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Nerve injury signalling

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Summary

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 determine 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 signalling pathways involved in stimulating the intrinsic growth capacity of injured neurons.

Introduction

The extremely polarized morphology of neurons (i.e. axon length extending for up to one meter) 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 CNS neurons, reactivate 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 prior to 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

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1990s in the mollusk *Aplysia californica* provided evidence for the existence of multiple injury signals functioning in a temporal sequence [4] (Figure 2): 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).

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 signalling mechanisms leading to the enhanced intrinsic growth capacity of peripheral neurons following injury.

Axonal injury signalling

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 signalling 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), interleukin-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 and colleagues [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 [16–18] 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* studies using compartmentalized cultures suggest a signaling endosome model in which the gp130/JAK complex is endocytosed and retrogradely transported to activate STAT3 in the cell body [22]. Interestingly, STAT3 activation through the Jak2 signalling pathway occurs in DRG neurons cell body after peripheral, but not central lesion [23,24], strongly supporting a role for STAT3 in neuronal regeneration. While STAT3 signalling promotes axonal re-growth, *in vitro* studies showed that suppressor of cytokine signaling (SOCS3) inhibits STAT3 [25] and SOCS3 levels are increased by peripheral injury [25,26]. While the influence of endogenous SOCS3 on axonal growth in peripheral neurons may be limited, SOCS3 may contribute to the lack of regeneration in CNS neurons [25]. The pathways leading to STAT3 activation are partially understood but the downstream targets of the cytokine-STAT3 signalling remains to be clearly defined.

Work over the past ten years has confirmed that axons have the capacity to locally synthesize proteins [27]. Axonal mRNA translation plays a role in axonal growth during development [27] and mature neurons use axonal mRNA translation to transfer injury signals to the nucleus of injured neurons. Following peripheral nerve injury, *de novo* synthesis of importin-beta [7] and vimentin [6] leads to the formation of an importin-activated Erk-vimentin complex that recruits the retrograde motor dynein, linking the nuclear import machinery to retrograde injury signaling [6]. Since a surprisingly large population of mRNAs localizes to sensory axons [28], future studies will reveal the possible role for other *de novo* synthesized proteins in injury signaling.

The positive injury signals identified so far share one common requirement, microtubule-dependent retrograde transport. Future studies will likely 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 signalling. 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 (Zhigang He). SMAD2 is down-regulated 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 PNS, but might be insufficient to initiate regeneration of CNS neurons.

Signalling 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 unraveled 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 resemble the conditioning injury in DRG neurons. Soluble factors released by activated macrophages, such as oncomodulin, are sufficient to promote RGC regeneration through a Ca²⁺/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 signalling

Role of cAMP

Elevation of cAMP levels in the soma following axonal injury to peripheral neurons contributes to initiation of axonal re-growth (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 (nerve growth factor-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 signalling 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 $\alpha7\beta1$, 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 come from the observation that c-Jun activation in DRGs is drastically greater following peripheral vs. central branch axotomy [52] and c-Jun activation persists until successful target re-innervation 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 [8,9]. 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 Bcl-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]. While the identity of the genes activated by injury is being unraveled, 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 GFR α 1 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 GFR α 1 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 over-expressing 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. 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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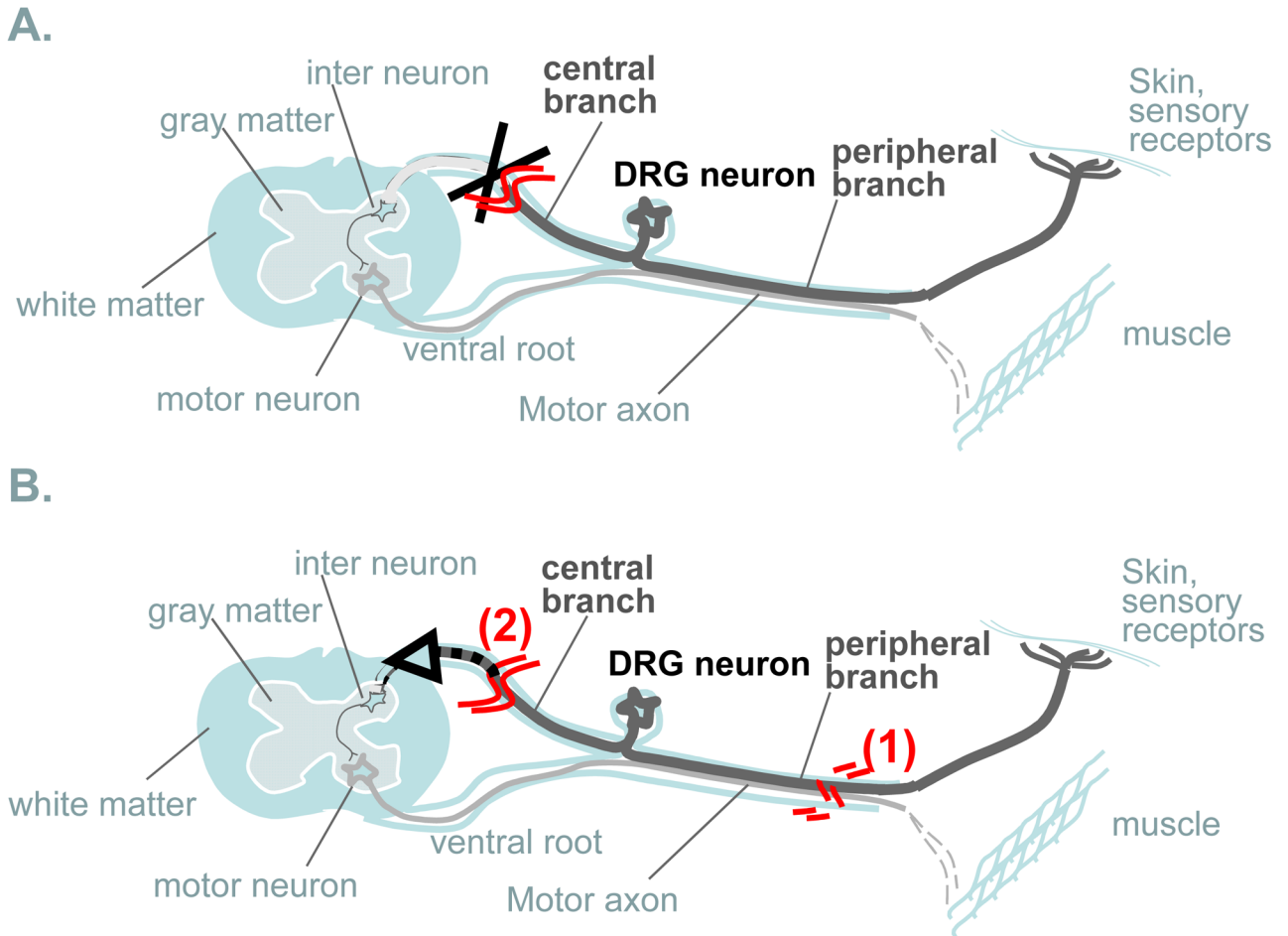


Figure 1. Signalling mechanisms

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.

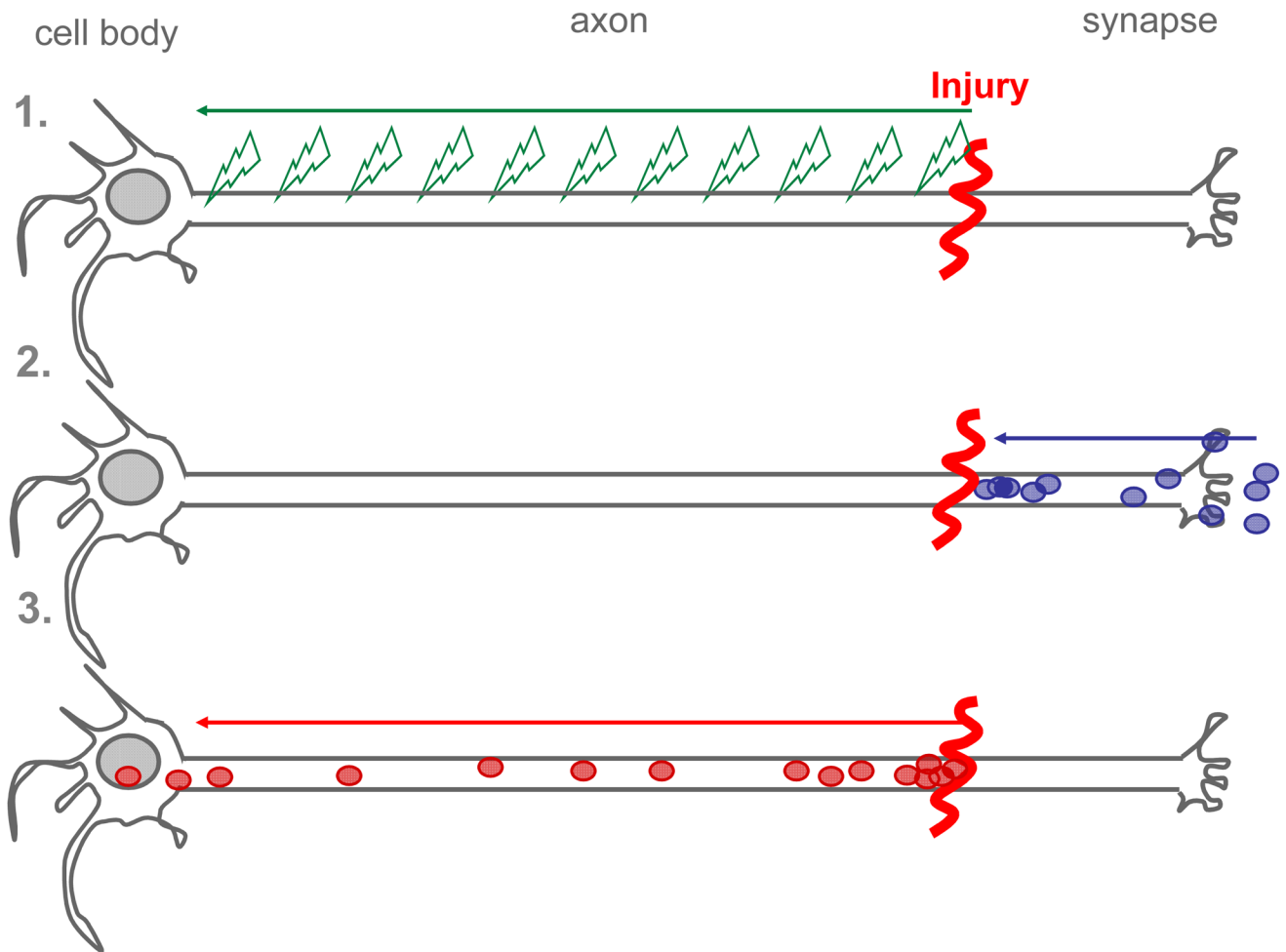


Figure 2. Conditioning injury paradigm

Primary sensory neurons within dorsal root ganglia (DRG) are particularly useful to study axonal regeneration. DRG neurons are unique in having two axonal branches; a long sensory CNS branch ascends the dorsal column in the spinal cord and a second branch projects through a peripheral nerve. Sensory axons in the adult spinal cord do not regenerate after injury (A), while peripheral injury result in a robust regenerative response. Regeneration of the central branch can be greatly enhanced by a prior injury to the peripheral branch, referred to as a “conditioning injury” (B). The conditioning injury suggests that distinct signaling mechanisms regulate responses to central vs. peripheral injury in DRG neurons and may contribute to their different abilities to axonal regrowth.

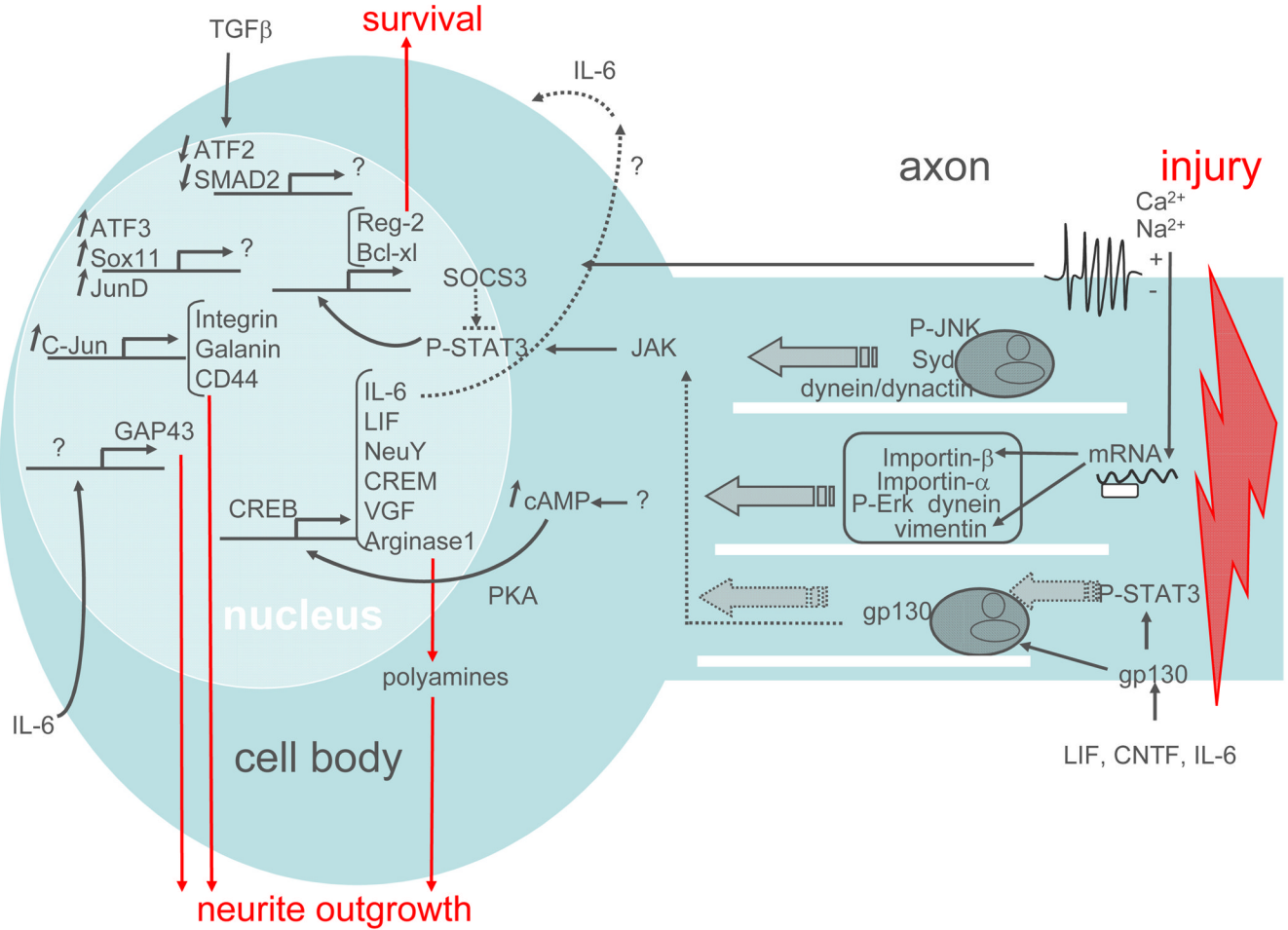


Figure 3. Activation of the intrinsic growth capacity by peripheral injury

Nerve injury triggers multiple signalling events in the axon, including membrane depolarization, JNK activation, mRNA translation, and cytokine-mediated STAT3 activation. These events lead to the microtubule-based retrograde transport of signaling molecules back to the cell body (shown by plain arrows). When these signalling molecules reach the cell body, they mediate the expression of a number of transcription factors that regulate the expression of genes involved in cell survival and neurite outgrowth. These downstream targets also include some components of the injury signal, such as IL-6 and LIF, which may amplify the injury signal via positive feedback.