well as spared axons in different models of stroke and spinal cord injury (for detailed reviews, see Refs. 39, 147). Comparable anatomical results as well as improved recovery of lost functions are also obtained by blocking NgR1 (33, 40, 133, 135) and the NgR1-associated protein LINGO-1 (52). However, differences were found with regard to the degree of axonal regeneration after spinal cord injury in independently generated Nogo-A, Nogo-A/B, and Nogo-A/B/C targeted or gene trap mutant mice (58, 64, 68, 106, 111, 146). Although two Nogo-A (111) and Nogo-A/B (58) mutant lines displayed increased axonal regeneration following a spinal cord dorsal hemisection, variable results with no significant effects were observed in three other Nogo-A/B and Nogo-A/B/C mutant lines (64, 146). Discrepancies were potentially due to strain background-dependent effects (27) and compensation by other Nogo isoforms or functionally related genes (66, 106).

Besides regenerative plasticity, acute neutralization as well as genetic deletion of Nogo-A have consistently proven effective in eliciting compensatory sprouting of spared fibers, i.e., non-regenerative plasticity, at different anatomical levels after injury. For example, after transection or elimination (by stroke) of the corticospinal tract (CST) that projects from the sensorimotor cortex to the spinal cord, a twofold increase in compensatory sprouting of unlesioned CST fibers was found in anti-Nogo-A- vs. control antibody-treated groups (39, 65, 91, 121, 147). Non-regenerative plasticity of spared fibers is thought to allow the formation of "detour" connections from cortical neurons to spinal motor neurons by either sprouting into denervated areas and thereby re-innervating the spinal cord deprived of its major cortical input or by contacting other nonlesioned axonal tracts. Rearrangement of the intact circuitry may provide an important substrate for plasticity and functional recovery in the adult CNS. Thus increasing the low degree of spontaneously occurring non-regenerative plasticity by inactivating the Nogo-A signaling axis may significantly improve the treatment of spinal cord injury and stroke.

Nogo-A Restricts Neuronal Plasticity in the Intact Adult CNS

Structural Plasticity and Fiber Growth in the Adult CNS

The observation that Nogo-A neutralization results in a marked increase of compensatory, non-regenerative sprouting after CNS injury raised the question as to whether Nogo-A also limits plasticity in the intact CNS. Initial studies showed that acute neutralization of Nogo-A by antibody injection resulted in marked sprouting of uninjured axons in the cerebellum and spinal cord of adult rats (10, 14) (FIGURE 3, *A–D*). Inactivation of Nogo-A in mature organotypic hippocampal slice cultures also led to an increase in the growth and complexity of pyramidal axons and dendrites (22, 141) (FIGURE 3, E AND F). Similar results were observed in NgR1/2/3 triple knockout mice (136). Using Nogo-A KO and Nogo-A overexpressing transgenic mice, Nogo-A was also recently found to negatively regulate the dendritic growth and complexity of Purkinje cells in the cerebellum (95). At the molecular level, these structural changes are accompanied by a concomitant upregulation of growth-associated markers and transcription factors, which suggests that Nogo-A may actively suppress anatomical plasticity in the adult CNS by a tonic downregulation of growth-associated gene expression (10, 14, 22, 140) (FIGURE 2). Indeed, transcriptional profiling of hippocampal or cerebellar slices treated with Nogo-A-neutralizing antibodies as well as proteomic profiling of the CNS of adult Nogo-A KO mice pointed to a marked regulation of the growth cone cytoskeleton machinery and of growth-associated transcription factors toward increased growth (22, 82, 140). Tonic growth inhibition might result from retrogradely transported inhibitory signals from the axons to the cell bodies. Inactivation of Nogo-A around mature

myelinated axons would thereby result in a disinhibition of the suppressed growth program, whereby the detailed mechanisms of action remain to be established (105). Interestingly, a recent study has shown that recombinant Nogo-A-Δ20 is internalized and subsequently retrogradely transported in signaling endosomes to neuronal cell bodies where it downregulates cAMP response element binding protein (CREB) phosphorylation, i.e., CREB activation (53). Given that CREB is known to be retrogradely activated by the neurotrophic nerve growth factor (NGF) (21), it is tempting to speculate that Nogo-A uses similar signaling platforms to counteract the effects of growth factor signaling and to repress the growth program at a transcriptional level.

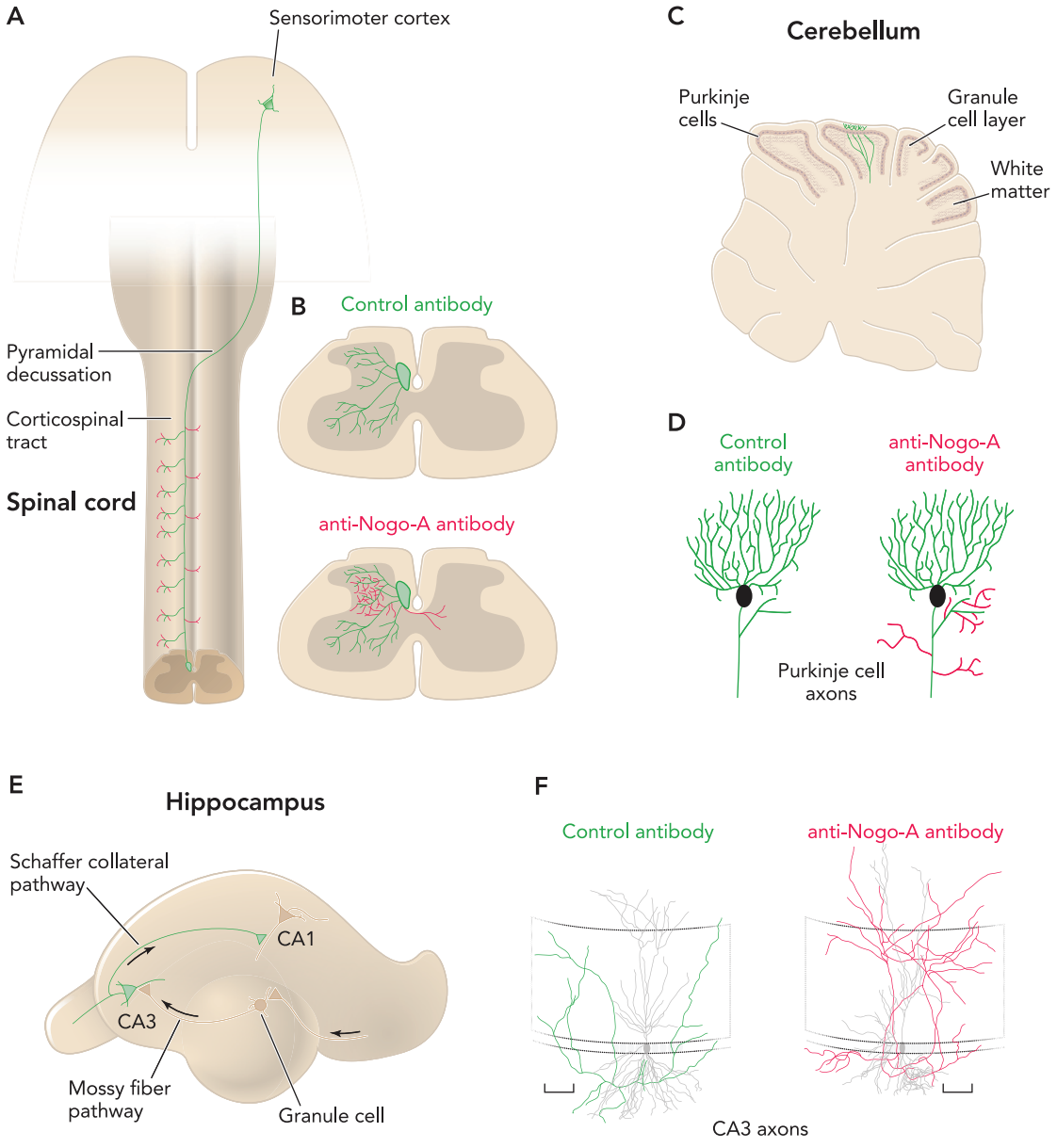


FIGURE 3. **Examples of restriction of axonal plasticity by Nogo-A in the intact adult CNS**
A: schematic representation of the corticospinal tract (CST; green) projecting from the sensorimotor cortex to the spinal cord. Anti-Nogo-A antibody induces sprouting of CST fibers ipsilaterally and, to a lesser extent, contralaterally across the midline in the noninjured spinal cord (red). *B*: magnification of the reorganization of CST projections occurring in the cervical enlargement in anti-Nogo-A- vs. control antibody-treated rats. *C*: schematic representation of the cerebellum. Purkinje cells extend axons (green) across the granule cell layer into the folium white matter. *D*: anti-Nogo-A antibody induces sprouting (red) of new collaterals from Purkinje cell axons within the granule cell layer. *E*: schematic representation of the hippocampus including two major synaptic pathways. Mossy fibers project from the granule cells to CA3 pyramidal neurons and Schaffer collateral fibers from CA3 to CA1 pyramidal neurons. *F*: anti-Nogo-A antibody induces sprouting of CA3 axons. Scale bar = 100 μm. *F* is reproduced from Ref. 141 with permission from the Society for Neuroscience.

Experience-Dependent Plasticity

Based on their role in limiting growth, Nogo-A and NgR1 were hypothesized to stabilize activity-dependent anatomical rearrangements and neuronal circuits during development, in particular at the end of the so-called "critical periods," which are characterized by a highly plastic fine-tuning of synaptic connections (55, 105). In the visual cortex, monocular deprivation of one eye results in an expansion of the ocular dominance regions of the non-deprived contralateral eye within a defined postnatal time window. This can be measured by electrophysiological recordings or optical imaging. Interestingly, the onset of myelination and Nogo-A expression in oligodendrocytes of the visual cortex tightly correlates with the termination of the critical period (3, 76). Genetic deletion of Nogo-A/-B or its receptors NgR1 or PirB induces an increase in adult optical dominance plasticity, suggesting that these proteins are involved in the closure of highly plastic developmental periods (76, 118). However, relatively little is known about the precise underlying mechanisms and the extent to which changes in structural connections and/or synaptic strengthening are involved.

Synaptic Plasticity, Long-Term Potentiation

A number of developmental axon guidance cues including repulsive molecules of the Ephrin/Eph and Semaphorin/Plexin families are found at synapses and have been shown to influence synaptic plasticity, in particular long-term potentiation (LTP) and long-term depression (LTD) (109). Nogo-A and NgR1 are also found pre- and postsynaptically in different brain regions including the hippocampus (7, 63, 71, 134). Antibody-mediated neutralization of Nogo-A or NgR1 in acute hippocampal slices induces an increase in LTP that is not due to a change in basal synaptic transmission or short-term plasticity (23) (FIGURE 4A). Accordingly, application of Nogo-66 or OMgp attenuates LTP in a NgR1-dependent manner (97). However, the genetic deletion of Nogo-A,

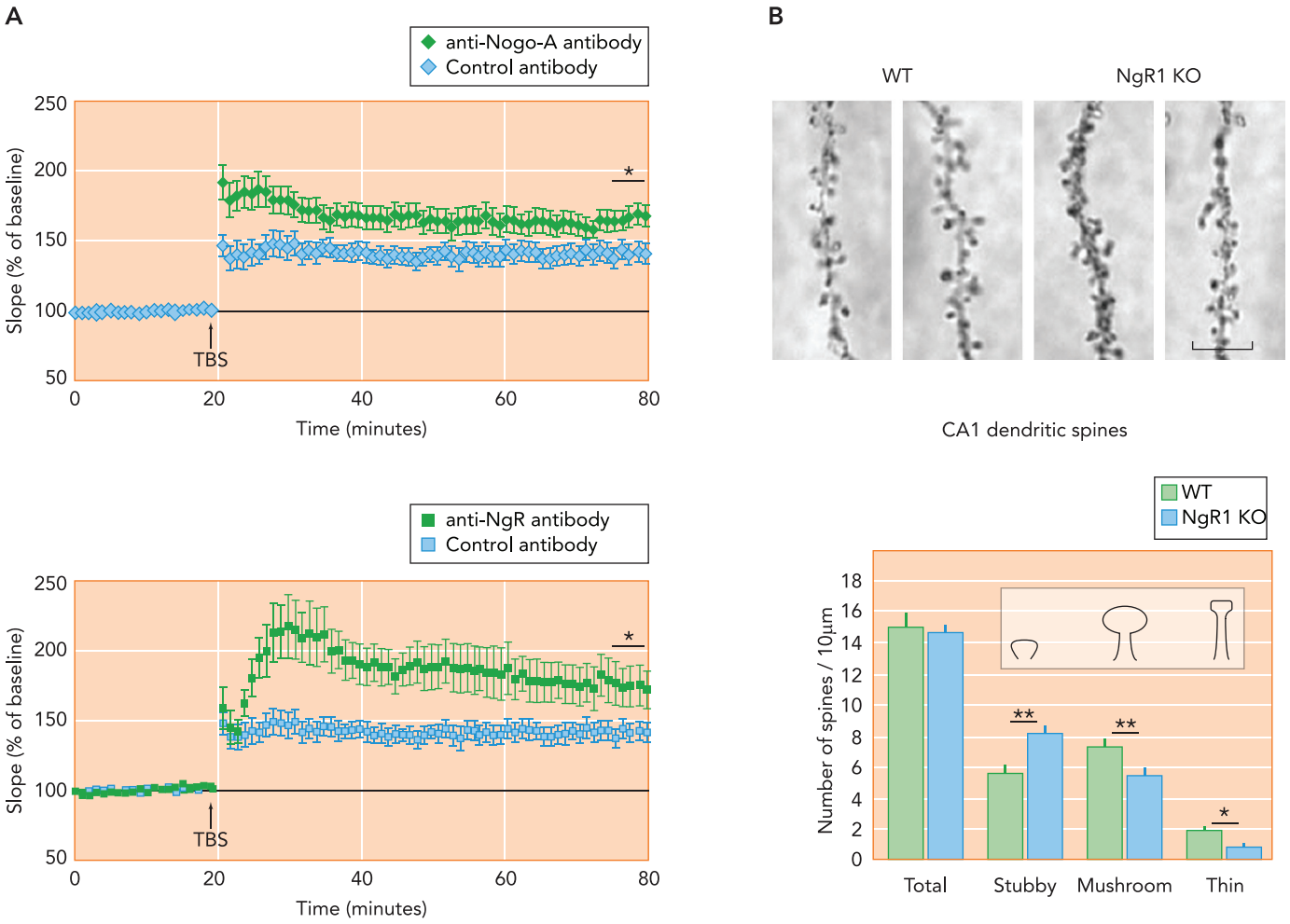


FIGURE 4. **Nogo-A restricts synaptic plasticity in the hippocampus**
A: LTP was induced in acute hippocampal slices at Schaffer collateral-CA1 synapses by theta burst stimulation (TBS; arrow). Nogo-A or NgR1 neutralization significantly increases LTP. *Significant difference ($P < 0.05$). *A* is reproduced from Ref. 63 with permission from the National Academy of Sciences. *B*: changes in dendritic spine morphology of CA1 pyramidal neurons. Genetic deletion of NgR1 induces morphological changes toward a more stubby- and less thin- or mushroom-shaped phenotype. Spine density remains unchanged. Significant difference: *$P < 0.05$; **$P < 0.001$. Scale bar = 5 µm. *B* is reproduced from Ref. 23 with permission from the Society for Neuroscience.

NgR1, or PirB does not significantly modulate LTP, which might be due to genetic and/or developmental compensatory mechanisms occurring in constitutive KO mutants (23, 56, 63, 97). NgR1 KO slices do show an increased enhancement of LTP in the presence of FGF2, suggesting that NgR1 signaling may antagonize growth factor-mediated promoting effects on synaptic plasticity (63). It remains to be investigated whether similar results are observed in Nogo-A and PirB KO mice. LTD is attenuated in NgR1 KO (56, 63) but not in Nogo-A or PirB KO mice, nor upon neutralization of Nogo-A. This shows disparate effects of Nogo-A and NgR1 on LTD (23, 97) (7). Together, these results suggest that acute blockade of Nogo-A/NgR1 signaling might not only increase synaptic plasticity but also promote learning at a circuit level. Interestingly, increased neuronal activity in regions linked to memory formation and storage leads to a downregulation of NgR1, and transgenic mice overexpressing NgR1 show impaired long-term spatial memory (56). Along this line, neutralization of Nogo-A in hippocampal slice cultures as well as genetic downregulation or deletion of NgR1 induces changes in pyramidal cell dendrite morphology and a shift in dendritic spine distribution toward a less mature and more plastic type. Overall, spine density is not altered (63, 141) (FIGURE 4B). Neuron-specific knockdown of NgR1 but not of Nogo-A in CA3 pyramidal cells reproduced these findings, indicating that the observed morphological changes relied on a receptor/ligand interaction rather than on a cell-autonomous effect of Nogo-A (141).

In the cerebellum, neuron-specific overexpression of Nogo-A in Purkinje cells induces a destabilization and loss of Purkinje axonal terminals on deep cerebellar nuclei neurons. This is accompanied by a severe deficit of motor coordination and motor learning (7). In the dendritic arbor, postsynaptic densities of parallel fiber (PF)-Purkinje cell synapses were longer in Nogo-A KO and shorter in mice overexpressing Nogo-A (95). This is accompanied by a negative regulation of the synaptic strength of PF-Purkinje cell synapses (95). Overall, these results suggest that Nogo-A signaling restricts different forms of synaptic strengthening and growth (CA3-CA1 LTP; PF-Purkinje cell synaptic strength) in the adult CNS.

Nogo-A Perturbs Cell Migration, Neurite Growth and Myelination During CNS Development

Nogo-A is expressed in the developing nervous system, in particular in postmitotic neurons and in many types of neurons during their axonal growth phase (50, 81, 94). Neuronal Nogo-A levels then decline progressively except for some types of neurons in highly plastic regions, and oligodendrocytes become the main site of Nogo-A synthesis during postnatal development and in adulthood (105). This pattern suggests functions of Nogo-A distinct from its role as a myelin-associated growth inhibitor (50). Anecdotally, an antibody originally designed to recognize growing axons in the developing olfactory bulb was found to specifically bind to Nogo-A (124). In vitro, genetic deletion or functional blockade of cell surface Nogo-A leads to an increase in neurite outgrowth, growth cone area, and motility in dissociated DRG neurons as well as to an increase in neurite fasciculation in DRG explants (82, 94). Similar results were also obtained by blocking NgR1 or downstream signaling molecules (82, 94). This suggests that axonal surface Nogo-A may have growth-, migration-, and fasciculation-related functions during development, possibly by acting as a repulsive or growth-restricting cue.

During embryonic corticogenesis, Nogo-A is expressed in migrating and postmigratory neurons as well as in radial glial cells (75, 80). Using Nogo-A and Nogo-A/-B/-C KO mice, two studies have shown that Nogo-A deletion transiently delays tangential and radial migration patterns of neuronal precursor cells in vivo, suggesting a pro-migratory effect of Nogo-A (75, 80). Similarly, in the adult CNS, Nogo-A was recently shown to promote tangential migration of Nogo-A-expressing neuroblasts from the subventricular zone (SVZ) toward the olfactory bulb (OB) by providing Nogo-A-Δ20-specific anti-adhesive signals (99). In contrast, the Nogo-66 domain of Nogo-A inhibits the migration of olfactory ensheating glial cells, which are themselves essential for the elongation of olfactory receptor axons, in vitro and in vivo (86, 116). Although it is difficult to understand how these results are reconciled, it was suggested that Nogo-A differentially modulates migration depending on the signaling domains and cellular systems (99). Along this line, whereas Nogo-A-Δ20 promotes the migration of neuroblasts toward the OB, Nogo-66 reduces the proliferation of neural stem cells in the SVZ before their migration, thereby also contributing to neurogenesis in the adult SVZ (99).

Because neuronal migration and axon guidance are tightly coordinated during development and often share similar substrates and cues, it was not surprising to find that neutralization of Nogo-A also results in misguidance phenotypes (73). Herein, Nogo-A inactivation led to miscrossed projections of optic nerve axons and spinal cord commissural axons during embryonic development (129, 130). In ovo antibody-mediated neutralization of Nogo-A in chicken embryos resulted in enhanced fasciculation and reduced branching of peripheral nerves leading to aberrant innervation patterns of the

hindlimb (94). Similar results were also observed in Nogo-A KO mouse embryos (94).

On the other hand, glial-derived Nogo-A is specifically involved in oligodendrocyte differentiation, channeling of CST axons down the spinal cord, and myelin formation in vivo (20, 93, 107, 144). Recently, Nogo-A was shown to participate as a repulsive signal in the competition of developing oligodendrocytes for axonal myelination and spatial distribution of myelin segments (internodes) (20). Internodes vary greatly in number and length in similar CNS axon tracts (20). Interestingly, Nogo-A-Δ20 but not Nogo-66 was found to inhibit intercellular interactions between oligodendrocytes competing for axon space, resulting in a spatial segregation of myelin internodes. In line with this, genetic deletion of Nogo-A resulted in an increased myelinogenic potential of oligodendrocytes in the developing cerebral cortex without altering global myelination patterns (20). This was accompanied by an increase in the number of oligodendrocyte precursor cells and a decrease of mature oligodendroytes (20). Consistent with a role of Nogo-A on oligodendrocyte differentiation, a transient delay in oligodendrocyte maturation was observed in the optic nerve of Nogo-A KO mice in vivo (93). In vitro, antibody-mediated neutralization of Nogo-A inhibited the differentiation of cortical OPCs (144).

Conclusions and Future Perspectives

Nogo-A was initially identified as a major myelin-associated inhibitor of axonal regeneration after CNS injury. Nogo-A binds to different cell surface receptors and activates signaling cascades that induce a collapse of the cytoskeleton and a down-regulation of the neuronal growth machinery. Although specific receptors for the Nogo-A-Δ20 domain remain to be identified, there is a general consensus on the existence of a multi-site/multi-subunit ligand/receptor complex consisting of structurally unrelated components. Long-term changes of Nogo-A signaling include a suppression of growth-related gene expression at the transcriptional level, albeit the exact mechanisms remain to be elucidated in details.

In the intact adult CNS, Nogo-A is thought to act as a stabilizer and regulator of neuronal networks by restricting various plastic processes, e.g., structural, activity- and experience-dependent plasticity. Increasing evidence also suggests that Nogo-A modulates migratory, growth, and contact-dependent processes during development. It will be important to understand to which extent these processes are affected by neuron-to-oligodendrocyte, neuron-to-neuron, or oligodendrocyte-to-oligodendrocyte interactions. Given the dual expression pattern and spatio-temporal regulation of Nogo-A, its receptor-binding promiscuity and multiple signaling domains, the cellular mechanisms by which Nogo-A exerts different physiological roles seem to be very complex and are far from being fully understood. Overall, Nogo-A/Nogo-A receptor interactions restrict growth-dependent processes, leading to a stabilization of the existing CNS circuitry. Lifting these brakes allows for the induction of extensive structural and functional rearrangements, e.g., after injury. ■

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References

1. Ahmed Z, Jacques SJ, Berry M, Logan A. Epidermal growth factor receptor inhibitors promote CNS axon growth through off-target effects on glia. *Neurobiol Dis* 36: 142–150, 2009.
2. Ahmed Z, Read ML, Berry M, Logan A. Satellite glia not DRG neurons constitutively activate EGFR but EGFR inactivation is not correlated with axon regeneration. *Neurobiol Dis* 39: 292–300, 2010.
3. Akbik F, Cafferty WB, Strittmatter SM. Myelin associated inhibitors: a link between injury-induced and experience-dependent plasticity. *Exp Neurol* 235: 43–52, 2012.
4. Alabed YZ, Grados-Munro E, Ferraro GB, Hsieh SH, Fournier AE. Neuronal responses to myelin are mediated by rho kinase. *J Neurochem* 96: 1616–1625, 2006.
5. Alabed YZ, Pool M, Ong Tone S, Fournier AE. Identification of CRMP4 as a convergent regulator of axon outgrowth inhibition. *J Neurosci* 27: 1702–1711, 2007.
6. Alabed YZ, Pool M, Ong Tone S, Sutherland C, Fournier AE. GSK3 beta regulates myelin-dependent axon outgrowth inhibition through CRMP4. *J Neurosci* 30: 5635–5643, 2010.
7. Aloy EM, Weinmann O, Pot C, Kasper H, Dodd DA, Rulicke T, Rossi F, Schwab ME. Synaptic destabilization by neuronal Nogo-A. *Brain Cell Biol* 35: 137–156, 2006.
8. Atwal JK, Pinkston-Gosse J, Syken J, Stawicki S, Wu Y, Shatz C, Tessier-Lavigne M. PirB is a functional receptor for myelin inhibitors of axonal regeneration. *Science* 322: 967–970, 2008.
9. Baldwin TA, Ostergaard HL. The protein-tyrosine phosphatase CD45 reaches the cell surface via golgi-dependent and -independent pathways. *J Biol Chem* 277: 50333–50340, 2002.
10. Bareyre FM, Haudenschild B, Schwab ME. Long-lasting sprouting and gene expression changes induced by the monoclonal antibody IN-1 in the adult spinal cord. *J Neurosci* 22: 7097–7110, 2002.
11. Bauer M, Pelkmans L. A new paradigm for membrane-organizing and -shaping scaffolds. *FEBS Lett* 580: 5559–5564, 2006.
12. Bogdanov M, Heacock PN, Dowhan W. A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition. *EMBO J* 21: 2107–2116, 2002.
13. Bonnon C, Goutebroze L, Denisenko-Nehrbass N, Girault JA, Faivre-Sarrailh C. The paranodal complex of F3/contactin and caspr/paranodin traffics to the cell surface via a non-conventional pathway. *J Biol Chem* 278: 48339–48347, 2003.

14. Buffo A, Zagrebelsky M, Huber AB, Skerra A, Schwab ME, Strata P, Rossi F. Application of neutralizing antibodies against NI-35/250 myelin-associated neurite growth inhibitory proteins to the adult rat cerebellum induces sprouting of uninjured purkinje cell axons. *J Neurosci* 20: 2275–2286, 2000.

15. Caroni P, Schwab ME. Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. *Neuron* 1: 85–96, 1988.

16. Caroni P, Schwab ME. Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. *J Cell Biol* 106: 1281–1288, 1988.

17. Chan CC, Khodarahmi K, Liu J, Sutherland D, Oschipok LW, Steeves JD, Tetzlaff W. Dose-dependent beneficial and detrimental effects of ROCK inhibitor Y27632 on axonal sprouting and functional recovery after rat spinal cord injury. *Exp Neurol* 196: 352–364, 2005.

18. Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Christ F, Schwab ME. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* 403: 434–439, 2000.

19. Chivatakarn O, Kaneko S, He Z, Tessier-Lavigne M, Giger RJ. The Nogo-66 receptor NgR1 is required only for the acute growth cone-collapsing but not the chronic growth-inhibitory actions of myelin inhibitors. *J Neurosci* 27: 7117–7124, 2007.

20. Chong SY, Rosenberg SS, Fancy SP, Zhao C, Shen YA, Hahn AT, McGee AW, Xu X, Zheng B, Zhang LI, Rowitch DH, Franklin RJ, Lu QR, Chan JR. Neurite outgrowth inhibitor Nogo-A establishes spatial segregation and extent of oligodendrocyte myelination. *Proc Natl Acad Sci USA* 109: 1299–1304, 2012.

21. Cox LJ, Hengst U, Gurskaya NG, Lukyanov KA, Jaffrey SR. Intra-axonal translation and retrograde trafficking of CREB promotes neuronal survival. *Nat Cell Biol* 10: 149–159, 2008.

22. Craveiro LM, Hakkoum D, Weinmann O, Montani L, Stoppini L, Schwab ME. Neutralization of the membrane protein Nogo-A enhances growth and reactive sprouting in established organotypic hippocampal slice cultures. *Eur J Neurosci* 28: 1808–1824, 2008.

23. Delekate A, Zagrebelsky M, Kramer S, Schwab ME, Korte M. NogoA restricts synaptic plasticity in the adult hippocampus on a fast time scale. *PNAS* 108: 2569–2574, 2011.

24. Deng K, Gao Y, Cao Z, Graziani EI, Wood A, Doherty P, Walsh FS. Overcoming amino-Nogo-induced inhibition of cell spreading and neurite outgrowth by 12-*O*-tetradecanoylphorbol-13-acetate-type tumor promoters. *J Biol Chem* 285: 6425–6433, 2010.

25. Dergham P, Ellezam B, Essagian C, Avedissian H, Lubell WD, McKerracher L. Rho signaling pathway targeted to promote spinal cord repair. *J Neurosci* 22: 6570–6577, 2002.

26. Dickendesher TL, Baldwin KT, Mironova YA, Koriyama Y, Raiker SJ, Askew KL, Wood A, Geoffroy CG, Zheng B, Liepmann CD, Katagiri Y, Benowitz LI, Geller HM, Giger RJ. NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. *Nat Neurosci* 15: 703–712, 2012.

27. Dimou L, Schnell L, Montani L, Duncan C, Simonen M, Schneider R, Liebscher T, Gullo M, Schwab ME. Nogo-A-deficient mice reveal strain-dependent differences in axonal regeneration. *J Neurosci* 26: 5591–5603, 2006.

28. Dodd DA, Niederoest B, Bloechlinger S, Dupuis L, Loeffler JP, Schwab ME. Nogo-A, -B, and -C are found on the cell surface and interact together in many different cell types. *J Biol Chem* 280: 12494–12502, 2005.

29. Domeniconi M, Cao Z, Spencer T, Sivasankaran R, Wang K, Nikulina E, Kimura N, Cai H, Deng K, Gao Y, He Z, Filbin M. Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron* 35: 283–290, 2002.

30. Douglas MR, Morrison KC, Jacques SJ, Leadbeater WE, Gonzalez AM, Berry M, Logan A, Ahmed Z. Off-target effects of epidermal growth factor receptor antagonists mediate retinal ganglion cell disinhibited axon growth. *Brain* 132: 3102–3121, 2009.

31. Duffy P, Schmandke A, Sigworth J, Narumiya S, Cafferty WB, Strittmatter SM. Rho-associated kinase II (ROCKII) limits axonal growth after trauma within the adult mouse spinal cord. *J Neurosci* 29: 15266–15276, 2009.

32. Erschbamer M, Pernold K, Olson L. Inhibiting epidermal growth factor receptor improves structural, locomotor, sensory, and bladder recovery from experimental spinal cord injury. *J Neurosci* 27: 6428–6435, 2007.

33. Fournier AE, Gould GC, Liu BP, Strittmatter SM. Truncated soluble Nogo receptor binds Nogo-66 and blocks inhibition of axon growth by myelin. *J Neurosci* 22: 8876–8883, 2002.

34. Fournier AE, GrandPre T, Strittmatter SM. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature* 409: 341–346, 2001.

35. Fournier AE, Takizawa BT, Strittmatter SM. Rho kinase inhibition enhances axonal regeneration in the injured CNS. *J Neurosci* 23: 1416–1423, 2003.

36. 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 Dis* 2: e198, 2011.

37. Gee HY, Noh SH, Tang BL, Kim KH, Lee MG. Rescue of DeltaF508-CFTR trafficking via a GRASP-dependent unconventional secretion pathway. *Cell* 146: 746–760, 2011.

38. Giger RJ, Hollis ER 2nd, Tuszynski MH. Guidance molecules in axon regeneration. *Cold Spring Harb Perspect Biol* 2: a001867, 2010.

39. Gonzenbach RR, Schwab ME. Disinhibition of neurite growth to repair the injured adult CNS: focusing on Nogo. *Cell Mol Life Sci* 65: 161–176, 2008.

40. GrandPre T, Li S, Strittmatter SM. Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature* 417: 547–551, 2002.

41. GrandPre T, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature* 403: 439–444, 2000.

42. Grieve AG, Rabouille C. Golgi bypass: skirting around the heart of classical secretion. *Cold Spring Harb Perspect Biol* 3: a005298, 2011.

43. Grunewald E, Kinnell HL, Porteous DJ, Thomson PA. GPR50 interacts with neuronal NOGO-A and affects neurite outgrowth. *Mol Cell Neurosci* 42: 363–371, 2009.

44. Harrison KD, Miao RQ, Fernandez-Hernando C, Suarez Y, Davalos A, Sessa WC. Nogo-B receptor stabilizes Niemann-Pick type C2 protein and regulates intracellular cholesterol trafficking. *Cell Metab* 10: 208–218, 2009.

45. Harrison KD, Park EJ, Gao N, Kuo A, Rush JS, Waechter CJ, Lehrman MA, Sessa WC. Nogo-B receptor is necessary for cellular dolichol biosynthesis and protein N-glycosylation. *EMBO J* 30: 2490–2500, 2011.

46. Hasegawa Y, Fujitani M, Hata K, Tohyama M, Yamagishi S, Yamashita T. Promotion of axon regeneration by myelin-associated glycoprotein and Nogo through divergent signals downstream of Gi/G. *J Neurosci* 24: 6826–6832, 2004.

47. He W, Shi Q, Hu X, Yan R. The membrane topology of RTN3 and its effect on binding of RTN3 to BACE1. *J Biol Chem* 282: 29144–29151, 2007.

48. Hsieh SH, Ferraro GB, Fournier AE. Myelin-associated inhibitors regulate cofilin phosphorylation and neuronal inhibition through LIM kinase and Slingshot phosphatase. *J Neurosci* 26: 1006–1015, 2006.

49. Hu F, Strittmatter SM. The N-terminal domain of Nogo-A inhibits cell adhesion and axonal outgrowth by an integrin-specific mechanism. *J Neurosci* 28: 1262–1269, 2008.

50. Huber AB, Weinmann O, Brosamle C, Oertle T, Schwab ME. Patterns of Nogo mRNA and protein expression in the developing and adult rat and after CNS lesions. *J Neurosci* 22: 3553–3567, 2002.

51. Huebner EA, Kim BG, Duffy PJ, Brown RH, Strittmatter SM. A multi-domain fragment of Nogo-A protein is a potent inhibitor of cortical axon regeneration via Nogo receptor 1. *J Biol Chem* 286: 18026–18036, 2011.

52. Ji B, Li M, Wu WT, Yick LW, Lee X, Shao Z, Wang J, So KF, McCoy JM, Pepinsky RB, Mi S, Relton JK. LINGO-1 antagonist promotes functional recovery and axonal sprouting after spinal cord injury. *Mol Cell Neurosci* 33: 311–320, 2006.

53. Joset A, Dodd DA, Halegoua S, Schwab ME. Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. *J Cell Biol* 188: 271–285, 2010.

54. Kajander T, Kuja-Panula J, Rauvala H, Goldman A. Crystal structure and role of glycans and dimerization in folding of neuronal leucine-rich repeat protein AMIGO-1. *J Mol Biol* 413: 1001–1015, 2011.

55. Kapfhammer JP, Schwab ME. Inverse patterns of myelination and GAP-43 expression in the adult CNS: neurite growth inhibitors as regulators of neuronal plasticity? *J Comp Neurol* 340: 194–206, 1994.

56. Karlen A, Karlsson TE, Mattsson A, Lundstromer K, Codeluppi S, Pham TM, Backman CM, Ogren SO, Aberg E, Hoffman AF, Sherling MA, Lupica CR, Hoffer BJ, Spenger C, Josephson A, Brene S, Olson L. Nogo receptor 1 regulates formation of lasting memories. *Proc Natl Acad Sci USA* 106: 20476–20481, 2009.

57. Kim H, Melen K, Osterberg M, von Heijne G. A global topology map of the Saccharomyces cerevisiae membrane proteome. *Proc Natl Acad Sci USA* 103: 11142–11147, 2006.

58. Kim JE, Li S, GrandPre T, Qiu D, Strittmatter SM. Axon regeneration in young adult mice lacking Nogo-A/B. *Neuron* 38: 187–199, 2003.

59. Kim JE, Liu BP, Park JH, Strittmatter SM. Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. *Neuron* 44: 439–451, 2004.

60. Koprivica V, Cho KS, Park JB, Yiu G, Atwal J, Gore B, Kim JA, Lin E, Tessier-Lavigne M, Chen DF, He Z. EGFR activation mediates inhibition of axon regeneration by myelin and chondroitin sulfate proteoglycans. *Science* 310: 106–110, 2005.

61. Lambert C, Prange R. Chaperone action in the posttranslational topological reorientation of the hepatitis B virus large envelope protein: implications for translocational regulation. *Proc Natl Acad Sci USA* 100: 5199–5204, 2003.

62. Lauren J, Hu F, Chin J, Liao J, Airaksinen MS, Strittmatter SM. Characterization of myelin ligand complexes with neuronal Nogo-66 receptor family members. *J Biol Chem* 282: 5715–5725, 2007.

63. Lee H, Raiker SJ, Venkatesh K, Geary R, Robak LA, Zhang Y, Yeh HH, Shrager P, Giger RJ. Synaptic function for the Nogo-66 receptor NgR1: regulation of dendritic spine morphology and activity-dependent synaptic strength. *J Neurosci* 28: 2753–2765, 2008.

64. Lee JK, Chan AF, Luu SM, Zhu Y, Ho C, Tessier-Lavigne M, Zheng B. Reassessment of corticospinal tract regeneration in Nogo-deficient mice. *J Neurosci* 29: 8649–8654, 2009.

65. Lee JK, Kim JE, Sivula M, Strittmatter SM. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. *J Neurosci* 24: 6209–6217, 2004.

66. Lee JK, Zheng B. Role of myelin-associated inhibitors in axonal repair after spinal cord injury. *Exp Neurol* 235: 33–42, 2012.

67. Levy D. Membrane proteins which exhibit multiple topological orientations. *Essays Biochem* 31: 49–60, 1996.

68. Liebscher T, Schnell L, Schnell D, Scholl J, Schneider R, Gullo M, Fouad K, Mir A, Rausch M, Kindler D, Hamers FP, Schwab ME. Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. *Ann Neurol* 58: 706–719, 2005.

69. Liu B, Chen H, Johns TG, Neufeld AH. Epidermal growth factor receptor activation: an upstream signal for transition of quiescent astrocytes into reactive astrocytes after neural injury. *J Neurosci* 26: 7532–7540, 2006.

70. Liu BP, Fournier A, GrandPre T, Strittmatter SM. Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *Science* 297: 1190–1193, 2002.

71. Liu YY, Jin WL, Liu HL, Ju G. Electron microscopic localization of Nogo-A at the postsynaptic active zone of the rat. *Neurosci Lett* 346: 153–156, 2003.

72. Lowery LA, Van Vactor D. The trip of the tip: understanding the growth cone machinery. *Nat Rev Mol Cell Biol* 10: 332–343, 2009.

73. Marin O, Valiente M, Ge X, Tsai LH. Guiding neuronal cell migrations. *Cold Spring Harb Perspect Biol* 2: a001834, 2010.

74. Martin PE, Blundell G, Ahmad S, Errington RJ, Evans WH. Multiple pathways in the trafficking and assembly of connexin 26, 32 and 43 into gap junction intercellular communication channels. *J Cell Sci* 114: 3845–3855, 2001.

75. Mathis C, Schroter A, Thallmair M, Schwab ME. Nogo-a regulates neural precursor migration in the embryonic mouse cortex. *Cereb Cortex* 20: 2380–2390, 2010.

76. McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM. Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. *Science* 309: 2222–2226, 2005.

77. Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, Levesque M, Allaire N, Perrin S, Sands B, Crowell T, Cate RL, McCoy JM, Pepinsky RB. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat Neurosci* 7: 221–228, 2004.

78. Miao RQ, Gao Y, Harrison KD, Prendergast J, Acevedo LM, Yu J, Hu F, Strittmatter SM, Sessa WC. Identification of a receptor necessary for Nogo-B stimulated chemotaxis and morphogenesis of endothelial cells. *Proc Natl Acad Sci USA* 103: 10997–11002, 2006.

79. Mimura F, Yamagishi S, Arimura N, Fujitani M, Kubo T, Kaibuchi K, Yamashita T. Myelin-associated glycoprotein inhibits microtubule assembly by a Rho-kinase-dependent mechanism. *J Biol Chem* 281: 15970–15979, 2006.

80. Mingorance-Le, Meur A, Zheng B, Soriano E, del Rio JA. Involvement of the myelin-associated inhibitor Nogo-A in early cortical development and neuronal maturation. *Cereb Cortex* 17: 2375–2386, 2007.

81. Mingorance A, Fontana X, Sole M, Burgaya F, Urena JM, Teng FY, Tang BL, Hunt D, Anderson PN, Bethea JR, Schwab ME, Soriano E, del Rio JA. Regulation of Nogo and Nogo receptor during the development of the entorhino-hippocampal pathway and after adult hippocampal lesions. *Mol Cell Neurosci* 26: 34–49, 2004.

82. Montani L, Gerrits B, Gehrig P, Kempf A, Dimou L, Wollscheid B, Schwab ME. Neuronal Nogo-A modulates growth cone motility via Rho-GTP/LIMK1/cofilin in the unlesioned adult nervous system. *J Biol Chem* 284: 10793–10807, 2009.

83. Nakaya N, Sultana A, Lee HS, Tomarev SI. Olfactomedin 1 interacts with the Nogo A receptor complex to regulate axon growth. *J Biol Chem* 287: 37171–37184, 2012.

84. Nash M, Pribiag H, Fournier AE, Jacobson C. Central nervous system regeneration inhibitors and their intracellular substrates. *Mol Neurobiol* 40: 224–235, 2009.

85. Niederost B, Oertle T, Fritsche J, McKinney RA, Bandtlow CE. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *J Neurosci* 22: 10368–10376, 2002.

86. Nocentini S, Reginensi D, Garcia S, Carulla P, Moreno-Flores MT, Wandosell F, Trepat X, Bribian A, del Rio JA. Myelin-associated proteins block the migration of olfactory ensheathing cells: an in vitro study using single-cell tracking and traction force microscopy. *Cell Mol Life Sci* 69: 1689–1703, 2012.

87. Oertle T, Huber C, van der Putten H, Schwab ME. Genomic structure and functional characterisation of the promoters of human and mouse nogo/rtn4. *J Mol Biol* 325: 299–323, 2003.

88. Oertle T, Merkler D, Schwab ME. Do cancer cells die because of Nogo-B? *Oncogene* 22: 1390–1399, 2003.

89. Oertle T, Schwab ME. Nogo and its paRTNers. *Trends Cell Biol* 13: 187–194, 2003.

90. Oertle T, van der Haar ME, Bandtlow CE, Robeva A, Burfeind P, Buss A, Huber AB, Simonen M, Schnell L, Brosamle C, Kaupmann K, Vallon R, Schwab ME. Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. *J Neurosci* 23: 5393–5406, 2003.

91. Papadopoulos CM, Tsai SY, Alsbiei T, O'Brien TE, Schwab ME, Kartje GL. Functional recovery and neuroanatomical plasticity following middle cerebral artery occlusion and IN-1 antibody treatment in the adult rat. *Ann Neurol* 51: 433–441, 2002.

92. Park JB, Yiu G, Kaneko S, Wang J, Chang J, He XL, Garcia KC, He Z. A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. *Neuron* 45: 345–351, 2005.

93. Pernet V, Joly S, Christ F, Dimou L, Schwab ME. Nogo-A and myelin-associated glycoprotein differently regulate oligodendrocyte maturation and myelin formation. *J Neurosci* 28: 7435–7444, 2008.

94. Petrinovic MM, Duncan CS, Bourikas D, Weinman O, Montani L, Schroeter A, Maerki D, Sommer L, Stoeckli ET, Schwab ME. Neuronal Nogo-A regulates neurite fasciculation, branching and extension in the developing nervous system. *Development* 137: 2539–2550, 2010.

95. Petrinovic MM, Hourez R, Aloy EM, Dewarrat G, Gall D, Weinmann O, Gaudias J, Bachmann LC, Schiffmann SN, Vogt KE, Schwab ME. Neuronal Nogo-A negatively regulates dendritic morphology and synaptic transmission in the cerebellum. *Proc Natl Acad Sci USA* 110: 1083–1088, 2013.

96. Prinjha R, Moore SE, Vinson M, Blake S, Morrow R, Christie G, Michalovich D, Simmons DL, Walsh FS. Inhibitor of neurite outgrowth in humans. *Nature* 403: 383–384, 2000.

97. Raiker SJ, Lee H, Baldwin KT, Duan Y, Shrager P, Giger RJ. Oligodendrocyte-myelin glycoprotein and Nogo negatively regulate activity-dependent synaptic plasticity. *J Neurosci* 30: 12432–12445, 2010.

98. Ramer LM, Borisoff JF, Ramer MS. Rho-kinase inhibition enhances axonal plasticity and attenuates cold hyperalgesia after dorsal rhizotomy. *J Neurosci* 24: 10796–10805, 2004.

99. Rolando C, Parolisi R, Boda E, Schwab ME, Rossi F, Buffo A. Distinct roles of nogo-a and nogo receptor 1 in the homeostatic regulation of adult neural stem cell function and neuroblast migration. *J Neurosci* 32: 17788–17799, 2012.

100. Schleiff E, Tien R, Salomon M, Soll J. Lipid composition of outer leaflet of chloroplast outer envelope determines topology of OEP7. *Mol Biol Cell* 12: 4090–4102, 2001.

101. Schmandke A, Strittmatter SM. ROCK and Rho: biochemistry and neuronal functions of Rho-associated protein kinases. *Neuroscientist* 13: 454–469, 2007.

102. Schnell L, Schwab ME. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. *Nature* 343: 269–272, 1990.

103. Schotman H, Karhinen L, Rabouille C. dGRASP-mediated noncanonical integrin secretion is required for Drosophila epithelial remodeling. *Dev Cell* 14: 171–182, 2008.

104. Schotman H, Karhinen L, Rabouille C. Integrins mediate their unconventional, mechanical-stress-induced secretion via RhoA and PINCH in Drosophila. *J Cell Sci* 122: 2662–2672, 2009.

105. Schwab ME. Functions of Nogo proteins and their receptors in the nervous system. *Nat Rev Neurosci* 11: 799–811, 2010.

106. Schwab ME. Nogo and axon regeneration. *Curr Opin Neurobiol* 14: 118–124, 2004.

107. Schwab ME, Schnell L. Channeling of developing rat corticospinal tract axons by myelin-associated neurite growth inhibitors. *J Neurosci* 11: 709–721, 1991.

108. Shao Z, Browning JL, Lee X, Scott ML, Shulga-Morskaya S, Allaire N, Thill G, Levesque M, Sah D, McCoy JM, Murray B, Jung V, Pepinsky RB, Mi S. TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. *Neuron* 45: 353–359, 2005.

109. Shen K, Cowan CW. Guidance molecules in synapse formation and plasticity. *Cold Spring Harb Perspect Biol* 2: a001842, 2010.

110. Shibata Y, Voss C, Rist JM, Hu J, Rapoport TA, Prinz WA, Voeltz GK. The reticulon and DP1/Yop1p proteins form immobile oligomers in the tubular endoplasmic reticulum. *J Biol Chem* 283: 18892–18904, 2008.

111. Simonen M, Pedersen V, Weinmann O, Schnell L, Buss A, Ledermann B, Christ F, Sansig G, van der Putten H, Schwab ME. Systemic deletion of the myelin-associated outgrowth inhibitor Nogo-A improves regenerative and plastic responses after spinal cord injury. *Neuron* 38: 201–211, 2003.

112. Sivasankaran R, Pei J, Wang KC, Zhang YP, Shields CB, Xu XM, He Z. PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. *Nat Neurosci* 7: 261–268, 2004.

113. Sparkes I, Tolley N, Aller I, Svozil J, Osterrieder A, Botchway S, Mueller C, Frigerio L, Hawes C. Five Arabidopsis reticulon isoforms share endoplasmic reticulum location, topology, and membrane-shaping properties. *Plant Cell* 22: 1333–1343, 2010.

114. Spillmann AA, Amberger VR, Schwab ME. High molecular weight protein of human central nervous system myelin inhibits neurite outgrowth: an effect which can be neutralized by the monoclonal antibody IN-1. *Eur J Neurosci* 9: 549–555, 1997.

115. Spillmann AA, Bandtlow CE, Lottspeich F, Keller F, Schwab ME. Identification and characterization of a bovine neurite growth inhibitor (bNI-220). *J Biol Chem* 273: 19283–19293, 1998.

116. Su Z, Cao L, Zhu Y, Liu X, Huang Z, Huang A, He C. Nogo enhances the adhesion of olfactory ensheathing cells and inhibits their migration. *J Cell Sci* 120: 1877–1887, 2007.

117. Sung JK, Miao L, Calvert JW, Huang L, Louis Harkey H, Zhang JH. A possible role of RhoA/Rho-kinase in experimental spinal cord injury in rat. *Brain Res* 959: 29–38, 2003.

118. Syken J, Grandpre T, Kanold PO, Shatz CJ. PirB restricts ocular-dominance plasticity in visual cortex. *Science* 313: 1795–1800, 2006.

119. Tan CL, Kwok JC, Patani R, Ffrench-Constant C, Chandran S, Fawcett JW. Integrin activation promotes axon growth on inhibitory chondroitin sulfate proteoglycans by enhancing integrin signaling. *J Neurosci* 31: 6289–6295, 2011.

120. Taylor CW, Dellis O. Plasma membrane IP3 receptors. *Biochem Soc Trans* 34: 910–912, 2006.

121. Thallmair M, Metz GA, Z'Graggen WJ, Raineteau O, Kartje GL, Schwab ME. Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions. *Nat Neurosci* 1: 124–131, 1998.

122. Thomas R, Favell K, Morante-Redolat J, Pool M, Kent C, Wright M, Daignault K, Ferraro GB, Montcalm S, Durocher Y, Fournier A, Perez-Tur J, Barker PA. LGI1 is a Nogo receptor 1 ligand that antagonizes myelin-based growth inhibition. *J Neurosci* 30: 6607–6612, 2010.

123. Tolley N, Sparkes I, Craddock CP, Eastmond PJ, Runions J, Hawes C, Frigerio L. Transmembrane domain length is responsible for the ability of a plant reticulon to shape endoplasmic reticulum tubules in vivo. *Plant J* 64: 411–418, 2010.

124. Tozaki H, Kawasaki T, Takagi Y, Hirata T. Expression of Nogo protein by growing axons in the developing nervous system. *Brain Res Mol Brain Res* 104: 111–119, 2002.

125. Vasudevan SV, Schulz J, Zhou C, Cocco MJ. Protein folding at the membrane interface, the structure of Nogo-66 requires interactions with a phosphocholine surface. *Proc Natl Acad Sci USA* 107: 6847–6851, 2010.

126. Venkatesh K, Chivatakarn O, Lee H, Joshi PS, Kantor DB, Newman BA, Mage R, Rader C, Giger RJ. The Nogo-66 receptor homolog NgR2 is a sialic acid-dependent receptor selective for myelin-associated glycoprotein. *J Neurosci* 25: 808–822, 2005.

127. Venkatesh K, Chivatakarn O, Sheu SS, Giger RJ. Molecular dissection of the myelin-associated glycoprotein receptor complex reveals cell type-specific mechanisms for neurite outgrowth inhibition. *J Cell Biol* 177: 393–399, 2007.

128. Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* 124: 573–586, 2006.

129. Wang J, Chan CK, Taylor JS, Chan SO. The growth-inhibitory protein Nogo is involved in midline routing of axons in the mouse optic chiasm. *J Neurosci Res* 86: 2581–2590, 2008.

130. Wang J, Wang L, Zhao H, Chan SO. Localization of an axon growth inhibitory molecule Nogo and its receptor in the spinal cord of mouse embryos. *Brain Res* 1306: 8–17, 2010.

131. Wang KC, Kim JA, Sivasankaran R, Segal R, He Z. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* 420: 74–78, 2002.

132. Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, Neve RL, He Z. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* 417: 941–944, 2002.

133. Wang X, Baughman KW, Basso DM, Strittmatter SM. Delayed Nogo receptor therapy improves recovery from spinal cord contusion. *Ann Neurol* 60: 540–549, 2006.

134. Wang X, Chun SJ, Treloar H, Vartanian T, Greer CA, Strittmatter SM. Localization of Nogo-A and Nogo-66 receptor proteins at sites of axon-myelin and synaptic contact. *J Neurosci* 22: 5505–5515, 2002.

135. Wang X, Duffy P, McGee AW, Hasan O, Gould G, Tu N, Harel NY, Huang Y, Carson RE, Weinzimmer D, Ropchan J, Benowitz LI, Cafferty WB, Strittmatter SM. Recovery from chronic spinal cord contusion after Nogo receptor intervention. *Ann Neurol* 70: 805–821, 2011.

136. Wills ZP, Mandel-Brehm C, Mardinly AR, McCord AE, Giger RJ, Greenberg ME. The nogo receptor family restricts synapse number in the developing hippocampus. *Neuron* 73: 466–481, 2012.

137. Wong ST, Henley JR, Kanning KC, Huang KH, Bothwell M, Poo MM. A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nat Neurosci* 5: 1302–1308, 2002.

138. Yamashita T, Tohyama M. The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nat Neurosci* 6: 461–467, 2003.

139. Yoo JS, Moyer BD, Bannykh S, Yoo HM, Riordan JR, Balch WE. Non-conventional trafficking of the cystic fibrosis transmembrane conductance regulator through the early secretory pathway. *J Biol Chem* 277: 11401–11409, 2002.

140. Zagrebelsky M, Buffo A, Skerra A, Schwab ME, Strata P, Rossi F. Retrograde regulation of growth-associated gene expression in adult rat Purkinje cells by myelin-associated neurite growth inhibitory proteins. *J Neurosci* 18: 7912–7929, 1998.

141. Zagrebelsky M, Schweigreiter R, Bandtlow CE, Schwab ME, Korte M. Nogo-A stabilizes the architecture of hippocampal neurons. *J Neurosci* 30: 13220–13234, 2010.

142. Zander H, Hettich E, Greiff K, Chatwell L, Skerra A. Biochemical characterization of the recombinant human Nogo-A ectodomain. *FEBS J* 274: 2603–2613, 2007.

143. Zhang L, Zheng S, Wu H, Wu Y, Liu S, Fan M, Zhang J. Identification of BLyS (B lymphocyte stimulator), a non-myelin-associated protein, as a functional ligand for Nogo-66 receptor. *J Neurosci* 29: 6348–6352, 2009.

144. Zhao XH, Jin WL, Ju G. An in vitro study on the involvement of LINGO-1 and Rho GTPases in Nogo-A regulated differentiation of oligodendrocyte precursor cells. *Mol Cell Neurosci* 36: 260–269, 2007.

145. Zheng B, Atwal J, Ho C, Case L, He XL, Garcia KC, Steward O, Tessier-Lavigne M. Genetic deletion of the Nogo receptor does not reduce neurite inhibition in vitro or promote corticospinal tract regeneration in vivo. *Proc Natl Acad Sci USA* 102: 1205–1210, 2005.

146. Zheng B, Ho C, Li S, Keirstead H, Steward O, Tessier-Lavigne M. Lack of enhanced spinal regeneration in Nogo-deficient mice. *Neuron* 38: 213–224, 2003.

147. Zorner B, Schwab ME. Anti-Nogo on the go: from animal models to a clinical trial. *Ann NY Acad Sci* 1198, *Suppl 1*: E22–E34, 2010.