Nerve injury signaling
Namiko Abe and Valeria Cavalli

Although neurons within the peripheral nervous system (PNS) have a remarkable ability to repair themselves after injury, neurons within the central nervous system (CNS) do not spontaneously regenerate. This problem has remained recalcitrant despite a century of research on the reaction of axons to injury. The balance between inhibitory cues present in the environment and the intrinsic growth capacity of the injured neuron determines the extent of axonal regeneration following injury. The cell body of an injured neuron must receive accurate and timely information about the site and extent of axonal damage in order to increase its intrinsic growth capacity and successfully regenerate. One of the mechanisms contributing to this process is retrograde transport of injury signals. For example, molecules activated at the injury site convey information to the cell body leading to the expression of regeneration-associated genes and increased growth capacity of the neuron. Here we discuss recent studies that have begun to dissect the injury-signaling pathways involved in stimulating the intrinsic growth capacity of injured neurons.

Addresses
Department of Anatomy and Neurobiology, Washington University in St. Louis, 660 S Euclid Avenue, St. Louis, MO 63110-1093, USA

Corresponding author: Cavalli, Valeria (cavalli@pcg.wustl.edu)

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Introduction
The extremely polarized morphology of neurons (i.e. axon length extending for up to 1 m) poses challenging problems for intracellular-signaling pathways. Information about distant injury, for example, has to be communicated to the cell body to initiate a proper regenerative response. Research on nerve regeneration has classically focused on identifying the inhibitory factors present in the environment, which include the glial scar and molecules such as Nogo and myelin-associated glycoprotein [1]. We know much less about the mechanisms that activate the intrinsic growth capacity of neurons following injury. Upon embryonic to adult transition, the intrinsic neuronal growth activity is repressed to allow for proper synaptic development. Injury to adult peripheral neurons, but not to central nervous system (CNS) neurons, reactivates the intrinsic growth capacity and allows regeneration to occur. Primary sensory neurons with cell bodies in the dorsal root ganglion (DRG) provide a useful model system to study the mechanisms that regulate regeneration. DRG neurons are pseudobipolar neurons and possess two axonal branches: a peripheral axon that regenerates when injured and a centrally projecting axon that does not regenerate following injury. Remarkably, injury to the peripheral branch before injury to the central branch promotes regeneration of central axons [2,3]. This phenomenon is referred to as the ‘conditioning lesion’ paradigm (Figure 1) and indicates that retrograde injury signals travel from the peripheral injury site back to the cell body to increase the intrinsic growth capacity of the neuron. An increased intrinsic growth state as a result of a preconditioning lesion may enable centrally injured axons to regenerate. A series of elegant studies in the early 1990s in the mollusk Aplysia californica provided evidence for the existence of multiple injury signals functioning in a temporal sequence [4]: injury-induced discharge of axonal potentials, interruption of the normal supply of retrogradely transported target-derived factors (also called negative injury signals) and retrograde injury signals traveling from the injury site back to the cell body (also called positive injury signals) (Figure 2).

The retrograde transport of injury signals is one of the essential cellular mechanisms leading to regeneration. Coordination between several injury-signaling pathways is necessary to regulate the appropriate genes to promote neuronal survival and increase the intrinsic growth state of injured neurons. In this review, we discuss recent studies that departed from the traditional focus on extrinsic factors and uncovered distinct signaling mechanisms leading to the enhanced intrinsic growth capacity of peripheral neurons following injury.

Axonal injury signaling
Positive injury signals
The positive injury signals identified thus far cover a broad array of functionally distinct proteins that include members of the mitogen-activated protein kinase family (MAPK), cytokines, and their downstream transcription factors, as well as locally translated importin, a main regulator of nuclear import and export.

Axonal transport of several kinases was initially suggested to play a role in relaying information from the nerve terminal to the cell body [5]. It is now known that axonal
injury induces local activation and retrograde transport of several MAPKs, including Erk [6**,7], the c-Jun N-terminal kinase (JNK) [8**,9], and the protein kinase G [10]. These studies strongly suggest that activation of kinases, in particular JNK and Erk and their interaction with the dynein/dynactin retrograde molecular motors is required for regeneration [6**,8**]. Transport of such injury signal is complicated by the fact that many kinases including JNK and Erk are activated by reversible phosphorylation and without proper protection this signal may not persist. A key question is then how to prevent deactivation of the signal during the long journey to the cell body. One elegant solution is to protect the signal with scaffolding proteins. For example, it has been recently shown that the intermediate filament vimentin interacts with phosphorylated Erk1 to protect it from dephosphorylation by calcium-dependent steric hindrance [11**]. Another mechanism proposed to protect dephosphorylation is storage within intraluminal vesicles of multivesicular bodies [12]. Indeed, kinases such as JNK can hitchhike on axonal vesicles [8**] and intraluminal vesicles are not always destined to lysosomes for degradation; they can also fuse back with the limiting membrane of late endosomes [13]. This process is hijacked by several toxins and viruses to reach the cell body and could similarly be exploited by signaling proteins. Combined with a protection mechanism against phosphatases during transport, activation and retrograde transport of MAPKs might play an important role in regeneration. The upstream signaling cascade leading to MAPK activation in the axon remains yet to be established.

In addition to MAPK, axonal injury activates several transcription factors through the local release of cytokines. These include the gp130 cytokines leukemia inhibitory factor (LIF), interleukine-6 (IL-6), and ciliary neurotrophic factor (CNTF). LIF and IL-6 are required for the increased growth state of DRG neurons following peripheral injury through activation of downstream genes such as GAP43 [14,15], although Cao et al. [16] reported that IL-6 knockout animals do not show defects in nerve regeneration. Upregulation of IL-6 in DRG cell bodies themselves following injury [16**,17,18**] raises the possibility of paracrine or autocrine action of IL-6, which may amplify a cytokine-induced retrograde signal. The gp130 cytokines signal through a common receptor, gp130, and the JAK-STAT pathway, which leads to STAT3 phosphorylation and translocation into the nucleus [19]. Although retrograde transport of locally activated STAT3 has been suggested [20,21], in vitro
Figure 2

Signaling mechanisms. The cell body of injured neurons must receive accurate and timely information on the site and extent of axonal damage in order to orchestrate an appropriate response leading to successful regeneration. Pioneering work from the laboratories of Ambron and Walters have led to the notion that three distinct signaling mechanisms may act in complementary and synergistic roles: (1) injury-induced discharge of axonal potentials, (2) interruption of the normal supply of retrogradely transported trophic factors or negative regulators of neuronal growth from the target, and (3) retrograde transport of activated proteins emanating at the injury site, termed positive injury signals.

The positive injury signals identified so far share one common requirement: microtubule-dependent retrograde transport. Future studies will probably identify new molecules involved in injury signaling. It is tempting to speculate that the combination of several positive injury signals might serve as an indicator of the extent and nature of damage.

**Negative injury signals**

Although loss of negative cues represents another important mechanism to sense injury, surprisingly little is known about this type of signaling. Once a neuron is connected with its target, target-derived signals must repress the intrinsic neuronal growth activity to allow for proper synaptic development. This repression has to be relieved to allow regeneration to occur. Although neurotrophins represent the ideal candidates, evidence for their role as...
negative signals following injury have not yet been established. One recently identified negative injury signal is the TGF beta/SMAD2/SMAD3 pathway. SMAD2 is downregulated following peripheral nerve injury, indicating that SMAD2-dependent gene transcription may restrict the axonal growth ability in healthy neurons and injury may relieve this inhibition. Whether SMAD2/SMAD3 contributes to the decreased regenerative ability of adult CNS neurons remains to be determined. The transcription factor ATF-2 is also rapidly suppressed in neurons following injury [29]. Similarly to SMAD2, ATF2-dependent gene transcription may repress neuronal growth capacity. Future studies are needed to explore the role of negative injury signals in axonal regeneration.

**Electrical activity**

Recent data suggest an important role of neural activity in regeneration. The transection of axons initiates a large depolarizing voltage discharge that travels back to the soma and triggers vigorous spiking activity and sustained depolarization [30]. This extensive electrical activity produces a strong calcium influx in both the axon and the soma. Propagation of this response requires the activation of voltage-gated sodium channels and is necessary for regeneration, since axotomy in the presence of tetrodotoxin reduces the regenerative process [30]. Calcium influx is also necessary for regeneration in vitro and is likely to act through protein kinases such as ERK or PKA [31]. In vivo studies showed that electrical stimulation accelerates motor [32] and sensory [33] axon outgrowth.
and increases intracellular cAMP levels in DRG neurons as effectively as the conditioning lesion [33]. However, electrical stimulation did not recapitulate all characteristics of axonal outgrowth, indicating that cAMP alone is not sufficient to trigger a complete regenerative response [33]. In marked contrast, electrical stimulation of CNS axons does not promote regeneration, even when provided a permissive growth environment through a peripheral nerve graft [34]. Electrical activity thus may play an important role as an early injury signal in the peripheral nervous system (PNS), but might be insufficient to initiate regeneration of CNS neurons.

**Signaling mechanisms in CNS axons**

Induction of retrograde injury signals has so far been demonstrated in peripheral neurons. Recent studies in CNS neurons of the retina have unravelled the existence of parallel mechanisms between CNS and PNS neurons and demonstrated that the growth capacity of CNS neurons can be enhanced. While retinal ganglion cells (RGCs) normally fail to regenerate their injured axons, lens injury activates macrophages and stimulates regeneration of RGCs [35] in a process that resembles the conditioning injury in DRG neurons. Soluble factors released by activated macrophages, such as oncomodulin, are sufficient to promote RGC regeneration through a Ca\(^{2+}\)/calmodulin-dependent pathway [35]. While oncomodulin promotes neurite outgrowth in cultured central and peripheral neurons [36], its role in sensory nerve regeneration has yet to be explored in vivo. Lens injury also induces upregulation of CNTF in retinal astrocytes, a process that is independent of macrophages, and leads to STAT3 activation in RGCs [37,38]. The cytokine-mediated activation of STAT3 is a central injury signaling mechanism in PNS neurons, suggesting another possible parallel between the responses of CNS and PNS neurons to injury. To elucidate molecular factors responsible for the poor regenerative capacity of the CNS neurons, it will be important to determine whether CNS neurons lack the ability to activate or transport injury signals, are unable to relieve the growth inhibition brought about during their maturation or are less responsive to cytokines and other injury induced stimuli.

**Somatic injury signaling**

**Role of cAMP**

Elevation of cAMP levels in the soma following axonal injury to peripheral neurons contributes to the initiation of axonal regrowth (for a recent review, see [39]). cAMP not only increases the growth capacity of injured neurons but also partly relieves CNS myelin inhibition. The increase in cAMP levels appears to be transient and initiates a series of signaling pathways involving PKA [40]. The effects of cAMP are transcription dependent [41] and require the transcription factor cAMP response element binding protein (CREB) [42]. Interestingly, CREB mRNA is present in developing axons and CREB translation and retrograde transport is triggered by the nerve growth factor (NGF) [43]. Whether CREB translation may play a role in injury signaling in adult neurons remains to be determined. Downstream targets of cAMP signaling include Arginase1, which mediates synthesis of polyamines [44], neuropeptide Y, CREM (cAMP response element modulator), VGF (NGF-inducible growth factor), and IL-6 [16,18]. Some of these genes were also identified in studies comparing the pattern of gene expression at different times after sciatic nerve transection [26,45], revealing a temporal hierarchy of gene activation following injury. Although cAMP analogs fail to activate the intrinsic growth state of RGCs [46] they potentiate the effect of lens injury [38], indicating that multiple pathways act in parallel to stimulate RGCs regeneration. Although a direct link between retrograde signaling in axons and elevation of cAMP in cell body of injured neurons is still lacking, these results strongly suggest that the intrinsic growth capacity of the CNS neurons can be enhanced under appropriate conditions.

**Transcription factors**

Initiation of a regeneration program requires that retrograde signals from the injury site alter transcription of multiple genes [41]. Members of the immediate-early genes family, including c-Jun and JunD [47,48], as well as members of the constitutive transcription factors CREB, STAT3, SOX11, and ATF3 [23,42,49,50] are elevated and in some cases also activated in DRG cell bodies after peripheral injury. The activation of c-Jun in the cell body is essential for the initiation of transcriptional changes required for successful axonal regeneration. Some of the identified c-Jun-dependent genes include integrin α7β1, CD44, and galanin [51]. Deletion of c-Jun in the nervous system, while causing little effect on axonal growth during development, leads to a marked defect in regeneration upon nerve transection [51]. The importance of c-Jun for regeneration also comes from the observation that c-Jun activation in DRGs is drastically greater following peripheral versus central branch axotomy [52] and c-Jun activation persists until successful target reinnervation has been achieved [53,54]. The time course of c-Jun induction depends on the distance between the injury site and the cell body [53], suggesting that JNK activation in the axon may lead to c-Jun expression in the cell body [8]. Similarly to c-Jun, ATF3, and STAT3 are induced in DRG neurons after peripheral, but not central injury [23,55]. Overexpression of ATF-3 in cultured neurons enhances neurite outgrowth [55] and transgenic expression of ATF3 can partially recapitulate a conditioning injury [56]. Conditional gene disruption of STAT3 indicates that this gene may contribute to the survival of motor neurons after peripheral nerve lesion through activation of motor neuron survival factors such as Reg-2 and Bel-xl [57], but a direct role on nerve regeneration per se has not been demonstrated. In vitro studies show that another transcription factor Sox11 is expressed at high
levels in developing and regenerating sensory neurons and regulates neurite outgrowth and cell survival [58]. Although the identity of the genes activated by injury is being unravelled, the overall sequence and coordination of transcriptional events that initiate and sustain a regeneration program awaits further studies.

**Neurotrophins**

Neurotrophic signaling is mostly known to play a role in neuronal survival during development. The function of neurotrophins has been recently extended to other aspects of neuronal function, including regeneration [59]. Upregulation of the glial-derived neurotrophic factor GDNF and one of its receptors GFRα1 in injured nerves suggest that GDNF provides neurotrophic support for injured DRG neurons [60]. GDNF delivery directly to DRG cell bodies facilitates the conditioning injury-induced growth promoting effect [61]. Although GDNF and GFRα1 are retrogradely transported in peripheral axons [62], a role of GDNF in injury signaling has not yet been examined. Fibroblast growth factor-2 (FGF-2) is another neurotrophic factor contributing to nerve regeneration [63]. FGF-2 is upregulated following injury both at the lesion site and in the cell bodies of peripheral nerves and transgenic mice overexpressing FGF2 show a greater increase in the number of regenerating axons after sciatic nerve transection [64]. The presence of neurotrophin signaling in injured nerve emphasizes the signaling crosstalk that is required to promote neuronal survival and regeneration.

**Conclusions**

A single signaling pathway is unlikely to fully mediate nerve regeneration. Several classes of injury signals may coexist to ensure precise information on the nature of the damage and its distance from the cell body (Figure 3). It is tempting to speculate that the difference in time between the arrival at the soma of the back propagating axonal depolarization — the first injury signal, and the later arrival of a positive injury signal might serve as an indicator of the distance of the injury site from the cell body. However, a clear link between the arrival of injury signals and specific gene activation is still missing. Ultimately, a direct comparison between injury-signaling mechanisms in CNS and PNS neurons might shed light on the poor regenerative capacity of CNS neurons. This knowledge will be essential to our understanding and ultimately treatment of many debilitating CNS disorders, since in addition to traumatic axonal damage resulting from spinal cord injury or stroke, axonal damage can also occur in many neurodegenerative diseases in which axonal pathologies interrupt the cell body/synapse connection.

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**References and recommended reading**

Paper of particular interests, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

This study identifies SOC3 as an inhibitor of STAT3 activation, and a negative regulator of neurite outgrowth. Their results support a signaling endosome model in which the LIF/C15/C15 cytokine signaling is triggered by axonal injury and that IL-6 is sufficient to mimic the conditioning effect on DRG neurite outgrowth. Contrary to the findings of Cafferty et al. [15], the authors of this study show that IL-6 is not necessary for the enhancement of nerve growth state by a peripheral conditioning injury.


This report links the beneficial effects of cAMP activity on axonal regeneration to cytokine signaling. The authors show that addition of cAMP into DRGs induces the expression of neuroepoietic cytokines IL-6 and LIF, which has been shown to be positive regulators of nerve regeneration.


The authors of this study use compartmentalized cultures of sympathetic neurons to investigate the mechanism of LIF-mediated retrograde signal activation. Their results support a signaling endosome model in which the LIF/C15/C15/Jak complex is internalized at the distal axon and retrogradely transported to mediate STAT3 activation in the cell body.


The authors demonstrate that electrical stimulation in the intact sciatic nerve mimics the conditioning-injury effect on DRG axon outgrowth, and that this effect of electrical stimulation is correlated with increased CAMP activity.


This study identified oncomodulin as a macrophage-derived secreted factor that promotes the regeneration of injured retinal ganglion cells. The study further shows that oncomodulin can stimulate neurite outgrowth in cultured DRG neurons.


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43. Cox LJ, Hengst U, Gurskaya NG, Lukyanov KA, Jaffrey SR:

The authors of this study show that axonal protein synthesis is required for NGF-dependent cell survival in developing sensory neurons. They further show that the transcription factor CREB is locally translated by NGF signaling and that newly synthesized CREB is retrogradely transported to the cell body.


Using transgenic mice that constitutively express ATF-3 in neurons, the authors of this study show that ATF-3 increases axonal growth capacity following a sciatic nerve conditioning injury.


This study provides extensive in vitro evidence for the role of Sox11 in the regulation of neuronal survival and neurite outgrowth in sensory neurons. DNA microarray analysis from neuroblastoma cells shows that Sox11 regulates the expression of apoptosis-related genes.


This study explores the role of GDNF in peripheral nerve regeneration. The authors show that peripheral nerve injury upregulates components of GDNF signaling, and that exogenous GDNF treatment enhances the effect of conditioning-injury on neurite outgrowth.


This carefully conducted study uses transgenic mice overexpressing FGF-2 to address the contribution of FGF-2 to nerve regeneration. The authors find that FGF-2 overexpression facilitates peripheral nerve regeneration following crush injury by regulating axonal growth, Schwann cell proliferation and axonal myelination.