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Lost in the jungle: new hurdles for optic nerve axon regeneration

Vincent Pernet and Martin E. Schwab

Brain Research Institute, University of Zürich, and Department of Health Sciences and Technology, ETH Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

The poor regenerative capacity of injured central nervous system (CNS) axons leads to permanent neurological deficits after brain, spinal cord, or optic nerve lesions. In the optic nerve, recent studies showed that stimulation of the cytokine or mammalian target of rapamycin (mTOR) signaling pathways potently enhances sprouting and regeneration of injured retinal ganglion cell axons in adult mice, but does not allow the majority of axons to reach their main cerebral targets. New analyses have revealed axon navigation defects in the optic nerve and at the optic chiasm under conditions of strong growth stimulation. We propose that a balanced growth stimulatory treatment will have to be combined with guidance factors and suppression of local growth inhibitory factors to obtain the full regeneration of long CNS axonal tracts.

Axonal regeneration can be stimulated via the signal transducer and activator of transcription (Stat)3 and mTOR pathways

Like other CNS tracts in higher vertebrates, the optic nerve cannot regenerate after traumatic injury. To study this phenomenon and to establish new regenerative strategies and potential treatments, the optic nerve crush is a model of choice. The optic nerve injury paradigm has been popularized in the 1980s by the laboratory of Albert Aguayo that took advantage of the easy access to retinal ganglion cells to test their survival and regenerative properties, for example, after neurotrophic factor injections in the eyeball [1], or after peripheral nerve graft transplantation onto the severed optic nerve stump in the orbit [2]. More recently, adeno-associated virus serotype 2 (AAV2) was shown to display specific tropism to and high rate of infectivity for retinal ganglion cells, allowing one to target and manipulate specific intracellular signaling molecules involved in CNS neuron survival and axonal regeneration *in vivo* [3,4]. The weak intrinsic capacity of adult neurons to reactivate a growth program after injury was proposed to be an important impediment to axonal regrowth [5] (for review, see [6]). Indeed, after birth, intrinsic growth repressors, for example, of the Krüppel-like factor (KLF) transcription factor family, are upregulated in the developing retina, while growth-inducing transcription factors are downregulated in retinal ganglion cells and other CNS neurons [7]. Likewise, the neuronal tissue environment is growth enhancing during development and switches to largely growth inhibitory in the adult brain and spinal cord, in particular, in the white matter [8,9]. The presence of growth-repressing molecules in the CNS tissue and within the neurons, and the lack of adequate stimulation and growth responses by adult neurons are currently thought to be the predominant causes of axon regeneration failure.

Although many families of molecules have been tested for stimulating effects on axonal regeneration, such as neurotrophins or cytokines, only a few of them are able to promote long axonal regrowth *in vivo* significantly. Many of these experiments were done using the optic nerve model. Robust axonal growth was first reported after experimental lens injury or injection of yeast cell walls (zymosan) in the rat eye [10,11]. The molecular mechanisms underlying these effects implicate macrophage/neutrophil-derived factors, for example, oncomodulin [12,13], and the release of cytokines such as ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) by reactive glial cells [14,15]. Indeed, the growth state of injured adult retinal ganglion cells could be increased by repeated injections of recombinant CNTF in the intraocular space [15], or directly by the intracellular activation of the downstream cytokine signaling component Stat3 in the retinal ganglion cells [15,16]. The effects of CNTF protein injections appeared relatively modest, probably because of the short half-life of the peptide, and stronger axonal sprouting and growth was obtained by the expression of AAV2–CNTF in retinal ganglion cells [17], or infection of Müller glia by an AAV variant called ShH10 carrying CNTF [18] (Figure 1A,B). Both procedures elicited long-distance regrowth of severed optic nerve fibers up to 4–5 mm, near or until the optic chiasm, but rarely beyond [18]. This extent of growth was comparable to that observed after the deletion of the suppressor of cytokine signaling (SOCS)3 gene [19], which encodes a protein involved in the negative feedback loop of Stat3 activation. Intracellularly, activation of cytokine receptors and Stat3 triggers a complex cascade of events; Stat3 influences the transcription of growth-associated genes, but also stabilizes the neuronal cytoskeleton [20,21]. For example, Stat3 can bind to and block the microtubule-destabilizing protein Stathmin and thereby promote microtubule elongation in neurons [20,21]. Stabilization of microtubules can enhance the growth of axons in the optic nerve and spinal cord [14,22].

Corresponding authors: Pernet, V. (pernet@hifo.uzh.ch); Schwab, M.E. (schwab@hifo.uzh.ch).

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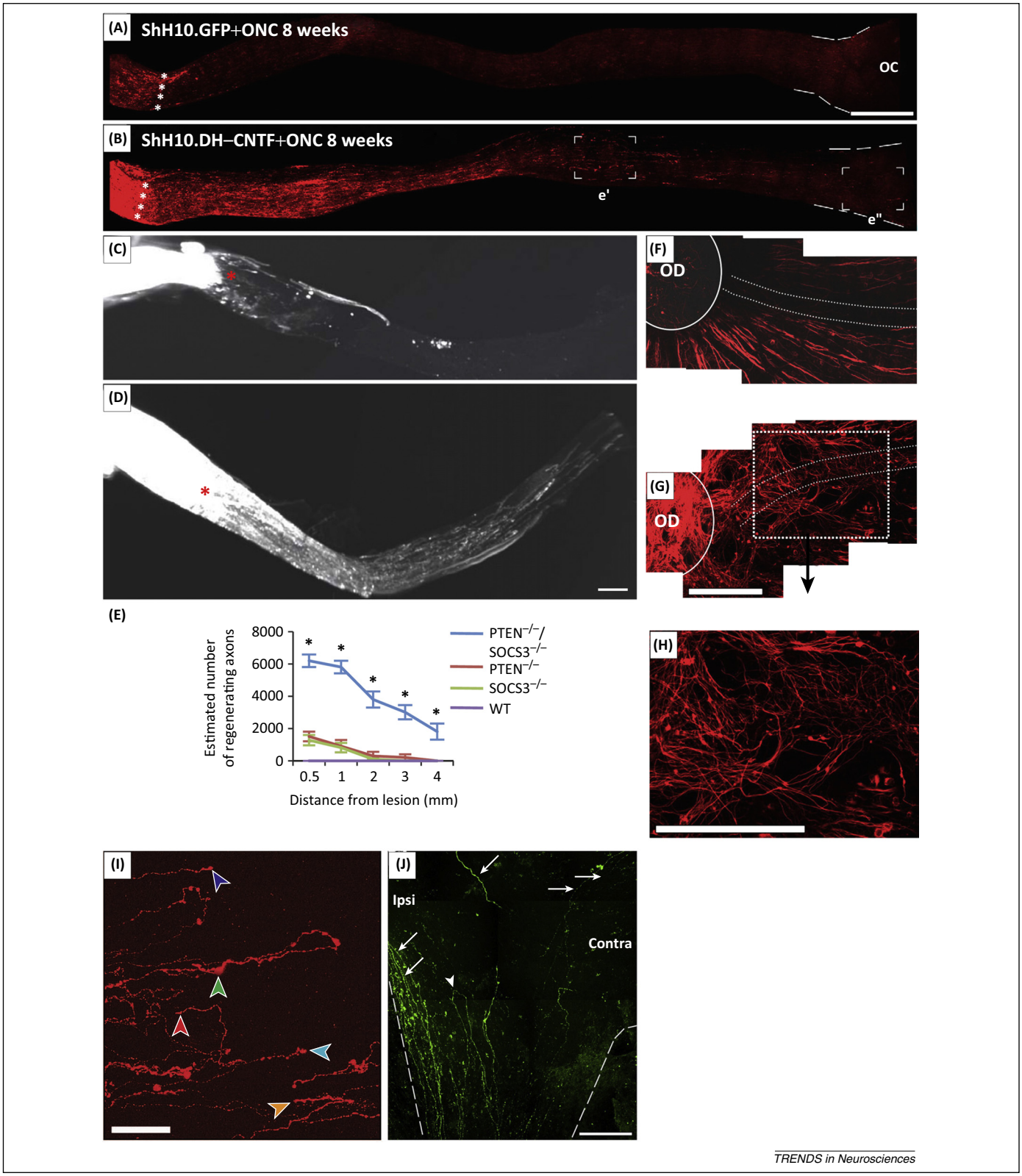

Figure 1. Anatomical abnormalities in long-distance axonal regeneration induced by the Stat3 and mTOR pathways. **(A,B)** On tissue sections, stimulation of RGCs by CNTF (ShH10.DH-CNTF) allowed many axons to extend in the mouse optic nerve 8 weeks after injury compared with control mice treated with ShH10.GFP virus. Few axons reached the OC. **(C,D)** SOCS3 and PTEN co-deletion in RGCs induced robust axonal regeneration 17 days after optic nerve crush. **(E)** Quantitatively, SOCS3/PTEN gene inactivation promoted stronger axonal regeneration than single gene ablation taken separately at 4 weeks post-lesion. However, the two-gene deletion revealed a drop-off in the number of growing axons in the middle of the optic nerve, that is, 2 mm past the lesion site. (F–H) Long-lasting stimulation of injured RGCs with CNTF caused massive aberrant axonal outgrowth at the surface of the retina and around the OD, 8 weeks after injury **(G)** compared with control injured mice **(F)**. In **(H)**, a high-magnification picture of β3 tubulin-stained axons shows disorganized axonal outgrowth. **(I)** Two weeks after optic nerve crush, the limited axonal regeneration induced by RGC transfection with Stat3 was correlated with a high number of axonal U-turns at the regeneration front, in the whole cleared optic nerve. **(J)** Continuous stimulation of injured RGCs with CNTF in the retina allowed few AAV2 GFP-labeled axons to reach the optic chiasm 6 months after injury. Axons remained abnormally ipsilateral and occasionally formed U-turns (arrowhead). Scale bars: (A,B) 400 μm; (C,D) 200 μm; (F–H) 200 μm; (I) 50 μm; (J) 100 μm. Adapted, with permission, from [18] (A,B,F–H,J), [28] (C,D), [30] (E), and [16] (I). AAV, adeno-associated virus; CNTF, ciliary neurotrophic factor; mTOR, mammalian target of rapamycin; OC, optic chiasm; OD, optic disk; ONC, oncomodulin; PTEN, phosphatase and tensin homolog; RGC, retinal ganglion cell; SOCS, suppressor of cytokine signaling; Stat, signal transducer and activator of transcription; WT, wild type.