

Journal Club

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Mind the GAP: A Role for Neurofibromin in Restricting Axonal Plasticity

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Review of Romero et al. (<http://www.jneurosci.org/cgi/content/full/27/8/2124>)

The axons of dorsal root ganglion (DRG) neurons project to cutaneous and soft tissue targets in the periphery, and centrally to the spinal cord and brainstem. The peripheral process can regenerate and reconnect with targets after injury, but the central process cannot regenerate beyond the PNS–CNS interface [the dorsal root entry zone (DREZ)]. The differential cellular responses to peripheral or dorsal root injury have contributed to our understanding of extrinsic and intrinsic barriers to regeneration in the adult CNS. In an interesting study published recently in *The Journal of Neuroscience*, Romero et al. (2007) used the dorsal root injury (DRI) model to determine whether neurofibromin affects axonal growth or sprouting in the CNS after injury. Neurofibromin, encoded by the neurofibromatosis type 1 (*Nf1*) tumor suppressor gene, contains a GTPase-activating protein (GAP)-related domain, which negatively regulates Ras GTPase activity and thereby prevents downstream Ras signaling (Fig. 1a) (Schubbert et al., 2007). Given that Ras

activation within neurons is associated with neurite elongation and cell survival (Fig. 1b), Romero et al. (2007) hypothesized that neurofibromin might suppress regeneration and/or sprouting of primary afferents after dorsal root injury.

The authors used a *LacZ* reporter gene to characterize the Cre-mediated inactivation of *Nf1* in DRGs of *Nf1*^{flox/flox}/Synapsin I-Cre (*Nf1*SynIKO) mice, which they had generated previously (Zhu et al., 2001). Cre was expressed in the majority of large-diameter tropomyosin-related kinase C (TrkC)-expressing proprioceptive DRG neurons, but not in calcitonin gene-related peptide (CGRP)-immunoreactive small-diameter nociceptors [Romero et al. (2007), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F1>)]. There was also a subset of *LacZ*-positive cells that did not express either CGRP or TrkC.

To determine whether conditional knock-out of *Nf1* affected recovery of proprioception after DRI, the authors tested for the recovery of abductor muscle function, and performed grid walking tests and paw print analyses [Romero et al. (2007), their Figs. 3 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F3>), 4 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F4>)]. Control DRI animals exhibited proprioceptive deficits that did not recover during the test period (up to 32 d postinjury). In contrast, *Nf1*SynIKO mice displayed accurate paw placement and regained normal toe spread by 24 d

postinjury. Importantly, the extent of recovery was equivalent between *Nf1*SynIKO mice that received lumbar 4 (L4)/L5 crush DRI (regeneration possible), and *Nf1*SynIKO mice in which the same roots were cut and ligated (precluding regeneration). Thus, a mechanism other than regeneration was responsible for recovery. The authors provided histological confirmation that proprioceptive afferents did not regenerate in *Nf1*SynIKO mice [Romero et al. (2007), their supplemental data 6 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/DC1>)].

One possible explanation for recovery in *Nf1*SynIKO mice is that axons from uninjured, adjacent DRGs show enhanced sprouting in the absence of neurofibromin signaling. Explant cultures of *Nf1*SynIKO DRG neurons exhibited increased axonal outgrowth *in vitro* [Romero et al. (2007), their Fig. 2 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F2>)]. To establish whether sensory neurons lacking neurofibromin had enhanced growth capacity *in vivo*, Romero et al. (2007) examined immunohistochemical localization of CGRP and cholera toxin B (CTB; a tracer used to visualize large-diameter afferents/motoneurons) in the gray matter of the lumbosacral spinal cord. The distribution of CGRP and CTB labeling was similar in the two strains without injury [Romero et al. (2007), their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F5>)]. DRI-

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induced sprouting from adjacent segments did occur in *Nf1*SynIKO mice, whereas CGRP-expression and CTB-transporting axons were nearly eliminated in the dorsal horn in control mice (unidentified time point). There was also an increased density of CTB tracing in the deep layers of the L4–L5 dorsal horn of *Nf1*SynIKO mice [Romero et al. (2007), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F6>)]. In the representative images, it seemed to us that CGRP-positive fibers may have sprouted as well, although the authors say not. Thus, neurofibromin suppresses the ability of intact DRG axons to sprout within the spinal cord in response to deafferentation, and this sprouting may contribute to functional recovery after DRI.

Cre recombinase is expressed in many CNS neurons in *Nf1*SynIKO mice. To determine whether conditional knock-out of *Nf1* affected sprouting by mechanisms intrinsic or extrinsic to DRGs, Romero et al. (2007) cocultured control and *Nf1*SynIKO spinal cords and DRGs in Matrigel [Romero et al. (2007), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/27/8/2124/F7>)]. Neurite outgrowth from *Nf1*SynIKO DRG neurons was greater than control DRGs and, interestingly, outgrowth from *Nf1*SynIKO DRG neurons was further increased in the presence of *Nf1*SynIKO spinal cords. This suggests that conditional knock-out of *Nf1* in DRG and spinal cord neurons synergistically enhances axonal sprouting from DRGs.

In their final experiment, the authors set out to establish which signaling pathways were affected by conditional *Nf1* deletion. Using Western blots, they found a significantly higher ratio of activated (phosphorylated) Erk to total Erk protein in the CNS and DRG of *Nf1*SynIKO mice. Although the authors suggest that the phosphatidylinositol 3 kinase-Akt-glycogen synthase kinase 3 β (GSK3 β) cascades may also be involved in the enhanced sprouting, there was no significant difference in the activation of either Akt or GSK3 β in the DRGs of *Nf1*SynIKO mice. Thus, endogenous neurofibromin likely represses axon sprouting by inhibiting Ras activation of the mitogen-activated protein (MAP) kinase kinase (MEK)–Erk MAP kinase pathway.

In summary, Romero et al. (2007) define a role for neurofibromin in the suppression of axonal plasticity after DRI. Although conditional knock-out of *Nf1*

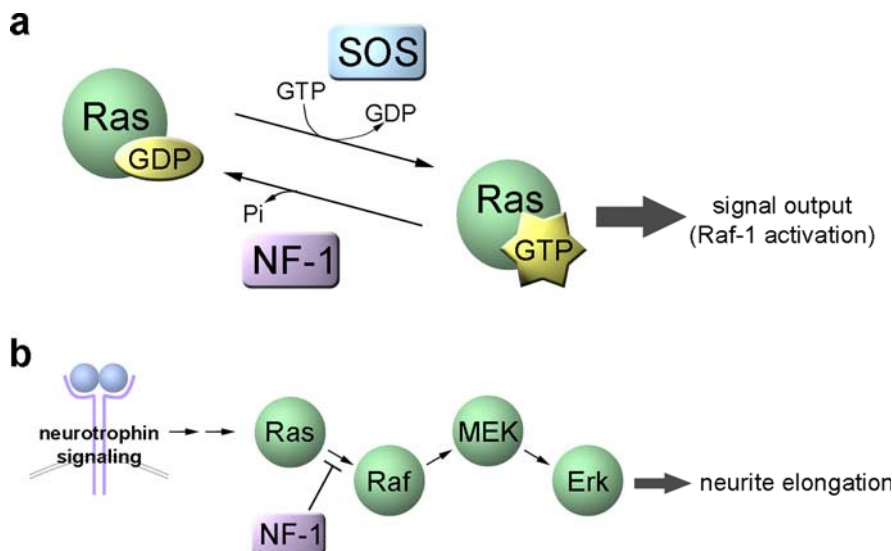


Figure 1. Role of neurofibromin (NF-1) in the regulation of intracellular Ras signaling within DRG neurons. **a**, When activated, the guanine-nucleotide exchange factor Son-of-Sevenless (SOS) displaces GDP from inactive Ras. This allows GTP, which is abundant in the cytosol, to bind Ras passively. GTP-bound Ras can then activate downstream effectors such as Raf-1. The GAP neurofibromin promotes Ras to convert GTP to GDP and thereby prevents downstream signaling (Schubbert et al., 2007). **b**, Although Ras activates various pathways, its involvement in the MAP kinase pathway has been studied most intensively. This pathway involves the dimerization of receptor tyrosine kinases (e.g., Trk receptors) that then activate SOS. After Ras activation, a phosphorylation cascade ensues, with subsequent activation of Raf-1, MEK, and Erk-1 and Erk-2, which ultimately results in the transcription of genes encoding Ets family transcription factors (Mor and Philips, 2006). Romero et al. (2007) showed that neurofibromin negatively regulates activation of the MEK–Erk pathway, which may underlie its plasticity-limiting influence on spared sensory projections to the deafferented spinal cord.

was not sufficient for regeneration of injured DRG axons across the gliotic scar at the DREZ, it did allow uninjured axons from adjacent DRGs to sprout ectopically into deafferented segments. This DRI-induced central sprouting is likely responsible for the recovery of proprioceptive function observed in *Nf1*SynIKO mice.

It is well accepted that spontaneous recovery after neural trauma depends on plasticity of spared neurons. As a result of the inherent challenges in promoting functionally relevant regeneration in the adult CNS, plasticity of spared systems may represent a realistic substrate for stimulating functional recovery. Because neurofibromin specifically limited injury-induced plasticity of spared neurons, it may be a therapeutic target, with the caveat that tissue-specific deletions of *Nf1* will be required to delineate the intrinsic and extrinsic effects.

It will be interesting to know whether sprouting of peripheral branches of DRG neurons has a role in the recovery of proprioception after DRI in *Nf1*SynIKO mice. The GAP-related domain of neurofibromin is not sufficient to rescue neural crest deficits in *Nf1* mutant mice (Ismat et al., 2006). Thus, it seems that other domains are required for the regulation of neurofibromin or for targeting to acti-

vated Ras. It will also be interesting to study DRI-induced axonal plasticity in mice that express Cre with more spatio-temporal resolution [e.g., SPRR1A (small proline-rich repeat protein 1A), which is expressed in DRG neurons after axonal injury (Bonilla et al., 2002)].

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